Biotechnology in Agriculture and Forestry 70

Jack M. Widholm · Jochen Kumlehn · Toshiyuki Nagata
Series Editors

Hiroshi Ezura
Tohru Ariizumi
Jordi Garcia-Mas
Jocelyn Rose *Editors*

Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops

Springer

Biotechnology in Agriculture and Forestry

Volume 70

Series editors
Prof. Dr. Jack M. Widholm (Managing Editor)
285A E.R. Madigan Laboratory, Department of Crop Sciences,
University of Illinois, 1201 W. Gregory, Urbana, IL 61801, USA

Dr. Jochen Kumlehn
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK),
Head, Plant Reproductive Biology, Corrensstr. 3,
06466 Gatersleben, Germany

Prof. Dr. Toshiyuki Nagata
Professor and Dean, Faculty of Biological Sciences and Applied Chemistry,
Hosei University, 3-7-2 Kajino-cho, Koganei-shi, Tokyo 184-8584,
Japan; Emeritus Professor of the University of Tokyo, 7-3-1 Hongo,
Bunkyo-ku, Tokyo 113-0033, Japan

More information about this series at http://www.springer.com/series/798

Hiroshi Ezura • Tohru Ariizumi •
Jordi Garcia-Mas • Jocelyn Rose
Editors

Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops

Editors
Hiroshi Ezura
Faculty of Life and Environmental Sciences
University of Tsukuba
Tsukuba, Japan

Tohru Ariizumi
Faculty of Life and Environmental Sciences
University of Tsukuba
Tsukuba, Japan

Jordi Garcia-Mas
IRTA, Center for Research in Agricultural Genomics CSIC-IRTA-UAB-UB
Barcelona, Spain

Jocelyn Rose
Plant Biology Section, School of Integrated Plant Science
Cornell University
Ithaca, New York
USA

ISSN 0934-943X
Biotechnology in Agriculture and Forestry
ISBN 978-3-662-48533-0 ISBN 978-3-662-48535-4 (eBook)
DOI 10.1007/978-3-662-48535-4

Library of Congress Control Number: 2015957962

Springer Berlin Heidelberg New York Dordrecht London
© Springer-Verlag Berlin Heidelberg 2016
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

Springer-Verlag GmbH Berlin Heidelberg is part of Springer Science+Business Media (www.springer.com)

Preface

The 8th Joint Conference on Solanaceae Genomics (SOL) and the 2nd International Cucurbit Genomics Initiative (ICuGI) were held in Kobe, Japan, from November 28 to December 2, 2011, organized by the 178th Committee on Plant Molecular Design, University-Industry Research Cooperation Societally Applied Scientific Linkage and Collaboration of Japan Society for the Promotion of Science (http://plantmdc.gene.tsukuba.ac.jp).

The annual SOL genomics workshop began after the meeting in Washington, DC, USA, on November 3, 2003, to initiate an international collaboration entitled the International Solanaceae Genome Project. The SOL achieved the whole-genome sequencing of tomato cv. Heinz 1706 in 2012, and the information and related tools are available through the SOL Genomics Network (http://solgenomics.net/). The ICuGI was initiated after the meeting in Barcelona, Spain, on June 30–July 1, 2005, as an international collaboration to establish genomic information and functional genomics tools for Cucurbit crops. Reference sequences of the cucumber (2009), melon (2012), and watermelon (2013) have been obtained. This information is available through the Cucurbit Genomics Database (http://www.icugi.org/cgi-bin/ICuGI/index.cgi).

The *Solanaceae* and *Cucurbitaceae* families include many edible vegetable crops that are among the most widely represented horticultural species. The conference provided many opportunities for scientists to interact with colleagues working in different and related areas and guided us toward elucidating the evolutionary history of, and the genetic diversity between, *Solanaceae* and *Cucurbitaceae*. We also believe that the joint conference provided us with approaches to addressing questions such as "What is the next step for plant genomics research?," "What can we learn from large volumes of sequencing data?," and "How can we use this information for plant improvement?" Thanks to the latest technical advancements in sequencing equipment and bioinformatics, we are now able to determine the genome sequences of cultivars, variations, and wild species and to investigate comprehensive gene fluctuations using whole-transcriptome shotgun sequencing (also called RNA-seq). The genome sequencing

projects of several key members are ongoing. One of the major goals of the conference was to explore the ideas, strategies, and methodologies by which we can use this information in our studies and eventually benefit human lives by improving global food security.

More than 300 delegates from over 22 countries attended the joint conference, and more than 200 presentations were made. These numbers were amazing for us when considering our situation after the disaster on March 11, 2011, in Japan. We believe the Joint Conference uplifted Japanese scientists and even Japanese society. Drawing from the presentations and related research, we invited several authors to prepare review chapters and prepared this volume for the book series.

I thank the authors of the chapters in this volume for their contributions and thoughtful insights regarding the current research and developments in this field. I hope that these chapters will serve as a valuable resource for advancing our basic and technical knowledge on *Solanaceae* and *Cucurbitaceae* research and breeding. Finally, I thank Prof. Toshiyuki Nagata, the Editor-in-Chief of this book series, for providing the timely opportunity to prepare this volume. The editing of this book was supported by my coeditors, Tohru Ariizumi (University of Tsukuba, Japan), Jordi Garcia-Mas (IRTA, Spain), and Joclyn K. C. Rose (Cornell University, USA).

Tsukuba, Japan H. Ezura

Contents

1 **Tomato Genome Sequence** 1
Shusei Sato and Satoshi Tabata

2 **Melon Genome Sequence** 15
Jordi Garcia-Mas and Pere Puigdomènech

3 **Databases for Solanaceae and Cucurbitaceae Research** 31
Masaaki Kobayashi, Hajime Ohyanagi, and Kentaro Yano

4 **DNA Markers in Solanaceae Breeding** 43
Hiroyuki Fukuoka

5 **DNA Markers in Cucurbitaceae Breeding** 59
Nobuko Fukino and Yoichi Kawazu

6 **Mutant Resources and TILLING Platforms in Tomato Research** . . . 75
Yoshihiro Okabe and Tohru Ariizumi

7 **Tomato Fruit Set and Its Modification Using Molecular Breeding Techniques** 93
Yoshihito Shinozaki and Kentaro Ezura

8 **Fruit Growth in Tomato and Its Modification by Molecular Breeding Techniques** 113
Lamia Azzi, Frédéric Gévaudant, Frédéric Delmas, Michel Hernould, and Christian Chevalier

9 **Sugar Accumulation in Tomato Fruit and Its Modification Using Molecular Breeding Techniques** 141
Chiaki Matsukura

10 **Fruit Ripening in Tomato and Its Modification by Molecular Breeding Techniques** 155
Kyoko Hiwasa-Tanase

11 Disease Resistance in Melon and Its Modification by Molecular Breeding Techniques 175
Michel Pitrat

12 Insect Resistance in Melon and Its Modification by Molecular Breeding 199
Catherine Dogimont and Nathalie Boissot

13 Genetic Engineering of Important Breeding Traits in Solanaceae and Cucurbitaceae 221
Satoko Nonaka and Hiroshi Ezura

14 Genome-Editing Technologies and Their Use in Tomato 239
Jeong-Eun Lee and Hiroshi Ezura

15 Toward In Silico Design and Engineering of Solanaceae and Cucurbitaceae Crops 251
Hiroshi Ezura

Index 259

Chapter 1
Tomato Genome Sequence

Shusei Sato and Satoshi Tabata

1.1 Introduction

The Solanaceae is a large family consisting of approximately 100 genera and 2500 species that grow in all habitats from rainforests to deserts (Knapp 2002). The Solanaceae family includes several plants of agronomic importance, including potato, eggplant, pepper, and tobacco, as well as tomato (*Solanum lycopersicum*). As well as its economic importance, tomato is considered to be a useful model plant species and has been the subject of extensive research, including genetic characterization. Tomato was consequently chosen as a target for genome sequencing. *S. lycopersicum* has a diploid genome of simple architecture that is approximately 900 Mb in size and is distributed across 12 chromosomes (Michaelson et al. 1991). Many Solanaceae species have highly syntenic genomes, each also with 12 chromosomes, and the reference genome sequence of the tomato thus provides a framework for the genomic analysis of Solanaceae plants in general and is a source of important information for molecular breeding.

In November 2003, the International Solanaceae Project (SOL; http://solgenomics.net/solanaceae-project/index.pl), a consortium initially involving researchers from ten countries, launched the tomato genome-sequencing project. The initial aim was to sequence gene-rich regions of the 12 chromosomes through high-quality sequencing of bacterial artificial chromosomes (BACs) that were

S. Sato (✉)
Kazusa DNA Research Institute, 2-6-7 Kazusa-Kamatari, Kisarazu, Chiba 292-0818, Japan

Graduate School of Life Sciences, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan
e-mail: shuseis@ige.tohoku.ac.jp

S. Tabata
Kazusa DNA Research Institute, 2-6-7 Kazusa-Kamatari, Kisarazu, Chiba 292-0818, Japan
e-mail: tabata@kazusa.or.jp

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_1

selected based on DNA markers mapped on the genome and accumulated end-sequence information (Mueller et al. 2005, and http://sgn.cornell.edu/). In 2008, a whole-genome-sequencing strategy was also adopted, with the aim of covering the entire genome. Alongside the sequencing efforts, DNA markers were evaluated and high-density genetic linkage maps were constructed to assist assembly of the whole-genome structure (Fulton et al. 2002; Frary et al. 2005; Shirasawa et al. 2010). The chloroplast and mitochondrial genomes were sequenced independently of the nuclear genome (Kahlau et al. 2006). The International Tomato Annotation Group (ITAG) subjected the obtained sequences to assembly and performed further analyses. Ultimately, researchers from 14 countries contributed to the project, and the results were published in 2012 (The Tomato Genome Consortium 2012).

In this chapter, we summarize the process of tomato genome sequencing and the features of the tomato genome revealed by the obtained sequence information.

1.2 Tomato Genome Sequencing

The tomato genome, estimated to be 900 Mb long, has rather simple architecture composed of pericentromeric heterochromatin and distal euchromatin. Pericentromeric heterochromatin, rich in repetitive sequences, is estimated to occupy three-quarters of the tomato genome. The remaining one-quarter (220 Mb) of the tomato genome consists of distal euchromatic segments; these regions were thought to contain more than 90 % of the genes prior to the project. Therefore, the strategy of the initial phase of the tomato genome-sequencing project was to sequence the euchromatic portions of the 12 chromosomes using a BAC-by-BAC sequencing approach. The tomato variety used for the sequencing project was the ‘Heinz 1706’ cultivar, provided by the Heinz Corporation (Pittsburgh, PA, USA). ‘Heinz 1706’ was chosen because the well-characterized *Hin*dIII BAC library available at the time of project inception was constructed from this cultivar (Budiman et al. 2000). Two *Eco*RI and *Mbo*I BAC libraries were also constructed, and end sequences of all three BAC libraries were analyzed. In this approach, molecular genetic markers were used to anchor seed BAC clones. The tiling path was generated by walking from seed clones in both directions using the analyzed BAC end-sequencing data. This BAC-by-BAC approach resulted in the sequencing of 117 Mb of tomato euchromatic regions with high accuracy (Mueller et al. 2005).

In 2008, the sequencing consortium adopted the selected BAC mixture (SBM) approach with the aim of accelerating progress (The Tomato Genome Consortium 2012). A total of 30,800 BAC clones were selected having considered the BAC end-sequence data accumulated in the initial phase of the project and the removal of BACs that had repetitive elements at their ends. The chosen BAC clones were pooled, and shotgun sequencing was performed using the Sanger sequencing method. A total of 4.2 million reads corresponding to 3.1 Gb were produced, and these sequences were assembled into contigs that covered 540 Mb of the genome

and encompassed >80 % of the previously registered tomato ESTs (http://www.kazusa.or.jp/tomato/). The success of the shotgun approach prepared the way for a next-generation sequencing (NGS) approach.

In 2009, the sequencing consortium decided to take advantage of the emerging NGS platforms and increase the scope of the project from euchromatic regions only to the whole tomato genome. Three NGS platforms, Roche/454, SOLiD, and Illumina, were used to generate 21 Gb, 64 Gb, and 82 Gb of sequence data, respectively. A de novo assembly of the 'Heinz 1706' genome was subsequently performed using the Sanger data (3.3 Gb, including ~200,000 BAC and fosmid paired-end sequences and 4.2 million SBM reads) and 454 data (21 Gb). Two programs, Newbler and CABOG, were used to generate independent assemblies; these were subsequently integrated. The structural accuracy of the de novo assembly was confirmed by mapping to paired-end sequences of the BAC and fosmid clones. The high coverage Illumina and SOLiD reads were used to improve overall base accuracy. As a result of read-mapping and error-base correction, high-base accuracy was achieved, resulting in only one base calling error per 29.4 kb and one indel error per 6.4 kb. Contig gaps were filled by integrating 117 Mb of BAC-clone Sanger sequences from the initial phase of the project. The resulting high-quality scaffolds were linked with two BAC-based physical maps and anchored using a high-density genetic map (Shirasawa et al. 2010), introgression-line mapping, and genome-wide BAC fluorescence in situ hybridization (FISH). The final tomato genome assembly consisted of 91 scaffolds covering 760 Mb. The scaffolds were then aligned with the 12 chromosomes, and most of the gaps were found to be restricted to pericentromeric regions (Table 1.1). The 21 Mb of sequences

Table 1.1 Status of tomato genome sequence (Assembly SL2.40)

Chromosome	Number of scaffolds	Cumulative scaffold length (bp)	Average GC %
chr1	9	90,303,444	33.7
chr2	7	49,917,694	33.6
chr3	13	64,839,514	34.0
chr4	6	64,063,812	33.7
chr5	3	65,021,238	34.0
chr6	8	46,040,936	34.0
chr7	4	65,268,321	34.1
chr8	9	63,031,857	34.1
chr9	10	67,661,191	34.1
chr10	6	64,833,805	34.0
chr11	6	53,385,525	34.1
chr12	10	65,485,353	34.2
Subtotal anchored scaffolds	91	759,852,690	34.0
Unanchored scaffolds (chr0)	3132	21,492,721	37.8
Total	3223	781,345,411	34.1

contained in the 3132 unanchored scaffolds were designated as chr0 and were primarily repetitive sequences (Table 1.1).

1.3 Features of Tomato Genome

1.3.1 Organization of Tomato Genome

Detailed analysis of the cytogenetic and genetic features of tomato genome organization was carried out based on the obtained tomato genome sequences anchored on the 12 chromosomes (pseudomolecules). By comparing the BAC-clone FISH results and the physical locations of these clones on pseudomolecules, it became clear that tomato pachytene chromosomes consist of prominent pericentromeric heterochromatin with $4-10\times$ more DNA per unit length than distal euchromatin (The Tomato Genome Consortium 2012). FISH analysis using Cot 100 DNA (including most repeats) as a probe demonstrated that the repeats are concentrated around centromeres and telomeres and within chromomeres. Using the positional information from FISH BAC probes, recombination nodule locations derived from cytological mapping were compared with the physical locations on the pseudomolecules. This revealed a much higher recombination frequency in distal euchromatin than in pericentromeric heterochromatin. This distribution was confirmed by the comparison of genetic distance and physical distance using molecular genetic markers.

Early RFLP mapping of random genomic clones led to the estimation that a large proportion of the tomato genome consists of low-copy, noncoding DNA (Zamir and Tanksley 1988). This is supported by DNA renaturation kinetics, which are consistent with predominantly low-copy DNA, despite the substantial proportion of the genome that is heterochromatic (Peterson et al. 1998). The number of repetitive sequences in the obtained tomato reference genome is far fewer than in the smaller, 740 Mb, sorghum genome, with ~4000 intact long terminal repeat (LTR) retrotransposons identified as opposed to the ~11,000 identified in sorghum (Paterson et al. 2009). The average insertion age (as estimated by base substitutions in LTR sequences) of the tomato LTR retrotransposons was older than that of sorghum [2.8 versus 0.8 million years (Myr) ago]. In addition, no high-copy full-length LTR retrotransposons were identified in tomato. The largest cluster contained just 581 members, with all the other clusters containing <100 members. Features of repetitive sequences in the tomato genome were also revealed by *k*-mer frequency analysis. *k*-mer frequencies are a repeat-library-independent, and thus unbiased, method for accessing the repetitive portion of a genome. When the frequencies of each 16-mer in the tomato genome sequence were calculated, only 24 % of the genome was found to be composed of 16-mers with frequencies that occur ≥ 10 times. This indicates that tomato has a distinctly lower repetitive element content

than the smaller sorghum genome, in which 41 % of the genome is composed of 16-mers with frequencies ≥10. These characteristics of the repeated portions of the tomato genome facilitated the creation of long scaffolds and the assignment of scaffold sequences to specific chromosomes.

1.3.2 Gene Structure

The tomato genome was annotated by the iTAG consortium. An integrated gene prediction pipeline based on EuGene (Foissac et al. 2008) and RNA-seq data was used which produced a consensus annotation of 34,727 protein-coding genes in tomato (iTAG v2.3: http://solgenomics.net/organism/Solanum_lycopersicum/genome). As a large amount of the RNA-seq data were accumulated by using NGS platforms, most of the predicted protein-coding genes (30,855) are supported by transcribed sequence information. More than 90 % of the predicted genes (31,741, with e-value <1e-3) are homologous to *A. thaliana* genes (TAIR10). Functional descriptions were putatively assigned to 78 % of the tomato proteins, and the remaining 22 % received a designation of "unknown protein." Small RNA data from three tomato libraries supported the prediction of 96 known miRNA genes in tomato, which is consistent with the copy number found in other model and non-model plant species investigated to date.

In order to survey conserved features in protein-coding genes, gene family clusters among different plant species were defined using OrthoMCL software (Li et al. 2003). The protein-coding genes of tomato, potato, *Arabidopsis*, rice, and *Vitis vinifera* (grape) were included in the analysis, and a total of 154,880 gene sequences from these five species were grouped into 23,208 gene groups ("families," each with at least two members). Of the 34,727 protein-coding genes predicted on the reference tomato genome, 25,885 were clustered in a total of 18,783 gene groups. Of these 18,783 gene groups, 8615 are common to all five genomes, 1727 are confined to eudicots (tomato, potato, grape, *Arabidopsis*), and 727 to plants with fleshy fruits (tomato, potato, grape) (The Tomato Genome Consortium 2012). A total of 5165 gene groups were identified as Solanaceae specific, while 562 were tomato specific and 679 were potato specific. Such genes provide candidates for further validation and exploration of diverse roles in species-specific traits, including fruit and tuber biogenesis.

1.3.3 Genome Triplication

The draft genome of grape (*V. vinifera*) indicated that no recent genome duplication had occurred, and this enabled the discovery of ancestral traits and features related

to the genetic organization of flowering plants (French-Italian Public Consortium for Grapevine Genome Characterization 2007). Further analysis revealed that whole-genome triplication contributed to the establishment of the grape genome and that this triplication is common to many dicot plants but is absent in monocots. To test the hypothesis that the whole-genome triplication in the rosid lineage, which includes grape and *Arabidopsis*, occurred in a common ancestor shared with tomato and other asterids (Tang et al. 2008), the tomato and grape genomes (French-Italian Public Consortium for Grapevine Genome Characterization 2007) were compared. A comparison of grape triplet chromosomes to the tomato genome inferred 1730 tomato-grape (asterid-rosid) homologous DNA segments. The distribution of synonymous nucleotide substitution rates (Ks) between corresponding gene pairs in duplicated blocks suggests that one polyploidization in tomato preceded the asterid-rosid divergence. Since each of the "triplets" of grape chromosomal segments matches optimally with a distinct homologous block in tomato, it can be inferred that tomato-grape genome structural divergence followed this triplication.

Comparison with the grape genome also reveals a more recent triplication in the tomato genome. While few individual tomato genes remain triplicated, about 73 % of tomato gene models are in blocks that are orthologous to one grape region, collectively covering 84 % of the grape gene space. Among grape genomic regions, 22.5 % have one orthologous region in tomato, 39.9 % have two, and 21.6 % have three. The most parsimonious explanation is that a whole-genome triplication occurred in the tomato lineage and was followed by widespread gene loss. Based on alignments of multiple tomato segments to single grape genome segments, the tomato genome can be partitioned into three nonoverlapping "subgenomes." The smaller number of tomato-tomato (501) compared with tomato-grape (1730) homologous segments is consistent with substantial gene loss and rearrangement following this additional polyploidy. Based on the Ks of triplicated genes, the tomato triplication is estimated at 71 Myr, and therefore, the majority of post-triplication gene loss predates the ~7.3 Myr tomato-potato divergence (Wu and Tanksley 2010).

These two genome triplication events shaped the evolution of genes involved in fleshy fruit development. Most of the genes were eliminated by widespread gene loss following the triplication events, with the duplicates that remained acquiring new and distinct functions. This group of genes includes pleiotropic transcription factors that are necessary for ethylene biosynthesis [*RIN* (Vrebalov et al. 2002), *CNR* (Manning et al. 2006)], enzymes necessary for ethylene biosynthesis and signaling (*ACS* (Nakatsuka et al. 1998), *ETR* (Klee and Giovannoni 2011)), red-light photoreceptors that are associated with fruit quality [PHYB1/PHYB2 (Pratt et al. 1995)], and enzymes necessary for lycopene biosynthesis [*PSY1*, *PSY2* (Giorio et al. 2008)] (Fig. 1.1).

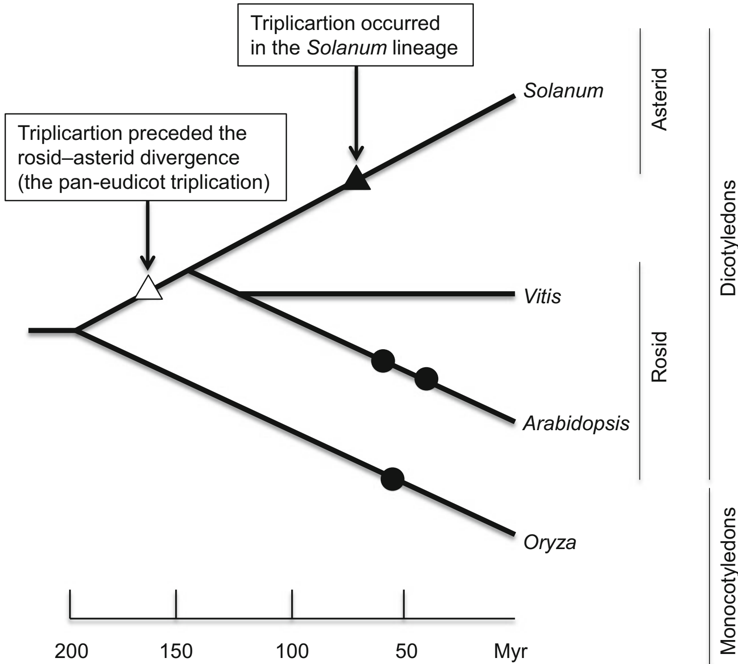


Fig. 1.1 Two triplication events in the *Solanum* genome. Reported polyploidization events in monocotyledon and eudicotyledon genomes. A *white triangle* indicates occurrence of a triplication event after divergence of dicotyledons from monocotyledons and before divergence of rosid and asterid (pan-eudicot triplication). The triplication event identified in the *Solanum* lineage (tomato and potato) is shown with a *black triangle*. *Black circles* indicate genome duplication reported in previous publications

1.4 Comparative Genomics of the Tomato Genome

1.4.1 Comparative Genome Analysis Against Potato

In the potato (*S. tuberosum*) genome-sequencing project (Potato Genome Sequencing Consortium 2011), which was published prior to the tomato genome, a homozygous doubled-monoploid potato clone was used for sequencing in order to overcome the highly heterozygous nature of most potato cultivars. A whole-genome shotgun sequencing approach was applied using different NGS platforms, primarily Illumina technology. A final assembly of 727 Mb was compiled from 96.6 Gb of raw sequences (Potato Genome Sequencing Consortium 2011).

Tomato and potato are estimated to have diverged ~7.3 Myr (Wu and Tanksley 2010). Sequence alignment of 71 Mb of euchromatic regions from the tomato reference genome to their counterparts in potato revealed 8.7 % nucleotide divergence with an average of one indel per 110 bp. The intergenic and repeat-rich heterochromatic sequences generally showed nucleotide divergence of >30 %

between the two species, consistent with the high-sequence diversity in these regions among different potato genotypes (Potato Genome Sequencing Consortium 2011). The chromosome pseudomolecules of the potato genome were updated by anchoring the scaffolds on the integrated genetic and physical reference map comprising nearly 2500 markers (Sharma et al. 2013). The dot plot alignments between the updated pseudomolecules of the potato genome and those of the tomato genome revealed 19 paracentric inversions including eight large inversions that were previously known from cytological studies.

In order to carry out a precise comparison between protein-coding genes of tomato and potato, the potato genome was re-annotated using the same pipeline as that used for tomato annotation. The annotation predicted 35,004 genes for potato, which is comparable to the number of genes (34,727) predicted for the tomato genome. By comparing the predicted genes in the tomato and potato genomes, 18,320 clearly orthologous tomato-potato gene pairs were identified (The Tomato Genome Consortium 2012). A total of 138 (0.75 %) gene pairs had significantly higher than average non-synonymous (Ka) vs. synonymous (Ks) nucleotide substitution rate ratios (ω), indicating diversifying selection, and many high ω-group genes were found to encode proteins that regulate biological processes, such as transcription factors. Conversely, 147 gene pairs (0.80 %) had significantly lower than average ω, indicating purifying selection, and most low-ω genes were found to be structural genes such as histone superfamily proteins and ribosomal proteins.

Comparison of the predicted genes also revealed genes conserved only in tomato or potato. Cytochrome P450 provides an example; several cytochrome P450 subfamilies show complete loss in tomato with respect to potato. Some of these losses, such as *CYP80N1* and *CYP82E4,* may be ecologically significant. Their absence may limit the biosynthesis of toxic glycoalkaloid and thus promote the development of a nutritionally attractive fruit that, in turn, enhances seed dispersal by animals (Cipollini and Levey 1997; Chakrabarti et al. 2007).

1.4.2 Comparative Genome Analysis of Tomato and Wild Relatives

The reference tomato genome sequence was obtained from ‘Heinz 1706’, a cultivated variety. To explore variation between cultivated tomato and the nearest wild tomato species, the tomato genome-sequencing consortium sequenced the *S. pimpinellifolium* genome (accession LA1589) using a whole-genome shotgun approach with Illumina technology (The Tomato Genome Consortium 2012). A final assembly of 739 Mb was generated from 39.3 Gb quality-trimmed sequences (43.7-fold coverage). Mapping the *S. pimpinellifolium* reads to the *S. lycopersicum* pseudomolecules revealed a nucleotide divergence of only 0.6 % (5.4 million SNPs), indicating a remarkably high level of genomic similarity between the two species. Correspondingly, no large structural variation was detected in gene-rich

euchromatic regions; however, a *k*-mer-based mapping strategy revealed that several pericentromeric regions containing coding sequences are absent in *S. pimpinellifolium*. The chromosome 1 indel contains a putative self-incompatibility locus, while the indel on chromosome 10 is segregated in the broader *S. pimpinellifolium* germplasm, suggesting the existence of an even greater reservoir of genetic variation among other isolates.

More than 90 % (32,955) of the predicted genes in the *S. lycopersicum* genome are present in the genome of *S. pimpinellifolium*. As expected from the pedigree of 'Heinz 1706', which has *S. pimpinellifolium* as one of its ancestors, putative *S. pimpinellifolium* introgressions were detected. Examination of the variation between the two species for 32,955 (92 %) of the iTAG annotated genes revealed 6659 identical genes and 3730 genes with only synonymous changes. Despite this high genic similarity, 68,683 SNPs from 22,888 genes are potentially disruptive to gene function, including non-synonymous changes, gain or loss of stop codons or essential splice sites, and indels causing frameshifts. In addition, 1550 genes either gained or lost a stop codon in *S. pimpinellifolium*. Since the identified SNPs can be used as markers for the whole *S. pimpinellifolium* genome, it will be possible to explore the biological relevance of this variation and its relationship to domestication and crop improvement. Within cultivated germplasms, particularly among the small-fruited cherry tomatoes, several chromosomal segments are more closely related to *S. pimpinellifolium* than to 'Heinz 1706', supporting previous observations on the recent admixture of these gene pools as a consequence of breeding (Ranc et al. 2008). 'Heinz 1706' itself has been reported to carry introgressions from *S. pimpinellifolium* (Ozminkowski 2004). Genomic regions with low divergence between *S. pimpinellifolium* and 'Heinz 1706' but with high divergence among domesticated cultivars were regarded as *S. pimpinellifolium* introgressions. Large introgressions were detected on both chromosomes 9 and 11, and both chromosomes have been implicated in the breeding of disease-resistance loci into 'Heinz 1706' using *S. pimpinellifolium* germplasm (Ozminkowski 2004).

1.5 Continuing Sequencing Efforts and Future Perspectives

NGS allowed the tomato genome-sequencing project, which began by using clone-by-clone Sanger technology of selected regions, to progress to the sequencing and assembly of the whole genome. The comprehensive datasets, which include large amounts of NGS data and BAC/cosmid end Sanger reads, alongside scrupulous attention-to-error correction, produced one of the highest-quality genome sequences to date (Assembly SL2.40). Nevertheless, the Tomato Genome Sequence Consortium is pursuing efforts to further improve the genome and reach "gold standard." These endeavors are currently focused upon gap closure and scaffold validation. A large number (~2000) of additional BAC clones have been sequenced

using NGS platforms with the aim of closing gaps within and between scaffolds. For smaller gaps of up to 1000 bp, an additional high-throughput method was developed using 454 technology and applied to gap closure. Scaffold validation was enhanced by adding >600 BAC clones to the tomato FISH map (SOL Newsletter April 2013, Issue 35: http://solgenomics.net/). The locations of the BAC clones were used both for estimating gap size between scaffolds and for validation and adjustment of the order and orientation of the scaffolds. Many of the localized BAC clones were selected from chr0 scaffolds (unanchored scaffolds), and the obtained FISH map data allowed these scaffolds to be mapped to pseudomolecules. The accumulated new data will be incorporated and the updated reference tomato genome information will be released as SL2.50 (Lucas Mueller, personal communication).

Extensive molecular marker analysis revealed that, as a result of domestication, genetic diversity in the cultivated tomato is much lower than in its wild relatives. The availability of a high-quality genome from the domesticated cultivar 'Heinz 1706' is facilitating the sequencing of additional cultivated and wild tomato ecotypes, with the aim of analyzing genetic variations and improving the data available for marker-associated breeding. One large-scale example of these ongoing projects is the "150 tomato genomes project" (http://www.tomatogenome.net). In this project, 84 ecotypes including 10 old varieties, 43 cultivated lines, and 30 wild accessions have been selected for sequencing. Moreover, some 60 F8 individuals of *S. pimpinellifolium* recombinant inbred lines (RILs) will also be sequenced with the aim of identifying recombination breakpoints at the sequence level. Popular cultivars in tomato experimental studies such as 'Ailsa Craig', 'Rutgers', 'M82', and 'Micro-Tom' will also be sequenced (Aoki et al. 2013; http://solgenomics.net/organism/1/view). Although most of these datasets are not currently publicly available, they will serve as excellent information resources for developing SNP markers and intraspecific maps.

In addition to cultivated and wild tomato ecotypes, hundreds of Solanaceae species will be sequenced using NGS technologies to create a common Solanaceae-based genomic framework that includes sequences and phenotypes of 100 genomes encompassing the phylogenetic diversity of Solanaceae group. This clade-oriented project, called "SOL-100," involves sequencing 100 different Solanaceae genomes and linking these sequences to the reference tomato sequence. The ultimate aim of this project is to explore key issues of plant biodiversity, genome conservation, and phenotypic diversification, and more information is available at the SOL Genomics Network (SGN) site (http://solgenomics.net/organism/sol100/view). At the time of writing (August 2013), genome-sequencing projects involving 25 Solanaceae species are ongoing (Table 1.2; http://solgenomics.net/organism/1/view), and the obtained results are beginning to emerge (Bombarely et al. 2012; Sierro et al. 2013).

The highly accurate 'Heinz 1706' reference genome sequence will, alongside genome sequences of *S. pimpinellifolium* and potato, pave the way for comparative and functional studies and for genomics-assisted breeding in Solanaceae. Additional sequencing and bioinformatics resources are currently being devoted to

Table 1.2 List of Solanaceae species analyzed in the SOL-100 project

Nicotiana tomentosiformis	*Solanum chilense*
Nicotiana benthamiana	*Solanum neorickii*
Nicotiana attenuata	*Solanum galapagense*
Nicotiana sylvestris	*Solanum pimpinellifolium*
Petunia axillaris	*Solanum pennellii*
Lycium barbarum	*Solanum huaylasense*
Capsicum annuum	*Solanum corneliomuelleri*
Withania somnifera	*Solanum chmielewski*
Iochroma cyaneum	*Solanum peruvianum*
Solanum tuberosum	*Solanum cheesmaniae*
Solanum retroflexum	*Solanum arcanum*
Solanum melongena	*Solanum habrochaites*
	Solanum lycopersicum

expand the Heinz 1706 sequence into a "gold standard." Extensive sequencing efforts on cultivated and wild tomato accessions will provide marker and gene pools of sufficient depth for crop improvement. Moreover, the SOL community aims to sequence and analyze 100 additional Solanaceae genomes (SOL100) and develop the needed translational tools. Along with the systematic development of material and information resources, genomic studies of cross-Solanaceae species analyses will bear considerable fruit in coming years.

References

Aoki K, Ogata Y, Igarashi K, Yano K, Nagasaki H, Kaminuma E, Toyoda A (2013) Functional genomics of tomato in a post-genome-sequencing phase. Breed Sci 63:14–20

Bombarely A, Rosli HG, Vrebalov J, Moffett P, Mueller LA, Martin GB (2012) A draft genome sequence of *Nicotiana benthamiana* to enhance molecular plant-microbe biology research. Mol Plant Microbe Interact 25:1523–1530

Budiman MA, Mao L, Wood TC, Wing RA (2000) A deep-coverage tomato BAC library and prospects toward development of an STC framework for genome sequencing. Genome Res 10:129–136

Chakrabarti M, Meekins KM, Gavilano LB, Siminszky B (2007) Inactivation of the cytochrome P450 gene CYP82E2 by degenerative mutations was a key event in the evolution of the alkaloid profile of modern tobacco. New Phytol 175:565–574

Cipollini ML, Levey DJ (1997) Secondary metabolites of fleshy vertebrate-dispersed fruits: adaptive hypotheses and implications for seed dispersal. Am Nat 150:346–372

Foissac S, Gouzy JP, Rombauts S, Mathé C, Amselem J, Sterck L, Van de Peer Y, Rouzé P, Schiex T (2008) Genome annotation in plants and fungi: EuGene as a model platform. Curr Bioinforma 3:87–97

Frary A, Xu Y, Liu J, Mitchell S, Tedeschi E, Tanksley S (2005) Development of a set of PCR-based anchor markers encompassing the tomato genome and evaluation of their usefulness for genetics and breeding experiments. Theor Appl Genet 111:291–312

French-Italian Public Consortium for Grapevine Genome Characterization (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449:463–467

Fulton TM, Van der Hoeven R, Eannetta NT, Tanksley SD (2002) Identification, analysis, and utilization of conserved ortholog set markers for comparative genomics in higher plants. Plant Cell 14:1457–1467

Giorio G, Stigliani AL, D'Ambrosio C (2008) Phytoene synthase genes in tomato (*Solanum lycopersicum* L.)—new data on the structures, the deduced amino acid sequences and the expression patterns. FEBS J 275:527–535

Kahlau S, Aspinall S, Gray JC, Bock R (2006) Sequence of the tomato chloroplast DNA and evolutionary comparison of solanaceous plastid genomes. J Mol Evol 63:194–207

Klee HJ, Giovannoni JJ (2011) Genetics and control of tomato fruit ripening and quality attributes. Annu Rev Genet 45:41–59

Knapp S (2002) Tobacco to tomatoes: a phylogenetic perspective on fruit diversity in the Solanaceae. J Exp Bot 53:2001–2022

Li L, Stoeckert CJ Jr, Roos DS (2003) OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Res 13:2178–2189

Manning K, Tör M, Poole M, Hong Y, Thompson AJ, King GJ, Giovannoni JJ, Seymour GB (2006) A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. Nat Genet 38:948–952

Michaelson MJ, Price HJ, Ellison JR, Johnston JS (1991) Comparison of plant DNA contents determined by Feulgen microspectrophotometry and laser flow cytometry. Am J Bot 78:183–188

Mueller LA, Tanksley SD, Giovannoni JJ, van Eck J, Stack S, Choi D, Kim BD, Chen M, Cheng Z, Li C, Ling H, Xue Y, Seymour G, Bishop G, Bryan G, Sharma R, Khurana J, Tyagi A, Chattopadhyay D, Singh NK, Stiekema W, Lindhout P, Jesse T, Lankhorst RK, Bouzayen M, Shibata D, Tabata S, Granell A, Botella MA, Giuliano G, Frusciante L, Causse M, Zamir D (2005) The tomato sequencing project, the first cornerstone of the International Solanaceae Project (SOL). Comp Funct Genomics 6:153–158

Nakatsuka A, Murachi S, Okunishi H, Shiomi S, Nakano R, Kubo Y, Inaba A (1998) Differential expression and internal feedback regulation of 1-aminocyclopropane-1-carboxylate synthase, 1-aminocyclopropane-1-carboxylate oxidase, and ethylene receptor genes in tomato fruit during development and ripening. Plant Physiol 118:1295–1305

Ozminkowski R (2004) Pedigree of variety Heinz 1706. Rep Tomato Genet Coop 54:26

Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A, Schmutz J, Spannagl M, Tang H, Wang X, Wicker T, Bharti AK, Chapman J, Feltus FA, Gowik U, Grigoriev IV, Lyons E, Maher CA, Martis M, Narechania A, Otillar RP, Penning BW, Salamov AA, Wang Y, Zhang L, Carpita NC, Freeling M, Gingle AR, Hash CT, Keller B, Klein P, Kresovich S, McCann MC, Ming R, Peterson DG, Mehboob-ur-Rahman, Ware D, Westhoff P, Mayer KF, Messing J, Rokhsar DS (2009) The Sorghum bicolor genome and the diversification of grasses. Nature 457:551–556

Peterson DG, Pearson WR, Stack SM (1998) Characterization of the tomato (*Lycopersicon esculentum*) genome using in vitro and in situ DNA reassociation. Genome 41:346–356

Potato Genome Sequencing Consortium (2011) Genome sequence and analysis of the tuber crop potato. Nature 475:189–195

Pratt LH, Cordonnier-Pratt MM, Hauser B, Caboche M (1995) Tomato contains two differentially expressed genes encoding B-type phytochromes, neither of which can be considered an ortholog of Arabidopsis phytochrome B. Planta 197:203–206

Ranc N, Muños S, Santoni S, Causse M (2008) A clarified position for *Solanum lycopersicum* var. cerasiforme in the evolutionary history of tomatoes (solanaceae). BMC Plant Biol 8:130

Sharma SK, Bolser D, de Boer J, Sønderkær M, Amoros W, Carboni MF, D'Ambrosio JM, de la Cruz G, Di Genova A, Douches DS, Eguiluz M, Guo X, Guzman F, Hackett CA, Hamilton JP, Li G, Li Y, Lozano R, Maass A, Marshall D, Martinez D, McLean K, Mejía N, Milne L,

Munive S, Nagy I, Ponce O, Ramirez M, Simon R, Thomson SJ, Torres Y, Waugh R, Zhang Z, Huang S, Visser RG, Bachem CW, Sagredo B, Feingold SE, Orjeda G, Veilleux RE, Bonierbale M, Jacobs JM, Milbourne D, Martin DM, Bryan GJ (2013) Construction of reference chromosome-scale pseudomolecules for potato: integrating the potato genome with genetic and physical maps. G3 (Bethesda) 3:2031–2047

Shirasawa K, Asamizu E, Fukuoka H, Ohyama A, Sato S, Nakamura Y, Tabata S, Sasamoto S, Wada T, Kishida Y, Tsuruoka H, Fujishiro T, Yamada M, Isobe S (2010) An interspecific linkage map of SSR and intronic polymorphism markers in tomato. Theor Appl Genet 121:731–739

Sierro N, Battey JN, Ouadi S, Bovet L, Goepfert S, Bakaher N, Peitsch MC, Ivanov NV (2013) Reference genomes and transcriptomes of *Nicotiana sylvestris* and *Nicotiana tomentosiformis*. Genome Biol 14:R60

Tang H, Bowers JE, Wang X, Ming R, Alam M, Paterson AH (2008) Synteny and collinearity in plant genomes. Science 320:486–488

The Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635–641

Vrebalov J, Ruezinsky D, Padmanabhan V, White R, Medrano D, Drake R, Schuch W, Giovannoni J (2002) A MADS-box gene necessary for fruit ripening at the tomato ripening-inhibitor (rin) locus. Science 296:343–346

Wu F, Tanksley SD (2010) Chromosomal evolution in the plant family Solanaceae. BMC Genomics 11:182

Zamir D, Tanksley SD (1988) Tomato genome is comprised largely of fast-evolving, low copy-number sequences. Mol Gen Genet 213:254–261

Chapter 2
Melon Genome Sequence

Jordi Garcia-Mas and Pere Puigdomènech

2.1 Introduction

Since the publication of the sequence of the genome of the model plant *Arabidopsis thaliana* in 2000 (The Arabidopsis Genome Initiative 2000), several international initiatives followed that completed the genome sequence of other plant species such as rice (The International Rice Genome Sequencing Project 2005), poplar (Tuskan et al. 2006), grapevine (The French-Italian Public Consortium for Grapevine Genome Characterization 2007) and papaya (Ming et al. 2008). The sequencing of all these genomes was performed using the Sanger technology. High sequencing costs for such genome initiatives hampered the start of new initiatives to sequence other genomes with potential scientific and economic interest. It was only after the implementation of next-generation sequencing (NGS) technologies (Shendure and Ji 2008) that an outburst of plant genome sequences was made available to the scientific community. In fact, the first draft plant genome sequence that was obtained using NGS technologies was a cucurbit, cucumber (*Cucumis sativus* L.) (Huang et al. 2009). Since 2009, the draft genome of many economically important plants is already available, including species with large genome size such as maize (Schnable et al. 2009). Draft sequences have also been obtained from some of the largest plant genomes such as barley (The International Barley Genome Sequencing Consortium 2012) with a 5.1 gigabase genome and Norway spruce (Nystedt et al. 2013) that has a 20 gigabase genome.

Melon (*Cucumis melo* L.) is a diploid ($2n = 2\times = 24$) species that belongs to the cucurbit family, which contains other important species such as cucumber,

J. Garcia-Mas (✉)
IRTA, Center for Research in Agricultural Genomics CSIC-IRTA-UAB-UB, Barcelona, Spain
e-mail: jordi.garcia@irta.cat

P. Puigdomènech
Center for Research in Agricultural Genomics CSIC-IRTA-UAB-UB, Barcelona, Spain

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_2

watermelon (*Citrullus lanatus*) and squash (*Cucurbita* spp.). Recent studies have discussed a possible origin of melon in Asia, as its close relative cucumber (Sebastian et al. 2010), or in Africa, based on chloroplast genome sequencing of distant varieties (Tanaka et al. 2013). Melon is a morphologically highly diverse species that has been divided into several botanical varieties included in two proposed subspecies, *melo* and *agrestis* (Pitrat 2008). The melon genome size is small and was estimated in 454 Mb after nuclear DNA content studies (Arumuganathan and Earle 1991). Melon has been proposed as a suitable model species for studying important biological processes such as sex determination (Boualem et al. 2008), phloem physiology (Zhang et al. 2010) and fruit ripening (Pech et al. 2008). Much effort has been done in the past years to obtain a set of genetic and genomic tools to assist breeding in melon, as mapping populations, genetic maps constructed with different types of molecular markers (Diaz et al. 2011), transcriptomes (Clepet et al. 2011) and mutant collections (Dahmani-Mardas et al. 2010). In the era of high-throughput sequencing of plant genomes, it was also important to provide the cucurbit scientific community with the genomes of melon (Garcia-Mas et al. 2012) and other related species as cucumber (Huang et al. 2009) and watermelon (Guo et al. 2012). The availability of the genome sequences of these three cucurbit species is expected to boost the improvement of breeding material in the following years.

2.2 Sequencing the Melon Genome: A Historical Perspective

Despite the worldwide economic importance of melon, the number of genetic and genomic tools available in the past years had been scarce. In 2005, the International Cucurbit Genomics Initiative (ICuGI) was established by several international research teams, with the main goal of obtaining different melon genomic tools and storing the information in a single location. As a result, the ICuGI webpage was constructed (http://www.icugi.org), which originally contained centralized information from melon genetic maps and expressed sequence tags (ESTs). New data have been added to ICuGI in the last years, including cucumber, watermelon and squash genomic data and the cucumber and watermelon draft genomes (http://www.icugi.org). Some of these melon genetic and genomic tools are briefly described here.

2.2.1 Genetic Maps

Many genetic maps obtained in different genetic backgrounds and using different types of molecular markers have been reported in melon. As a result of the ICuGI initiative, a saturated consensus genetic map that integrated eight independent previously published genetic maps was built, containing 1592 markers and

370 QTLs that controlled 62 traits (Diaz et al. 2011). Some QTLs for the same trait obtained in different experiments were shown to map in similar genomic locations. This genetic map is now considered the initial reference map for melon. More recently, a new genetic map was built using a double haploid line (DHL) mapping population derived from PI 161375 (Songhwan charmi, ssp. *agrestis*) (SC) and the Piel de Sapo line T111 (ssp. *melo*) (PS), which contains 602 SNP markers (Esteras et al. 2013; Garcia-Mas et al. 2012). This genetic map has been used to anchor the melon genome sequence to chromosomes (see below).

2.2.2 ESTs and RNA-Seq

The ICuGI initiative also aimed at increasing the number of ESTs from different plant tissues and genetic backgrounds. A melon transcriptome containing all published melon ESTs obtained with Sanger sequencing and representing 24,444 unigenes is available at http://www.icugi.org (Clepet et al. 2011), which includes 1382 full-length transcripts. An oligo-based microarray containing 17,510 of the above-mentioned unigenes was developed (Mascarell-Creus et al. 2009), which was used to perform transcriptome analysis in melons infected with *watermelon mosaic virus* (Gonzalez-Ibeas et al. 2012) and *Monosporascus cannonballus* (Roig et al. 2012). More recently, RNA-seq data have been generated from different melon genotypes and tissues, using 454 pyrosequencing (Blanca et al. 2011b; Corbacho et al. 2013; Portnoy et al. 2011) or SOLiD sequencing (Blanca et al. 2012). In some of these works, SSR and SNP markers have been mined from the sequences, representing a valuable source of genetic markers that can be applied to melon breeding programmes.

2.2.3 Mutant Collections

Induced mutations in genes of agronomic interest from mutant populations can be efficiently screened using Targeting Induced Local Lesion in Genomes (TILLING) (Till et al. 2003). Several mutant populations have been developed in different genetic backgrounds representing the *cantalupensis* and the *inodorus* melon types (Dahmani-Mardas et al. 2010; Gonzalez et al. 2011; Tadmor et al. 2007), which have been used to identify mutations in target genes as the sex determination genes *a* (*andromonoecious*) and *g* (*gynoecious*), among others (Boualem et al. 2008; Dahmani-Mardas et al. 2010; Martin et al. 2009).

2.2.4 BAC Libraries

At least five different BAC libraries have been reported in melon, which have been used for positional cloning of agronomically important genes (e.g. the sex determination genes *a* and *g* and the resistance genes *Fom-2* and *nsv*) and for improving the genome assembly (Boualem et al. 2008; Garcia-Mas et al. 2012; Gonzalez et al. 2010b; Luo et al. 2001; Martin et al. 2009; van Leeuwen et al. 2003). Previous to the sequencing of the melon genome, a pool of 57 BAC clones from two of these BAC libraries, which represented 1.5 % of the genome, was sequenced with 454 pyrosequencing, allowing obtaining a preliminary view of the genome structure (Gonzalez et al. 2010a). Also, the availability of BAC-end sequences from two of these BAC libraries (Gonzalez et al. 2010b) has proven to be extremely useful for the efficient assembly of the melon genome (Garcia-Mas et al. 2012).

2.3 MELONOMICS: Sequencing the Melon Genome

In 2009, the MELONOMICS project started, a Spanish public–private initiative that aimed at obtaining a draft of the melon genome using NGS technologies with a whole-genome shotgun strategy. The sequenced DNA material was obtained from the doubled haploid line DHL92, which was derived from a hybrid between PI 161375 (SC) and the Piel de Sapo line T111 (PS) (Garcia-Mas et al. 2012) (http://melonomics.net).

2.3.1 Genome Assembly

The chosen technology for genome sequencing was 454 pyrosequencing (Roche). 14.8 million shotgun and 7.7 million paired-end reads from 3-kb, 8-kb and 20-kb paired-end libraries were produced, respectively. Additionally, 53,203 BAC-end sequences from two BAC libraries obtained from DHL92 were also used in the assembly of the genome (Gonzalez et al. 2010b). Melon chloroplast and mitochondrial genomes were assembled and filtered before genome assembly (Rodriguez-Moreno et al. 2011). Strikingly, melon mitochondria contains one of the largest (2.74 Mb) genomes reported in plants. The melon genome assembly v3.5 spans 375 Mb, which represents 83 % of the 454 Mb estimated melon genome size. The unassembled genome fraction most probably contains repetitive DNA sequences. The melon genome assembly v3.5 contains 1594 scaffolds and 29,865 contigs, 90 % of the assembly is contained in 78 scaffolds and the N50 is 4.68 Mb. A comparison of these data with other NGS-sequenced plant genomes confirmed the good quality of the melon genome assembly, probably attributable to the use of the 454 Titanium technology, which yields longer reads than those produced using

Illumina sequencing, combined with the efficient scaffolding obtained with the Sanger BAC-end sequences. Nowadays, the most efficient way of sequencing a medium-size plant genome is probably the one based in the use of a combination of NGS technologies (454 pyrosequencing, Illumina-Solexa, SOLiD) and Sanger sequencing. The melon genome assembly v3.5 was corrected with low-coverage Illumina reads from DHL92, as 454 pyrosequencing frequently introduces sequencing errors in homopolymer regions.

2.3.2 Genome Anchoring

The anchoring of the genome assembly to chromosomes was performed using the SC × PS DHL genetic map, which contains 602 SNPs. It allowed placing 316 Mb of the genome assembly (87 scaffolds, 84 % of the assembly) to the 12 melon linkage groups. Of these, 71 scaffolds (292 Mb, 78 % of the assembly) were correctly oriented (http://melonomics.net). Mapped SNPs between SC and PS were previously identified from a melon 454 transcriptome (Blanca et al. 2011b) and genotyped using the Illumina GoldenGate genotyping method (Esteras et al. 2013). Genetic markers from two additional genetic maps in the SC × PS (Gonzalo et al. 2005) and the PI 414723 × Dulce (Oliver unpublished) genetic backgrounds were also used to resolve the order and orientation of some scaffolds. During the genome anchoring, 5 chimerical scaffolds were detected and manually corrected, yielding a final version of the assembly containing 1599 scaffolds and 29,865 contigs.

An improved version of the melon genome anchoring has been recently obtained, where approximately 95 % of the genome assembly has been positioned in the 12 melon linkage groups. Briefly, after selecting the 150 largest genome scaffolds, which represent 95 % of the assembly, SNPs were mined from scaffolds that were not anchored in the first published version. The resequenced genomes of SC and PS (see below) were used for SNP mining. An F2 mapping population of 150 individuals in the same genetic background (SC × PS) was used for the genetic map construction with higher mapping resolution (Argyris et al. 2015).

2.3.3 Transposon Content in the Melon Genome

For an efficient annotation of the gene content in a plant genome, it is necessary to previously identify and mask the fraction of transposable elements (TEs) included in the genome assembly. A total of 73,787 copies of the two major types of TEs (retrotransposons and DNA transposons) were annotated using ab initio and homology-based methods, which accounted for 19.7 % of the genome assembly. LTR retrotransposons of the *copia* and *gypsy* superfamilies were the most abundant retrotransposon classes, accounting for 5.5 and 7.2 % of the genome assembly,

respectively. The CACTA superfamily (1.6 %) was the most abundant class of DNA transposons. It is worth noting that the fraction of TEs found in the melon genome assembly is probably an underestimation due to the stringent search parameters used and the high proportion of repetitive sequences present in the unassembled genome fraction. The distribution of the annotated TEs showed a complementary pattern to the gene fraction, being more frequently found in centromeric and pericentromeric regions.

The insertion time of major LTR retrotransposon families was obtained after sequence comparison of each pair of LTRs in complete elements. A major peak of retrotransposon amplification was found around 2 Myr. This is well after the divergence of melon and cucumber, which has been estimated around 10 Myr (Sebastian et al. 2010). In contrast, in the Gy14 cucumber genome sequence, only 1.5 % of the assembled genome was annotated as retrotransposons after applying the same criteria for transposon annotation in both species. All these data suggest that one of the main reasons for the differences in genome size between melon (454 Mb) and cucumber (367 Mb) may be attributed to a recent burst of TE amplification in melon, which is not observed in cucumber.

2.3.4 Genome Annotation

After masking the repeat content in the melon genome assembly, a combination of ab initio and evidence-based approaches (homology to protein databases and EST evidence) was used to annotate the protein-coding genes. The estimation obtained is that the melon genome contains 27,427 protein-coding genes, which is in a general agreement with the number of genes reported in cucumber (26,682), watermelon (23,440) and other plant genomes. In total, 69.1 % of these genes are supported by ESTs or show homology to protein databases. However, still 30 % of the annotated genes are simply based on ab initio predictions. Only when new melon RNA-seq data from different plant tissues and varieties is available, then the evidence-based protein-coding gene number might increase, although as it has happened in other species, some of the gene predictions may not be experimentally confirmed.

2.3.5 Phylome Database

A phylogenomic analysis was conducted with melon and 22 plant species using the complete set of annotated protein-coding genes, resulting in the characterization of the melon phylome. Among the 22 plant species represented in the melon phylome, there are algae (4), mosses (2), monocots (5) and dicots (12). In total, 22,218 maximum-likelihood (ML) phylogenetic trees were obtained, which can be retrieved at PhylomeDB (http://phlylomedb.org). The melon phylome was important to help assisting the functional annotation of the protein-coding genes and

represents an excellent tool to identify orthologous and paralogous sequences among the plant kingdom.

The phylome analysis was also used to infer whole-genome duplication (WGD) events in the melon genome. The results obtained suggest that melon has not suffered a recent WGD since the ancestral paleohexaploidization described for eudicots after the divergence of monocots and dicots (Paterson et al. 2010), although some expanded gene families were observed (Garcia-Mas et al. 2012).

2.3.6 Noncoding RNAs

The melon genome sequence allowed the annotation of the noncoding RNA (ncRNA) repertoire. Among the 1253 ncRNA genes identified, 140 potential miRNAs were described. Of these, 80 miRNAs were shown to be expressed when compared with a previous characterization of the melon small RNAome by 454 pyrosequencing (Gonzalez-Ibeas et al. 2011). In this previous work, ten small RNA (sRNA) libraries were constructed from several plant tissues and miRNAs from known families, and new candidate miRNAs were characterized. Several ncRNAs were distributed in clusters in the melon genome (Garcia-Mas et al. 2012).

2.3.7 Disease Resistance Genes

Among the 411 annotated melon genes putatively involved in disease resistance, 81 belong to the plant nucleotide-binding site (NBS)–leucine-rich repeat (LRR) class (R-genes). R-genes are known to be involved in conferring resistance to a wide range of plant pathogens as virus, bacteria, fungi, insects and nematodes (Ellis et al. 2000). As already reported in other plant species, melon R-genes are also arranged in clusters. Two major clusters of R-genes were indentified in chromosomes V and IX, where the resistance genes *Vat* (Dogimont et al. 2008) and *Fom-1* (Brotman et al. 2013) are located, respectively.

The number of R-genes in melon is low when compared with those found in other species as *Arabidopsis* (212) and grape (302). This may be a general trend in cucurbits, as the number of annotated R-genes in cucumber (61) (Huang et al. 2009; Wan et al. 2013; Yang et al. 2013) and watermelon (44) (Guo et al. 2012) is also low. A recent work has suggested that the low number of R-genes found in cucurbit species may be due to frequent loss of R-gene lineages and deficient duplications in extant R-gene lineages (Lin et al. 2013). Resequencing of watermelon wild and cultivated germplasm has permitted to hypothesize that a large proportion of R-genes may have been lost during the domestication process in this species (Guo et al. 2012).

2.3.8 Resequencing of Melon Genomes

Once a reference draft genome is available for a given species, then it is possible to resequence individual genomes and map the reads to the reference genome in a simple way, which allows performing additional studies, among them the assessment of genetic diversity in the species at the whole-genome level and the identification of domestication and improvement genes (Hufford et al. 2012). The recent publication of the watermelon genome sequence included the resequencing of 20 accessions belonging to three different subspecies, allowing the identification of putative genomic regions that may have suffered selection after domestication (Guo et al. 2012).

The two parental lines of DHL92, SC and PS, were resequenced, which allowed the reconstruction of the DHL92 genome, which contains 17 recombination events (Garcia-Mas et al. 2012). More than one million SNPs were identified between SC and PS, and SNP subsets have already been validated in different genomic studies (Sanseverino et al. 2015). More recently, additional melon accessions from both the *melo* and *agrestis* subspecies have been resequenced, including PI 124112 (*momordica* type) and Védrantais (*cantalupensis* type). Preliminary analysis of the resequence data has allowed characterizing the genomic variation at the level of SNPs, short indels and structural variation (SV), as well as estimating the nucleotide diversity along the chromosomes and the distribution of TE polymorphism (Sanseverino et al. 2015).

2.4 The Era of Plant Genome Sequences: The Cucurbits

The Cucurbitaceae family includes four major species with a significant impact for human nutrition and that together are second only after Solanaceae for their economic importance among horticultural species worldwide. These species are melon, cucumber, watermelon and pumpkins and squashes, including zucchini. The first three species originated in Asia or Africa, while the last ones have a Central American origin. At this moment, reference draft genome sequences from cucumber, melon and watermelon have already been published, thus allowing the first comparative studies among them. The main features of the cucurbit sequenced genomes are described in Table 2.1.

Most of genomic data that was produced in cucurbits in the past years was from melon (see Sect. 2.2); however, the first reference genome published was that of cucumber (Huang et al. 2009). It was a draft obtained from DNA of the "Chinese long" inbred line 9930, essentially using Illumina reads and Sanger sequences that allowed 69.5 % coverage of the genome, with a scaffold N50 of 1.1 Mb, but contained in more than 47,000 scaffolds. The sequence allowed identifying 26,682 genes including a low proportion of genes related to pathogen resistance. It also did not find any evidence for recent genome duplication. The sequence

Table 2.1 Main characteristics of the draft genome sequences of melon, cucumber and watermelon. In the cucumber 9930 genome sequence, assembly versions 1.0 and 2.0 are included

Species	Assembled genome (in Mb) (% genome)	N50 contig size (kb)	N50 scaffold size (Mb)	Annotated genes	Transposable elements (% genome)	NBS-LRR predicted genes	Reference
Melon (DHL92)	375 (83.3)	18.2	4.68	27,427	19.7	81	(Garcia-Mas et al. 2012)
Cucumber (9930)							
assemVer 1.0	243.5 (69.5)		1.14	26,682	24	61	(Huang et al. 2009)
assemVer 2.0	197.2 (56.2)	37.9	1.40	23,248	–	–	(Li et al. 2011b)
Cucumber (Borszczagowski)	224 (64)	27.1	2.32	26,587	–	–	(Wóycicki et al. 2011)
Cucumber (Gy14)	203 (58)	37.6	0.99	21,491	–	70	www.phytozome.net
Watermelon (97103)	353.5 (83.2)	26.38	2.38	23,440	45.2	44	(Guo et al. 2012)

(–) Indicates data not reported in the genome source

assembly and annotation was lately improved through RNA-seq data (Li et al. 2011b). Another cucumber sequence was published from the Northern Europe cucumber cultivar ‘Borszczagowski’ using 454 and Sanger sequence from BAC ends (Wóycicki et al. 2011). The sequence had lower genome coverage, and it was compared with the Asian 9930 cucumber genome, finding several chromosomal rearrangements between both cultivars. A third cucumber sequence is available through the Phytozome database from the gynoecious Gy14 cucumber cultivar (http://www.phytozome.net). Gy14 was mostly used in most comparative analyses with melon, as both genomes were sequenced using the same NGS technology (454 pyrosequencing).

The genome of melon was published in 2012 and has been described in Sect. 2.3. Following the publication of the melon genome, the sequence of the watermelon genome appeared (Guo et al. 2012). The published draft genome was obtained by Illumina sequencing from the Chinese inbred line 97103, and it represented coverage of 83.2 % of the watermelon genome and a N50 of 2.38 Mb. 23,440 genes were predicted from the genome assembly. In this article, the reference sequence was compared with the results from sequence data of 20 different accessions that included cultivated and semiwild cultivars from Africa, Asia and America. The variability observed allowed a classification of the cultivars and discovery of SNPs. Evolutionary relationships between the varieties and analysis of the population substructures were also provided.

After a comparison of the genome data reported in melon, cucumber and watermelon (Table 2.1), the melon and watermelon genomes reached the highest proportion of assembly of the estimated genome size, around 83 %. Regarding the quality of the genome assembly, measured using the N50 scaffold size, data suggests that the melon assembly is of higher quality, probably due to the use of 454 pyrosequencing. The number of annotated protein-coding genes is similar in all three species, ranging from 21,491 in Gy14 cucumber to 27,427 in melon. However, gene annotations in all three species have to be improved through incorporation of additional RNA-seq data. Regarding the TE fraction of the cucurbit genomes, it shows a wide range between 19.7 % in melon to 45.2 % in watermelon. However, it is clear that different TE annotation procedures were used in all species. A TE annotation was performed in the 9930 and Gy14 cucumber genomes using the same pipeline used in melon (Garcia-Mas et al. 2012), yielding a different number than the one reported in Huang et al. (2009), suggesting that the TE genome content data reported in the three species has to be taken with caution. Genome data also suggests that the R-gene content in the three cucurbit genomes is low, ranging between 44 and 81, as it has been discussed in Sect. 2.4.

In *Cucurbita*, there is still not a draft genome sequence available. The most complete genomic data come from RNA-seq obtained by using the 454 pyrosequencing technology (Blanca et al. 2011a) and that allowed the discovery of a large number of SNPs that are useful for plant genotyping applications (Esteras et al. 2012).

2.4.1 Comparative Genomics of Cucurbit Genomes

Sequence similarities between melon and cucumber were already suggested when SSR primers from one species amplified genomic regions in the other species (Danin-Poleg et al. 2001). The existence of synteny between melon and cucumber was evident in some genomic regions, as it was shown after mapping the *Psm* locus in cucumber using a set of melon genetic markers (Al-Faifi et al. 2008). Comparison of the eIF4E and eIF(iso)4E loci regions in melon and cucumber using BAC and fosmid sequences revealed extensive sequence conservation in these intervals (Meyer et al. 2008). The cucumber genomic region containing the scab resistance gene *Ccu* also showed high levels of microsynteny with the melon orthologous region (Kang et al. 2011).

When full draft genome sequences were available, the complete picture of the genomes could be constructed. It showed that chromosome structures were largely kept between the different genomes although extensive chromosomal rearrangements had occurred. They also confirmed that no large genome duplications have occurred within the Cucurbitaceae family or in its specific ancestor besides the one reported in the origin of eudicots (Paterson et al. 2010). Genome data allowed constructing a general map of genome colinearity between the three species, although showing a high degree of complexity for the chromosome evolution (Guo et al. 2012). Comparisons between the melon and the Gy14 and 9930 cucumber genomes suggest that essentially cucumber chromosome 7 corresponds to melon chromosome I, and the additional six cucumber chromosomes have originated after fusions of melon chromosome pairs, but with extensive additional chromosome reorganizations (Garcia-Mas et al. 2012; Huang et al. 2009; Li et al. 2011a). From these data, it is also clear that the melon genome has expanded when compared to cucumber, especially in pericentromeric regions and most probably attributable to transposable element expansions. An example is illustrated in the melon LG IV distal region of 8.5 Mb, which in cucumber corresponds to a 5 Mb distal region of chromosome 3, suggesting an expansion in the melon genome near the centromeric regions (Garcia-Mas et al. 2012).

2.5 How Useful Is the Melon Genome Sequence?

There is no doubt that the availability of a draft genome for a plant species is the starting point of new studies that will permit better understanding of several important biological processes and at the same time performing plant breeding at a faster speed.

The availability of the gene repertoire of melon permits the analysis of genes that could be relevant to interesting genetic characters. This is the case for the biosynthesis of secondary metabolites that produce fruit aromas and pigments (Gonda et al. 2013; Tadmor et al. 2010), sugar accumulation in fruit (Dai et al. 2011) and

climacteric fruit ripening (Vegas et al. 2013). Another important group of genes are those that have similarity to resistance genes (R-genes), which has already been discussed in Sect. 2.3. As an example, the melon genome sequence has recently been useful to assist in cloning the *Fom-1* and *Prv* genes for resistance to *Fusarium oxysporum* races 0 and 2 and *Papaya ring-spot virus*, respectively (Brotman et al. 2013). In the last years, melon has also been used as a model system to shed light to the sex determination in cucurbits. The characterization of the *a* and *g* genes and the availability of the genomes of three cucurbit species (Boualem et al. 2008; Martin et al. 2009) will permit a better understanding of the control and regulation of this important trait. Also, the study of signalling and transport of macromolecules through phloem will benefit from the availability of the cucurbit genomes (Turnbull and Lopez-Cobollo 2013).

On the other hand, resequencing of melon germplasm has already provided an enormous amount of SNP markers, which can be used in high-throughput genotyping platforms for germplasm selection in breeding programmes (Esteras et al. 2013) (Sanseverino et al. 2015). In the near future, significant collection of wild and cultivated melon accessions with known origin will be resequenced, which will allow to identify genomic regions and candidate genes responsible for the domestication and improvement of this important plant species. Also, the availability of melon, cucumber and watermelon genomes provides the possibility of translating information acquired in one species to the others. The recent developments in NGS have allowed de novo sequencing of three cucurbit genomes since 2009. In the next years, mainly due to new advances in genome sequencing technologies, we will have access to the genome of hundreds/thousands of cultivars and reference genomes of several other cucurbit species. This will surely change the way we study the biology of cucurbits in the following years.

References

Al-Faifi S, Meyer J, Garcia-Mas J, Monforte A, Havey M (2008) Exploiting synteny in Cucumis for mapping of Psm: a unique locus controlling paternal mitochondrial sorting. Theor Appl Genet 117:523–529

Argyris JM, Ruiz-Herrera A, Madriz-Masis P, Sanseverino W, Morata J, Pujol M, Ramos-Onsins SE, Garcia-Mas J (2015) Use of targeted SNP selection for an improved anchoring of the melon (Cucumis melo L.) scaffold genome assembly. BMC Genomics 16:4

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Report 9:208–218

Blanca J, Cañizares J, Roig C, Ziarsolo P, Nuez F, Pico B (2011a) Transcriptome characterization and high throughput SSRs and SNPs discovery in Cucurbita pepo (Cucurbitaceae). BMC Genomics 12:104

Blanca JM, Cañizares J, Ziarsolo P, Esteras C, Mir G, Nuez F, Garcia-Mas J, Picó M (2011b) Melon transcriptome characterization: simple sequence repeats and single nucleotide polymorphisms discovery for high throughput genotyping across the species. Plant Genome 4:118–131

Blanca J, Esteras C, Ziarsolo P, Perez D, Fernandez-Pedrosa V, Collado C, Rodriguez de Pablos R, Ballester A, Roig C, Cañizares J, Picó B (2012) Transcriptome sequencing for SNP discovery across Cucumis melo. BMC Genomics 13:280

Blanca JM, Cañizares J, Ziarsolo P, Esteras C, Mir G, Nuez F, Garcia-Mas J, Picó M (2011c) Melon transcriptome characterization: simple sequence repeats and single nucleotide polymorphisms discovery for high throughput genotyping across the species. Plant Genome 4:118–131

Boualem A, Fergany M, Fernandez R, Troadec C, Martin A, Morin H, Sari M-A, Collin F, Flowers J, Pitrat M, Purugganan M, Dogimont C, Bendahmane A (2008) A conserved mutation in an ethylene biosynthesis enzyme leads to andromonoecy in melons. Science 321:836–838

Brotman Y, Normantovich M, Goldenberg Z, Zvirin Z, Kovalski I et al (2013) Dual resistance of melon to Fusarium oxysporum races 0 and 2 and to Papaya ring-spot virus is controlled by a pair of head-to-head-oriented NB-LRR genes of unusual architecture. Mol Plant 6:235–238

Clepet C, Joobeur T, Zheng Y, Jublot D, Huang M et al (2011) Analysis of expressed sequence tags generated from full-length enriched cDNA libraries of melon. BMC Genomics 12:252

Corbacho J, Romojaro F, Pech J-C, Latche A, Gomez-Jimenez M (2013) Transcriptomic events involved in melon mature-fruit abscission comprise the sequential induction of cell-wall degrading genes coupled to a stimulation of endo and exocytosis. PLoS One 8:e58363

Dahmani-Mardas F, Troadec C, Boualem A, Lévêque S, Alsadon A, Aldoss A, Dogimont C, Bendahmane A (2010) Engineering melon plants with improved fruit shelf life using the TILLING approach. PLoS One 5:e15776

Dai N, Cohen S, Portnoy V, Tzuri G, Harel-Beja R et al (2011) Metabolism of soluble sugars in developing melon fruit: a global transcriptional view of the metabolic transition to sucrose accumulation. Plant Mol Biol 76:1–18

Danin-Poleg Y, Reis N, Tzuri G, Katzir N (2001) Development and characterization of microsatellite markers in Cucumis. Theor Appl Genet 102

Diaz A, Fergany M, Formisano G, Ziarsolo P, Blanca J et al (2011) A consensus linkage map for molecular markers and quantitative trait loci associated with economically important traits in melon (Cucumis melo L.). BMC Plant Biol 11:111

Dogimont C, Chovelon V, Tual S, Boissot N, Rittener V, Giovinazzo V, Bendahmane A (2008) Molecular diversity at the Vat/Pm-W resistance locus in melon. In: Pitat M (ed) IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae. Avignon, Institut National de la Recherche Agronomique

Ellis J, Dodds P, Pryor T (2000) Structure, function and evolution of plant disease resistance genes. Curr Opin Plant Biol 3:278–284

Esteras C, Gomez P, Monforte A, Blanca J, Vicente-Dolera N, Roig C, Nuez F, Pico B (2012) High-throughput SNP genotyping in Cucurbita pepo for map construction and quantitative trait loci mapping. BMC Genomics 13:80

Esteras C, Formisano G, Roig C, Diaz A, Blanca J, Garcia-Mas J, Gomez-Guillamon M, Lopez-Sese A, Lazaro A, Monforte A, Pico B (2013) SNP genotyping in melons: genetic variation, population structure, and linkage disequilibrium. Theor Appl Genet 126:1285–1303

Garcia-Mas J, Benjak A, Sanseverino W, Bourgeois M, Mir G et al (2012) The genome of melon (Cucumis melo L.). Proc Natl Acad Sci U S A 109:11872–11877

Gonda I, Lev S, Bar E, Sikron N, Portnoy V, Davidovich-Rikanati R, Burger J, Schaffer A, Tadmor Y, Giovannonni J, Huang M, Fei Z, Katzir N, Fait A, Lewinsohn E (2013) Catabolism of L-methionine in the formation of sulfur and other volatiles in melon (Cucumis melo L.) fruit. Plant J 74:458–472

Gonzalez V, Benjak A, Henaff E, Mir G, Casacuberta J, Garcia-Mas J, Puigdomenech P (2010a) Sequencing of 6.7 Mb of the melon genome using a BAC pooling strategy. BMC Plant Biol 10:246

Gonzalez V, Rodriguez-Moreno L, Centeno E, Benjak A, Garcia-Mas J, Puigdomenech P, Aranda M (2010b) Genome-wide BAC-end sequencing of Cucumis melo using two BAC libraries. BMC Genomics 11:618

Gonzalez M, Xu M, Esteras C, Roig C, Monforte A, Troadec C, Pujol M, Nuez F, Bendahmane A, Garcia-Mas J, Pico B (2011) Towards a TILLING platform for functional genomics in Piel de Sapo melons. BMC Res Notes 4:289

Gonzalez-Ibeas D, Blanca J, Donaire L, Saladié M, Mascarell-Creus A, Cano-Delgado A, Garcia-Mas J, Llave C, Aranda M (2011) Analysis of the melon (Cucumis melo) small RNAome by high-throughput pyrosequencing. BMC Genomics 12:393

Gonzalez-Ibeas D, Cañizares J, Aranda M (2012) Microarray analysis shows that recessive resistance to Watermelon mosaic virus in melon is associated with the induction of defense response genes. Mol Plant Microbe Interact 25:107–118

Gonzalo M, Oliver M, Garcia-Mas J, Monfort A, Dolcet-Sanjuan R, Katzir N, Arus P, Monforte A (2005) Simple-sequence repeat markers used in merging linkage maps of melon (Cucumis melo L.). Theor Appl Genet 110:802–811

Guo S, Zhang J, Sun H, Salse J, Lucas W et al (2012) The draft genome of watermelon (Citrullus lanatus) and resequencing of 20 diverse accessions. Nat Genet 45:51–58

Huang S, Li R, Zhang Z, Li L, Gu X et al (2009) The genome of the cucumber, Cucumis sativus L. Nat Genet 41:1275–1281

Hufford MB, Xu X, vanHeerwaarden J, Pyhäjärvi T, Chia J-M et al (2012) Comparative population genomics of maize domestication and improvement. Nat Genet 44:808–811

Kang H, Weng Y, Yang Y, Zhang Z, Zhang S, Mao Z, Cheng G, Gu X, Huang S, Xie B (2011) Fine genetic mapping localizes cucumber scab resistance gene Ccu into an R gene cluster. Theor Appl Genet 122:795–803

Li D, Cuevas H, Yang L, Li Y, Garcia-Mas J, Zalapa J, Staub J, Luan F, Reddy U, He X, Gong Z, Weng Y (2011a) Syntenic relationships between cucumber (Cucumis sativus L.) and melon (C. melo L.) chromosomes as revealed by comparative genetic mapping. BMC Genomics 12:396

Li Z, Zhang Z, Yan P, Huang S, Fei Z, Lin K (2011b) RNA-Seq improves annotation of protein-coding genes in the cucumber genome. BMC Genomics 12:540

Lin X, Zhang Y, Kuang H, Chen J (2013) Frequent loss of lineages and deficient duplications accounted for low copy number of disease resistance genes in Cucurbitaceae. BMC Genomics 14:335

Luo M, Wang Y, Frisch D, Joobeur T, Wing R, Dean R (2001) Melon bacterial artificial chromosome (BAC) library construction using improved methods and identification of clones linked to the locus conferring resistance to melon Fusarium wilt (Fom-2). Genome 44:154–162

Martin A, Troadec C, Boualem A, Rajab M, Fernandez R, Morin H, Pitrat M, Dogimont C, Bendahmane A (2009) A transposon-induced epigenetic change leads to sex determination in melon. Nature 461:1135–1138

Mascarell-Creus A, Cañizares J, Vilarrasa-Blasi J, Mora-Garcia S, Blanca J et al (2009) An oligo-based microarray offers novel transcriptomic approaches for the analysis of pathogen resistance and fruit quality traits in melon (Cucumis melo L.). BMC Genomics 10:467

Meyer J, Deleu W, Garcia-Mas J, Havey M (2008) Construction of a fosmid library of cucumber (Cucumis sativus) and comparative analyses of the eIF4E and eIF(iso)4E regions from cucumber and melon (Cucumis melo). Mol Genet Genomics 279:473–480

Ming R, Hou S, Feng Y, Yu Q, Dionne-Laporte A et al (2008) The draft genome of the transgenic tropical fruit tree papaya (Carica papaya Linnaeus). Nature 452:991–996

Nystedt B, Street NR, Wetterbom A, Zuccolo A, Lin Y-C et al (2013) The Norway spruce genome sequence and conifer genome evolution. Nature 497:579–584

Paterson A, Freeling M, Tang H, Wang X (2010) Insights from the comparison of plant genome sequences. Annu Rev Plant Biol 61:349–372

Pech JC, Bouzayen M, Latche A (2008) Climacteric fruit ripening: ethylene-dependent and independent regulation of ripening pathways in melon fruit. Plant Sci 175:114–120

Pitrat M (2008) Melon (*Cucumis melo* L.). In: Prohens J, Nuez F (eds) Handbook of crop breeding vol I: vegetables. Springer, New York, pp 283–315

Portnoy V, Diber A, Pollock S, Karchi H, Lev S, Tzuri G, Harel-Beja R, Forer R, Portnoy V, Lewinsohn E, Tadmor Y, Burger J, Schaffer A, Katzir N (2011) Use of non-normalized, non-amplified cDNA for 454-based RNA sequencing of fleshy melon fruit. Plant Genome 4:36–46

Rodriguez-Moreno L, Gonzalez V, Benjak A, Marti M, Puigdomenech P, Aranda M, Garcia-Mas J (2011) Determination of the melon chloroplast and mitochondrial genome sequences reveals that the largest reported mitochondrial genome in plants contains a significant amount of DNA having a nuclear origin. BMC Genomics 12:424

Roig C, Fita A, Rios G, Hammond J, Nuez F, Pico B (2012) Root transcriptional responses of two melon genotypes with contrasting resistance to Monosporascus cannonballus (Pollack et Uecker) infection. BMC Genomics 13:601

Sanseverino W, Hénaff E, Vives C, Pinosio S, Burgos-Paz W, Morgante M, Ramos-Onsins SE, Garcia-Mas J, Casacuberta JM (2015) Transposon insertion, structural variations and SNPs contribute to the evolution of the melon genome. Mol Biol Evol (in press)

Schnable P, Ware D, Fulton R, Stein J, Wei F et al (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112–1115

Sebastian P, Schaefer H, Telford I, Renner S (2010) Cucumber (Cucumis sativus) and melon (C. melo) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. Proc Natl Acad Sci U S A 107:14269–14273

Shendure J, Ji H (2008) Next-generation DNA sequencing. Nat Biotechnol 26:1135–1145

Tadmor Y, Katzir N, Meir A, Yaniv-Yaakov A, Sa'ar U, Baumkoler F, Lavee T, Lewinsohn E, Schaffer A, Burger J (2007) Induced mutagenesis to augment the natural genetic variability of melon (Cucumis melo L.). Isr J Plant Sci 55:159–169

Tadmor Y, Burger J, Yaakov I, Feder A, Libhaber S, Portnoy V, Meir A, Tzuri G, Sa'ar U, Rogachev I, Aharoni A, Abeliovich H, Schaffer A, Lewinsohn E, Katzir N (2010) Genetics of flavonoid, carotenoid, and chlorophyll pigments in melon fruit rinds. J Agric Food Chem 58:10722–10728

Tanaka K, Akashi Y, Fukunaga K, Yamamoto T, Aierken Y, Nishida H, Long CL, Yoshino H, Sato Y-I, Kato K (2013) Diversification and genetic differentiation of cultivated melon inferred from sequence polymorphism in the chloroplast genome. Breed Sci 63:183–196

The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 408:796–815

The French-Italian Public Consortium for Grapevine Genome Characterization (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449:463–467

The International Barley Genome Sequencing Consortium (2012) A physical, genetic and functional sequence assembly of the barley genome. Nature 491:711–716

The International Rice Genome Sequencing Project (2005) The map-based sequence of the rice genome. Nature 436:793–800

Till B, Reynolds S, Greene E, Codomo C, Enns L, Johnson J, Burtner C, Odden A, Young K, Taylor N, Henikoff J, Comai L, Henikoff S (2003) Large-scale discovery of induced point mutations with high-throughput TILLING. Genome Res 13:524–530

Turnbull C, Lopez-Cobollo R (2013) Heavy traffic in the fast lane: long-distance signalling by macromolecules. New Phytol 198:33–51

Tuskan G, Difazio S, Jansson S, Bohlmann J, Grigoriev I et al (2006) The genome of black cottonwood, Populus trichocarpa (Torr. & Gray). Science 313:1596–1604

van Leeuwen H, Monfort A, Zhang H-B, Puigdomenech P (2003) Identification and characterisation of a melon genomic region containing a resistance gene cluster from a constructed BAC library. Microcolinearity between Cucumis melo and Arabidopsis thaliana. Plant Mol Biol 51:703–718

Vegas J, Garcia-Mas J, Monforte A (2013) Interaction between QTLs induces an advance in ethylene biosynthesis during melon fruit ripening. Theor Appl Genet 126:1531–1544

Wan H, Yuan W, Bo K, Shen J, Pang X, Chen J (2013) Genome-wide analysis of NBS-encoding disease resistance genes in Cucumis sativus and phylogenetic study of NBS-encoding genes in Cucurbitaceae crops. BMC Genomics 14:109

Wóycicki R, Witkowicz J, Gawronski P, Dabrowska J, Lomsadze A et al (2011) The genome sequence of the North-European cucumber (Cucumis sativus L.) unravels evolutionary adaptation mechanisms in plants. PLoS One 6(7):e22728

Yang L, Li D, Li Y, Gu X, Huang S, Garcia-Mas J, Weng Y (2013) A 1,681-locus consensus genetic map of cultivated cucumber including 67 NB-LRR resistance gene homolog and ten gene loci. BMC Plant Biol 13:53

Zhang B, Tolstikov V, Turnbull C, Hicks L, Fiehn O (2010) Divergent metabolome and proteome suggest functional independence of dual phloem transport systems in cucurbits. Proc Natl Acad Sci U S A 107:13532–13537

Chapter 3
Databases for Solanaceae and Cucurbitaceae Research

Masaaki Kobayashi, Hajime Ohyanagi, and Kentaro Yano

3.1 Outline of the Omics Databases in Plants

Currently, the vast majority of plant science information is available via the Internet. It covers publications, experimental resources, protocols and results, analysis tools, and so on. In this section, major databases for general plant sciences will be briefly introduced.

The primary sequence data are provided by the International Nucleotide Sequence Databases (INSD) (Nakamura et al. 2013) and Universal Protein Resource (UniProt) (The UniProt Consortium 2014). The INSD, maintained by the DNA Data Bank of Japan (DDBJ) (Kosuge et al. 2014), the European Nucleotide Archive (ENA) (Pakseresht et al. 2014), and GenBank (Benson et al. 2014), stores nucleotide sequence data. UniProt provides high-quality information on protein sequences and their biological functions. Through the INSD and UniProt, sequence data for Solanaceae and Cucurbitaceae crops are also accessible.

M. Kobayashi • K. Yano (✉)
Bioinformatics Laboratory, School of Agriculture, Meiji University, Kawasaki, Japan
e-mail: kyano@meiji.ac.jp

H. Ohyanagi
Bioinformatics Laboratory, School of Agriculture, Meiji University, Kawasaki, Japan

Computational Bioscience Research Center, King Abdullah University of Science and Technology, Thuwal, Kingdom of Saudi Arabia

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_3

3.1.1 Information and Tools from NCBI

On the website of the National Center for Biotechnology Information (NCBI) (NCBI Resource Coordinators 2014), sequence and annotation data stored in the INSD are easily accessible. From the sequence databases nucleotide, EST, and protein in NCBI, which can be specified with a pull-down selector on the top menu, each sequence entity (record, entry) can be retrieved by a text-based keyword search. For a keyword search, each phrase should be in quotes (e.g., "Solanum lycopersicum"); a field-specific search function is also available. For example, sequences of tomato can be retrieved by the query "Solanum lycopersicum" [ORGN]. NCBI provides useful field search functions, such as [ORGN] for the source of the sequence records, [ECNC] for the Enzyme Commission (EC) number (Webb 1992), and [GENE] for the gene name (see http://www.ncbi.nlm.nih.gov/books/NBK49540/).

NCBI's database of expressed sequence tags (dbESTs) (Boguski et al. 1993) and UniGene database (UniGene) (Wheeler et al. 2003) provide information on expressed sequence tags (ESTs) from a broad variety of organisms. The UniGene database contains information on protein similarities, gene expression, cDNA clones, and genomic locations with a list of accession numbers of ESTs. The UniGene database is regularly updated on a weekly or a monthly basis.

Besides sequence data, NCBI also provides information on genomes and genes. The genome database provides information on genome sequencing and annotation projects. For example, a genome map viewer and information on genome sequencing projects for tomato can be browsed by the query "Solanum lycopersicum" [ORGN]. With the gene database, information on genomic structure, positions on the genome, and literature is provided.

The Basic Local Alignment Search Tool (BLAST) algorithm (Boratyn et al. 2013) assists in retrieving sequences homologous to a query sequence. By taking advantage of the web interfaces for a BLAST search, sequences homologous to a nucleotide or protein query sequence in the database can be comprehensively identified. In the case of the NCBI web tool for BLAST, a single database should be specified from the preset databases. In the case of a stand-alone BLAST search, by installing the program distributed from NCBI's FTP site, a custom sequence database, for example, a sequence database for transcription factors in tomato, can be constructed with a set of arbitrary sequences on each personal computer or server.

3.1.2 The Gene Index Databases

The Dana-Farber Cancer Institute (DFCI) maintains a gene index database (http://compbio.dfci.harvard.edu/tgi/) to provide comprehensive information on expressed genes in animals, plants, protists, and fungi. It contains a nonredundant consensus sequence set, called a tentative consensus (TC), generated by assembling and clustering methods (Lee et al. 2005). In the database, information on variants and functional and structural annotations is also available. The database stores

information on homologous protein sequences, open reading frames (ORFs), gene ontology (GO) terms, single nucleotide polymorphisms (SNPs), alternative splicing sequences, cDNA libraries, EC numbers of the International Union of Biochemistry and Molecular Biology, Kyoto Encyclopedia of Genes and Genomes (KEGGs) metabolic pathways (Kanehisa et al. 2014), unique 70-mer oligonucleotide sequences, and orthologs in other organisms. The current version of gene indices provides omics information on plants such as tomato, *Nicotiana benthamiana*, tobacco, eggplant, potato, pepper, petunia, and coffee.

3.1.3 Sequence Data with Next-Generation Sequencing Technologies

Genomic DNA and cDNA sequencing data obtained from next-generation sequencing (NGS) technologies have been rapidly accumulated in the public databases (Wheeler et al. 2008), i.e., the NCBI Sequence Read Archive (SRA) (NCBI Resource Coordinators 2014), the ENA of The European Bioinformatics Institute—Part of the European Molecular Biology Laboratory (EMBL-EBI) (Pakseresht et al. 2014), and the DDBJ Sequence Read Archive (DRA) (Kosuge et al. 2014). Genomic DNA sequence data can be employed to determine genome sequences by de novo assembly or to identify DNA polymorphisms including SNPs and simple sequence repeats (SSRs) by reference mapping methodologies. With cDNA or mRNA sequencing data (RNA-Seq data) generated under multiple experimental conditions, gene expression profiles across these conditions can be obtained. To obtain the gene expression profiles, genome sequences or unigene sequences (a nonredundant sequence set of transcripts derived from ESTs and draft full-length sequences) are required as reference sequences.

3.2 Genome and Transcriptome Data

The families Solanaceae (nightshade-related species) and Cucurbitaceae include numbers of agronomically and economically significant flowering plants. Among the Solanaceae, tomato (*Solanum lycopersicum*) is one of the most important agricultural crops in human culture and history. Together with the Brassicaceae and Fabaceae families, the Solanaceae family has been widely used for evolutionary analysis. The family Cucurbitaceae, sometimes called the gourd family, consists of over a hundred genera. It includes cucumber, watermelon, melon, and pumpkin, as well as other food plants.

To date, Solanaceae and Cucurbitaceae plants have been employed as model plants. Major databases are being maintained to provide omics information on these model plants. The databases maintained by genome sequencing projects generally provide information on genome and transcriptome data, since both types of data are often required for structural and functional annotation of the genome. In this

Table 3.1 Web databases for Solanaceae and Cucurbitaceae

Name of database	URL
International Nucleotide Sequence Databases Collaboration (INSDC)	http://insdc.org/
Universal Protein Resource (UniProt)	http://www.uniprot.org/
DNA Data Bank of Japan (DDBJ)	http://www.ddbj.nig.ac.jp/
European Nucleotide Archive (ENA)	http://www.ebi.ac.uk/ena/
GenBank	http://www.ncbi.nlm.nih.gov/genbank/
National Center for Biotechnology Information (NCBI)	http://www.ncbi.nlm.nih.gov/
NCBI's database of expressed sequence tags (dbESTs)	http://www.ncbi.nlm.nih.gov/dbEST/
UniGene database (UniGene)	http://www.ncbi.nlm.nih.gov/unigene
Basic Local Alignment Search Tool (BLAST)	http://blast.ncbi.nlm.nih.gov/Blast.cgi
Dana-Farber Cancer Institute (DFCI) Gene Index Project	http://compbio.dfci.harvard.edu/tgi/
Kyoto Encyclopedia of Genes and Genomes (KEGGs)	http://www.genome.jp/kegg/pathway.html
NCBI Sequence Read Archive (SRA)	http://www.ncbi.nlm.nih.gov/sra
DDBJ Sequence Read Archive (DRA)	http://trace.ddbj.nig.ac.jp/dra/index.html
Sol Genomics Network (SGN)	http://solgenomics.net/
TOMATOMICS	http://bioinf.mind.meiji.ac.jp/tomatomics/
MiBASE	http://www.pgb.kazusa.or.jp/mibase/
KaFTom	http://www.pgb.kazusa.or.jp/kaftom/
Solanum lycopersicum project in PGSB	http://pgsb.helmholtz-muenchen.de/plant/tomato/
National BioResource Project (NBRP) TOMATO	http://tomato.nbrp.jp/indexEn.html
Potato Genome Sequence Consortium (PGSC)	http://www.potatogenome.net/index.php/Main_Page
CuGenDB	http://www.icugi.org/cgi-bin/ICuGI/index.cgi
MELONOMICS	https://www.melonomics.net/
MeloGene	http://www.melogene.net/
CucurbiGene	http://www.cucurbigene.net/
Plant Metabolic Network (PMN)	http://www.plantcyc.org/
SolCyc	http://solcyc.solgenomics.net/
KaPPA-View4	http://kpv.kazusa.or.jp/
Platform for RIKEN Metabolomics (PRIMe)	http://prime.psc.riken.jp/
KNApSAcK	http://kanaya.naist.jp/KNApSAcK/
KNApSAcK core system	http://kanaya.naist.jp/knapsack_jsp/top.html
SHared Information of GENetic resources (SHIGEN) project	http://www.shigen.nig.ac.jp/indexja.htm
Tomato Genetic Resource Center (TGRC)	http://tgrc.ucdavis.edu/index.aspx
National BioResource Project (NBRP)	http://www.nbrp.jp/
PhylomeDB	http://phylomedb.org/

(continued)

Table 3.1 (continued)

Name of database	URL
The Arabidopsis Information Resource (TAIR)	http://www.arabidopsis.org/
The Rice Annotation Project Database (RAP-DB)	http://rapdb.dna.affrc.go.jp/
MSU Rice Genome Annotation Project Database	http://rice.plantbiology.msu.edu/
InParanoid	http://inparanoid.sbc.su.se/cgi-bin/index.cgi
OrthoMCL	http://orthomcl.org/orthomcl/
InterProScan	http://www.ebi.ac.uk/interpro/search/sequence-search
UniProt Knowledgebase (UniProtKB)	http://www.uniprot.org/uniprot/
Plant Omics Data Center (PODC)	http://bioinf.mind.meiji.ac.jp/podc/

section, leading databases providing large-scale genome and transcriptome data in tomato, potato, tobacco, cucumber, melon, and other species are briefly introduced (Table 3.1).

3.2.1 Tomato

The Sol Genomics Network (SGN) (http://solgenomics.net), funded by the NSF and USDA CSREES and hosted at the Boyce Thompson Institute of Cornell University, NY, USA (Bombarely et al. 2011), represents one of the major versatile Solanaceae databases. It stores and serves complete genomic DNA sequences of potato (Potato Genome Sequencing Consortium 2011) and cultivated tomato (Tomato Genome Consortium 2012). It also archives the draft genome DNA sequences of wild tomato, *Nicotiana benthamiana*, and other species. Information on these genomes and annotations is available via the GBrowse genome browser and FTP server for the database. The SGN also covers resources for the SOL-100 project (a comprehensive genome sequencing project) and other Solanaceae genome and annotation information. Transcriptome data such as microarray data, ESTs, cDNA clones, and unigenes for tomato, potato, pepper, *Nicotiana* species, petunia, and coffee are accessible from the SGN website. Each record (entry in the database) has been integrated and assigned internal SGN identifiers.

The TOMATOMICS database (http://bioinf.mind.meiji.ac.jp/tomatomics/), now open to the public, is an omics database designed especially for tomato. Aside from genome sequences and annotations, it integrates various types of biological information on tomato such as ESTs, nucleotide variant information (SNPs and insertion/deletions or InDels) among inbred lines, DNA markers, microarray data, gene expression networks, and metabolic pathways. It has been constructed and maintained by Meiji University, Japan. TOMATOMICS contains information on ESTs, draft full-length sequences (high-throughput cDNA sequences or HTCs), and unigenes (a nonredundant sequence set derived from ESTs and HTCs) provided from the MiBASE (Yano et al. 2006c) and KaFTom databases (Aoki et al. 2010). In this database, unigenes are called Kazusa tomato unigenes (KTUs). The current

version of the database provides information on KTU version 4 (KTU4). The ESTs and HTCs were generated from the "Micro-Tom" model plant (Aoki et al. 2010; Tsugane et al. 2005; Yamamoto et al. 2005). SNPs/InDels stored in TOMATOMICS were derived from analyses of ESTs and genomes. With EST analysis, SNPs in transcripts were detected among inbred lines (Yano et al. 2006c). By using NGS technology, over 1.2 million SNP sites between the Heinz 1706 and Micro-Tom genomes were detected (Kobayashi et al. 2014). In TOMATOMICS, users can browse comprehensive SNP information with the GBrowse. For the genome sequence of Micro-Tom, TOMATOMICS also provides BAC-end sequence data via GBrowse (Asamizu et al. 2012). The sequences and functional annotation data for Micro-Tom assist in designing effective strategies for elucidating of molecular mechanisms involved in each trait and biological process by using experimental resources (mutant lines and cDNA clones) from Micro-Tom. The Micro-Tom experimental resources are distributed by the National BioResource Project (NBRP) of Japan (http://tomato.nbrp.jp/indexEn.html) (Yamazaki et al. 2010) (see Sect. 3.3).

In addition, the Solanum lycopersicum project of PGSB (http://pgsb.helmholtz-muenchen.de/plant/tomato/) maintains its own integrated database for tomato.

3.2.2 *Potato*

The potato (*Solanum tuberosum*) genome was sequenced by the Potato Genome Sequencing Consortium (PGSC), an international group of scientists from 14 countries, in 2011 (Potato Genome Sequencing Consortium 2011). Information on the genome sequence, including annotations, has been served primarily by the consortium's website (http://www.potatogenome.net/index.php/Main_Page). The SGN mirrors and integrates the potato information and provides it on their website.

3.2.3 *Tobacco*

Regarding tobacco (genus *Nicotiana*), a couple of draft genome sequence datasets have been provided. Genome sequence data on the experimental model plant *Nicotiana benthamiana* are available from SGN (Bombarely et al. 2011).

3.2.4 *Other Species*

The Cucurbit Genomics Database (CuGenDB) (http://www.icugi.org/cgi-bin/ICuGI/index.cgi) is an integrative database of the Cucurbitaceae. From the CuGenDB, information on the genomes of cucumber (Huang et al. 2009) and watermelon (Guo et al. 2013) is available. Beijing Genomics Institute (BGI) also

examined and published genome resequencing data on a broad variety of cucumber lines in late 2013 (Qi et al. 2013). The CuGenDB contains a massive amount of cucumber genome information. The genomic sequences of newly sequenced cultivars are also available in CuGenDB or its associated website (http://cmb.bnu.edu.cn/Cucumis_sativus_v20/resequence/). The draft genome sequences of an elite Chinese watermelon inbred line were published in 2013 (Guo et al. 2013). Genomic data and genome resequencing information on 20 watermelon accessions are available in the CuGenDB.

The CuGenDB also provides information such as annotations, ESTs, pathways, nucleotide variants, SSRs, and genetic maps. ESTs stored in CuGenDB were generated from melon, cucumber, watermelon, and *Cucurbita pepo* (Ando and Grumet 2010; Blanca et al. 2011a, b; Clepet et al. 2011; Guo et al. 2010, 2011; Levi et al. 2006). Data on these ESTs and unigenes are downloadable from the database. BLAST searches against the ESTs and CDSs can be performed. In the current version of CuGenDB, information on SSR markers for melon and pathway data for melon, cucumber, watermelon, and *Cucurbita pepo* are accessible.

Moreover, the CucurbiGene database serves transcriptome data from *Cucurbita pepo* derived from de novo assembly of next-generation sequences (Blanca et al. 2011a).

The genome sequence, physical map, annotations, and transcriptome sequences of melon are available from the MELONOMICS (https://www.melonomics.net/) (Blanca et al. 2011b; Garcia-Mas et al. 2012; González et al. 2010). Additionally, the MeloGene (http://www.melogene.net/) offers GBrowse interface including unigenes derived from EST assembly, SSRs, and SNPs among several botanical varieties obtained by mapping their next-generation sequences against genome (Blanca et al. 2011b, 2012; Esteras et al. 2013; Gonzalez-Ibeas et al. 2007).

3.3 Data on Metabolic Pathways and Compounds

Many metabolic pathway databases are provided from the Plant Metabolic Network (PMN) (http://www.plantcyc.org). These databases contain information on genes, enzymes, compounds, reactions, and pathways involved in primary and secondary metabolism. SolCyc (http://solcyc.solgenomics.net), one of the databases maintained by the PMN, provides metabolic data in the Solanaceae, including tomato (LycoCyc), pepper (CapCyc), petunia (PetCyc), potato (PotatoCyc), and tobacco (TobaccoCyc). The information on biochemical pathways for melon (MelonCyc), cucumber (CucCyc), watermelon (WmnCyc), and *Cucurbita pepo* is available from CuGenDB.

KEGG stores information on the molecular interaction and reaction networks involved in metabolic pathways (Ogata et al. 1998). In KEGG, metabolites and ligands can be searched by keyword. The web page containing the retrieved data shows graphical pathway maps.

Information on tomato metabolic pathways is also available from the KaPPA-View4 database (Sakurai et al. 2011). In KaPPA-View4, not only a browser for metabolic pathways is available but also an analysis function for custom gene expression data. In addition, in the viewer for the metabolic pathways in KaPPA-View4, gene expression data from the MiBASE and TOMATOMICS databases are shown. Using the microarray search function in TOMATOMICS, microarray expression data can be retrieved with hyperlinks to KaPPA-View4. With these hyperlinks, upregulated and downregulated genes and correlations in expression are graphically shown in the KaPPA-View4 viewer.

Metabolic data for tomato and cucumber are freely available from public databases. Spectral data of metabolites measured by nuclear magnetic resonance (NMR), gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), and capillary electrophoresis/mass spectrometry (CE/MS) are accessible from the Platform for RIKEN Metabolomics (PRIMe) (http://prime.psc.riken.jp/). In the PRIMe, spectral data can be searched for by keywords for compound name, PubChem ID, KEGG ID, and chemical formula. The KNApSAcK database (http://kanaya.naist.jp/KNApSAcK/) stores multiple species-wide metabolite data (Nakamura et al. 2014). Metabolite data are available through the keyword search function by names of organisms including tomato, potato, pepper, petunia, tobacco, melon, cucumber, and *Cucurbita* in the KNApSAcK core system (http://kanaya.naist.jp/knapsack_jsp/top.html).

3.4 Information on Experimental Resources

An infrastructure of experimental resources such as seeds and DNA clones facilitates more efficient research strategies. The SHared Information of GENetic resources (SHIGEN) project (http://www.shigen.nig.ac.jp) provides information on experimental materials and databases for various organisms including tomato. The experimental materials (i.e., frozen embryos, plant seeds, cultured cells, DNA clones, live animal stocks, etc.) in SHIGEN are available upon request. The Tomato Genetic Resource Center (TGRC) (http://tgrc.ucdavis.edu) also provides information on tomato genetic resources, including wild relatives.

Information on DNA resources in tomato is accessible through web databases. The information pages for BAC and EST clones in the SGN (Bombarely et al. 2011) contain hyperlinks to order the clones when they are freely available. The NBRP (Yamazaki et al. 2010) in Japan has also established a bioresource infrastructure for many model organisms, including tomato. The tomato NBRP has enhanced the research infrastructure with mutant collections and DNA resources generated from Micro-Tom. Information on the mutant lines is accessible from the TOMATOMA database (Saito et al. 2011). Information on cDNA clones containing full-length cDNA is available from the TOMATOMICS database (Kobayashi et al. 2014).

3.5 Omics Information and Biological Knowledge on Specific Characters in the Solanaceae and Cucurbitaceae

A wealth of omics information allows comprehensive analysis of the genome, transcriptome, metabolome, and other omes. A large-scale analysis, especially comparison of omics information among inbred lines and/or species, provides clues to understand the molecular mechanisms behind specific traits in the Solanaceae and Cucurbitaceae. Comparisons with model plants such as *Arabidopsis* and rice have been widely performed to find species-specific characters.

For example, a set of expressed genes in tomato that have no counterpart in *Arabidopsis* has been identified (Yano et al. 2006b). Omics information in *Arabidopsis* and rice is available from databases such as the Arabidopsis Information Resource (TAIR) (Lamesch et al. 2012), the Rice Annotation Project Database (RAP-DB) (Sakai et al. 2013), and the MSU Rice Genome Annotation Project Database (Ouyang et al. 2007). Ortholog information is useful to compare genes and proteins among species. Information on orthologs is provided in the InParanoid (Ostlund et al. 2010) and OrthoMCL databases (Chen et al. 2006). In terms of orthology determination, the protein phylogenic trees provided by the database PhylomeDB (http://phylomedb.org/) are significant (Huerta-Cepas et al. 2014). With the interface for the phylogenic trees, protein domains are graphically shown along with their lineage relationship. The current version of the PhylomeDB offers phylogenetic trees for melon, cucumber, cacao, and other 20 model plants.

Gene expression networks (GENs) permit genome-wide views of the similarities of gene expression profiles. In GENs, nodes represent genes, and two nodes with significantly similar expression profiles are connected by an edge. Therefore, a gene set with similar expression profiles (a gene module) can be simultaneously identified from a graphical viewer of GENs. The genes with similar expression profiles can be used as candidates for being involved in the same biological process. With accumulating data on genome sequences and transcriptomes (gene expression) in plants, GEN analyses have been widely used to discover genes and elucidate their biological functions.

The construction of GENs from large-scale expression data becomes difficult while GENs demonstrate substantial usefulness. This is mainly due to the current method for assessment of similarities in expression profiles by Pearson correlation coefficients (PCCs). With the increments in the numbers of genes and samples (experimental conditions) for the analysis with PCC, CPU resources (CPUs, memory, and calculation time) required for the analysis are drastically increased to compute PCCs for gene and/or sample pairs. While the accumulation of data promises to improve the accuracy of the computational analysis, the calculation becomes difficult with large-scale datasets. To handle large-scale omics data, such as NGS and microarray data, with the personal computers or workstations generally

used in laboratories, new bioinformatics and statistical approaches should be developed.

To date, one approach to quickly mine genes with similar expression profiles from large-scale transcriptome data has been introduced (Yano et al. 2006a; Hamada et al. 2011; Manickavelu et al. 2012). The method allows detection of genes with similar expression profiles and construction of GENs even with the use of personal computers. The method can be performed by GUI software distributed by Meiji University (http://bioinf.mind.meiji.ac.jp/lab/index.php?catid=15&blogid=1).

Comparisons of GENs among plant species uncover new biological knowledge. Comparative analysis provides information on species-specific genes and gene modules controlling specific traits in each species. Besides identifying similarities of gene expression profiles, accurate functional annotations also facilitate discovery of genes and elucidation of their biological functions. The current annotations provided from most databases have been assigned based on sequence similarity analysis. Sequence similarity searches by programs such as BLAST and InterProScan (Quevillon et al. 2005) permit genome-wide analysis to quickly predict biological functions of genes. On the other hand, accurate annotations are difficult to be assigned, based on such high-throughput homology search methods. To resolve this issue and provide accurate functional annotations for proteins, the UniProt Knowledgebase (UniProtKB) (The UniProt Consortium 2014) has collected high-quality information based on manual annotations with the literature and curator-evaluated computational analysis. Manual annotation is a time-consuming and labor-intensive procedure requiring expert knowledge and training, but these more sophisticated descriptions of the functions and interactions of genes, gene products, and biological conditions will undoubtedly more effectively reveal complicated biological mechanisms and behaviors. Combined with an analysis tool for comparison of GENs among species including tomato, the Plant Omics Data Center (PODC) database (http://bioinf.mind.meiji.ac.jp/podc/) is being maintained to provide information on both automated and manual annotations of genes (Ohyanagi et al. 2015). Such web services with graphical and intuitive interfaces enable both understanding and making use of species-specific characters in the Solanaceae and Cucurbitaceae.

References

Ando K, Grumet R (2010) Transcriptional profiling of rapidly growing cucumber fruit by 454-pyrosequencing analysis. J Am Soc Hortic Sci 135:291–302

Aoki K, Yano K, Suzuki A et al (2010) Large-scale analysis of full-length cDNAs from the tomato (*Solanum lycopersicum*) cultivar Micro-Tom, a reference system for the Solanaceae genomics. BMC Genomics 11:210

Asamizu E, Shirasawa K, Hirakawa H et al (2012) Mapping of Micro-Tom BAC-end sequences to the reference tomato genome reveals possible genome rearrangements and polymorphisms. Int J Plant Genomics 2012:437026

Benson DA, Clark K, Karsch-Mizrachi I et al (2014) GenBank. Nucleic Acids Res 42:D32–D37

Blanca J, Cañizares J, Roig C et al (2011a) Transcriptome characterization and high throughput SSRs and SNPs discovery in *Cucurbita pepo* (Cucurbitaceae). BMC Genomics 12:104

Blanca J, Cañizares J, Ziarsolo P et al (2011b) Melon transcriptome characterization: simple sequence repeats and single nucleotide polymorphisms discovery for high throughput genotyping across the species. Plant Genome 4:118–131

Blanca J, Esteras C, Ziarsolo P et al (2012) Transcriptome sequencing for SNP discovery across Cucumis melo. BMC Genomics 13:280

Boguski MS, Lowe TM, Tolstoshev CM (1993) dbEST—database for "expressed sequence tags". Nat Genet 4:332–333

Bombarely A, Menda N, Tecle IY et al (2011) The Sol Genomics Network (solgenomics.net): growing tomatoes using Perl. Nucleic Acids Res 39:D1149–D1155

Boratyn GM, Camacho C, Cooper PS et al (2013) BLAST: a more efficient report with usability improvements. Nucleic Acids Res 41:W29–W33

Chen F, Mackey AJ, Stoeckert CJ Jr et al (2006) OrthoMCL-DB: querying a comprehensive multi-species collection of ortholog groups. Nucleic Acids Res 34:D363–D368

Clepet C, Joobeur T, Zheng Y et al (2011) Analysis of expressed sequence tags generated from full-length enriched cDNA libraries of melon. BMC Genomics 12:252

Esteras C, Formisano G, Roig C et al (2013) SNP genotyping in melons: genetic variation, population structure, and linkage disequilibrium. Theor Appl Genet 126(5):1285–1303

Garcia-Mas J, Benjak A, Sanseverino W et al (2012) The genome of melon (Cucumis melo L.). Proc Natl Acad Sci U S A 109(29):11872–11877

González VM, Garcia-Mas J, Arús P et al (2010) Generation of a BAC-based physical map of the melon genome. BMC Genomics 11:339

Gonzalez-Ibeas D, Blanca J, Roig C et al (2007) MELOGEN: an EST database for melon functional genomics. BMC Genomics 8:306

Guo S, Zheng Y, Joung JG et al (2010) Transcriptome sequencing and comparative analysis of cucumber flowers with different sex types. BMC Genomics 11:384

Guo S, Liu J, Zheng Y et al (2011) Characterization of transcriptome dynamics during watermelon fruit development: sequencing, assembly, annotation and gene expression profiles. BMC Genomics 12:454

Guo S, Zhang J, Sun H et al (2013) The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. Nat Genet 45:51–58

Hamada K, Hongo K, Suwabe K et al (2011) OryzaExpress: an integrated database of gene expression networks and omics annotations in rice. Plant Cell Physiol 52:220–229

Huang S, Li R, Zhang Z et al (2009) The genome of the cucumber, *Cucumis sativus* L. Nat Genet 41:1275–1281

Huerta-Cepas J, Capella-Gutiérrez S, Pryszcz LP et al (2014) PhylomeDB v4: zooming into the plurality of evolutionary histories of a genome. Nucleic Acids Res 42:D897–D902

Kanehisa M, Goto S, Sato Y et al (2014) Data, information, knowledge and principle: back to metabolism in KEGG. Nucleic Acids Res 42:D199–D205

Kobayashi M, Nagasaki H, Garcia V et al (2014) Genome-wide analysis of intraspecific DNA polymorphism in 'Micro-Tom', a model cultivar of tomato (*Solanum lycopersicum*). Plant Cell Physiol 55:445–454

Kosuge T, Mashima J, Kodama Y et al (2014) DDBJ progress report: a new submission system for leading to a correct annotation. Nucleic Acids Res 42:D44–D49

Lamesch P, Berardini TZ, Li D et al (2012) The Arabidopsis Information Resource (TAIR): improved gene annotation and new tools. Nucleic Acids Res 40:D1202–D1210

Lee Y, Tsai J, Sunkara S et al (2005) The TIGR Gene Indices: clustering and assembling EST and known genes and integration with eukaryotic genomes. Nucleic Acids Res 33:D71–D74

Levi A, Davis A, Hernandez A et al (2006) Genes expressed during the development and ripening of watermelon fruit. Plant Cell Rep 25:1233–1245

Manickavelu A, Kawaura K, Oishi K et al (2012) Comprehensive functional analyses of expressed sequence tags in common wheat (*Triticum aestivum*). DNA Res 19:165–177

Nakamura Y, Cochrane G, Karsch-Mizrachi I (2013) The international nucleotide sequence database collaboration. Nucleic Acids Res 41:D21–D24
Nakamura Y, Afendi FM, Parvin AK et al (2014) KNApSAcK metabolite activity database for retrieving the relationships between metabolites and biological activities. Plant Cell Physiol 55:e7
NCBI Resource Coordinators (2014) Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 42:D7–D17
Ogata H, Goto S, Fujibuchi W et al (1998) Computation with the KEGG pathway database. Biosystems 47:119–128
Ohyanagi H, Takano T, Terashima S et al (2015) Plant Omics Data Center: an integrated web repository for interspecies gene expression networks with NLP-based curation. Plant Cell Physiol 56:e9
Ostlund G, Schmitt T, Forslund K et al (2010) InParanoid 7: new algorithms and tools for eukaryotic orthology analysis. Nucleic Acids Res 38:D196–D203
Ouyang S, Zhu W, Hamilton J et al (2007) The TIGR rice genome annotation resource: improvements and new features. Nucleic Acids Res 35:D883–D887
Pakseresht N, Alako B, Amid C et al (2014) Assembly information services in the European Nucleotide Archive. Nucleic Acids Res 42:D38–D43
Potato Genome Sequencing Consortium (2011) Genome sequence and analysis of the tuber crop potato. Nature 475:189–195
Qi J, Liu X, Shen D et al (2013) A genomic variation map provides insights into the genetic basis of cucumber domestication and diversity. Nat Genet 45:1510–1515
Quevillon E, Silventoinen V, Pillai S et al (2005) InterProScan: protein domains identifier. Nucleic Acids Res 33:W116–W120
Saito T, Ariizumi T, Okabe Y et al (2011) TOMATOMA: a novel tomato mutant database distributing Micro-Tom mutant collections. Plant Cell Physiol 52:283–296
Sakai H, Lee SS, Tanaka T et al (2013) Rice Annotation Project Database (RAP-DB): an integrative and interactive database for rice genomics. Plant Cell Physiol 54:e6
Sakurai N, Ara T, Ogata Y et al (2011) KaPPA-View4: a metabolic pathway database for representation and analysis of correlation networks of gene co-expression and metabolite co-accumulation and omics data. Nucleic Acids Res 39:D677–D684
The UniProt Consortium (2014) Activities at the Universal Protein Resource (UniProt). Nucleic Acids Res 42:D191–D198
Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635–641
Tsugane T, Watanabe M, Yano K et al (2005) Expressed sequence tags of full-length cDNA clones from the miniature tomato (*Lycopersicon esculentum*) cultivar Micro-Tom. Plant Biotechnol 22:161–165
Webb EC (1992) Enzyme nomenclature. Academic Press, San Diego, CA
Wheeler DL, Church DM, Federhen S et al (2003) Database resources of the National Center for Biotechnology. Nucleic Acids Res 31:28–33
Wheeler DL, Barrett T, Benson DA et al (2008) Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 36:D13–D21
Yamamoto N, Tsugane T, Watanabe M et al (2005) Expressed sequence tags from the laboratory-grown miniature tomato (*Lycopersicon esculentum*) cultivar Micro-Tom and mining for single nucleotide polymorphisms and insertions/deletions in tomato cultivars. Gene 356:127–134
Yamazaki Y, Akashi R, Banno Y et al (2010) NBRP databases: databases of biological resources in Japan. Nucleic Acids Res 38:D26–D32
Yano K, Imai K, Shimizu A et al (2006a) A new method for gene discovery in large-scale microarray data. Nucleic Acids Res 34:1532–1539
Yano K, Tsugane T, Watanabe M et al (2006b) Non-biased distribution of tomato genes with no counterparts in *Arabidopsis thaliana* in expression patterns during fruit maturation. Plant Biotechnol 23:199–202
Yano K, Watanabe M, Yamamoto N et al (2006c) MiBASE: a database of a miniature tomato cultivar Micro-Tom. Plant Biotechnol 23:195–198

Chapter 4
DNA Markers in Solanaceae Breeding

Hiroyuki Fukuoka

4.1 Introduction

It goes without saying that DNA markers are valuable and useful tools for basic genetic research and applied breeding. Continuous efforts have been made by a number of researchers since the 1980s to develop DNA markers and to construct linkage maps for solanaceous crops such as tomato, potato, pepper, and eggplant. This information would allow researchers to locate genes for the traits of interest at specific chromosome positions, which is a standard procedure in selectable DNA marker development for marker-assisted selection to introduce agronomically important traits in desired cultivars. Isolation of the genes of interest by map-based cloning would also be the first step toward understanding the molecular biological basis of the traits. A number of genes have been isolated by using a map-based approach (especially in tomato), and some of them are used as "complete linkage" markers in practical breeding (Jones et al. 1994; Thomas et al. 1997; Kawchuk et al. 2001; Fradin et al. 2009). Availability of a linkage map and a set of DNA markers evenly distributed throughout the genome makes it possible to map and to evaluate the contribution of the individual genes that control quantitatively inherited traits. Most agronomically important traits, such as harvest quality, yield, and tolerance to biotic and abiotic stresses, are thought to be quantitative and controlled by multiple genes that influence the traits with varying relative contributions. Therefore, the quantitative trait locus (QTL) analysis of the traits (a combined approach using molecular and statistical genetics) provides a more solid theoretical basis for breeding programs and helps breeders to understand the genetic background of practically important traits.

H. Fukuoka (✉)
NARO Institute of Vegetable and Tea Science, National Agriculture and Food Research Organization, Kusawa, Japan
e-mail: h.fukuoka@affrc.go.jp

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_4

With the recent rapid development of the next-generation sequencing (NGS) technologies, whole-genome sequencing becomes much more common and accessible to research in non-model species. Full reference genome sequences of potato and tomato have been recently released (The Potato Genome Sequencing Consortium 2011; The Tomato Genome Consortium 2012); in these projects, high-density molecular marker linkage maps were important, as they provided the starting points for bacterial artificial chromosome (BAC)-by-BAC sequencing and the information on anchoring the assembled genome scaffolds to the genetic maps to construct pseudomolecules for each chromosome. Even in less explored species such as pepper and eggplant, genetic linkage maps constructed using high-density molecular markers would provide fundamental tools for the accumulation of genomic information. DNA markers based on polymorphisms in gene sequences (exons, introns, 5′- and 3′-untranslated regions [UTRs], and neighboring regulatory sequences) mapped on the linkage maps are useful for analysis of genome-wide syntenic relationships among related species. In particular, DNA markers developed on the basis of the "conserved ortholog set" (COS) concept introduced by Fulton et al. (2002), or derived concepts, have been widely used for comparative analysis of solanaceous genomes (Doganlar et al. 2002; Wu et al. 2009a, b, c). These DNA markers are powerful tools that allow applying complete genome sequence information for tomato and potato to other solanaceous species.

In this review, the history, principles, and perspectives of the DNA marker technology and related genomic technologies are summarized from the point of view of their application in practical breeding of tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), and eggplant (*S. melongena* L.). Tomato will be mainly covered as a model species for the solanaceous crops because more information on molecular breeding in this species is available. Because of its different breeding due to vegetative propagation, topics related to another important solanaceous crop, potato (*S. tuberosum* L.), will not be covered here, and the reader is referred to recent comprehensive reviews (Milbourne et al. 2007; Ortega and Lopez-Vizcon 2012).

4.2 Detection of Polymorphisms for DNA Marker Development and Linkage Map Construction

4.2.1 Pre-PCR Age

A variety of methods have been developed to detect DNA polymorphisms and to use them as DNA markers. The earliest attempts to develop DNA markers and to construct a genetic linkage map in tomato were similar to those in other plant species, such as maize (Helentjaris et al. 1986), rice (McCouch et al. 1988), barley (Saghai-Maroof et al. 1984), and pea (Polans et al. 1985). These attempts were based on restriction fragment length polymorphisms (RFLPs), detected by

hybridization of labeled DNA probes with the genomic DNA digested by restriction enzymes, separated by gel electrophoresis, and transferred to nitrocellulose or nylon membranes. RFLP analysis is time and labor consuming, and therefore, the total throughput is relatively low. However, the first tomato high-density molecular linkage map, construction of which involved quite a large effort, comprised 1030 RFLP markers (Tanksley et al. 1992). At the same time, a common marker set was used to construct a potato map; a comparative analysis of the positions and the order of the markers allowed to determine the precise breakpoints corresponding to five chromosomal rearrangements that led to the divergence of the genomes of the two species. The interspecific comparative analysis of the two genomes was possible due to the advantageous characteristics of RFLPs, such as codominance, sequence-tagged nature, and transferability among related species. This first DNA marker-based comparative analysis of solanaceous genomes was followed by a number of comparative genomic analyses in solanaceous species. Fulton et al. (2002) identified more than 1000 highly conserved single- or low-copy genes in tomato and *Arabidopsis*. These COS genes, used as RFLP probes, provide useful anchor markers for comparison of genome organization and analysis of syntenic relationships among related species (Doganlar et al. 2002). The cleaved amplified polymorphic sequence (CAPS) markers can be regarded as a variation of the RFLP principle; the CAPS method detects mutations in restriction enzyme recognition sites in the DNA sequences amplified by PCR. A number of CAPS markers derived from the COS genes (COSII markers) have been developed (Wu et al. 2006) and used for comparative analysis of genome organization in tomato, pepper (Wu et al. 2009a), and eggplant (Wu et al. 2009b).

4.2.2 PCR-Based Arbitrary DNA Markers

In the 1990s, PCR-based DNA polymorphism analysis rapidly prevailed in the research community as it was less labor and time consuming than RFLP analysis. DNA markers based on random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) principles are more efficient for obtaining a large number of polymorphic markers. For example, more than 1000 common markers were obtained for tomato and its wild relative *Solanum pennellii* by using only 22 *Eco*RI + *Mse*I primer combinations (Haanstra et al. 1999). The first eggplant DNA marker linkage map was constructed by using RAPD and AFLP markers developed for an intraspecific F_2 mapping population (Nunome et al. 2001). However, the molecular markers based on the arbitrary PCR approach have limitations because the results vary depending on the brand and the lot of DNA polymerase, the purity and quantity of template DNA preparations, and the model of thermal cycling apparatus (data not shown). In addition, PCR products of the same size may correspond to different loci in various mapping populations; therefore, most marker fragments are unsuitable for studying other cross combinations. For more precise and reproducible results, polymorphic fragments have to be

cloned, sequenced, and converted into sequence-tagged site (STS) markers, also known as sequence-characterized amplified region (SCAR) markers. The weakness of these markers is that the amplified fragments by arbitrary primers are preferentially derived from repetitive sequences; therefore, the marker distribution is extremely biased toward heterochromatic regions. The strong point of such markers is that hundreds to thousands of loci can be mapped with a relatively small experimental effort; this has been employed for the development of selectable DNA markers by bulk segregant analysis. DNA markers associated with agronomically important traits such as *tomato yellow leaf curl virus* (TYLCV) resistance in tomato (Chagué et al. 1997; Agrama and Scott 2006), fertility restoration in pepper (Zhang et al. 2000), and *Fusarium* wilt resistance in eggplant (Toppino et al. 2008) have been successfully developed by using this strategy.

4.2.3 Microsatellites

Microsatellites or simple sequence repeats (SSRs), another class of PCR-based markers, are quite different from the random PCR markers described above. Microsatellites have several advantages for DNA marker development: they are multi-allelic and highly polymorphic within a species, have codominant inheritance, and are sequence tagged. Genotype data for several hundred markers for a mapping population of reasonable size (e.g., 96 F_2 individuals) can be obtained automatically within a few days by using multi-capillary DNA sequencing (which allows researchers to easily distinguish each allele combination of at least 2-bp difference in length) accompanied by multiplexed PCR with multicolor primer labeling. In eggplant, more than 1000 SSR markers have been isolated from SSR-enriched genomic libraries, and an SSR-based linkage map (EW2009) has been constructed by using an intraspecific F_2 population (Nunome et al. 2009). An SSR-based high-density linkage map including 372 SSR markers has also been published recently for pepper (Sugita et al. 2013). In tomato, more than 5000 SSRs have been computationally identified in BAC-end sequences and ESTs, and more than 1200 additional SSR markers have been mapped to one of the standard tomato linkage map EXPEN2000 (Ohyama et al. 2009; Shirasawa et al. 2010). Due to their multi-allelic and highly polymorphic nature even among closely related breeding materials and modern cultivars, SSR markers evenly distributed throughout the whole genome would be useful tools for rapid mapping and selectable marker development for traits of interest. Yet, it is well known that genomic SSRs are mainly located in heterochromatic regions, and therefore, covering the whole genome solely with randomly isolated genomic SSR markers would require enormous efforts and thus would be inefficient (Ohyama et al. 2009; Shirasawa et al. 2010; Fukuoka et al. 2012). However, the accumulation of whole-genome sequence data for solanaceous crops can be expected to enable the development of region-targeted SSR markers, which should overcome these difficulties.

4.2.4 Single Nucleotide Polymorphisms

Single nucleotide polymorphisms (SNPs) are genetic variations at the level of single nucleotides; therefore, DNA markers based on SNPs are expected to be much denser than other markers. A number of SNP genotyping methods have been reported, including those based on allele-specific PCR (Sommer et al. 1992), single-base extension (Syvanen 2001), probe hybridization (Liviak et al. 1995; Piatek et al. 1998), and other approaches (reviewed by Kwok 2001). Large-scale SNP genotyping platforms such as Infinium (Illumina), GoldenGate (Illumina), Axiom (Affymetrix), and MassARRAY (Sequenom) are now commercially available, and thousands of SNP markers can be genotyped simultaneously.

As described above, sometimes genomic SSRs alone are not sufficient for breeding and genetic research purposes because of their positional bias, but they are useful for materials with low DNA polymorphism. In eggplant, 571 SNPs found in the coding and noncoding (introns and UTRs) regions of functional genes have been genotyped by using a modified Tm-shift PCR method (Wang et al. 2005; Fukuoka et al. 2008) and mapped along with 329 genome SSRs, as well as some CAPS and STS markers, by using two intraspecific F_2 mapping populations (Fukuoka et al. 2012). An integrated map (LWA2010) comprised 12 linkage groups (equal to the basic chromosome number) with a total map length of 1285 cM; genomic coverage of LWA2010 was 1.5× that of the previously reported SSR-based map EW2009 (Nunome et al. 2009). Thus, genic SNP markers are helpful for covering the genome regions where SSR markers tend to be underrepresented. In this work (Fukuoka et al. 2012), SNPs between the parental lines of mapping populations were screened by direct Sanger sequencing of intron-containing genomic sequences and UTRs amplified by PCR. Most of the target genes were selected from *Solanum* orthologous (SOL) gene sets (i.e., sets of orthologous unigenes from eggplant, tomato, and potato). Thus, SOL markers could be mapped on both eggplant and tomato linkage maps and provided useful information on overall relationships between the genomes of the two species. Once a trait of interest has been mapped by using the SOL markers, corresponding tomato genome information can be used for fine mapping and gene isolation. Genic SNP markers will be helpful in applying the rich genomic and genetic information available for tomato to eggplant and pepper.

In a more comprehensive study on tomato, Sim et al. (2012a) developed a large SNP genotyping array based on the Infinium platform, genotyped 7720 SNPs, and constructed high-density genetic maps with 3503–4410 SNPs by using three F_2 mapping populations derived from interspecific crosses between cultivated tomato (*Solanum lycopersicum* LA0925 or cv. 'Moneymaker') and its wild relatives (*S. pennellii* LA0716 or *S. pimpinellifolium* LA0121). These maps are among the highest-density genetic maps available at present for tomato. In this study, the candidate SNPs were mainly identified by comparison of large-scale EST sequences from four cultivated tomato accessions (NC84175, Fla.7600, OH08-6405, and OH9242), an *S. lycopersicum* var. *cerasiforme* accession (PI 114490),

and an *S. pimpinellifolium* accession (PI 128216) (Hamilton et al. 2012). Therefore, a considerable proportion of SNPs represents polymorphisms between species rather than between cultivated tomato accessions. By using the same genotyping arrays, my research group successfully generated reliable genotype data for 6929 out of 7720 markers, but approximately half of them (3407 markers, or 49 %) were monomorphic within 18 heirloom and modern F_1 cultivars of Western and Japanese origin. We also found that distribution of the polymorphic markers was biased toward specific regions of linkage maps (H. Fukuoka et al. unpublished data). In tomato, most of the reported linkage maps have been obtained by using interspecific mapping populations because of the low genetic diversity within modern tomato cultivars in comparison with its wild relatives. However, it is also important to develop a large set of markers, which are highly polymorphic among closely related cultivars and breeding materials, to precisely evaluate genetic segregation of agronomically important traits; for this purpose, segregating populations derived from intraspecific crosses are often essential. Shirasawa et al. (2010) performed SNP mining in silico using approximately 230,000 ESTs available in the public databases SGN (http://solgenomics.net) and MiBASE (http://www.pgb.kazusa.or.jp/mibase/). In total, these authors found 5607 SNPs, genotyped 1536 of them by using the GoldenGate assay platform, and mapped more than 600 SNPs along with the genome-based SSR and EST-SSR markers; this was the first high-density SNP genetic map constructed by using intraspecific mapping populations of cultivated tomato.

Another set of 1536 intraspecific SNP markers has been recently developed in tomato (Shirasawa et al. 2013). The whole genomes of six cultivated tomato lines were sequenced by using a short-read next-generation DNA sequencer (5500xl SOLiD). From ~1.5 million SNP candidates identified by mapping to the tomato reference genome sequence (SL2.40), 1536 SNPs were selected for the GoldenGate assay. Among 1293 successfully genotyped SNPs, 1248 were polymorphic in 663 cultivated tomato accessions. The developed marker set should be useful for gene identification and for the development of genome-based breeding technologies in cultivated tomato. With the rapid progress in massively parallel sequencing, NGS will likely become the mainstream approach for SNP discovery even in the species for which full genome sequences are not available. In pepper, the transcriptome of cv. 'Yolo Wonder' has been sequenced by using long-read (~300 bp) NGS (Roche 454), and ~580,000 reads have been assembled into ~84,000 unique sequences (Nicolai et al. 2012). The transcriptome of another cultivar, 'Criollo de Morelos 334', has been sequenced by short-read (~100 bp) NGS (Illumina Genome Analyzer II) and mapped to the reference 'Yolo Wonder' sequence to identify more than ~12,000 reliable SNPs. Ashrafi et al. (2012) compared transcriptomes of three pepper cultivars and identified more than 22,000 high-quality SNPs. In eggplant, Barchi et al. (2011) identified ~10,000 SNPs by using the restriction-site-associated DNA (RAD) approach. Combining the selected sequencing target of reduced genome redundancy (cDNA or RAD-tag) with high-throughput sequencing has become a standard approach for SNP discovery; in the near future, it will likely be replaced by whole-genome comparative resequencing

of cultivars and germplasms of interest, made possible by the fast-growing performance of NGS technologies in terms of high throughput and low cost.

4.3 DNA Marker Development for Marker-Assisted Selection

It is widely recognized that the traits loosely fall into two categories: qualitative and quantitative. Most agronomically important traits, such as yield, biotic and abiotic stress tolerance, growth habit, quality, shape, and the appearance, size, and other characteristics of the product that is harvested, are quantitative traits, controlled by a number of genes and influenced by environmental factors. The locations and the relative contributions of the QTLs involved in the expression of quantitative traits can be estimated by a combined analysis of DNA marker genotypes and phenotypic values in segregating populations. In tomato, a large number of reported mapped QTLs for disease and abiotic stress resistance and flower-, fruit-, and yield-related characteristics have been summarized (Foolad 2007). To date, however, few QTLs are used in tomato breeding programs, in part because most of them were identified by using interspecific mapping populations and therefore do not reflect the phenotypic differences among tomato cultivars and breeding lines, and cannot be directly used in practical breeding. Some traits measured by using numerical indices, such as viral disease resistance (Chagué et al. 1997) and soluble solid contents in fruits (Fridman et al. 2000), were initially treated as quantitative traits and were measured by numerical indices, but have been eventually found to be controlled mainly by single major Mendelian loci, and thus are suitable for marker-assisted breeding.

Whereas many agronomically important traits are quantitative, there are also many important traits that are controlled by one gene or a small number of major genes and are thus suitable for marker-assisted selection. Genes controlling vertical resistance to pathogens are the major examples (Foolad and Panthee 2012). Such qualitative traits can be selected by phenotype in the field or greenhouse, but the availability of a selectable DNA marker makes it easy to select promising individuals even at the seedling stage. The ability to select for only one trait is of little appeal for breeders, but the benefits of reduced cost and effort become obvious if simultaneous selection for multiple qualitative traits is possible. In addition, marker-assisted selection is quite effective for recessive traits because it makes it possible to distinguish between homozygous and heterozygous plants.

Many attempts to develop selectable DNA markers in solanaceous crops, especially in tomato, have been reported, and some of these markers are used for commercial breeding. Most of them are markers for disease resistance genes, such as genes conferring resistance to *tobacco mosaic virus* (*Tm-2*^{a}; Dax et al. 1994; Ohmori et al. 1995), *Fusarium* wilt (*I-1, I-2* and *I-3*; Sarfatti et al. 1989, 1991; Simons et al. 1998; Lim et al. 2006), leaf mold caused by *Cladosporium fulvum* (*Cf-2*; Dickinson et al. 1993), root knot caused by

Meloidogyne spp. (*Mi*; Williamson et al. 1994), *Verticillium* wilt (*Ve-1*; Kawchuk et al. 1998; Diwan et al. 1999), and gray leaf spot caused by *Stemphylium* spp. (*Sm*; Behare et al. 1991). These genes have been introduced into cultivated tomato from related wild species, and are surrounded by considerable flanking segments of the wild chromosomes, where it is relatively easy to find DNA polymorphisms to be used as DNA markers. Once fine mapping of the genes is completed, map-based cloning is feasible in tomato because of its relatively small genome size (~950 Mb). Genes for bacterial leaf spot resistance (*Pto*; Martin et al. 1993), *Tospovirus* resistance (*Sw-5*; Brommonschenkel and Tanksley 1997), and *Fusarium* wilt resistance (*I-2*; Simons et al. 1998) were successfully isolated by map-based cloning in the early years. In the following years, genes responsible for more than 30 traits, including disease resistance and fruit characters, were isolated in tomato (reviewed by Foolad 2007). More recently, resistance genes *Ty-1* and *Ty-3* for tomato yellow leaf curl virus disease caused by TYLCV, which is one of the most invasive and severe diseases that affects tomato production worldwide, have been isolated by fine mapping and map-based cloning (Verlaan et al. 2013). These genes will be used as the ultimate markers and will replace adjacent linked markers in the near future.

In pepper and eggplant, selectable DNA marker development for agronomically important traits has been less advanced than in tomato; future progress may depend on the progress of linkage map construction and whole-genome sequencing in those species. Among almost 300 genes for various characteristics described in pepper by extensive genetic studies (Wang 2006), disease resistance has been the first trait that elicited interest in marker-assisted pepper breeding, as it was in tomato. Matsunaga et al. (2003) reported a selectable marker for L^4, the most universal gene conferring *Tobamovirus* resistance, including resistance to *tobacco mosaic virus* and *pepper mild mottle virus*; later, it became possible to select another allele, L^3, by using a DNA marker (Sugita et al. 2004). DNA markers for *tomato spotted wilt virus* resistance (Moury et al. 2000; Jahn et al. 2000), *cucumber mosaic virus* resistance (Kang et al. 2010), and *Potyvirus* resistance (Arnedo-Andrés et al. 2002; Kang et al. 2005) have also been developed. Pungency due to capsaicinoid accumulation in the pepper fruit, which determines its flavor, is probably the most characteristic and important trait. A single dominant locus, *Pun1* (formerly *C*), controls the presence or absence of pungency, and the responsible gene has been isolated and characterized by using a candidate gene strategy (Blum et al. 2003; Stewart et al. 2005). The nonpungent allele *pun1* is a loss-of-function mutation resulting from a large deletion at this locus; therefore, it can be easily distinguished by DNA marker technology. However, even in pungent chili peppers with the functional *Pun1* gene, the pungency level and capsaicinoid content vary widely. Despite the presence of the functional *Pun1* gene, Japanese chili pepper cultivars such as ‘Manganji’ and ‘Shishitou’ are used as vegetables, as they have a very low capsaicinoid content (Saritnum et al. 2008). Therefore, capsaicinoid content appears to be a quantitatively inherited trait and would be rather difficult to target for DNA marker selection. Eggplant genomics is the least explored among those of the three solanaceous vegetables; therefore, few traits that attract practical breeders

have been genetically characterized to date. DNA marker development for *Fusarium* wilt resistance is one of the few examples. Toppino et al. (2008) and Mutlu et al. (2008) have reported random PCR-based markers for resistance to this disease, which are derived from *S. melongena* or its wild relatives. On the RAD marker-based linkage map reported by Barchi et al. (2012), the random PCR-based selectable marker developed by Toppino et al. (2008) was mapped between two sequence-tagged SNP and SSR markers in the linkage group E2. Another notable example comes from the work by Miyatake et al. (2012), who used an intraspecific F_2 population derived from a cross between a parthenocarpic line 'AE-P03' and a non-parthenocarpic breeding line 'EPL1' to map a major gene controlling parthenocarpic fruit development on the linkage group E8. By using two SSR markers, parthenocarpic offspring was successfully selected regardless of the non-parthenocarpic parents. Parthenocarpy is a very valuable trait for tomato, pepper, and eggplant production, and this work enables for the first time a systematic breeding program that uses DNA markers for parthenocarpic cultivars of solanaceous species. The assay for parthenocarpy will be rather difficult if the size and architecture of the fruit differ to a large extent between the two parental lines of the mapping population. Intraspecific cross-based genetic analysis is a key element in successful gene mapping, even though it is difficult to obtain sufficient numbers of polymorphic DNA markers to cover the whole genome.

Until recently, finding DNA polymorphisms around the target loci was a major concern for developing DNA markers for qualitative traits in solanaceous crops. However, as discussed above, the technology for accumulating the genome-wide information for intraspecific DNA polymorphisms is advancing rapidly and is becoming more accessible for breeders and applied geneticists. Therefore, finding materials with practically important traits and their precise evaluation is now more important than polymorphic DNA marker development as such.

4.4 Next-Generation Technology for Marker-Assisted Breeding

Until not long ago, it was believed that a large-scale development of molecular markers enables mapping not only major genes but also quantitatively inherited traits more precisely and with higher marker density. Highly confident genetic analysis of quantitatively inherited traits, however, requires sophisticated development of segregating populations such as recombinant inbred lines and chromosome segment substitution lines, and several rounds of reiterative phenotyping until QTLs are identified and characterized at a level satisfactory for practical breeding, as demonstrated by the genetic dissection of the heading date in rice (Yano et al. 2001; Ebana et al. 2011). Further, pyramiding of many minor QTLs into one line by DNA marker selection is too complex and difficult for breeders to conduct without a guarantee that they will obtain exceptional cultivars, which,

unfortunately, is not currently possible. This is probably one of the major reasons why marker-assisted breeding has been used for a limited set of targets, such as vertical disease resistance and other qualitative traits controlled by a few major genes.

The genome-wide association study (GWAS) approach has been popular in human genetics (reviewed by McCarthy et al. 2008) and has recently emerged as a powerful tool in plants (Yu and Buckler 2006; Hamblin et al. 2011). The GWAS approach allows researchers to develop markers that are significantly associated with the traits by analyzing genetically unrelated individuals with and without the phenotypes of interest by using a large number of DNA markers. Whereas this approach ideally requires millions of markers, the sufficient number depends on the extent of linkage disequilibrium (LD), in other words, on the experimental design and the materials to be analyzed. In maize, the extent of LD within a global germplasm collection has been estimated from 1 to 10 kb (Yan et al. 2009). In rice, LD decays quickly within 10 kb for *indica* and related wild species, whereas it extends to 50 kb in *japonica* (Xu et al. 2012). Using 663 tomato accessions, Shirasawa et al. (2013) detected highly biased LD decays between euchromatic (58 kb) and heterochromatic regions (13.8 Mb). Using phenotype data for 23 traits registered in the NIAS Genebank database (http://www.gene.affrc.go.jp) and marker genotype data obtained by using 1248 SNP markers, they identified a total of nine SNP loci significantly associated with eight morphological traits (inflorescence branching, determinate plant habit, plant height, number of leaves between inflorescences, fruit size, locule number, green shoulder on immature fruit, and the color of the fruit epidermis). Rodríguez et al. (2011) also detected a strong association of *SUN*, *OVATE*, *LC*, and *FAS* alleles with variation in fruit shape, classified into eight categories. On the other hand, no significant association could be detected for 15 of the 23 traits tested by Shirasawa et al. (2013). One of the important factors is the accuracy of the phenotype data because most of the data in the NIAS Genebank database was scored on 1–5 or 1–10 scales, rather than on the basis of actual measurements. Because the scale standards may vary between individual investigators, the accuracy is unlikely to be sufficient for GWAS. The other reason could be that whereas 1248 SNPs were employed in GWAS, LD extension in the gene-rich euchromatin region (58 kb) was too short to be covered by the SNP density employed (1 SNP/213 kb in euchromatin). In contrast, tomato cultivars exhibit low rates of DNA polymorphism and high LD (Sim et al. 2012b), which would reduce the mapping resolution of GWAS. Ranc et al. (2012) described GWAS using the cherry-type tomato, whose genome appears to contain elements from both a cultivated tomato accession and *S. pimpinellifolium*, to overcome the low resolution of association mapping in cultivated tomato. Although there is still plenty of room for improvement, GWAS is a promising strategy for detecting genetic factors controlling agronomically important traits and development of selectable DNA markers by using genetic resource accessions in solanaceous crops.

The availability of a large amount of genome-wide DNA polymorphism information from next-generation sequencing and genotyping will help practical applications of genomic-based breeding strategies such as genomic selection (GS,

Meuwissen et al. 2001). GS has been used in animal breeding (Hayes et al. 2009) and subsequently in plant breeding (Jannink et al. 2010; Lorenz et al. 2011; Iwata et al. 2011, 2013). GS predicts the genetic potential (i.e., the breeding value) of breeding lines in a population by analyzing the association between their phenotypes and high-density marker scores caused by LD between markers and responsible genes or QTLs. GS is powerful because its prediction model incorporates all marker information, thereby reducing the effects of biased marker selection on estimates and capturing more information on the effects of minor QTLs (Heffner et al. 2009). Because of these characteristics, GS is expected to be efficient even for low-heritability polygenic traits and to provide a breakthrough in practical realization of much-needed marker-assisted breeding for quantitative traits.

4.5 Conclusion

The genetic linkage maps based on DNA marker technology have been and will continue to be an important tool for anchoring short genome sequences to chromosomes in whole-genome sequencing of tomato and other solanaceous crops. Biparental genetic analysis using DNA marker linkage maps has provided useful information for developing selectable markers for practical breeding, especially for traits (such as vertical disease resistance) controlled by a single gene or a few major genes. Next-generation technologies (such as massively parallel sequencing and array-based SNP typing platforms) will be a driving force for the development and practical realization of the next-generation breeding methodologies (such as GWAS-based marker development and GS) based on comprehensive whole-genome polymorphism information for breeding lines and genetic resources in solanaceous crops.

References

Agrama HA, Scott JW (2006) Quantitative trait loci for *Tomato yellow leaf curl virus* and *tomato mottle virus* resistance in tomato. J Am Soc Hortic Sci 131:267–272

Arnedo-Andrés MS, Gil-Ortega R, Luis-Arteaga M, Hormaza JI (2002) Development of RAPD and SCAR markers linked to the *Pvr4* locus for resistance to *PVY* in pepper (*Capsicum annuum* L.). Theor Appl Genet 105:1067–1074

Ashrafi H, Hill T, Stoffel K, Kozik A, Yao J, Chin-Wo SR, Van Deynze A (2012) *De novo* assembly of the pepper transcriptome (*Capsicum annuum*): a benchmark for *in silico* discovery of SNPs, SSRs and candidate genes. BMC Genomics 13:571

Barchi L, Lanteri S, Portis E, Acquadro A, Valè G, Toppino L, Rotino GL (2011) Identification of SNP and SSR markers in eggplant using RAD tag sequencing. BMC Genomics 12:304

Barchi L, Lanteri S, Portis E, Valè G, Volante A et al (2012) A RAD tag derived marker based eggplant linkage map and the location of QTLs determining anthocyanin pigmentation. PLoS One 7:e43740. doi:10.1371/journal.pone.0043740

Behare J, Laterrot H, Sarfatti M, Zamir D (1991) Restriction fragment length polymorphism mapping of the *Stemphylium* resistance gene in tomato. Mol Plant Microbe Interact 4:489–492

Blum E, Mazourek M, O'Connell M, Curry J, Thorup T, Liu K, Jahn M, Paran I (2003) Molecular mapping of capsaicinoid biosynthesis genes and quantitative trait loci analysis for capsaicinoid content in *Capsicum*. Theor Appl Genet 108:79–86

Brommonschenkel S, Tanksley S (1997) Map-based cloning of the tomato genomic region that spans the *Sw-5* tospovirus resistance gene in tomato. Mol Gen Genet 256:121–126

Chagué V, Mercier JC, Guénard M, Courcel AD, Vedel F (1997) Identification of RAPD markers linked to a locus involved in quantitative resistance to TYLCV in tomato by bulked segregant analysis. Theor Appl Genet 95:671–677

Dax E, Livneh O, Edelbaum O, Kedar N, Gavish N, Karchi H, Milo J, Sela I, Rabinowitch HD (1994) A random amplified polymorphic DNA (RAPD) molecular marker for the $Tm\text{-}2^a$ gene in tomato. Euphytica 74:159–163

Dickinson MJ, Jones DA, Jones JD (1993) Close linkage between the *Cf-2*/*Cf-5* and *Mi* resistance loci in tomato. Mol Plant Microbe Interact 6:341–347

Diwan N, Fluhr R, Eshed Y, Zamir D, Tanksley SD (1999) Mapping of *Ve* in tomato: a gene conferring resistance to the broad-spectrum pathogen, *Verticillium dahliae* race 1. Theor Appl Genet 98:315–319

Doganlar S, Frary A, Daunay MC, Lester RN, Tanksley SD (2002) A comparative genetic linkage map of eggplant (*Solanum melongena*) and its implications for genome evolution in the Solanaceae. Genetics 161:1697–1711

Ebana K, Shibaya T, Wu J, Matsubara K, Kanamori H et al (2011) Uncovering of major genetic factors generating naturally occurring variation in heading date among Asian rice cultivars. Theor Appl Genet 122:1199–1210

Foolad MR (2007) Genome mapping and molecular breeding of tomato. Intl J Plant Genomics 2007: Article ID 64358 doi:10.1155/2007/64358

Foolad MR, Panthee DR (2012) Marker-assisted selection in tomato breeding. Crit Rev Plant Sci 31:93–123

Fradin EF, Zhang Z, Juarez-Ayala JC, Castroverde CDM, Nazar RN, Robb J, Liu CM, Thomma BPHJ (2009) Genetic dissection of *Verticillium* wilt resistance mediated by tomato *Ve1*. Plant Physiol 150:320–332

Fridman E, Pleban T, Zamir D (2000) A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. Proc Natl Acad Sci U S A 97:4718–4723

Fukuoka H, Miyatake K, Negoro S, Nunome T, Ohyama A, Yamaguchi H (2008) Development of a routine procedure for single nucleotide polymorphism marker design based on the Tm-shift genotyping method. Breed Sci 58:461–464

Fukuoka H, Miyatake K, Nunome T, Negoro S, Shirasawa K, Isobe S, Asamizu E, Yamaguchi H, Ohyama A (2012) Development of gene-based markers and construction of an integrated linkage map in eggplant by using *Solanum* orthologous (SOL) gene sets. Theor Appl Genet 125:47–56

Fulton TM, Van der Hoeven R, Eannetta NT, Tanksley SD (2002) Identification, analysis, and utilization of conserved ortholog set markers for comparative genomics in higher plants. Plant Cell 14:1457–1467

Haanstra JPW, Wye C, Verbakel H, Meijer-Dekens F, van den Berg P, Odinot P, van Heusden AW, Tanksley S, Lindhout P, Peleman J (1999) An integrated high-density RFLP-AFLP map of tomato based on two *Lycopersicon esculentum* × *L. pennellii* F_2 populations. Theor Appl Genet 99:254–271

Hamblin MT, Buckler ES, Jannink JL (2011) Population genetics of genomics-based crop improvement methods. Trends Genet 27:98–106

Hamilton JP, Sim S, Stoffel K, van Deynze A, Buell GR, Francis D (2012) Single nucleotide polymorphism discovery in cultivated tomato via sequencing by synthesis. Plant Genome 5:17–29

Hayes J, Bowman PJ, Chamberlain AJ, Goddard ME (2009) Invited review: genomic selection in dairy cattle: progress and challenges. J Dairy Sci 92:433–443

Heffner EL, Sorrells ME, Jannink JL (2009) Genomic selection for crop improvement. Crop Sci 49:1–12

Helentjaris T, Slocum M, Wright S, Schaefer A, Nienhuis J (1986) Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor Appl Genet 72:761–769

Iwata H, Hayashi T, Tsumura Y (2011) Prospects for genomic selection in conifer breeding: a simulation study of *Cryptomeria japonica*. Tree Genet Genomes 7:747–758

Iwata H, Hayashi T, Terakami S, Takada N, Sawamura Y, Yamamoto T (2013) Potential assessment of genome-wide association study and genomic selection in Japanese pear *Pyrus pyrifolia*. Breed Sci 63:125–140

Jahn M, Paran I, Hoffmann K, Radwanski ER, Livingstone KD, Grube RC, Aftergoot E, Lapidot M, Moyer J (2000) Genetic mapping of the *Tsw* locus for resistance to the *Tospovirus Tomato spotted wilt virus* in *Capsicum* spp. and its relationship to the *Sw-5* gene for resistance to the same pathogen in tomato. Mol Plant Microbe Interact 13:673–689

Jannink JL, Lorenz AJ, Iwata H (2010) Genomic selection in plant breeding: from theory to practice. Brief Funct Genomics 9:166–177

Jones DA, Thomas CM, Hammond-Kosack KE, Balint-Kurti PJ, Jones JD (1994) Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. Science 266:789–793

Kang BC, Yeam I, Frantz JD, Murphy JF, Jahn MM (2005) The *pvr1* locus in *Capsicum* encodes a translation initiation factor eIF4E that interacts with *Tobacco etch virus* VPg. Plant J 42:392–405

Kang WH, Hoang NH, Yang HB, Kwon JK, Jo SH, Seo JK, Kim KH, Choi D, Kang BC (2010) Molecular mapping and characterization of a single dominant gene controlling *CMV* resistance in peppers (*Capsicum annuum* L.). Theor Appl Genet 120:1587–1596

Kawchuk LM, Hachey J, Lynch DR (1998) Development of sequence characterized DNA markers linked to a dominant *Verticillium* wilt resistance gene in tomato. Genome 41:91–95

Kawchuk L, Hachey J, Lynch DR, Klcsar F, van Rooijen G et al (2001) Tomato *Ve* disease resistance genes encode cell surface-like receptors. Proc Natl Acad Sci U S A 98:6511–6515

Kwok PY (2001) Methods for genotyping single nucleotide polymorphisms. Annu Rev Genomics Hum Genet 2:235–258

Lim GTT, Wang GP, Hemming MN, Basuki S, McGrath DJ, Carroll BJ, Jones DA (2006) Mapping the *I-3* gene for resistance for *Fusarium* wilt in tomato: application of an I-3 marker in tomato improvement and progress towards the cloning of *I-3*. Australas Plant Pathol 35:671–680

Liviak KJ, Marmaro J, Todd JA (1995) Towards fully automated genome-wide polymorphism screening. Nat Genet 9:341–342

Lorenz AJ, Chao S, Asoro FG, Heffner EL, Hayashi T, Iwata H, Smith KP, Sorrells ME, Jannink JL (2011) Genomic selection in plant breeding: knowledge and prospects. Adv Agron 110:77–123

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436

Matsunaga H, Saito T, Hirai M, Nunome T, Yoshida T (2003) DNA markers linked to *pepper mild mottle virus* (*PMMoV*) resistant locus (L^4) in Capsicum. J Jpn Soc Hortic Sci 72:218–220

McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ioannidis JPA, Hirschhorn JN (2008) Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 9:356–369

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829

Milbourne D, Pande B, Bryan GJ (2007) Potato. In: Kole C (ed) Genome mapping and molecular breeding in plants, vol 3, Pulses, sugar and tuber crops. Springer, Heidelberg, pp 205–236

Miyatake K, Saito T, Negoro S, Yamaguchi H, Nunome T, Ohyama A, Fukuoka H (2012) Development of selective markers linked to a major QTL for parthenocarpy in eggplant (*Solanum melongena* L.). Theor Appl Genet 124:1403–1413

Moury B, Pflieger S, Blattes A, Lefebvre V, Palloix A (2000) A CAPS marker to assist selection of tomato spotted wilt virus (TSWV) resistance in pepper. Genome 43:137–142

Mutlu N, Boyaci FH, Göçmen M, Abak K (2008) Development of SRAP, SRAP-RGA, RAPD and SCAR markers linked with a *Fusarium* wilt resistance gene in eggplant. Theor Appl Genet 117:1303–1312

Nicolai M, Pisani C, Bouchet JP, Vuylsteke M, Palloix A (2012) Discovery of a large set of SNP and SSR genetic markers by high-throughput sequencing of pepper (*Capsicum annuum*). Genet Mol Res 11:2295–2300

Nunome T, Ishiguro K, Yoshida T, Hirai M (2001) Mapping of fruit shape and color development traits in eggplant (*Solanum melongena* L.) based on RAPD and AFLP markers. Breed Sci 51:19–26

Nunome T, Negoro S, Kono I, Kanamori H, Miyatake K, Yamaguchi H, Ohyama A, Fukuoka H (2009) Development of SSR markers derived from SSR-enriched genomic library of eggplant (*Solanum melongena* L.). Theor Appl Genet 119:1143–1153

Ohmori T, Murata M, Motoyoahi F (1995) Identification of RAPD markers linked to the *Tm-2* locus in tomato. Theor Appl Genet 90:307–311

Ohyama A, Asamizu E, Negoro S, Miyatake K, Yamaguchi H, Tabata S, Fukuoka H (2009) Characterization of tomato SSR markers developed using BAC-end and cDNA sequences from genome databases. Mol Breed 23:685–691

Ortega F, Lopez-Vizcon C (2012) Application of molecular marker-assisted selection (MAS) for disease resistance in a practical potato breeding programme. Potato Res 55:1–13

Piatek AS, Tyagi S, Pol AC, Telenti A, Miller LP, Kramer FR, Allend D (1998) Molecular beacon sequence analysis for detecting drug resistance in *Mycobacterium tuberculosis*. Nat Biotechnol 16:359–363

Polans NO, Weeden NF, Thompson WF (1985) Inheritance, organization, and mapping of *rbcS* and *cab* multigene families in pea. Proc Natl Acad Sci U S A 82:5083–5087

Ranc N, Muños S, Xu J, Le Paslier MC, Chauveau A, Bounon R, Rolland S, Bouchet JP, Brunel D, Causse M (2012) Genome-wide association mapping in tomato (*Solanum lycopersicum*) is possible using genome admixture of *Solanum lycopersicum var. cerasiforme*. G3 2:853–864

Rodríguez GR, Muños S, Anderson C, Sim SC, Michel A, Causse M, Gardener BB, Francis D, van der Knaap E (2011) Distribution of *SUN*, *OVATE*, *LC*, and *FAS* in the tomato germplasm and the relationship to fruit shape diversity. Plant Physiol 156:275–285

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci U S A 81:8014–8018

Sarfatti M, Katan J, Fluhr R, Zamir D (1989) An RFLP marker in tomato linked to the *Fusarium oxysporum* resistance gene I2. Theor Appl Genet 78:755–759

Sarfatti M, Abu-Abied M, Katan J, Zamir D (1991) RFLP mapping of *I1*, a new locus in tomato conferring resistance against Fusarium oxysporum f. sp. *lycopersici* race 1. Theor Appl Genet 82:22–26

Saritnum O, Minami M, Matsushima K, Minamiyama Y, Hirai M, Baba T, Bansho H, Nemoto K (2008) Inheritance of few-pungent trait in chili pepper ‘S3212’ (*Capsicum frutescens*). J Jpn Soc Hortic Sci 77:265–269

Shirasawa K, Asamizu E, Fukuoka H, Ohyama A, Sato S et al (2010) An interspecific linkage map of SSR and intronic polymorphism markers in tomato. Theor Appl Genet 121:731–739

Shirasawa K, Fukuoka H, Matsunaga H, Kobayashi Y, Kobayashi I, Hirakawa H, Isobe S, Tabata S (2013) Genome-wide association studies using single nucleotide polymorphism markers developed by re-sequencing of the genomes of cultivated tomato. DNA Res 20:593–603

Sim SC, Durstewitz G, Plieske J, Wieseke R, Ganal MW et al (2012a) Development of a large SNP genotyping array and generation of high-density genetic maps in tomato. PLoS One 7:e40563. doi:10.1371/journal.pone.0040563

Sim SC, Van Deynze A, Stoffel K, Douches DS, Zarka D et al (2012b) High-density SNP genotyping of tomato (*Solanum lycopersicum* L.) reveals patterns of genetic variation due to breeding. PLoS One 7:e45520. doi:10.1371/journal.pone.0045520

Simons G, Groenendijk J, Wijbrandi J, Reijans M, Groenen J et al (1998) Dissection of the Fusarium *I-2* gene cluster in tomato reveals six homologs and one active gene copy. Plant Cell 10:1055–1068

Sommer SS, Groszbach AR, Bottema CD (1992) PCR amplification of specific alleles (PASA) is a general method for rapidly detecting known single-base changes. Biotechniques 12:82–87

Stewart C Jr, Kang BC, Liu K, Mazourek M, Moore SL, Yoo EY, Kim BD, Paran I, Jahn MM (2005) The *Pun1* gene for pungency in pepper encodes a putative acyltransferase. Plant J 42:675–688

Sugita T, Yamaguchi K, Sugimura Y, Nagata R, Yuji K, Kinoshita T, Todoroki A (2004) Development of SCAR markers linked to L^3 gene in *Capsicum*. Breed Sci 54:111–115

Sugita T, Semi Y, Sawada H, Utoyama Y, Hosomi Y, Yoshimoto E, Haehata Y, Fukuoka H, Nagata R, Ohyama A (2013) Development of simple sequence repeat markers and construction of a high-density linkage map of *Capsicum annuum*. Mol Breed 31:909–920

Syvanen AC (2001) From gels to chips: “minisequencing” primer extension for analysis of point mutations and single nucleotide polymorphisms. Hum Mutat 13:1–10

Tanksley SD, Ganal MW, Prince JP, DeVicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

The Potato Genome Sequencing Consortium (2011) Genome sequence and analysis of the tuber crop potato. Nature 475:189–195

The Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635–641

Thomas CM, Jones DA, Parniske M, Harrison K, Balint-Kurti PJ, Hatzixanthis K, Jones JD (1997) Characterization of the tomato *Cf-4* gene for resistance to *Cladosporium fulvum* identifies sequences that determine recognitional specificity in *Cf*-4 and *Cf*-9. Plant Cell 9:2209–2224

Toppino L, Valè G, Rotino GL (2008) Inheritance of *Fusarium* wilt resistance introgressed from *Solanum aethiopicum Gilo* and *Aculeatum* groups into cultivated eggplant (*S. melongena*) and development of associated PCR-based markers. Mol Breed 22:237–250

Verlaan MG, Hutton SF, Ibrahem RM, Kormelink R, Visser RGF, Scott JW, Edwards JD, Bai Y (2013) The tomato yellow leaf curl virus resistance genes *Ty-1* and *Ty-3* are allelic and code for DFDGD-class RNA-dependent RNA polymerases. PLoS Genet 9:e1003399. doi:10.1371/journal.pgen.1003399

Wang D (2006) The genes of *Capsicum*. HortSci 41:1169–1187

Wang J, Chuang K, Ahluwalia M, Patel S, Umblas N, Mirel D, Higuchi R, Germer S (2005) High-throughput SNP genotyping by single-tube PCR with Tm-shift primers. Biotechniques 39:885–893

Williamson VM, Ho JY, Wu FF, Miller N, Kaloshian I (1994) A PCR-based marker tightly linked to the nematode resistance gene, *Mi*, in tomato. Theor Appl Genet 87:757–763

Wu F, Mueller LA, Crouzillat D, Petiard V, Tanksley SD (2006) Combining bioinformatics and phylogenetics to identify large sets of single-copy orthologous genes (COSII) for comparative, evolutionary and systematic studies: a test case in the euasterid plant clade. Genetics 174:1407–1420

Wu F, Eannetta NT, Xu Y, Durrett R, Mazourek M, Jahn MM, Tanksley SD (2009a) A COSII genetic map of the pepper genome provides a detailed picture of synteny with tomato and new

insights into recent chromosome evolution in the genus *Capsicum*. Theor Appl Genet 118:1279–1293
Wu F, Eannetta NT, Xu Y, Tanksley SD (2009b) A detailed synteny map of the eggplant genome based on conserved ortholog set II (COSII) markers. Theor Appl Genet 118:927–935
Wu F, Eanetta NT, Xu Y, Plieske J, Ganal M, Pozzi C, Bakaher N, Tanksley SD (2009c) COSII genetics maps of two diploid *Nicotiana* species provide a detailed picture of synteny with tomato and insights into chromosome evolution in tetraploid *N. tabacum*. Theor Appl Genet 120:809–827
Xu X, Liu X, Ge S, Jensen JD, Hu F et al (2012) Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. Nat Biotechnol 30:105–111
Yan J, Shah T, Warburton ML, Buckler ES, McMullen MD, Crouch J (2009) Genetic characterization and linkage disequilibrium estimation of a global maize collection using SNP markers. PLoS One 4(12):e8451. doi:10.1371/journal.pone.0008451
Yano M, Kojima S, Takahashi Y, Lin H, Sasaki T (2001) Genetic control of flowering time in rice, a short-day plant. Plant Physiol 127:1425–1429
Yu J, Buckler ES (2006) Genetic association mapping and genome organization of maize. Curr Opin Biotechnol 17:155–160
Zhang B, Huang S, Yang G, Guo J (2000) Two RAPD markers linked to a major fertility restorer gene in pepper. Euphytica 113:155–161

Chapter 5
DNA Markers in Cucurbitaceae Breeding

Nobuko Fukino and Yoichi Kawazu

5.1 Introduction

The family Cucurbitaceae comprises ca. 130 genera and 900 species (Jeffrey 1980). *Citrullus*, *Cucumis*, *Cucurbita*, and *Lagenaria*, which are four major cucurbitaceous genera, include many economically important vegetable crops grown on arable land throughout the world: cucumber (*Cucumis sativus*; $2n = 14$), melon (*Cucumis melo*; $2n = 24$), watermelon (*Citrullus lanatus*; $2n = 22$), and pumpkins and squash (*Cucurbita pepo*, *Cucurbita maxima*, *Cucurbita moschata*; $2n = 40$). Cucurbit plants are trailing or vining, tendril-bearing, and frost-sensitive annuals (Wehner and Maynard 2003). They are mostly monoecious and require various insects, especially bees, for pollination. Within fruits of each species, there is a wide assortment of sizes, shapes, color variants, flesh textures, flavors, etc. (Sitterly 1972). Breeders are continually developing improved varieties that are better adapted to a wider range of growing conditions, have multiple-disease resistance, offer labor-saving and high-yielding abilities, and meet the fluctuating demands of economics and consumers.

Marker-assisted selection (MAS) has earned an important position in crop breeding because conventional breeding is time-consuming and vulnerable to changes in environmental conditions. An important part of the infrastructure supporting MAS is genetic linkage maps. Especially, genetic linkage maps constructed with codominant and transferable DNA markers are anticipated as powerful tools for development of DNA markers for MAS. In recent years, the draft genome sequences of cucumber, melon, and watermelon have been released.

N. Fukino (✉) • Y. Kawazu
NARO Institute of Vegetable and Tea Science, National Agriculture and Food Research Organization, Tsu, Japan
e-mail: nbk@affrc.go.jp

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_5

Numerous DNA markers with positions in the linkage map or genome were developed using the genome sequence. These increasing genomic resources have promoted the construction of high-density genetic linkage maps and the identification of genomic regions controlling agronomically important traits.

This review, which specifically addresses melon, cucumber, watermelon, pumpkin, and squash, summarizes attempts at constructing genetic linkage maps and developing DNA markers for MAS, along with advancing genomic resources to support them.

5.2 DNA Markers in Melon Breeding

The first linkage map of melon, based on 23 genes that control disease resistance, flower biology, or vegetative characteristics, was published in 1991 (Pitrat 1991). Genetic linkage maps that contain DNA markers such as RFLPs, RAPDs, and AFLPs were subsequently constructed (Baudracco-Arnas and Pitrat 1996; Wang et al. 1997). These DNA markers used for primary linkage maps have been gradually replaced by SSRs and SNPs (Gonzalo et al. 2005; Fukino et al. 2008a; Deleu et al. 2009) because SSRs and SNPs present many advantages: they are highly polymorphic, codominant, transferable, and high-throughput molecular markers. During the last two decades, several molecular linkage maps of melon that contain many agronomically important phenotypic traits have been developed using different mapping populations and molecular markers (Ezura and Fukino 2009). Mapping populations contain recombinant inbred lines (RILs), doubled haploid lines (DHLs), and near isogenic lines (NILs) for the advanced analysis of quantitative traits such as disease resistance (Perchepied et al. 2005; Fukino et al. 2008a; Essafi et al. 2009), fruit quality (Monforte et al. 2004; Cuevas et al. 2009; Harel-Beja et al. 2010), and yield (Zalapa et al. 2007). One hundred sixty genes were reported in the 2011 gene list for melon (Dogimont 2011), but many have been neither cloned nor mapped with codominant and transferable markers (Table 5.1). In many cases, comparison of the maps has been difficult because most markers (mostly dominant markers) cannot be transferred from one mapping population to others. However, the International Cucurbit Genomics Initiative (ICuGI: http://www.icugi.org/) has merged eight maps after adding anchor SSRs to individual maps (Diaz et al. 2011). The consensus map spans 1150 cM across the 12 melon linkage groups and comprises 1592 markers (640 SSRs, 330 SNPs, 252 AFLPs, 239 RFLPs, 89 RAPDs, 15 IMAs, 16 indels, and 11 morphological traits). In fact, 370 QTLs controlling 62 traits (i.e., disease resistance, fruit shape, fruit weight, and sugar content) from 18 previously reported mapping experiments using genetically diverse parental genotypes were also integrated into the consensus map. All the individual maps, the integrated map, marker, and QTL information are available at ICuGI web site. The integrated map will be a valuable tool for inferring the positions of genes and QTLs in new linkage maps

Table 5.1 Major genes that have been cloned or mapped with codominant and transferable markers in melon

Gene symbol	Character	Reference
Disease and pest resistance		
Pm-2 F	Powdery mildew resistance	Zhang et al. (2012a)
Pm-w[a]		Dogimont et al. (2007)
Pm-AN		Wang et al. (2011)
Fom-1[a]	Fusarium wilt resistance	Oumouloud et al. (2008), Tezuka et al. (2010), Brotman et al. (2013)
Fom-2[a]		Joobeur et al. (2004)
nsv[a]	*Melon necrotic spot virus* resistance	Nieto et al. (2006)
zymv	*Zucchini yellow mosaic virus* resistance	Harel-Beja et al. (2010)
Prv[a]	*Papaya ringspot virus* resistance	Brotman et al. (2005), Brotman et al. (2013)
Vat[a]	Virus aphid transmission resistance	Dogimont et al. (2003)
Others		
a[a]	Andromonoecious	Boualem et al. (2008)
g[a]	Gynoecious	Martin et al. (2009)
p	Pentamerous	Oliver et al. (2001)
pH	pH (acidity) of the mature fruit flesh	Harel-Beja et al. (2010)
gf	Green flesh color	Monforte et al. (2004), Fukino et al. (2012)
wf	White flesh	Fukino et al. (2008a), Cuevas et al. (2009)
mt-2	Spots on the rind	Boissot et al. (2010)
s-2	Sutures on the rind	Diaz et al. (2011)
slb	Short lateral branching	Fukino et al. (2012)

[a]Genes which have been cloned

developed from different populations and for prompting the Cucurbitaceae research community to conduct next-generation genomic and genetic studies.

Other genomic resources of melon have been increased dramatically. Genome (Garcia-Mas et al. 2012) and organelle sequences (Rodriguez-Moreno et al. 2011), physical maps (González et al. 2010a), BAC-end sequences (González et al. 2010b), and transcriptomes (Blanca et al. 2011b) have been reported and made available at the MELONOMICS web site (https://melonomics.net/). In addition, ca. 24000 unigene sequences and SSRs and SNPs derived from them have been released at the ICuGI web site (Clepet et al. 2011). In the context of increasing resources, SNP genotyping of ca. 70 melon genotypes chosen to cover a broad range of diversity (Blanca et al. 2012; Esteras et al. 2013), mapping of the pH trait by combining microarray technology with a bulk segregation approach (Sherman

et al. 2013), and association mapping of fruit traits (Tomason et al. 2013) have been reported.

The number of *R*-genes in melon, cucumber, and watermelon is markedly lower than those of other species. Many *R*-genes are in clusters in the genome (Garcia-Mas et al. 2012; Lin et al. 2013). *Vat* and *Fom*-1 genes, which, respectively, confer resistance to virus aphid transmission and Fusarium wilt, were found to colocalize with *R*-gene clusters. Furthermore, the fact that most genes and QTLs for powdery mildew resistance reported to date (Perchepied et al. 2005; Fukino et al. 2008a; Yuste-Lisbona et al. 2010; Ning et al. 2014) locate in a similar region of the linkage groups II, V, and XII might be attributed to this property of *R*-genes in the melon genome.

As for mitochondrial genomes, melon, as is true with cucumber, both of which belong to *Cucumis*, has outstanding characteristics: they are severalfold larger than those of other species and are transmitted paternally (Matsuura 1995; Havey et al. 1998). By taking advantage of this characteristic, bulked seed purity tests of F_1 hybrids using DNA markers designed from repetitive DNA sequences and other markers have been reported (Lilly and Havey 2001; Akashi et al. 2008; Kato et al. 2011). Watanabe et al. (2012) verified the usefulness of the method. Subsequently, they concluded that markers derived from mitochondria are useful for primary screening and that markers developed from the nuclear genome might be necessary for the final confirmation because maternal or hybrid type of mitochondrial markers appeared in F_1 hybrid with low frequency. Sequence polymorphisms of chloroplast genome have been used to study the maternal lineage of melon accessions (Tanaka et al. 2013).

5.3 DNA Markers in Cucumber Breeding

Since the 1930s, about 170 cucumber genes have been reported (Call and Wehner 2010–2011). Many studies have been conducted of the linkage analysis of these genes (Fanourakis and Simon 1987; Walters et al. 2001). With the advance of molecular marker technology, considerable research has been devoted to the construction of genetic linkage maps with morphological traits (i.e., resistance to scab, downy mildew, *Zucchini yellow mosaic virus* (ZYMV), and *Papaya ringspot virus*, determinate, little leaf, female, spine color, and tuberculate fruit) and QTL analysis of horticultural traits and disease resistance (Kennard et al. 1994; Park et al. 2000; Fazio et al. 2003b; Sakata et al. 2006; Sun et al. 2006; Liu et al. 2008; Yuan et al. 2008; Zhang et al. 2012b). The utility of molecular markers linked to these genes or QTLs in MAS has been reported (Horejsi et al. 2000; Fazio et al. 2003a; Fan et al. 2006). Molecular markers linked to angular leaf spot resistance have also been reported (Olczak-Woltman et al. 2009). However, the broad application of these markers is usually difficult because they are mostly dominant markers and because no consensus map is available for them.

Recently, many SSR markers and linkage maps of RILs based on them have been reported (Fukino et al. 2008b; Ren et al. 2009; Cavagnaro et al. 2010; Yang et al. 2012). This marker information enabled construction of an SSR-based genetic linkage map with horticultural traits (Miao et al. 2011), a 1681-locus consensus map (Yang et al. 2013), fine mapping of genes controlling scab resistance (*Ccu*; Kang et al. 2011), ZYMV resistance (Amano et al. 2013), tuberculate fruit (*Tu*; Zhang et al. 2010), quantity of β-carotene (Bo et al. 2011), as well as QTL analysis of resistance to powdery mildew (Fukino et al. 2013; He et al. 2013) and downy mildew (Zhang et al. 2013; Yoshioka et al. 2014). Furthermore, genotyping of more than 3000 cucumber accessions with 23 SSRs distributed evenly across the cucumber genetic map revealed the genetic structure of cucumber and allowed the construction of a core set of 115 accessions capturing more than 77 % of the total allelic diversity observed (Lv et al. 2012).

The draft genome sequence of the Chinese fresh market-type inbred line '9930' (Huang et al. 2009), the North American pickling type inbred line 'Gy14' (http://cucumber.vcru.wisc.edu/), and the North-European Borszczagowski cultivar (line 'B10'; Wóycicki et al. 2011) has been released. Furthermore, a genomic variation map generated by deep resequencing of 115 cucumber lines sampled from 3342 accessions worldwide has been reported (Qi et al. 2013). By comparing the whole genome between European and Asian cucumber cultivars, chromosomal rearrangements between cucumber varieties were identified (Wóycicki et al. 2011). In addition, approximately 93000 unigenes are available at ICuGI web site. Marker sequences are available from Cucumber Genome Database (http://cucumber.genomics.org.cn). Chromosome rearrangement between *C. sativus* var. *sativus* and *C. sativus* var. *hardwickii* (wild cucumber) was revealed by comparing their genetic linkage maps (Miao et al. 2011) and by adopting a cytological approach (Yang et al. 2012). Integration of molecular, genetic, and cytological maps has been accomplished for all seven chromosomes (Han et al. 2009, 2011; Sun et al. 2013). This information is expected not only to provide a framework for cucumber genome assembly but also to provide a solid foundation for cucumber genetic and genomic research such as map-based gene isolation, comparative genomics, and evolutionary studies (Han et al. 2011).

C. hystrix is a wild *Cucumis* species possessing many valuable traits such as resistance to the root-knot nematode, downy mildew, gummy stem blight, and Fusarium wilts as well as tolerance to low light and temperature (Lou et al. 2013). Introgression lines have been obtained from an interspecific hybridization of *C. sativus* and *C. hystrix* (Zhou et al. 2009). Moreover, QTL mapping of downy mildew resistance (Pang et al. 2013) and gummy stem blight (Lou et al. 2013) has been conducted. These studies are expected to support the development of DNA markers for MAS and accelerate the development of new cultivars from introgression lines.

5.4 DNA Markers for Watermelon Breeding

The first linkage map for watermelon (*Citrullus lanatus*) was reported using isozymes and seed protein (Navot and Zamir 1986). A progeny of an interspecific cross of *C. lanatus* and *C. colocynthis* was used, revealing four linkage groups. Then Navot et al. (1990) constructed an isozyme-based map containing seven linkage groups spanning 354 cM. They reported isozyme markers linked to red color and bitterness of the fruit. Hashizume et al. (1996) constructed a linkage map with RAPDs, isozymes, and RFLPs using a progeny of a cultivated inbred line and a wild form, which contained 11 linkage groups spanning 524 cM. They reported RAPD markers linked to exocarp color and flesh color. Then Hashizume et al. (2003) constructed a linkage map using 477 RAPDs, 53 RFLPs, 23 ISSRs, and 1 isozyme marker, and 554 loci were mapped to 11 linkage groups that extended for 2384 cM. They performed QTL analysis for hardness of rind, soluble solid concentration of the flesh, flesh color (red and yellow), and rind color, and five QTLs for the four traits were detected. Levi et al. (2006) used AFLP, SRAP, and SSR markers as well as ISSR and RAPD markers to extend a genetic linkage map for watermelon. They constructed a linkage map which contained 360 DNA markers on 19 linkage groups that extended 1976 cM, with average distance of 5.8 cM between markers.

Disease resistance is extremely important for crop production. ZYMV, an aphid-born potyvirus, is an important virus affecting cucurbit crops. Mutations in the eukaryotic translation initiation factors such as eukaryotic initiation factors (eIF)4E and eIF4G reportedly engender resistance to specific RNA viruses in many plant systems (Maule et al. 2007). Ling et al. (2009) reported that eIF4E is closely associated with ZYMV resistance in watermelon. A CAPS marker derived from an SNP in the genomic sequence of watermelon eIF4E was developed. Powdery mildew caused by the fungus *Podosphaera xanthii* is also an important disease affecting cucurbit crops. Kim et al. (2013) reported genetic inheritance of resistance to powdery mildew and a molecular marker linked to the resistance. They found that the resistance of a cultivar 'Arka Manik' is conditioned by a single incomplete dominant gene. Furthermore, they identified a RAPD marker linked (3.6 cM) to the resistance, which was converted to CAPS and SNP markers.

Ren et al. (2012) constructed a genetic linkage map using Illumina GA sequencing technology and an elite line and a wild accession. The genetic map consists of 11 linkage groups with a mean marker interval of 0.8 cM, which includes 698 SSRs, 219 insertion-deletion, and 36 structure variation markers. The first SNP maps for watermelon were constructed using Roche 454 Sequencing and three populations from elite cultivars and wild accessions (Sandlin et al. 2012). The lengths of the genetic maps were, respectively, 1438, 1514, and 1144 cM with average marker distances of 3.8, 4.2, and 3.4 cM. A consensus map that was constructed using the three maps contained 378 SNP markers with average distance of 5.1 cM between markers. Phenotypic data were collected for fruit weight, fruit length, fruit width, fruit shape index, rind thickness, and Brix. Then 40 QTLs were identified in the

three populations for the six traits. The information will facilitate the development of DNA markers for MAS in watermelon breeding. The draft genome sequence of the east Asia watermelon cultivar '97103' and genome resequencing of 20 watermelon accessions representing three *C. lanatus* subspecies (subsp. *lanatus*, subsp. *mucosospermus*, and subsp. *vulgaris*) was reported (Guo et al. 2013). They reported genomic regions that were selected preferentially during domestication and many disease resistance genes that were lost during domestication. They performed the watermelon, cucumber, and pumpkin phloem transcriptomes and found that about 36 % of their transcripts were shared. They also performed RNA sequencing of both the flesh and rind at four stages of fruit development in the inbred line 97103 and identified 3046 and 558 genes that are differentially expressed in the flesh and rind, respectively, during fruit development. They also found 5352 genes that are differentially expressed between the flesh and rind in at least one of the four stages. The watermelon genome sequences and annotation, as well as 75000 unigenes and information of markers (SNPs and SSRs) designed from them, are available at the ICuGI web site. Marker sequences are also available at Cucumber Genome Database. A large amount of the information is expected to accelerate the development of DNA markers for watermelon breeding.

5.5 DNA Markers for Pumpkin and Squash (*Cucurbita*) Breeding

The first linkage map of *Cucurbita* ($2n = 40$) was reported in which isozyme markers and F_2 of the interspecific cross of *C. maxima* and *C. ecuadorensis* were used. The map contained 11 isozyme loci in five linkage groups (Weeden and Robinson 1986). Lee et al. (1995) used RAPD markers to build a linkage map of *Cucurbita* using F_2 of the interspecific cross of *C. pepo* and *C. moschata*. The map contained 28 markers in five linkage groups. Then Brown and Myers (2002) developed a genetic map with RAPD using a BC_1 progeny of *C. pepo* and *C. moschata*. The map contained 148 RAPD markers in 28 linkage groups. Loci controlling five morphological traits (*B* gene for fruit which turn yellow before anthesis, *M* gene for silver mottling of leaves, rind color on mature fruit, fruit shape, and the depth of the indentations between primary leaf veins) were placed on their map. Zraidi et al. (2007) constructed genetic maps using RAPD, AFLP, SSR, and two F_2 populations of *C. pepo*. A total of 332 and 323 markers were mapped in over 21 linkage groups. They found markers linked to the locus *n* (naked seed), affecting lignification of the seed coat, and markers linked to genes for resistance to ZYMV. Gong et al. (2008) developed many *Cucurbita* SSR markers using SSR-enriched partial genomic libraries and updated a previous *C. pepo* map. The updated map included 178 SSRs, 244 AFLPs, 230 RAPDs, and 5 SCARs and two morphological traits: hull-less seed (*h*) and bush growth (*B*). The map included 20 linkage groups with a map density of 2.9 cM. Kabelka and Young (2010) specifically examined

squash silverleaf caused by the silverleaf whitefly. A resistant zucchini line and a susceptible zucchini cultivar were screened with 1152 RAPD and 432 SSR markers to identify polymorphisms. Using F_2 and BC_1 progeny segregating for squash silverleaf disorder resistance, molecular markers associated with the resistance were developed.

By virtue of the recent development of high-throughput sequencing technologies, a large collection of EST-derived SSR and SNP in *C. pepo* was generated (Blanca et al. 2011a; Esteras et al. 2012). Blanca et al. (2011a) reported the generation of 49610 *Cucurbita* unigenes assembled de novo from about 500000 ESTs obtained from roots, leaves, and flowers of two *C. pepo* cultivars ('Zucchini MU16' and 'Scallop UPV196') using Roche/454 GS FLX Titanium massive parallel pyrosequencing technology. From the unigene dataset, 1882 unigenes with SSR motifs and 9043 SNPs were identified. Esteras et al. (2012) used the dataset of 9043 EST-SNPs and constructed an SNP-based genetic map of *C. pepo* using a population derived from the cross of the two cultivars described above. Fifty quantitative traits such as fruit weight and 12 qualitative traits such as flesh color were measured or scored. Then QTL analysis was performed. Forty-eight QTLs were detected for 31 quantitative traits. Then 11 QTLs were detected for 11 qualitative traits. Fifteen major QTLs ($R^2 > 25$ %) were detected for flowering traits (associated with late flowering and maleness tendency) and for immature and mature fruit traits (associated with fruit length and rind and flesh color). To validate the QTL effects and the utility of the linked markers for breeding purposes, genotypic and phenotypic data of the two backcross populations were analyzed for the detected QTLs. The effects of 11 of the 15 major QTLs detected in the F_2 were verified in the backcrosses. In addition, six minor QTLs ($R^2 > 10$ %) and eight QTLs involved in qualitative traits were also validated in the backcrosses. These QTLs are useful for *Cucurbita* breeding programs.

5.6 Syntenic Relation Among Cucumber, Melon, and Watermelon

Conserved microsynteny between melon and cucumber has been studied: one was around eIF4E and iso(4E) associated with recessively inherited resistances to potyviruses in many plants (Meyer et al. 2008). Another was around *Psm* gene, which controls sorting of paternally transmitted mitochondrial DNA (Al-Faifi et al. 2008).

Syntenic relations between chromosomes of melon ($2n = 24$) and cucumber ($2n = 14$) by broad-based comparative genetic mapping have suggested that cucumber chromosomes might result from chromosome fusion from a 24-chromosome progenitor species (Li et al. 2011).

As described in Sect. 5.2, melon, cucumber, and watermelon have fewer *R*-genes than other species. Most *R*-genes are distributed unevenly. They are located in

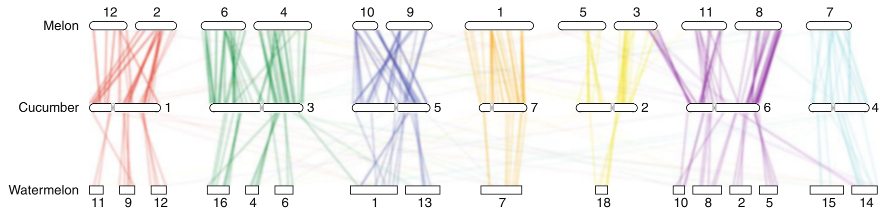


Fig. 5.1 Comparative genomic analysis of cucurbits (Huang et al. 2009). Comparative analysis of the melon and watermelon genetic maps with the cucumber sequence map. Cucumber, melon, and watermelon have 7, 12, and 11 pairs of chromosomes, respectively. The version of the watermelon genetic map shown in this figure is organized into 18 genetic groups. Reprinted by permission from Macmillan Publishers Ltd: Nature Genetics 41: 1275–1281, copyright 2009

clusters in the genome. Comparative analysis revealed high-degree sequence homology and synteny in chromosomal locations of *R*-genes in the melon, cucumber, and watermelon genomes (Lin et al. 2013; Yang et al. 2013). Lin et al. (2013) concluded that the scarcity of *R*-genes in Cucurbitaceae species was attributable to the frequent loss of *R*-gene lineages and infrequent duplications in extant lineages. They presented an integrated map of *R* loci in Cucurbitaceae.

An alignment of genomes showed a high level of synteny in *Cucumis* (Fig. 5.1; Huang et al. 2009; Garcia-Mas et al. 2012; Guo et al. 2013; Yang et al. 2014). The complicated syntenic patterns illustrated as mosaic chromosome-to-chromosome orthologous relations unveiled a high degree of complexity of chromosomal evolution and rearrangement in the Cucurbitaceae family (Guo et al. 2013). Conserved synteny among Cucurbitaceae will not only facilitate the investigation of genome evolution and dynamics but also enable the use of genetic information among related species in gene isolation and molecular tagging experiments (Li et al. 2011).

5.7 Conclusions

First linkage maps of Cucurbitaceae were reported in 1980s. Non-DNA markers such as isozymes or phenotypic traits were first used for construction of linkage maps. Then DNA markers such as RFLP, RAPD, and AFLP were developed. These markers have been replaced gradually by SSRs and SNPs, which are now the predominant markers for genetic analysis because they are highly polymorphic, codominant, transferable, and high-throughput molecular markers. Many major genes for agronomically important traits have been mapped or cloned, and DNA markers that are applicable to MAS have been reported. Furthermore, consensus maps of melon, cucumber, and watermelon have been constructed. Nevertheless, quantitative traits remain elusive which have not been mapped because evaluations of quantitative traits are difficult. Many replications will be necessary to obtain reliable evaluated values. Mapping populations such as RILs, NILs, and DHLs have

been developed and used for analyzing such traits. The lines, which are genetically homozygous, can be distributed and replicated for experiments in different laboratories and environments and are expected to be useful for the analysis of quantitative traits because they reduce the environmental component of total phenotypic variance. However, high-throughput sequencing technologies have been developed in recent years. We can now obtain a large amount of genome sequence information of non-model crops. Massive sequencing will enable the discovery of a large amount of DNA markers such as SSRs and SNPs, which will accelerate the development of DNA markers for MAS. It is expected that conserved synteny among Cucurbitaceae family will support the use of genetic information among related species for marker development and gene isolation.

References

Akashi Y, Fukino N, Uraki S, Tanaka K, Aierken Y, Nishida H, Matsumoto S, Osawa R, Kato K (2008) Bulked seed purity test of F_1 hybrids of melon, by the use of paternally inherited DNA markers. Breed Res 10(Suppl 2):97 (in Japanese)

Al-Faifi S, Meyer JD, Garcia-Mas J, Monforte AJ, Havey MJ (2008) Exploiting synteny in *Cucumis* for mapping of *Psm*: a unique locus controlling paternal mitochondrial sorting. Theor Appl Genet 117:523–529. doi:10.1007/s00122-008-0796-1

Amano M, Mochizuki A, Kawagoe Y, Iwahori K, Niwa K, Svoboda J, Maeda T, Imura Y (2013) High-resolution mapping of zym, a recessive gene for Zucchini yellow mosaic virus resistance in cucumber. Theor Appl Genet 126:2983–2993. doi:10.1007/s00122-013-2187-5

Baudracco-Arnas S, Pitrat M (1996) A genetic map of melon (*Cucumis melo* L.) with RFLP, RAPD, isozyme, disease resistance and morphological markers. Theor Appl Genet 93:57–64

Blanca J, Cañizares J, Roig C, Ziarsolo P, Nuez F, Picó B (2011a) Transcriptome characterization and high throughput SSRs and SNPs discovery in *Cucurbita pepo* (Cucurbitaceae). BMC Genomics 12:104

Blanca JM, Cañizares J, Ziarsolo P, Esteras C, Mir G, Nuez F, Garcia-Mas J, Picó MB (2011b) Melon transcriptome characterization: simple sequence repeats and single nucleotide polymorphisms discovery for high throughput genotyping across the species. Plant Genome J 4:118. doi:10.3835/plantgenome2011.01.0003

Blanca J, Esteras C, Ziarsolo P, Pérez D, Fernández-Pedrosa V, Collado C, de Pablos RR, Ballester A, Roig C, Cañizares J, Picó B (2012) Transcriptome sequencing for SNP discovery across *Cucumis melo*. BMC Genomics 13:280. doi:10.1186/1471-2164-13-280

Bo K, Song H, Shen J, Qian C, Staub JE, Simon PW, Lou Q, Chen J (2011) Inheritance and mapping of the *ore* gene controlling the quantity of β-carotene in cucumber (*Cucumis sativus* L.) endocarp. Mol Breed 30:335–344. doi:10.1007/s11032-011-9624-4

Boissot N, Thomas S, Sauvion N, Marchal C, Pavis C, Dogimont C (2010) Mapping and validation of QTLs for resistance to aphids and whiteflies in melon. Theor Appl Genet 121:9–20. doi:10.1007/s00122-010-1287-8

Boualem A, Fergany M, Fernandez R, Troadec C, Martin A et al (2008) A conserved mutation in an ethylene biosynthesis enzyme leads to andromonoecy in melons. Science 321:836–838. doi:10.1126/science.1159023

Brotman Y, Kovalski I, Dogimont C, Pitrat M, Portnoy V, Katzir N, Perl-Treves R (2005) Molecular markers linked to papaya ring spot virus resistance and *Fusarium* race 2 resistance in melon. Theor Appl Genet 110:337–345. doi:10.1007/s00122-004-1845-z

Brotman Y, Normantovich M, Goldenberg Z, Zvirin Z, Kovalski I et al (2013) Dual resistance of melon to *Fusarium oxysporum* races 0 and 2 and to *Papaya ring-spot virus* is controlled by a pair of head-to-head-oriented NB-LRR genes of unusual architecture. Mol Plant 6:235–238. doi:10.1093/mp/sss121

Brown RN, Myers JR (2002) A genetic map of squash (*Cucurbita* ssp.) with randomly amplified polymorphic DNA markers and morphological markers. J Am Soc Hortic Sci 127:568–575

Call AD, Wehner TC (2010–2011) Gene list 2010 for cucumber. Cucurbit Genet Coop Rep 33–34:69–103

Cavagnaro PF, Senalik DA, Yang L, Simon PW, Harkins TT, Kodira CD, Huang S, Weng Y (2010) Genome-wide characterization of simple sequence repeats in cucumber (*Cucumis sativus* L.). BMC Genomics 11:569. doi:10.1186/1471-2164-11-569

Clepet C, Joobeur T, Zheng Y, Jublot D, Huang M, Truniger V, Boualem A, Hernandez-Gonzalez ME, Dolcet-Sanjuan R, Portnoy V, Mascarell-Creus A, Cano-Delgado AI, Katzir N, Bendahmane A, Giovannoni JJ, Aranda MA, Garcia-Mas J, Fei Z (2011) Analysis of expressed sequence tags generated from full-length enriched cDNA libraries of melon. BMC Genomics 12:252. doi:10.1186/1471-2164-12-252

Cuevas HE, Staub JE, Simon PW, Zalapa JE (2009) A consensus linkage map identifies genomic regions controlling fruit maturity and beta-carotene-associated flesh color in melon (*Cucumis melo* L.). Theor Appl Genet 119:741–756. doi:10.1007/s00122-009-1085-3

Deleu W, Esteras C, Roig C, Gonzalez-To M, Fernandez-Silva I et al (2009) A set of EST-SNPs for map saturation and cultivar identification in melon. BMC Plant Biol 9:90. doi:10.1186/1471-2229-9-90

Diaz A, Fergany M, Formisano G, Ziarsolo P, Blanca J et al (2011) A consensus linkage map for molecular markers and quantitative trait loci associated with economically important traits in melon (*Cucumis melo* L.). BMC Plant Biol 11:111. doi:10.1186/1471-2229-11-111

Dogimont C (2011) 2011 Gene list for melon. Cucurbit Genet Coop Rep 33–34:104–133

Dogimont C, Bendahmane A, Pitrat M, Burget BE, Hagen L, Le Menn A, Pauquet J, Rousselle P, Caboche M, Chovelon V (2003) Gene resistant to *Aphis gossypii*. WO 2004/072109

Dogimont C, Chovelon V, Tual S, Boissot N, Rittener V, Giovinazzo N, Bendahmane A (2007) Molecular determinants of recognition specificity at the aphid and powdery mildew Vat/Pm-W resistance locus in melon. In: XIII International Congress MPMI 2007, Sorrento, Italy, p 375

Essafi A, Diaz-Pendon JA, Moriones E, Monforte AJ, Garcia-Mas J, Martin-Hernandez AM (2009) Dissection of the oligogenic resistance to *Cucumber mosaic virus* in the melon accession PI 161375. Theor Appl Genet 118:275–284. doi:10.1007/s00122-008-0897-x

Esteras C, Gómez P, Monforte AJ, Blanca J, Vicente-Dólera N, Roig C, Nuez F, Picó B (2012) High-throughput SNP genotyping in *Cucurbita pepo* for map construction and quantitative trait *loci* mapping. BMC Genomics 13:80

Esteras C, Formisano G, Roig C, Diaz A, Blanca J et al (2013) SNP genotyping in melons: genetic variation, population structure, and linkage disequilibrium. Theor Appl Genet 126:1285–1303. doi:10.1007/s00122-013-2053-5

Ezura H, Fukino N (2009) Research tools for functional genomics in melon (*Cucumis melo* L.): current status and prospects. Plant Biotechnol 26:359–368

Fan Z, Robbins MD, Staub JE (2006) Population development by phenotypic selection with subsequent marker-assisted selection for line extraction in cucumber (*Cucumis sativus* L.). Theor Appl Genet 112:843–855. doi:10.1007/s00122-005-0186-x

Fanourakis NE, Simon PW (1987) Analysis of genetic linkage in the cucumber. J Hered 78:238–242

Fazio G, Chung SM, Staub JE (2003a) Comparative analysis of response to phenotypic and marker-assisted selection for multiple lateral branching in cucumber (*Cucumis sativus* L.). Theor Appl Genet 107:875–883. doi:10.1007/s00122-003-1313-1

Fazio G, Staub JE, Stevens MR (2003b) Genetic mapping and QTL analysis of horticultural traits in cucumber (*Cucumis sativus* L.) using recombinant inbred lines. Theor Appl Genet 107:864–874. doi:10.1007/s00122-003-1277-1

Fukino N, Ohara T, Monforte AJ, Sugiyama M, Sakata Y, Kunihisa M, Matsumoto S (2008a) Identification of QTLs for resistance to powdery mildew and SSR markers diagnostic for powdery mildew resistance genes in melon (*Cucumis melo* L.). Theor Appl Genet 118:165–175. doi:10.1007/s00122-008-0885-1

Fukino N, Yoshioka Y, Kubo N, Hirai M, Sugiyama M, Sakata Y, Matsumoto S (2008b) Development of 101 novel SSR markers and construction of an SSR-based genetic linkage map of cucumber (*Cucumis sativus* L.). Breed Sci 58:475–483

Fukino N, Ohara T, Sugiyama M, Kubo N, Hirai M, Sakata Y, Matsumoto S (2012) Mapping of a gene that confers short lateral branching (*slb*) in melon (*Cucumis melo* L.). Euphytica 187:133–143. doi:10.1007/s10681-012-0667-3

Fukino N, Yoshioka Y, Sugiyama M, Sakata Y, Matsumoto S (2013) Identification and validation of powdery mildew (*Podosphaera xanthii*)-resistant loci in recombinant inbred lines of cucumber (*Cucumis sativus* L.). Mol Breed 32:267–277. doi:10.1007/s11032-013-9867-3

Garcia-Mas J, Benjak A, Sanseverino W, Bourgeois M, Mir G et al (2012) The genome of melon (*Cucumis melo* L.). Proc Natl Acad Sci U S A 109:11872–11877. doi:10.1073/pnas.1205415109

Gong L, Stift G, Kofler R, Pachner M, Lelley T (2008) Microsatellites for the genus *Cucurbita* and an SSR-based genetic linkage map of *Cucurbita pepo* L. Theor Appl Genet 117:37–48

González VM, Garcia-Mas J, Arus P, Puigdomenech P (2010a) Generation of a BAC-based physical map of the melon genome. BMC Genomics 11:339. doi:10.1186/1471-2164-11-339

González VM, Rodriguez-Moreno L, Centeno E, Benjak A, Garcia-Mas J, Puigdomenech P, Aranda MA (2010b) Genome-wide BAC-end sequencing of *Cucumis melo* using two BAC libraries. BMC Genomics 11:618. doi:10.1186/1471-2164-11-618

Gonzalo MJ, Oliver M, Garcia-Mas J, Monfort A, Dolcet-Sanjuan R, Katzir N, Arus P, Monforte AJ (2005) Simple-sequence repeat markers used in merging linkage maps of melon (*Cucumis melo* L.). Theor Appl Genet 110:802–811. doi:10.1007/s00122-004-1814-6

Guo S, Zhang J, Sun H, Salse J, Lucas WJ et al (2013) The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. Nat Genet 45:51–58. doi:10.1038/ng.2470

Han Y, Zhang Z, Liu C, Liu J, Huang S, Jiang J, Jin W (2009) Centromere repositioning in cucurbit species: implication of the genomic impact from centromere activation and inactivation. Proc Natl Acad Sci U S A 106:14937–14941. doi:10.1073/pnas.0904833106

Han Y, Zhang Z, Huang S, Jin W (2011) An integrated molecular cytogenetic map of *Cucumis sativus* L. chromosome 2. BMC Genet 12:18. doi:10.1186/1471-2156-12-18

Harel-Beja R, Tzuri G, Portnoy V, Lotan-Pompan M, Lev S et al (2010) A genetic map of melon highly enriched with fruit quality QTLs and EST markers, including sugar and carotenoid metabolism genes. Theor Appl Genet 121:511–533. doi:10.1007/s00122-010-1327-4

Hashizume T, Shimamoto I, Harusima Y, Yui M, Sato T, Imai T, Hirai M (1996) Construction of a linkage map for watermelon (*Citrullus lanatus*) using random amplified polymorphic DNA (RAPD). Euphytica 90:265–273

Hashizume T, Shimamoto I, Hirai M (2003) Construction of a linkage map and QTL analysis of horticultural traits for watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] using RAPD, RFLP and ISSR markers. Theor Appl Genet 106:779–785

Havey MJ, McCreight JD, Rhodes B, Taurick G (1998) Differential transmission of the *Cucumis* organellar genomes. Theor Appl Genet 97:122–128

He X, Li Y, Pandey S, Yandell BS, Pathak M, Weng Y (2013) QTL mapping of powdery mildew resistance in WI 2757 cucumber (*Cucumis sativus* L.). Theor Appl Genet 126:2149–2161

Horejsi T, Staub JE, Thomas C (2000) Linkage of random amplified polymorphic DNA markers to downy mildew resistance in cucumber (*Cucumis sativus* L.). Euphytica 115:105–113

Huang S, Li R, Zhang Z, Li L, Gu X et al (2009) The genome of the cucumber, *Cucumis sativus* L. Nat Genet 41:1275–1281. doi:10.1038/ng.475

Jeffrey C (1980) A review of the Cucurbitaceae. Bot J Linn Soc 81:233–247

Joobeur T, King JJ, Nolin SJ, Thomas CE, Dean RA (2004) The Fusarium wilt resistance locus *Fom-2* of melon contains a single resistance gene with complex features. Plant J 39:283–297. doi:10.1111/j.1365-313X.2004.02134.x

Kabelka EA, Young K (2010) Identification of molecular markers associated with resistance to squash silverleaf disorder in summer squash (*Cucurbita pepo*). Euphytica 173:49–54

Kang H, Weng Y, Yang Y, Zhang Z, Zhang S, Mao Z, Cheng G, Gu X, Huang S, Xie B (2011) Fine genetic mapping localizes cucumber scab resistance gene *Ccu* into an *R* gene cluster. Theor Appl Genet 122:795–803. doi:10.1007/s00122-010-1487-2

Kato K, Tanaka K, Yamamoto T, Fukino N, Kodani S, Akashi Y, Matsumoto S, Ohsawa R, Nishida H (2011) MtIREP, a new marker to detect structural polymorphism in mitochondrial genome of melon and cucumber. In: SOL & ICuGI 2011, Kobe, Japan, p 51

Kennard W, Poetter K, Dijkhuizen A, Meglic V, Staub J, Havey M (1994) Linkages among RFLP, RAPD, isozyme, disease-resistance, and morphological markers in narrow and wide crosses of cucumber. Theor Appl Genet 89:42–48. doi:10.1007/BF00226980

Kim KH, Ahn SG, Hwang JH, Choi YM, Moon HS, Park YH (2013) Inheritance of resistance to powdery mildew in the watermelon and development of a molecular marker for selecting resistant plants. Hortic Environ Biotechnol 54(2):134–140

Lee YH, Jeon HJ, Hong KH, Kim BD (1995) Use of random amplified polymorphic DNA for linkage group analysis in an interspecific cross hybrid F_2 generation of *Cucurbita*. J Kor Soc Hortic Sci 36:323–330

Levi A, Thomas CE, Trebitsh T, Salman A, King J, Karalius J, Newman M, Reddy OUK, Xu Y, Zhang X (2006) An extended linkage map for watermelon based on SRAP, AFLP, SSR, ISSR, and RAPD markers. J Amer Soc Hort Sci 131(3):393–402

Li D, Cuevas HE, Yang L, Li Y, Garcia-Mas J et al (2011) Syntenic relationships between cucumber (*Cucumis sativus* L.) and melon (*C. melo* L.) chromosomes as revealed by comparative genetic mapping. BMC Genomics 12:396. doi:10.1186/1471-2164-12-396

Lilly J, Havey M (2001) Small, repetitive DNAs contribute significantly to the expanded mitochondrial genome of cucumber. Genetics 159:317–328

Lin X, Zhang Y, Kuang H, Chen J (2013) Frequent loss of lineages and deficient duplications accounted for low copy number of disease resistance genes in Cucurbitaceae. BMC Genomics 14:335. doi:10.1186/1471-2164-14-335

Ling KS, Harris KR, Meyer JDF, Levi A, Guner N, Wehner TC, Bendahmane A, Havey MJ (2009) Non-synonymous single nucleotide polymorphisms in the watermelon eIF4E gene are closely associated with resistance to *Zucchini yellow mosaic virus*. Theor Appl Genet 120:191–200

Liu L, Yuan X, Cai R, Pan J, He H, Yuan L, Guan Y, Zhu L (2008) Quantitative trait loci for resistance to powdery mildew in cucumber under seedling spray inoculation and leaf disc infection. J Phytopathol 156:691–697. doi:10.1111/j.1439-0434.2008.01427.x

Lou L, Wang H, Qian C, Liu J, Bai Y, Chen J (2013) Genetic mapping of gummy stem blight (*Didymella bryoniae*) resistance genes in *Cucumis sativus-hystrix* introgression lines. Euphytica 192:359–369. doi:10.1007/s10681-013-0860-z

Lv J, Qi J, Shi Q, Shen D, Zhang S et al (2012) Genetic diversity and population structure of cucumber (*Cucumis sativus* L.). PLoS One 7:e46919. doi:10.1371/journal.pone.0046919

Martin A, Troadec C, Boualem A, Rajab M, Fernandez R, Morin H, Pitrat M, Dogimont C, Bendahmane A (2009) A transposon-induced epigenetic change leads to sex determination in melon. Nature 461:1135–1138. doi:10.1038/nature08498

Matsuura S (1995) Paternal inheritance of mitochondrial DNA in cucumber (*Cucumis sativus* L.). Cucurbit Genet Coop Rep 18:31–33

Maule A, Caranta C, Boulton M (2007) Sources of natural resistance to plant viruses: status and prospects. Mol Plant Pathol 8:223–231

Meyer JDF, Deleu W, Garcia-Mas J, Havey MJ (2008) Construction of a fosmid library of cucumber (*Cucumis sativus*) and comparative analyses of the eIF4E and eIF(iso)4E regions from cucumber and melon (*Cucumis melo*). Mol Genet Genomics 279:473–480. doi:10.1007/s00438-008-0326-5

Miao H, Zhang S, Wang X, Zhang Z, Li M et al (2011) A linkage map of cultivated cucumber (*Cucumis sativus* L.) with 248 microsatellite marker loci and seven genes for horticulturally important traits. Euphytica 182:167–176. doi:10.1007/s10681-011-0410-5

Monforte AJ, Oliver M, Gonzalo MJ, Alvarez JM, Dolcet-Sanjuan R, Arus P (2004) Identification of quantitative trait loci involved in fruit quality traits in melon (*Cucumis melo* L.). Theor Appl Genet 108:750–758. doi:10.1007/s00122-003-1483-x

Navot N, Zamir D (1986) Linkage relationships of 19 protein coding genes in watermelon. Theor Appl Genet 72:274–278

Navot N, Sarfatti M, Zamir D (1990) Linkage relationships of genes affecting bitterness and flesh color in watermelon. J Hered 81:162–165

Nieto C, Morales M, Orjeda G, Clepet C, Monfort A et al (2006) An *eIF4E* allele confers resistance to an uncapped and non-polyadenylated RNA virus in melon. Plant J 48:452–462. doi:10.1111/j.1365-313X.2006.02885.x

Ning X, Wang X, Gao X, Zhang Z, Zhang L, Yan W, Li G (2014) Inheritances and location of powdery mildew resistance gene in melon Edisto47. Euphytica 195:345–353. doi:10.1007/s10681-013-1000-5

Olczak-Woltman H, Bartoszewski G, Madry W, Niemirowicz-Szczytt K (2009) Inheritance of resistance to angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*) in cucumber and identification of molecular markers linked to resistance. Plant Pathol 58:145–151. doi:10.1111/j.1365-3059.2008.01911.x

Oliver M, Garcia-Mas J, Cardús M, Pueyo N, López-Sesé AI, Arroyo M, Gómez-Paniagua H, Arús P, de Vincente MC (2001) Construction of a reference linkage map for melon. Genome 44:836–845

Oumouloud A, Arnedo-Andres MS, Gonzalez-Torres R, Alvarez JM (2008) Development of molecular markers linked to the *Fom-1* locus for resistance to Fusarium race 2 in melon. Euphytica 164:347–356. doi:10.1007/s10681-008-9664-y

Pang X, Zhou X, Wan H, Chen J (2013) QTL mapping of downy mildew resistance in an introgression line derived from interspecific hybridization between cucumber and *Cucumis hystrix*. J Phytopathol 161:536–543. doi:10.1111/jph.12103

Park YH, Sensoy S, Wye C, Antonise R, Peleman J, Havey MJ (2000) A genetic map of cucumber composed of RAPDs, RFLPs, AFLPs, and loci conditioning resistance to papaya ringspot and zucchini yellow mosaic viruses. Genome 43:1003–1010

Perchepied L, Bardin M, Dogimont C, Pitrat M (2005) Relationship between loci conferring downy mildew and powdery mildew resistance in melon assessed by quantitative trait loci mapping. Phytopathology 95:556–565. doi:10.1094/phyto-95-0556

Pitrat M (1991) Linkage groups in *Cucumis melo* L. J Hered 82:406–411

Qi J, Liu X, Shen D, Miao H, Xie B et al (2013) A genomic variation map provides insights into the genetic basis of cucumber domestication and diversity. Nat Genet 45:1510–1515

Ren Y, Zhang Z, Staub J, Cheng Z, Li X, Lu J, Miao H, Kang H, Xie B, Gu X (2009) An integrated genetic and cytogenetic map of the cucumber genome. PLoS One 4:1–8. doi:10.1371/journal.pone.0005795

Ren Y, Zhao H, Kou Q, Jiang J, Guo S et al (2012) A high resolution genetic map anchoring scaffolds of the sequenced watermelon genome. PLoS One 7(1):e29453

Rodriguez-Moreno L, Gonzalez VM, Benjak A, Marti MC, Puigdomenech P, Aranda MA, Garcia-Mas J (2011) Determination of the melon chloroplast and mitochondrial genome sequences reveals that the largest reported mitochondrial genome in plants contains a significant amount of DNA having a nuclear origin. BMC Genomics 12:424. doi:10.1186/1471-2164-12-424

Sakata Y, Kubo N, Morishita M, Kitadani E, Sugiyama M, Hirai M (2006) QTL analysis of powdery mildew resistance in cucumber (*Cucumis sativus* L.). Theor Appl Genet 112:243–250. doi:10.1007/s00122-005-0121-1

Sandlin K, Prothro J, Heesacker A, Khalilian N, Okashah R et al (2012) Comparative mapping in watermelon [*Citrullus lanatus* (Thunb.) Matsum. et Nakai]. Theor Appl Genet 125:1603–1618

Sherman A, Eshed R, Harel-Beja R, Tzuri G, Portnoy V et al (2013) Combining bulk segregation analysis and microarrays for mapping of the pH trait in melon. Theor Appl Genet 126:349–358. doi:10.1007/s00122-012-1983-7

Sitterly WR (1972) Breeding for disease resistance in cucurbits. Annu Rev Phytopathol 10:471–490. doi:10.1146/annurev.py.10.090172.002351

Sun Z, Staub JE, Chung SM, Lower RL (2006) Identification and comparative analysis of quantitative trait loci associated with parthenocarpy in processing cucumber. Plant Breed 125:281–287. doi:10.1111/j.1439-0523.2006.01225.x

Sun J, Zhang Z, Zong X, Huang S, Li Z, Han Y (2013) A high-resolution cucumber cytogenetic map integrated with the genome assembly. BMC Genomics 14:461. doi:10.1186/1471-2164-14-461

Tanaka K, Akashi Y, Fukunaga K, Yamamoto T, Aierken Y, Nishida H, Long CL, Yoshino H, Sato Y, Kato K (2013) Diversification and genetic differentiation of cultivated melon inferred from sequence polymorphism in the chloroplast genome. Breed Sci 63:183–196. doi:10.1270/jsbbs.63.183

Tezuka T, Waki K, Kuzuya M, Ishikawa T, Takatsu Y, Miyagi M (2010) Development of new DNA markers linked to the Fusarium wilt resistance locus *Fom-1* in melon. Plant Breed 130:261–267. doi:10.1111/j.1439-0523.2010.01800.x

Tomason Y, Nimmakayala P, Levi A, Reddy UK (2013) Map-based molecular diversity, linkage disequilibrium and association mapping of fruit traits in melon. Mol Breed 31:829–841. doi:10.1007/s11032-013-9837-9

Walters SA, Shetty NV, Wehner TC (2001) Segregation and linkage of several genes in cucumber. J Amer Soc Hort Sci 126:442–450

Wang YH, Thomas CE, Dean RA (1997) A genetic map of melon (*Cucumis melo* L.) based on amplified fragment length polymorphism (AFLP) markers. Theor Appl Genet 95:791–798

Wang X, Li G, Gao X, Xiong L, Wang W, Han R (2011) Powdery mildew resistance gene (*Pm-AN*) located in a segregation distortion region of melon LGV. Euphytica 180:421–428. doi:10.1007/s10681-011-0406-1

Watanabe Y, Izumida A, Katsumata K, Hiramoto T, Hasegawa M, Suzuki T (2012) Verification of usefulness as a large scale examination for hybridity using mitochondrial genome marker inherited paternally in *Cucumis melo* L. Breed Res 14(Suppl 2):64 (in Japanese)

Weeden NF, Robinson RW (1986) Allozyme segregation ratios in the interspecific cross *Cucurbita maxima* x *C. ecuadorensis* suggest that hybrid breakdown is not caused by minor alterations in chromosome structure. Genetics 114:593–609

Wehner TC, Maynard DN (2003) Cucurbitaceae (vine crops). In: Encyclopedia of life. Nature Publishing

Wóycicki R, Witkowicz J, Gawronski P, Dabrowska J, Lomsadze A et al (2011) The genome sequence of the North-European cucumber (*Cucumis sativus* L.) unravels evolutionary adaptation mechanisms in plants. PLoS One 6:e22728. doi:10.1371/journal.pone.0022728

Yang L, Koo DH, Li Y, Zhang X, Luan F, Havey MJ, Jiang J, Weng Y (2012) Chromosome rearrangements during domestication of cucumber as revealed by high-density genetic mapping and draft genome assembly. Plant J 71:895–906. doi:10.1111/j.1365-313X.2012.05017.x

Yang L, Li D, Li Y, Gu X, Huang S, Garcia-Mas J, Weng Y (2013) A 1,681-locus consensus genetic map of cultivated cucumber including 67 NB-LRR resistance gene homolog and ten gene loci. BMC Plant Biol 13:53

Yang L, Koo DH, Li D, Zhang T, Jiang J et al (2014) Next-generation sequencing, FISH mapping, and synteny-based modeling reveal mechanisms of decreasing dysploidy in Cucumis. Plant J 77:16–30. doi:10.1111/tpj.12355

Yoshioka Y, Sakata Y, Sugiyama M, Fukino N (2014) Identification of quantitative trait loci for downy mildew resistance in cucumber (*Cucumis sativus* L.). Euphytica 198(2):265–276. doi:10.1007/s10681-014-1102-8

Yuan XJ, Li XZ, Pan JS, Wang G, Jiang S et al (2008) Genetic linkage map construction and location of QTLs for fruit-related traits in cucumber. Plant Breed 127:180–188. doi:10.1111/j.1439-0523.2007.01426.x

Yuste-Lisbona FJ, Capel C, Sarria E, Torreblanca R, Gómez-Guillamón ML, Capel J, Lozano R, López-Sesé AI (2010) Genetic linkage map of melon (*Cucumis melo* L.) and localization of a major QTL for powdery mildew resistance. Mol Breed 27:181–192. doi:10.1007/s11032-010-9421-5

Zalapa JE, Staub JE, McCreight JD, Chung SM, Cuevas H (2007) Detection of QTL for yield-related traits using recombinant inbred lines derived from exotic and elite US Western Shipping melon germplasm. Theor Appl Genet 114:1185–1201. doi:10.1007/s00122-007-0510-8

Zhang W, He H, Guan Y, Du H, Yuan L, Li Z, Yao D, Pan J, Cai R (2010) Identification and mapping of molecular markers linked to the tuberculate fruit gene in the cucumber (Cucumis sativus L.). Theor Appl Genet 120:645–654. doi:10.1007/s00122-009-1182-3

Zhang C, Ren Y, Guo S, Zhang H, Gong G, Du Y, Xu Y (2012a) Application of comparative genomics in developing markers tightly linked to the *Pm-2F* gene for powdery mildew resistance in melon (*Cucumis melo* L.). Euphytica 190:157–168. doi:10.1007/s10681-012-0828-4

Zhang WW, Pan JS, He HL, Zhang C, Li Z, Zhao JL, Yuan XJ, Zhu LH, Huang SW, Cai R (2012b) Construction of a high density integrated genetic map for cucumber (*Cucumis sativus* L.). Theor Appl Genet 124:249–259. doi:10.1007/s00122-011-1701-x

Zhang SP, Liu MM, Miao H, Zhang SQ, Yang YH, Xie BY, Wehner TC, Gu XF (2013) Chromosomal mapping and QTL analysis of resistance to downy mildew in *Cucumis sativus*. Plant Dis 97:245–251. doi:10.1094/pdis-11-11-0941-re

Zhou X-H, Qian C-T, Lou Q-F, Chen J-F (2009) Molecular analysis of introgression lines from *Cucumis hystrix* Chakr. to *C. sativus* L. Sci Hortic 119:232–235. doi:10.1016/j.scienta.2008.08.011

Zraidi A, Stift G, Pachner M, Shojaeiyan A, Gong L, Lelley T (2007) A consensus map for *Cucurbita pepo*. Mol Breed 20:375–388

Chapter 6
Mutant Resources and TILLING Platforms in Tomato Research

Yoshihiro Okabe and Tohru Ariizumi

6.1 Introduction

Genetic analysis of tomato is becoming increasingly powerful and efficient, as in other model plants like *Arabidopsis* and rice, due to the wealth of genetic resources that are available including resource centers containing natural accessions, introgression lines (ILs), mutant lines, full-length cDNAs, and DNA markers. In addition, extensive genetic and genomic data are available, including the full genome sequence of several tomato accessions and wild relatives (Saito et al. 2011; Aoki et al. 2010; Shirasawa et al. 2013). The availability of the tomato genome allows identification of novel genes with the potential to influence commercially important tomato traits. Increasing the availability and diversity of mutant lines will facilitate tomato functional genomics, and novel tomato varieties with improved agricultural traits will be developed in parallel with functional analysis. In this chapter, we discuss recent advances in the development of mutant resources, with a focus on Targeting Induced Local Lesions In Genomes (TILLING) platforms and Micro-Tom as a model system for tomato mutant research.

6.2 Micro-Tom: A Toolkit for Tomato Research

Developing a large library of mutant strains for model organism research can present several logistical challenges. Ideally, plants should be easy to manipulate and carry traits such as small size, short generation time, good proliferation, and

Y. Okabe • T. Ariizumi (✉)
Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tenno-dai, Tsukuba 305-8572, Japan
e-mail: okabe.yoshihiro.gp@u.tsukuba.ac.jp; ariizumi.toru.ge@u.tsukuba.ac.jp

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_6

high efficiency of genetic transformation. Micro-Tom, a dwarf tomato variety, fulfills these conditions. Micro-Tom was originally developed as an ornamental variety (Scott and Harbaugh 1989), but it is increasingly used as a model tomato system due to its experimental tractability. Micro-Tom is a semidwarf variety that reaches a final height of 20–30 cm. Three recessive mutations underlie the dwarfism trait: *dwarf* (*d*), *self-pruning* (*sp*), and *miniature* (*mnt*). The *D* gene encodes a cytochrome P450 protein involved in brassinosteroid (BR) biosynthesis (Bishop et al. 1996). Plants with the *d* mutation fail to synthesize BR normally. *SP* is the ortholog of *Arabidopsis TERMINAL FLOWER 1* (*TFL1*) and *Antirrhinum CENTRORADIALIS* (*CEN*) and directs continuous growth of the shoot apical meristem (Pnueli et al. 1998). The *sp* mutation results in a clear determinate phenotype. The *mnt* mutation is associated with a short internode phenotype that is likely due to a reduction in gibberellin (GA) signaling (Martí et al. 2006); however, the gene responsible for this phenotype has not yet been identified. Micro-Tom plants are dwarf, bushy, and determinate and have dark-green leaves, deformed simple leaflets, and short internodes. The small size allows Micro-Tom to be cultivated at high densities of up to 1357 plants/m^2 (Meissner et al. 1997), although environmental conditions have a significant impact on plant vigor and development (Ariizumi T, unpublished data). The Micro-Tom phenotype provides a number of experimental advantages. First, plants can be raised to maturity under fluorescence lighting, allowing year-round indoor cultivation as for *Arabidopsis*. Second, the generation time from sowing to seed harvesting is short, at 70–90 days. Third, *Agrobacterium* transformation in Micro-Tom is well established and has a relatively high efficiency compared to other model plants (Sun et al. 2006), and finally, Micro-Tom can be crossed to many tomato varieties and related *Solanum* spp. This allows facile transfer of Micro-Tom-derived mutations to other varieties. For example, the capacity for interspecific crossing allows generation of F_2 mapping populations between Micro-Tom mutants and wild relatives such as *S. pimpinellifolium* to perform positional cloning of candidate genes. In addition, genetic linkage maps comprising simple sequence repeats (SSRs), cleavage amplified polymorphic sequence (CAPS), and derived cleavage amplified polymorphic sequence (dCAPS) markers are available between Micro-Tom and Ailsa Craig or M82, allowing high-resolution mapping of interspecific F_2 populations (Shirasawa et al. 2010a, b).

6.3 Mutant Resources in Tomato

6.3.1 Induced Mutant Populations Generated Through Chemical and Physical Mutagenesis

More than 83,000 tomato accessions are contained within seed banks worldwide; the most extensive of these repositories is the Tomato Genetic Resource Center in

California (TGRC: www.tgrc.ucdavis.edu) (Bauchet and Causse 2012). In addition to tomato genetic lines such as wild relatives, ILs, domesticated varieties, and spontaneous mutants, several tomato groups have also collected induced mutant lines. An estimated ~60,000 induced mutant tomato lines are currently available for tomato studies worldwide, potentially covering almost all the genes in the tomato genome.

Classical breeding techniques have been used throughout human agricultural history, leading to the domestication of numerous plants and animals. Classical breeding involves the crossing of accessions carrying different breeding traits followed by repetitive backcrossing into the original background. Approximately 7,500 cultivated tomato varieties have been generated through classical approaches (Bauchet and Causse 2012). A number of agronomically desirable traits have been derived from wild species, particularly those pertaining to disease resistance and stress tolerance. Wild species contain a wealth of natural variation as a result of ongoing spontaneous mutation, natural outcrossing, and recombination. Wild traits are of great potential importance for the genetic improvement of cultivated tomatoes, but interspecific crosses between wild and cultivated tomatoes are often hampered by low efficiency. In particular, high interspecific incompatibility resulting in a hybrid lethal phenotype is often observed when the cultivated tomato is used as the pollen donor (Bedinger et al. 2011). Although the hybrid lethality problem can sometimes be sidestepped by the use of molecular embryo rescue techniques, incompatibility significantly reduces the opportunities for exploiting wild tomato genetic resources.

Hybrid lethality is not a concern when using induced mutant cultivated tomato lines as a source of genetic variation because direct movement of desirable traits can be achieved using standard crossing techniques. Induced mutation also allows the development of different mutations within the same gene, forming an allelic series of strains with a variety of genotypic and phenotypic modifications. Allelic series are useful not only for analyzing gene function but also for breeding programs. Chemical- and radiation-based mechanisms are often used for the generation of a large population containing random mutations in genomic DNA. Mutants can also be generated through transposon tagging, as described subsequently. Commonly used chemical mutagens include alkylating agents such as ethyl methanesulfonate (EMS) and *N*-methyl-*N*-nitrosourea (MNU). EMS and MNU generally cause single nucleotide transitions and only rarely induce nucleotide transversion. Exposure to fast neutrons is an alternative to chemical mutagenesis and generally induces single and multiple nucleotide deletions randomly across the whole genome. Fast neutrons also stimulate insertions and produce a higher rate of single nucleotide substitution than deletion (Belfield et al. 2011). Gamma-ray irradiation also induces random nucleotide deletions as well as large deletions up to ~10 kb (Morita et al. 2009).

Several groups have been instrumental in producing tomato mutant populations by EMS treatment, gamma-ray irradiation, and fast-neutron irradiation. Menda et al. (2004) produced ~6,000 EMS mutant lines and 7000 fast-neutron mutant lines in the M82 background. These are described and distributed through an

accompanying database: "The Genes That Make Tomatoes" (http://zamir.sgn.cornell.edu/mutants/). Similarly, 5,508 EMS mutant lines were developed in Red Setter, as detailed in the LycoTILL database (http://www.agrobios.it/tilling/; Minoia et al. 2010), 8225 EMS mutant lines were produced using TPPADASU (Gady et al. 2009), and more than 5000 EMS mutant lines were generated in Heinz 1706 (http://tilling.ucdavis.edu/index.php/Tomato_Tilling). Two groups used Micro-Tom as a genetic background for mutagenesis. Saito et al. (2011) developed 8,598 EMS and 6,422 gamma-ray irradiation mutant lines, which are freely available through the "TOMATOMA" resource (http://tomatoma.nbrp.jp/), which also holds data regarding the visible phenotypes of the individual mutants such as fruit color, fruit size, and fruit shape. TOMATOMA currently contains ~1,500 EMS mutants and 164 gamma-ray irradiated mutant lines with phenotypic mutations. The visible phenotypes of these mutants were scored and classified into 15 major phenotypic categories and 48 subcategories. Micro-Tom was also used by INRA-Bordeaux (France), where ~8,000 EMS mutant lines have been developed so far and 30,000 annotations are available for more than 150 phenotypic categories (Just et al. 2013) in the associated Micro-Tom Mutant Database (MMDB, not yet publicly available).

6.3.2 Insertional Mutant Lines Using T-DNA Tagging System

Transfer DNA (T-DNA) tagging approaches have been widely used for functional gene analysis and generation of mutant populations in several species, including *Arabidopsis*, rice, soybean, maize, and other many crop plants (Alonso et al. 2003; Mathieu et al. 2009; Brutnell 2002; Jeong et al. 2002). An advantage of tagging mutagenesis is that the sequences flanking the insertion sites can be identified using simple PCR techniques such as thermal asymmetric interlaced (TAIL–PCR). Four groups produced large numbers of T-DNA insertion lines in tomato. Meissner et al. (2000) used the maize *Activator* (*Ac*)/*Dissociator* (*Ds*) transposon system. This technique enables rapid production of many independent mutant lines from a limited number of parental lines (even from a single line) and facilitates identification of flanking sequences. The *Ac*/*Ds* system was applied to Micro-Tom and produced 2,932 lines with 2–3 *Ds* insertions per line (~7,500 *Ds* insertions in total). Tagged populations were also developed using promoter and enhancer trapping systems with luciferase (LUC) and *β*-glucuronidase (GUS) reporter genes. When reporters are inserted into or near to the promoters or enhancers of genes, the reporter is activated when the gene is expressed, providing information regarding the expression pattern of the tagged gene. Pineda et al. (2010) developed more than 2,000 T-DNA tagged tomato lines with GUS insertion in the genetic background cv. SLDC2. One of these was a new mutant, *Arlequin* (*Alq*), in which succulent sepals were homeotically converted into fleshy fruit organs; this allowed the isolation of a new component, *ALQ*/*TAGL1*, which controlled fruit ripening.

Mathews et al. (2003) generated 10,427 independent activation-tagged lines of Micro-Tom to identify metabolic pathways regulators. Of these, 1,338 lines (12.83 %) exhibited one or more visible phenotypic variations. In addition to evaluating T1 progeny selected in the T0 generation, T1 progeny from 1,014 randomly selected activation-tagged lines that did not exhibit visible phenotypes in the T0 generation were also evaluated. Possible loss-of-function traits were observed in 103 lines (10.16 %). Recently, Carter et al. (2013) utilized the *Ac*/*Ds* system to generate a tagged population in the M82 background. The *Ds* elements carried four tandem copies of the cauliflower mosaic virus 35S enhancer region, which activated the native promoters of genes adjacent to the T-DNA insertion. This system allowed the production of activation-tagged dominant mutants showing visible phenotypes in the T0 generation through the transcriptional activation of host genes and knockout mutants in the T1 or later generations as a consequence of the T-DNA insertions. A population of 509 independent transposant lines carrying *Ds* insertions was developed, and the adjacent sequences were mapped to the tomato genome. Insertion sites were found predominantly in upstream promoter regions (within 1 kb of the nearest gene) and were distributed across all 12 chromosomes. Genes adjacent to *Ds* insertion sites were overexpressed at distances up to 4.5–12 kb from the insertion site, demonstrating the efficiency of the *Ds* insertion system for generating transformants with elevated gene expression. Flanking sequence and T1 visual phenotypic data are available through the accompanying website http://hortmutants.vbi.vt.edu.

6.4 TILLING

TILLING is an alternative reverse genetic tool for the identification of chemically induced mutations in animal and plant species and has developed into a high-throughput system for the rapid identification of allelic variants from large numbers of mutant lines (McCallum et al. 2000a, b). TILLING has a number of advantages over other reverse genetics methods, as described below. Chemical mutagens such as EMS or MNU can induce point mutations randomly across the whole genome. This characteristic allows the isolation of a broad range of mutant alleles with various mutation types (e.g., missense, nonsense, and splice junction) from a relatively small mutant population of ~10^3 individuals (Emmanuel and Levy 2002). TILLING is a non-transgenic approach and is not subject to the stringent regulatory requirements required of transgenic technologies. Mutant alleles isolated by TILLING can therefore be used directly in breeding programs as markers for target traits and germplasms. TILLING platforms have been developed in numerous species, including *Arabidopsis*, *Lotus japonicus*, wheat, soybean, barley, maize, melon, and tomato. Through the increasing availability of crop genome sequences and induced mutant resources, it is now possible to engineer agronomically important traits in crop species using TILLING.

6.4.1 Varieties Used in Tomato TILLING

Tomato TILLING platforms have been developed in a variety of genetic backgrounds including M82, Micro-Tom, Red Setter, and TPAADASU (Table 6.1). In addition to these platforms, the group of University of California-Davis (UC Davis) in the USA recently developed another tomato TILLING platform using EMS- or MNU-mutagenized populations of cultivar "Heinz 1706," which was used for the international tomato genome sequencing project (Tomato Genome Consortium 2012, http://tilling.ucdavis.edu/index.php/Tomato_Tilling).

6.4.2 Mutagens and Mutation Frequency in TILLING Populations of Tomato

EMS mutagenesis has been used for mutational breeding and functional gene studies in several crop species such as wheat, barley, soybean, rice, and tomato. In previously developed tomato TILLING populations, EMS mutagenesis was performed using a dosage of 0.5–1.0 %. Mutation with 0.5 % EMS generated one mutation per 574–1710 kb, while 1.0 % EMS treatment corresponded to one mutation per 322–737 kb (Table 6.1). The lethality dose (LD) and fertility rate of 0.5 % EMS-mutagenized M_1 populations were LD_{15}–LD_{35} and 40.3–82.3 %, respectively, while the lethality and fertility rates of 1.0 % EMS-mutagenized M_1 populations were LD_2–LD_{63} and 42.8–87.9 %, respectively, suggesting that 1.0 % EMS treatment induced more point mutations than 0.5 % EMS treatment. Importantly for maintenance of mutations in progeny, treatment with 1.0 % EMS successfully generated mutant lines without high M_1 lethality or diminished M2 seed levels. By contrast, higher concentrations of EMS (e.g., 1.5 % EMS) significantly decreased fertility rate and seed number in M_1 plants (Saito et al. 2011).

A large-scale study of TILLING in *Arabidopsis* (Greene et al. 2003) indicated that ~10 mutated coding region alleles are needed to reliably obtain loss-of-function alleles (e.g., null, leaky allele) for functional study. The optimal mutation frequency to obtain one loss-of-function allele in a TILLING population consisting of 5,000 mutant lines is estimated as >500 kb per mutation. Although high mutation frequencies are generally required for functional studies, low mutation frequencies may be desirable in breeding programs because multiple rounds of backcrossing are often required to remove additional undesirable mutations before agronomic use.

6.4.3 Spectrum of Induced Mutations

The spectrum and density of induced mutations in TILLING screens vary with different mutagens and plant species (Tadele et al. 2009). EMS is a mutagenic

Table 6.1 Overview of tomato TILLING platforms

Variety	Country	Population size	Mutagenesis	Mutation frequency	Screened gene(s)	References
M82	France	4759	0.5 % EMS	1/574 kb	*eIF4E*, *DET1*, *COP1-like*, *DDB1a*, *COP10*, *NAM*, *ACO1*, *E8*, *DHS*, *Rab11a*, *PG*, *MET1*, *Exp1 CRTISO*, *CUL4*	Piron et al. (2010)
Micro-Tom	Japan	2180	0.5 % EMS	1/1710 kb	*ETR1-ETR6*, *GABA-T1*, *GABA-T3 SSADH*, *PL*, *ARF7*, *PIN4*, *TAP3*, *NOR*, *RIN*	Okabe et al. (2011, 2013; Okabe Y, personal communication)
		872	1.0 % EMS	1/737 kb		
		5272	1.0 % EMS	1/850 kb		
Micro-Tom	France	7296	1.0 % EMS + 1.0 % EMS	1/663 kb	*GGP2*, *GME1*, *GMP2*	Just et al. (2013), Baldet et al. (2013)
Red Setter	Italy	3885	0.7 % EMS	1/574 kb	*Rab11a*, *PG*, *Exp1*, *RIN*, *GR*, *Lcy-a*, *Lcy-b*	Minoia et al. (2010)
		1284	1.0 % EMS	1/322 kb		
TPPADASU	Netherlands	8225	1.0 % EMS	1/737 kb	*ARF7*, *ProDH*, *PSY1*, *Sus2*	Gady et al. (2009)
Moneymaker	France	5000	0.75 % EMS	–	–	http://www-urgv.versailles.inra.fr/tilling/tomato.htm
Heinz 1706	USA	1000	110 mM EMS	1/455 kb	–	http://tilling.ucdavis.edu/index.php/Tomato_Tilling
		>4000		–		

alkylating agent that alkylates guanine residues; this leads to mispairing during replication and production of G/C to A/T transitions (Vidal et al. 1995). In *Arabidopsis* and wheat, more than 99 % of mutations identified by TILLING corresponded to GC/AT transition (Greene et al. 2003; Slade et al. 2005). However, in plant species, including barley, rice, soybean, and tomato (Caldwell et al. 2004; Till et al. 2007; Cooper et al. 2008; Minoia et al. 2010), several non-GC/AT mutations were observed in the TILLING screens. Minoia et al. (2010) reported that transversions (AT/TA, GC/CG, AT/CG) were frequently identified in tomato mutant populations. In an additional screen of Micro-Tom and M82 mutant populations, approximately 90 % of mutations were GC/AT transitions (Okabe et al. 2011; Piron et al. 2010).

Single-base substitutions in the protein-coding region are classified as silent, missense, or truncation (nonsense) mutations. A study of non-synonymous mutations induced by EMS in *Arabidopsis* estimated that 5 % of truncations and 50 % of missense mutations affected the biological function of the protein and, hence, the phenotype of the plants (Greene et al. 2003). The respective distributions of silent, missense, and nonsense mutations in tomato were 22.3, 68.6, and 9.1 % in a Micro-Tom mutant population (Okabe Y, unpublished data) and 36.6, 58.6, and 4.1 % in an M82 mutant population (Piron et al. 2010).

6.4.4 Mutation Detection

A gel-based screening method using a LI-COR 4300 DNA analyzer (LI-COR, Lincoln, USA) combined with a mismatch-specific endonuclease (CEL I nuclease) has been frequently used for TILLING due to its cost-effectiveness and detection sensitivity. CEL I nuclease, which was extracted and purified from celery (Till et al. 2006), has a substrate preference of C/C ≥ C/A, C/T ≥ G/G > A/C A/A ~ T/C > T/G, G/T, G/A, A/G > T/T (Oleykowski et al. 1998). By contrast with CEL I, *Arabidopsis* endonuclease 1 (ENDO1) recognizes all types of mismatch DNA, which improves the efficiency of detection for non-GC/AT types of mutation. ENDO1 has been used in several TILLING platforms (Triques et al. 2007; Minoia et al. 2010; Dahmani-Mardas et al. 2010; Piron et al. 2010; Okabe et al. 2011), but no direct comparisons between CEL1 and ENDO1 efficiency have been performed. However, in addition to its higher mismatch sensitivity, ENDO1 is more stable and can be easily isolated through histidine tagging and Ni-affinity column purification (Triques et al. 2007, 2008). Alternative nonenzymatic TILLING techniques have also been developed. High-resolution DNA melting analysis (HRM) has been used for TILLING and EcoTILLING in tomato, wheat, *Brassica rapa*, and *Medaka* (Gady et al. 2009; Ishikawa et al. 2010; Botticella et al. 2011; Lochlainn et al. 2011). HRM typically uses melting-curve analysis of target PCR products intercalated with a fluorescent dye such as LCgreen Plus+, which is more sensitive in the detection of heteroduplex DNA than SYBR Green. A LightScanner discriminates melting temperature differences between heteroduplex and homoduplex

Table 6.2 Mutant alleles identified by TILLING in tomato

Mutant allele	Genetic background	Trait	References
SleIF4E	M82	Virus resistance	Piron et al. (2010)
Sldet1	M82	Increased pigment and nutrient content	Jones et al. (2012)
Sltmf	M82	Early flowering, a solitary flower	MacAlister et al. (2012)
Sletr1-1 *Sletr1-2*	Micro-Tom	Reduced ethylene sensitivity, delayed fruit ripening, prolonged fruit shelf life	Okabe et al. (2011)
Slggp2	Micro-Tom	Decreased ascorbate	Baldet et al. (2013)
Slgmp2	Micro-Tom	Decreased ascorbate	Baldet et al. (2013)
Slpsy1	TPPADASU	Decreased carotenoid content	Gady et al. (2012)
Slcyc-B	Red Setter	Increased lycopene content	Silletti et al. (2013)
Slerf1	Red Setter	Reduced phenolics content	Di Matteo et al. (2013)

DNA. When compared to LI-COR/ENDO1 analysis, HRM may provide an increased likelihood of detecting mutations. HRM provides a number of advantages in flexibility and cost-effectiveness when used to examine short regions of interest, such as functional domains, motifs, and segmented short exons, or when considering large numbers of individuals. These advantages include the use of short amplicons (200–500 bp), unlabeled standard primers, and simple, efficient procedures. Another cost-effective TILLING method, which used standard agarose gel electrophoresis combined with CEL I nuclease, was employed in soybean (Hoshino et al. 2010). Anai (2012) reported that the soybean agarose gel-based TILLING system enabled a single person to screen 6,000 mutant lines per day (Table 6.2).

Technological advantages have allowed sequencing approaches to be used for the detection of induced mutations and SNPs in large-scale mutant populations in recent years. TILLING systems using next-generation sequencing (NGS) were employed in crop species such as rice, wheat, and tomato (Rigola et al. 2009; Tsai et al. 2011, 2013). Rigola et al. (2009) described a TILLING and EcoTILLING approach using NGS in tomato. In this approach, named Keypoint, DNA samples were isolated from 3008 M_2 families (15,000 M_2 plants), and a 3D pooling strategy was used to create 28 pooled samples; 12 or 8 DNA samples were placed on each axis (e.g., x-, y-, and z-axes with 12, 8, and 8 samples, respectively). A Roche 454 GS FLX system was used to sequence the pooled samples in a screen for mutations in the 287 bp targeting region of the *SlelF4E* gene. Two novel *SlelF4E* alleles were successfully identified.

The Illumina GA II sequencing system was also recently used alongside a multidimensional pooling strategy (2D or 3D pooling) to identify individual mutant

lines and mutations without extra deconvolution or sequencing of the pools. Tsai et al. (2011, 2013) used this system to identify rare low-frequency mutations in rice and wheat mutant populations. Using this system, 1,600,000 kb of sequence (e.g., 40 million reads each of 40 bases in length or 20 million reads each of 80 bases in length) were sequenced in a single run (Tsai et al. 2011). The dataset was incorporated and processed using the Coverage Aware Mutation program with Bayesian analysis (CAMBa, Missirian et al. 2011). The high sensitivity of the Illumina GA II platform enabled the identification of a number of additional mutations from previously characterized mutant populations in rice (Till et al. 2007) and wheat (Uauy et al. 2009), and these mutations have since been validated using conventional TILLING. NGS-based TILLING systems may exhibit higher sensitivity for mutation detection (up to the ratio of 1:192 mutant to wild-type alleles) (Tsai et al. 2011), than conventional high-sensitivity LI-COR/ENDO1 analysis (up to the ratio of 1:60 mutant to wild-type alleles) (Triques et al. 2007), indicating that TILLING systems coupled to NGS are potent alternatives to conventional systems for the identification of rare mutations.

6.4.5 Mutant Tomato Alleles Isolated by TILLING

TILLING approaches were used to identify novel alleles from tomato EMS mutant populations. Piron et al. (2010) identified the *SleIF4E1* allele from a novel potyvirus-resistant mutant by screening the genes for translation initiation factors eIF4E and eIF4G. The *SleIF4E1* allele had a splice-junction mutation, g1485a, and encoded a truncated mRNA. This caused impairment of SleIF4E1cap-binding activity, which is required for virus infection, and conferred resistance to Potato virus Y (PVY-LYE90) and Pepper mottle virus (PepMov strain Texas) without accompanying growth defects. Jones et al. (2012) isolated mutants in the *Solanum lycopersicum DEETIOLATED 1* (*SlDET1*) gene from a M82 EMS-mutagenized population developed by Menda et al. (2004). *SlDET1* is allelic to *high pigment 2* (*hp2*) (Mustilli et al. 1999) and encodes a component of the tomato light signal transduction pathway. Carotenoid and phenylpropanoid levels were increased in ripe fruits of *SlDET1* mutants, and the nutritional quality of the crop was enhanced. Two phytoene synthase (*Psy1*) mutants [*SlPsy1* P192L (leaky) and W180stop (null)] were isolated from a TPAADASU EMS-mutagenized population by Gady et al. (2012). PSY1 catalyzes a rate-limiting step in the carotenoid pathway and acts as a fruit-specific phytoene synthase (Fray and Grierson 1993; Rodríguez-Villalón et al. 2009). The phenotype of the *SlPsy1* null allele resembled the *yellow-flesh* (*r*) mutant (Fray and Grierson 1993), which had pale-yellow fruit and was caused by a recessive mutation that led to disruption of PSY1 activity. Other *SlPsy1* mutant alleles have also been isolated from the M82 mutant population (Kachanovsky et al. 2012). In a recent study, a novel tomato mutant, *terminating flower* (*tmf*), was isolated that exhibited an early flowering phenotype and converted the multiflowered inflorescence into a solitary flower. The mutation was mapped to a

gene encoding an ALOG (*Arabidopsis* LIGHT-SENSITIVE HYPOCOTYL 1, *Oryza* G1) family protein (MacAlister et al. 2012). A second allele, *tmf-2*, which also produced a primary inflorescence with a single flower and enlarged leaf-like sepals, was isolated using M82 TILLING. Di Matteo et al. (2013) used a Red Setter population to isolate a *Solanum lycopersicum ethylene-responsive factor 1* (*SlERF1*) mutant that exhibited lower levels of phenolics in the fruits. Subsequent expression analysis of genes involved in sequestration of phenolics in the *Slerf1* line showed that ERF1 played a role in accumulation of phenolics in tomato fruit. A new *Cyc-B* allele (DNA, a949g; protein, R317G) was also identified using a Red Setter TILLING platform (Silletti et al. 2013). *Cyc-B* encodes a chromoplast-specific lycopene β-cyclase that converts lycopene to β-carotene (Ronen et al. 2000). *Cyc-B* a949g is allelic to *old-gold* (*og*) and *old-gold-crimson* (og^c), which induce frameshift mutations in the coding region of the *Cyc-B* gene and impair the enzyme activity of lycopene β-cyclase. Carotenoid profiles were altered in the *Cyc-B* mutant in petals and fruit and led to an increased lycopene content due to the negative effect by the Cyc-B mutant protein activity.

Novel ascorbate-deficient mutants were isolated in Micro-Tom through a TILLING screen of three ascorbate biosynthetic genes, GDP-D-mannose pyrophosphorylase (*GMP*), GDP-D-mannose epimerase (*GME*), and GDP-L-galactose phosphorylase (*GGP*), using EMS-mutagenized populations from the University of Tsukuba (Japan) and INRA-Bordeaux (France) (Baldet et al. 2013; Just et al. 2013). GGP catalyzes the commitment step of the ascorbate biosynthetic pathway and is involved in the regulation of ascorbate biosynthesis in plants (Bulley et al. 2012; Linster and Clarke 2008). GMP and GME are involved in the synthesis of precursors (e.g., D-mannose, L-galactose) of ascorbic acid and key cell wall components such as mannans and rhamnogalacturonans (Voxeur et al. 2011; Gilbert et al. 2009). Mutations in the *Slgmp2* and *Slggp2* alleles affected the enzymes involved in reduction of ascorbate content and decoloration of the leaf surface under natural solar radiation. The ascorbate-deficient mutants could therefore be valuable tools in deciphering the link between cell wall biosynthesis and ascorbate biosynthesis in tomato. In addition, we previously isolated novel mutant alleles (*Sletr1-1* and *Sletr1-2*) of an ethylene receptor gene, *Solanum lycopersicum ETHYLENE RESPONSE 1* (*SlETR1*), from a Micro-Tom mutant population (Okabe et al. 2011). The *Sletr1* mutations affected transmembrane domains (P51L, V69D) that are important for ethylene binding (Schaller and Bleecker 1995; Wang et al. 2006). The mutant alleles exhibited ethylene insensitivity, delayed fruit ripening, and extended fruit shelf life. The ethylene sensitivity and impairment of ripening in the *Sletr1* mutant distinguished it from the *Never ripe* (*Nr*) phenotype (Lanahan et al. 1994) and indicated that the SlETR1 tomato ethylene receptor was important for accelerating fruit ripening alongside other ethylene receptors such as NR, SlETR4, and SlETR6. Novel parental germplasms were developed for the improvement of fruit shelf life through molecular breeding (Okabe et al. 2011, 2012). The *Indole Acetic Acid 9* (*IAA9*) gene is a member of the *Aux/IAA* transcription factor family that negatively regulates auxin response as a central component of the auxin-signaling pathway. Downregulation or loss of function of *IAA9* causes

parthenocarpic fruit development (Wang et al. 2005, 2009; Saito et al. 2011). A mutant screen combining forward and reverse genetics approaches successfully isolated several *Sliaa9 alleles* (Saito et al. 2011; personal communication) that exhibited parthenocarpic fruit set and fused leaf phenotypes.

In summary, several studies recently employed TILLING platforms to isolate novel mutant alleles in tomato, demonstrating the utility of this technology for developing breeding lines with desirable agronomic traits. Reverse genetics in tomato using TILLING have been established through the cooperative efforts of the tomato research community.

6.5 Perspectives

Micro-Tom has become an attractive model for functional genomics studies in recent decades, and genomic tools and resources such as whole genome sequences, DNA markers, mutant lines, and TILLING techniques are freely available in both Micro-Tom and the other main tomato model M82.

Induced mutant populations often contain novel mutations that are not found within the currently available natural populations. The broad allelic diversity in induced mutant populations allows precise assessment of gene function through the comparison of mutant phenotypes in allelic lines. Induced mutant populations also potentially harbor valuable allelic traits for use in breeding programs. As mutations associated with agronomically important traits are often accompanied with deviation from normal growth, the capacity to precisely isolate the mutation responsible for a desirable trait is particularly important. For example, two *Sletr1* mutant alleles were identified by Okabe et al. (2011), and the severe allele *Sletr1-1* produced mature fruits with highly increased shelf life; however, the long shelf life was accompanied by an undesirable ripening phenotype in which mature fruits were orange rather than red. The weaker *Sletr1-2* allele also exhibited an increased shelf life but produced mature fruit with a ripe red color. Such “moderate” or “weak” alleles are often more attractive for breeding programs, and the variety inherent in an induced mutation population therefore provides great potential for the development of desirable breeding traits.

The major aims of tomato breeding are to improve yield, shelf life, tolerance to abiotic and biotic stresses, and content of desirable nutrients such as lycopene, amino acids, and sugars. These improvements are desirable for both the processed and fresh tomato markets. The production of innovative new varieties carrying these important traits is of key importance for scientists and breeders, and expansion of mutant resources will be greatly beneficial in this endeavor.

Acknowledgment This work was supported by Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry, Japan (grant no. 26013A).

References

Alonso JM, Stepanova AN, Leisse TJ, Kim CJ, Chen H, Shinn P, Stevenson DK, Zimmerman J, Barajas P, Cheuk R et al (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. Science 301:653–657, Erratum in: Science 301:1849

Anai T (2012) Potential of a mutant-based reverse genetic approach for functional genomics and molecular breeding in soybean. Breed Sci 61:462–467

Aoki K, Yano K, Suzuki A, Kawamura S, Sakurai N, Suda K, Kurabayashi A, Suzuki T, Tsugane T, Watanabe M et al (2010) Large-scale analysis of full-length cDNAs from the tomato (*Solanum lycopersicum*) cultivar Micro-Tom, a reference system for the Solanaceae genomics. BMC Genomics 11:210

Baldet P, Brès C, Okabe Y, Mauxion JP, Just D, Bournonville C, Ferrand C, Mori M, Ezura H, Rothan C (2013) Investigating the role of vitamin C in tomato through TILLING identification of ascorbate-deficient tomato mutants. Plant Biotechnol 30:309–314

Bauchet G, Causse M (2012). Genetic diversity in tomato (*Solanum lycopersicum*) and its wild relatives. In: Caliskon M (ed) Genetic diversity in plants. InTech. ISBN: 978-953-51-0185-7

Bedinger PA, Chetelat RT, McClure B, Moyle LC, Rose JKC, Stack SM, Knaap E, Baek YS, Lopez-Casado G, Covey PA, Kumar A et al (2011) Interspecific reproductive barriers in the tomato clade: opportunities to decipher mechanisms of reproductive isolation. Sex Plant Reprod 24:171–187

Belfield EJ, Gan X, Mithani A, Brown C, Jiang C, Franklin K, Alvey E, Wibowo A, Jung M, Bailey K et al (2011) Genome-wide analysis of mutations in mutant lineages selected following fast-neutron irradiation mutagenesis of *Arabidopsis thaliana*. Genome Res 22:1306–1315

Bishop GJ, Harrison K, Jones JD (1996) The tomato *Dwarf* gene isolated by heterologous transposon tagging encodes the first member of a new cytochrome P450 family. Plant Cell 8:959–969

Botticella E, Sestili F, Hernandez-Lopez A, Phillips A, Lafiandra D (2011) High resolution melting analysis for the detection of EMS induced mutations in wheat *SBEIIa* genes. BMC Plant Biol 11:156

Brutnell TP (2002) Transposon tagging in maize. Funct Integr Genomics 2:4–12

Bulley S, Wright M, Rommens C, Yan H, Rassam M, Lin-Wang K, Andre C, Brewster D, Karunairetnam S, Allan AC, Laing WA (2012) Enhancing ascorbate in fruits and tubers through over-expression of the L-galactose pathway gene GDP-L-galactose phosphorylase. Plant Biotechnol J 10:390–397

Caldwell DG, McCallum N, Shaw P, Muehlbauer GJ, Marshall DF, Waugh R (2004) A structured mutant population for forward and reverse genetics in barley (*Hordeum vulgare* L.). Plant J 40:143–150

Carter JD, Pereira A, Dickerman AW, Veilleux RE (2013) An active *Ac*/*Ds* transposon system for activation tagging in tomato cultivar M82 using clonal propagation. Plant Physiol 162:145–156

Cooper JL, Till BJ, Laport RG, Darlow MC, Kleffner JM, Jamai A, El-Mellouki T, Liu S, Ritchie R, Nielsen N et al (2008) TILLING to detect induced mutations in soybean. BMC Plant Biol 8:9

Dahmani-Mardas F, Troadec C, Boualem A, Lévêque S, Alsadon AA, Aldoss AA, Dogimont C, Bendahmane A (2010) Engineering melon plants with improved fruit shelf life using the TILLING approach. PLoS One 5:e15776

Di Matteo A, Ruggieri V, Sacco A, Rigano MM, Carriero F, Bolger A, Fernie AR, Frusciante L, Barone A (2013) Identification of candidate genes for phenolics accumulation in tomato fruit. Plant Sci 205–206:87–96

Emmanuel E, Levy AA (2002) Tomato mutants as tools for functional genomics. Curr Opin Plant Biol 5:112–117

Fray RG, Grierson D (1993) Identification and genetic analysis of normal and mutant phytoene synthase genes of tomato by sequencing, complementation and co-suppression. Plant Mol Biol 22:589–602

Gady AL, Hermans FW, Van de Wal MH, van Loo EN, Visser RG, Bachem CW (2009) Implementation of two high through-put techniques in a novel application: detecting point mutations in large EMS mutated plant populations. Plant Methods 5:13

Gady AL, Vriezen WH, Van de Wal MH, Huang P, Bovy AG, Visser RG, Bachem CW (2012) Induced point mutations in the *phytoene synthase 1* gene cause differences in carotenoid content during tomato fruit ripening. Mol Breed 29:801–812

Gilbert L, Alhagdow M, Nunes-Nesi A, Quemener B, Guillon F, Bouchet B, Faurobert M, Gouble B, Page D, Garcia V et al (2009) GDP-D-mannose 3,5-epimerase (GME) plays a key role at the intersection of ascorbate and non-cellulosic cell-wall biosynthesis in tomato. Plant J 60:499–508

Greene EA, Codomo CA, Taylor NE, Henikoff JG, Till BJ, Reynolds SH, Enns LC, Burtner C, Johnson JE, Odden AR et al (2003) Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in *Arabidopsis*. Genetics 164:731–740

Hoshino T, Takagi Y, Anai T (2010) Novel *GmFAD2-1b* mutant alleles created by reverse genetics induce marked elevation of oleic acid content in soybean seeds in combination with *GmFAD2-1a* mutant alleles. Breed Sci 60:419–425

Ishikawa T, Kamei Y, Otozai S, Kim J, Sato A, Kuwahara Y, Tanaka M, Deguchi T, Inohara H, Tsujimura T et al (2010) High-resolution melting curve analysis for rapid detection of mutations in a *Medaka* TILLING library. BMC Mol Biol 11:70

Jeong DH, An S, Kang HG, Moon S, Han JJ, Park S, Lee HS, An K, An G (2002) T-DNA insertional mutagenesis for activation tagging in rice. Plant Physiol 130:1636–1644

Jones MO, Piron-Prunier F, Marcel F, Piednoir-Barbeau E, Alsadon AA, Wahb-Allah MA, Al-Doss AA, Bowler C, Bramley PM, Fraser PD, Bendahmane A (2012) Characterisation of alleles of tomato light signalling genes generated by TILLING. Phytochemistry 79:78–86

Just D, Garcia V, Ferrand C, Bres C, Mauxion JP, Petit J, Jorly J, Assali J, Boumonville C, Ferrand C et al (2013) Micro-Tom mutants for functional analysis of target genes and discovery of new alleles in tomato. Plant Biotechnol 30:225–231

Kachanovsky DE, Filler S, Isaacson T, Hirschberg J (2012) Epistasis in tomato color mutations involves regulation of *phytoene synthase 1* expression by cis-carotenoids. Proc Natl Acad Sci U S A 109:19021–19026

Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ (1994) The *never ripe* mutation blocks ethylene perception in tomato. Plant Cell 6:521–530

Linster CL, Clarke SG (2008) L-Ascorbate biosynthesis in higher plants: the role of VTC2. Trends Plant Sci 13:567–573

Lochlainn SO, Amoah S, Graham NS, Alamer K, Rios JJ, Kurup S, Stoute A, Hammond JP, Ostergaard L, King GJ et al (2011) High Resolution Melt (HRM) analysis is an efficient tool to genotype EMS mutants in complex crop genomes. Plant Methods 7:43

MacAlister CA, Park SJ, Jiang K, Marcel F, Bendahmane A, Izkovich Y, Eshed Y, Lippman ZB (2012) Synchronization of the flowering transition by the tomato *TERMINATING FLOWER* gene. Nat Genet 44:1393–1398

Martí E, Gisbert C, Bishop GJ, Dixon MS, García-Martínez JL (2006) Genetic and physiological characterization of tomato cv. Micro-Tom. J Exp Bot 57:2037–2047

Mathews H, Clendennen SK, Caldwell CG, Liu XL, Connors K, Matheis N, Schuster DK, Menasco DJ, Wagoner W, Lightner J et al (2003) Activation tagging in tomato identifies a transcriptional regulator of anthocyanin biosynthesis, modification, and transport. Plant Cell 15:1689–1703

Mathieu M, Winters EK, Kong F, Wan J, Wang S, Eckert H, Luth D, Paz M, Donovan C, Zhang Z et al (2009) Establishment of a soybean (*Glycine max* Merr. L) transposon-based mutagenesis repository. Planta 229:279–289

McCallum CM, Comai L, Greene EA, Henikoff S (2000a) Targeted screening for induced mutations. Nat Biotechnol 18:455–457

McCallum CM, Comai L, Greene EA, Henikoff S (2000b) Targeting induced local lesions in genomes (TILLING) for plant functional genomes. Plant Physiol 123:439–442

Meissner R, Jacobson Y, Melamed S, Levyatuv S, Shalev G, Ashri A, Elkind Y, Levy A (1997) A new model system for tomato genetics. Plant J 12:1465–1472

Meissner R, Chague V, Zhu Q, Emmanuel E, Elkind Y, Levy AA (2000) Technical advance: a high throughput system for transposon tagging and promoter trapping in tomato. Plant J 22:265–274

Menda N, Semel Y, Peled D, Eshed Y, Zamir D (2004) *In silico* screening of a saturated mutation library of tomato. Plant J 38:861–872

Minoia S, Petrozza A, D'Onofrio O, Piron F, Mosca G, Sozio G, Cellini F, Bendahmane A, Carriero F (2010) A new mutant genetic resource for tomato crop improvement by TILLING technology. BMC Res Notes 3:69

Missirian V, Comai L, Filkov V (2011) Statistical mutation calling from sequenced overlapping DNA pools in TILLING experiments. BMC Bioinf 12:287

Morita R, Kusaba M, Iida S, Yamaguchi H, Nishio T, Nishimura M (2009) Molecular characterization of mutations induced by gamma irradiation in rice. Genes Genet Syst 84:361–370

Mustilli AC, Fenzi F, Ciliento R, Alfano F, Bowler C (1999) Phenotype of the tomato *high pigment-2* mutant is caused by a mutation in the tomato homolog of *DEETIOLATED1*. Plant Cell 11:145–157

Okabe Y, Asamizu E, Saito T, Matsukura C, Ariizumi T, Brès C, Rothan C, Mizoguchi T, Ezura H (2011) Tomato TILLING technology: development of a reverse genetics tool for the efficient isolation of mutants from Micro-Tom mutant libraries. Plant Cell Physiol 52:1994–2005

Okabe Y, Asamizu E, Ariizumi T, Shirasawa K, Tabata S, Ezura H (2012) Availability of Micro-Tom mutant library combined with TILLING in molecular breeding of tomato fruit shelf-life. Breed Sci 62:202–208

Okabe Y, Ariizumi T, Ezura H (2013) Updating the Micro-Tom TILLING platform. Breed Sci 63:42–48

Oleykowski CA, Bronson Mullins CR, Godwin AK, Yeung AT (1998) Mutation detection using a novel plant endonuclease. Nucleic Acids Res 26:4597–4602

Pineda B, Giménez-Caminero E, García-Sogo B, Antón MT, Atarés A, Capel J, Lozano R, Angosto T, Moreno V (2010) Genetic and physiological characterization of the arlequin insertional mutant reveals a key regulator of reproductive development in tomato. Plant Cell Physiol 51:435–447

Piron F, Nicolaï M, Minoïa S, Piednoir E, Moretti A, Salgues A, Zamir D, Caranta C, Bendahmane A (2010) An induced mutation in tomato eIF4E leads to immunity to two potyviruses. PLoS One 5:e11313

Pnueli L, Carmel-Goren L, Hareven D, Gutfinger T, Alvarez J, Ganal M, Zamir D, Lifschitz E (1998) The *SELF-PRUNING* gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of *CEN* and *TFL1*. Development 125:1979–1989

Rigola D, van Oeveren J, Janssen A, Bonné A, Schneiders H, van der Poel HJ, van Orsouw NJ, Hogers RC, de Both MT, van Eijk MJ (2009) High-throughput detection of induced mutations and natural variation using KeyPoint technology. PLoS One 4:e4761

Rodríguez-Villalón A, Gas E, Rodríguez-Concepción M (2009) Phytoene synthase activity controls the biosynthesis of carotenoids and the supply of their metabolic precursors in dark-grown *Arabidopsis* seedlings. Plant J 60:424–435

Ronen G, Carmel-Goren L, Zamir D, Hirschberg J (2000) An alternative pathway to beta-carotene formation in plant chromoplasts discovered by map-based cloning of beta and old-gold color mutations in tomato. Proc Natl Acad Sci U S A 97:11102–11107

Saito T, Ariizumi T, Okabe Y, Asamizu E, Hiwasa-Tanase K, Fukuda N, Mizoguchi T, Yamazaki Y, Aoki K, Ezura H (2011) TOMATOMA: a novel tomato mutant database distributing Micro-Tom mutant collections. Plant Cell Physiol 52:283–296

Schaller GE, Bleecker AB (1995) Ethylene-binding sites generated in yeast expressing the *Arabidopsis ETR1* gene. Science 270:1809–18011

Scott JW, Harbaugh BK (1989) MICRO-TOM—a miniature dwarf tomato. Agricultural Experiment Station, Institute of Food and Agricultural Sciences, University of Florida, Circular, 1989, S-370:1–6

Shirasawa K, Isobe S, Hirakawa H, Asamizu E, Fukuoka H, Just D, Rothan C, Sasamoto S, Fujishiro T, Kishida Y et al (2010a) SNP discovery and linkage map construction in cultivated tomato. DNA Res 17:381–391

Shirasawa K, Asamizu E, Fukuoka H, Ohyama A, Sato S, Nakamura Y, Tabata S, Sasamoto S, Wada T, Kishida Y et al (2010b) An interspecific linkage map of SSR and intronic polymorphism markers in tomato. Theor Appl Genet 121:731–739

Shirasawa K, Fukuoka H, Matsunaga H, Kobayashi Y, Kobayashi I, Hirakawa H, Isobe S, Tabata S (2013) Genome-wide association studies using single nucleotide polymorphism markers developed by re-sequencing of the genomes of cultivated tomato. DNA Res 20:593–603

Silletti MF, Petrozza A, Stigliani AL, Giorio G, Cellini F, D'Ambrosio C, Carriero F (2013) An increase of lycopene content in tomato fruit is associated with a novel *Cyc-B* allele isolated through TILLING technology. Mol Breeding 31:665–674

Slade AJ, Fuerstenberg SI, Loeffler D, Steine MN, Facciotti D (2005) A reverse genetic, nontransgenic approach to wheat crop improvement by TILLING. Nat Biotechnol 23:75–81

Sun HJ, Uchii S, Watanabe S, Ezura H (2006) A highly efficient transformation protocol for Micro-Tom, a model cultivar for tomato functional genomics. Plant Cell Physiol 47:426–431

Tadele Z, Mba C, Till BJ (2009) TILLING for mutations in model plants and crops. In: Jain SM, Brar DS (eds) Molecular techniques in crop improvement. Springer, The Netherlands, pp 308–332

Till BJ, Zerr T, Comai L, Henikoff S (2006) A protocol for TILLING and EcoTILLING in plants and animals. Nat Protoc 1:2465–2477

Till BJ, Cooper J, Tai TH, Colowit P, Greene EA, Henikoff S, Comai L (2007) Discovery of chemically induced mutations in rice by TILLING. BMC Plant Biol 7:19

Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635–641

Triques K, Sturbois B, Gallais S, Dalmais M, Chauvin S, Clepet C, Aubourg S, Rameau C, Caboche M, Bendahmane A (2007) Characterization of *Arabidopsis thaliana* mismatch specific endonucleases: application to mutation discovery by TILLING in pea. Plant J 51:1116–1125

Triques K, Piednoir E, Dalmais M, Schmidt J, Le Signor C, Sharkey M, Caboche M, Sturbois B, Bendahmane A (2008) Mutation detection using ENDO1: application to disease diagnostics in humans and TILLING and Eco-TILLING in plants. BMC Mol Biol 9:42

Tsai H, Howell T, Nitcher R, Missirian V, Watson B, Ngo KJ, Lieberman M, Fass J, Uauy C, Tran RK et al (2011) Discovery of rare mutations in populations: TILLING by sequencing. Plant Physiol 156:1257–1268

Tsai H, Missirian V, Ngo KJ, Tran RK, Chan SR, Sundaresan V, Comai L (2013) Production of a high-efficiency TILLING population through polyploidization. Plant Physiol 161:1604–1614

Uauy C, Paraiso F, Colasuonno P, Tran RK, Tsai H, Berardi S, Comai L, Dubcovsky J (2009) A modified TILLING approach to detect induced mutations in tetraploid and hexaploid wheat. BMC Plant Biol 9:115

Vidal A, Abril N, Pueyo C (1995) DNA repair by Ogt alkyltransferase influences EMS mutational specificity. Carcinogenesis 16:817–821

Voxeur A, Gilbert L, Rihouey C, Driouich A, Rothan C, Baldet P, Lerouge P (2011) Silencing of the GDP-D-mannose 3,5-epimerase affects the structure and cross-linking of the pectic polysaccharide rhamnogalacturonan II and plant growth in tomato. J Biol Chem 286:8014–8020

Wang H, Jones B, Li Z, Frasse P, Delalande C, Regad F, Chaabouni S, Latché A, Pech JC, Bouzayen M (2005) The tomato *Aux/IAA* transcription factor *IAA9* is involved in fruit development and leaf morphogenesis. Plant Cell 17:2676–2692

Wang W, Esch JJ, Shiu SH, Agula H, Binder BM, Chang C, Patterson SE, Bleecker AB (2006) Identification of important regions for ethylene binding and signaling in the transmembrane domain of the ETR1 ethylene receptor of *Arabidopsis*. Plant Cell 18:3429–3442

Wang H, Schauer N, Usadel B, Frasse P, Zouine M, Hernould M, Latché A, Pech JC, Fernie AR, Bouzayen M (2009) Regulatory features underlying pollination-dependent and -independent tomato fruit set revealed by transcript and primary metabolite profiling. Plant Cell 21:1428–1452

Chapter 7
Tomato Fruit Set and Its Modification Using Molecular Breeding Techniques

Yoshihito Shinozaki and Kentaro Ezura

7.1 Introduction

Breeding objectives have become more multifaceted over the centuries, but yield performance remains the most important trait for numerous crops. In fruit crops, yield is determined by the number and weight of fruits, and so efficient fruit set is essential for achieving good yield. Fruit set is a developmental process in which ovaries differentiate into fruits, and it is generally stimulated by successful pollination and fertilization, leading to embryo and seed development. Fruit set in tomato (*Solanum lycopersicum*) is very sensitive to environmental conditions and in particular to temperature because pollination is inhibited under excessively low or high temperatures (Iwahori and Takahashi 1963; Charles and Harris 1972). To avoid unfavorable conditions and achieve multiple cropping, greenhouse production is adapted to optimal conditions, but the maintenance of suitable temperatures is costly and consumes large amounts of energy. Greenhouse production also poses another problem for pollination in tomato, since closed greenhouses are barriers for wind- or pollinator-dependent flower vibration, which stimulates pollen diffusion from the anthers and hence self-pollination. Greenhouse growers often use insect pollinators, such as bumblebees, or hormone treatments to facilitate fruit set, but these methods are costly or time-consuming.

Parthenocarpy, or fruit set without pollination and fertilization, is a valuable trait for efficient tomato fruit production, in particular under unfavorable conditions for pollination and fertilization, such as during hot summers and cold winter weathers. Furthermore, parthenocarpic fruits are seedless, which is also generally desirable for both processing and fresh market production. In the processing industry, tomato seeds are usually removed from the paste, and seedless fruits often have longer shelf

Y. Shinozaki (✉) • K. Ezura
Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan
e-mail: shinozaki@gene.tsukuba.ac.jp

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_7

life than seeded fruits because seeds may produce ethylene, a ripening hormone (Varoquaux et al. 2000; Fei et al. 2004; Martinelli et al. 2009).

There is considerable evidence that fruit set is tightly controlled by several plant hormones, such as auxin, gibberellin (GA), and cytokinin (CK) (Gillaspy et al. 1993; Ruan et al. 2012; Ariizumi et al. 2013; McAtee et al. 2013), and application of these phytohormones to unpollinated tomato ovaries can induce parthenocarpic fruit set (Serrani et al. 2007a; Matsuo et al. 2012). In addition, several parthenocarpic tomato varieties have been developed, but most of the genes responsible for the traits remain unknown. The ovaries of major parthenocarpic varieties contain high amounts of GAs, even before pollination, and it has therefore been suggested that their parthenocarpy may be caused by altered hormonal activities (de Jong et al. 2009a). Tomato mutants and transgenic plants exhibiting parthenocarpy have also been developed, based on altered expression of hormone-related genes. Studies with *Arabidopsis thaliana* and tomato have led to model of fruit set mediated by auxin and GA (McAtee et al. 2013), wherein successful pollination and fertilization triggers the synthesis of auxin in the ovule. Auxin is then transported to the pericarp, where it induces de novo GA synthesis and the newly synthesized GA promotes pericarp expansion. In the early stages of tomato fruit development, CKs are also synthesized and they function to stimulate cell division (Matsuo et al. 2012). Application of *N*-(2-chloro-4-pyridyl)-*N*-phenylurea (CPPU), a synthetic CK, to unpollinated tomato ovaries can induce parthenocarpic fruit development, and auxin and GA concentrations in the ovaries will transiently increase after CPPU treatment (Ding et al. 2013). Thus, modification of genes associated with CK biosynthesis and/or signaling pathway may lead to further understanding of the role of CK and the cross talk between CK and other plant hormones in fruit set.

Even though parthenocarpy represents a potentially valuable trait for many fruit crops, parthenocarpic varieties have been widely cultivated for only a few species (e.g., cucumber and banana) because high-quality fruits and a high yield are seldom combined with the parthenocarpic trait (Varoquaux et al. 2000; Pandolfini et al. 2002). This limitation is also true for tomato. In addition, the environment in which the fruits are cultivated has a major influence on parthenocarpy, and so the consistent control of the phenotypes in a commercial setting has proven difficult. These barriers have made it difficult to develop parthenocarpic varieties that stably produce high-quality fruits. Molecular techniques, such as genetic modification and marker-assisted selection (MAS), represent powerful tools for the development of new practical varieties showing parthenocarpy in the form of efficient introduction, selection, and pyramiding of useful genes. Here, we review studies of tomato fruit set and parthenocarpy, focusing particularly on parthenocarpic genes, loci, and variations associated with plant hormones and other components (Table 9.1). We also provide suggestions for genetically improving tomato fruit set using molecular breeding strategies. The induction of fruit set by modification of plant hormone-related genes is described, and we review other genetic modifications leading to fruit formation, such as MADS-box transcription factors, anther ablation, and the

Table 7.1 Genetic modifications, mutations, and variations conferring parthenocarpic fruit set in tomato

Gene/locus symbol	Defined function	Genetic modification/ mutation/variation	Parthenocarpic fruit phenotypes (compared to seeded control fruits)	Vegetative and reproductive phenotypes	References
DefH9-iaaM	Auxin biosynthesis	Expression of *iaaM* under an ovary-specific promoter	Normal size and weight, normal or increased soluble solid, increased beta-carotene content	Normal vegetative organs	Ficcadenti et al. (1999), Rotino et al. (2005)
DefH9-RI-iaaM	Auxin biosynthesis	Moderate expression of *DefH9-iaaM*	Decreased weight, increased fruit number, normal yield, increased beta-carotene content	Normal vegetative organs	Pandolfini et al. (2002)
INO-iaaM	Auxin biosynthesis	Expression of *iaaM* under an ovary-specific promoter	Normal size and weight, increased linoleic acid and succinic acid	–	Martinelli et al. (2009)
TPRP-F1::rolB	Auxin response	Expression of *rolB* under an ovary- and young-fruit-specific promoter	Normal size, shape, and jelly, increased weight and soluble solids, lower yield, decreased ripening time	–	Carmi et al. (2003)
DefH9-rolB	Auxin response	Expression of *rolB* under an ovary- and young-fruit-specific promoter	Increased size, increased soluble solids and several nutrient metabolites	–	Martinelli et al. (2009)
INO-rolB	Auxin response	Expression of *rolB* under an ovary- and young-fruit-specific promoter	Increased size and several nutrient metabolites	–	Martinelli et al. (2009)
AUCSIA	Auxin biosynthesis or transport	RNAi silencing of *AUCSIA1* and *AUCSIA2*	Decreased size and weight	Fused and curled leaves	Molesini et al. (2009)
SlPIN4	Auxin transport	Constitutive silencing (RNAi)	Facultative and obligate parthenocarpy, depending on the lines	Normal vegetative organs	Mounet et al. (2012)

(continued)

Table 7.1 (continued)

Gene/locus symbol	Defined function	Genetic modification/ mutation/variation	Parthenocarpic fruit phenotypes (compared to seeded control fruits)	Vegetative and reproductive phenotypes	References
SlIAA9	Auxin signaling	Constitutive silencing (antisense)	Normal	Reduced leaf complexity	Wang et al. (2005)
		Three loss-of-function mutations induced by EMS treatment or γ-ray irradiation	Different parthenocarpic rates, fruit sizes, and ripening times among lines	Reduced leaf complexity	Saito et al. (2011)
SlARF7	Auxin signaling	Constitutive silencing (RNAi)	Heart shaped and a thicker pericarp, seedlike structure formation	Curled leaves, randomized growth orientation	de Jong et al. (2009b, 2011)
AtARF8	Auxin signaling	Constitutive overexpression of a mutated *A. thaliana ARF8*	Normal size, weight, and shape	–	Goetz et al. (2007)
SlTIR1	Auxin signaling	Constitutive overexpression of the *S. lycopersicum TIR1*	–	Dwarf, reduced leaf complexity, increased leaf size, increased petiole diameter	Ren et al. (2011)
CcGA20ox1	GA synthesis	Constitutive overexpression of the citrus hybrid Carrizo citrange *GA20ox1*	Decreased size, normally filled locular tissue	Taller height, non-serrated leaf, longer style, delayed flowering	García-Hurtado et al. (2012)
SlDELLA	GA response	Constitutive silencing (antisense)	Decreased size, elongated shape, elongated cells and decreased number of cells in pericarp, lower growth of locular tissue	Taller height; elongated flower trusses, style, and stylar hair primordia; blunt growth of stigma	Martí et al. (2007)
		Loss-of-function mutation	Decreased weight, increased fruit number, lower yield, increased soluble solids	Altered branching pattern, longer hypocotyls and internodes, thinner stem, delayed flowering, shorter roots	Bassel et al. (2008), Carrera et al. (2012)

SlTPR1	Ethylene signaling	Constitutive overexpression of the *S. lycopersicum TPR1*	Normal ripening, often fused parthenocarpic fruits	Dwarf, epinasty, pleiotropically altered leaf morphology	Lin et al. (2008)
TM29	Flower development	Constitutive silencing (cosuppression or antisense)	Increased size, delayed ripening, misshapen due to growth of internal tissues	Normal vegetative organs, floral homeotic conversion	Ampomah-Dwamena et al. (2002)
TM5	Flower development	Constitutive silencing (antisense)	Frequent parthenocarpy	Normal vegetative organs, floral homeotic conversion	Pnueli et al. (1994)
TM6	Flower development	Constitutive silencing (RNAi)	Occasional parthenocarpy	Normal vegetative organs, floral homeotic conversion	de Martino et al. (2006)
TAP3	Flower development	AC/DS transposon-inserted mutation	Occasional parthenocarpy	Normal vegetative organs, floral homeotic conversion	de Martino et al. (2006)
		Frameshift mutation (*sl-Pr*) or mutation in promoter region (*sl-LA0269*)	Modified floral organs remaining attached to fruits	Normal vegetative organs, floral homeotic conversion with more (*sl-Pr*) or less (*sl-LA0269*) severity	Quinet et al. (2014)
		Constitutive silencing (antisense)	Carpel-like stamens remaining attached to fruits with different severity correlated with the silencing level	Normal vegetative organs, floral homeotic conversion with different severity correlated to the silencing level	Quinet et al. (2014)
PsEND1::barnase	Cytotoxic ribonuclease	Expression of *barnase* under an anther-specific promoter	Decreased size, increased fruit number, normal yield, increased soluble solids and several nutrient metabolites, lower acidity	Normal vegetative organs, early anther ablation	Medina et al. (2013)
StSy	Resveratrol biosynthesis	Constitutive overexpression of the grape *StSy*	Slightly decreased size, increased soluble antioxidants (ascorbate and glutathione)	Normal vegetative organs	Giovinazzo et al. (2005), Ingrosso et al. (2011)
CHS	Flavonoid biosynthesis	Constitutive silencing (RNAi against *CHS* family genes)	Decreased size, misshapen in several lines, normal sugar and organic acid	Normal vegetative organs	Schijlen et al. (2007)

(continued)

Table 7.1 (continued)

Gene/locus symbol	Defined function	Genetic modification/ mutation/variation	Parthenocarpic fruit phenotypes (compared to seeded control fruits)	Vegetative and reproductive phenotypes	References
pat	Parthenocarpy	Natural variation ("*Soressi*" and "*Montfavet 191*")	Decreased size due to decreased cell enlargement	Aberrant anther and ovule development	Mazzucato et al. (1998)
pat-2	Parthenocarpy	Natural variation ("*Severianin*")	Almost normal size	Different yields and plant vigor depending on genetic background	Philouze et al. (1988), Philouze and Maisonneuve (1978)
pat-3/*pat-4*	Parthenocarpy	Natural variation ("*RP75*/ *59*")	Considerably decreased size	–	Philouze (1989)
pat4.1/ *pat5.1*	Parthenocarpy	Introgression line (IL5-1)	Normal size, lower partheno-carpic rate than *pat4.2*/*pat9.1*	–	Gorguet et al. (2008)
pat4.2/ *pat9.1*	Parthenocarpy	Natural variation (IVT-line 1)	Decreased size, higher parthe-nocarpic rate than *pat4.1*/*pat5.1*	–	Gorguet et al. (2008)

–, not described; *ARF*, *auxin response factor*; *AUCSIA*, *AUXIN CUM SILENCING ACTION*; *CHS*, *chalcone synthase*; *DefH9*, *Deficiens homolog 9*; EMS, ethyl methyl sulfonate; *END1*, *ENDOTHECIUM 1*; GA, gibberellin; *INO*, *INNER NO OUTER*; *PIN*, *PIN-FORMED*; RNAi, RNA interference; *TAP3*, *Tomato APETALLA3*; *TM*, *TOMATO MADS-box*; *StSy*, *stilbene synthase*

flavonoid synthesis pathways. Parthenocarpic variation developed through inter-variety or interspecies crossing is also discussed.

7.2 Genetic Modifications and Mutations Influencing Fruit Set

7.2.1 Modifying the Expression of Genes Related to Auxin Synthesis, Responses, and Transport

The effect of auxin-related compounds on fruit set was first described in the early twentieth century (Gustafson 1936, 1937, 1939), and several natural and synthetic auxins were subsequently shown to induce parthenocarpic fruit set (Ho and Hewitt 1986). For example, application of the synthetic auxin, 4-chlorophenoxyacetic acid (4-CPA), can induce parthenocarpic fruits with comparable size to pollination-induced fruits (Bünger-Kibler and Bangerth 1982). More recently, several reports have described the induction of tomato parthenocarpy by altering the expression of several genes related to auxin metabolism and signaling (de Jong et al. 2009a; Ariizumi et al. 2013). For example, a chimeric gene, *DefH9-iaaM*, was used in early trials to produce transgenic parthenocarpic fruits in Solanaceae. This chimeric gene comprises the following two components: (1) the promoter of *Deficiens homolog 9* (*DefH9*), which is an *Antirrhinum majus* MADS-box gene that is expressed specifically in the ovule, and (2) *iaaM*, derived from *Pseudomonas syringae*, which can induce auxin biosynthesis via synthesis of the indoleacetic acid (IAA) precursor indolacetamide. The expression of *DefH9-iaaM* was detected in the developing buds of the transgenic plants (Ficcadenti et al. 1999), which showed normal vegetative growth and developed fruits from emasculated flowers at a rate similar to that of pollinated flowers from non-transformed control plants. The fresh weights and soluble solid concentrations of the parthenocarpic fruits were unchanged or higher than the seeded fruits of control plants; however, the fruits produced by several transgenic lines were misshapen, likely because of excessive auxin levels (Pandolfini et al. 2002). To solve this problem, Pandolfini et al. (2002) altered the 5′ untranslated leader region (5′ ULR) of *iaaM* (*DefH9-RI-iaaM*), leading to a reduction in the translation efficiency of *iaaM* mRNA and, consequently, reducing the IAA content in flower buds compared with that in buds of the *DefH9-iaaM* transgenic plants. This resulted in optimal parthenocarpy, and when fruit productivity and quality of the *DefH9-RI-iaaM* transgenic plants were then evaluated under open-field conditions (Rotino et al. 2005), no obvious differences in fruit quality were detected, with the exception of a higher β-carotene level. The fresh weight of individual transgenic fruits was lower, but was compensated for by an increased fruit number, resulting in a yield comparable with that of parental non-transgenic lines. Expression of *iaaM* under the promoter of the ovule-specific *INNER NO OUTER* (*INO*) gene, which is expressed in only one cell layer in the

ovule outer integument, has also been shown to induce parthenocarpy (Martinelli et al. 2009), but with fruits showing no significant morphological abnormalities.

Another chimeric gene, comprising *Agrobacterium rhizogenes rolB* fused to the ovary- and young-fruit-specific promoter, *TPRP-F1*, has also been used to generate parthenocarpic fruits (Carmi et al. 2003). Tomato plants transformed with *rolB* showed auxin-responsive phenotypes, although the underlying molecular mechanism is not yet known. The highest expression of *rolB* under the control of the *TPRP-F1* promoter was detected in early developing fruits. The transgenic plants developed seedless fruits with a size and morphology comparable to those of seeded fruits from the parental lines, but fruit yield and several other qualities were different in greenhouse-cultivated plants. Expression of *rolB* under the control of the *DefH9* and *INO* promoters also conferred parthenocarpy, but altered fruit qualities were observed (Martinelli et al. 2009).

AUXIN CUM SILENCING ACTION (*AUCSIA*) was identified as a gene that was repressed in parthenocarpic flower buds of *DefH9-iaaM* and *DefH9-RI-iaaM* transgenic plants (Molesini et al. 2009). The tomato genome has two *AUCSIA* genes (*AUCSIA1* and *AUCSIA2*) that encode small polypeptides. RNA interference (RNAi)-mediated simultaneous suppression of *AUCSIA1* and *AUCSIA2* has been shown to result in parthenocarpy and an approximately 100-fold increase in total IAA content in the buds. The parthenocarpic fruit size and weight were smaller than those of wild-type fertilized fruits. *AUCSIA1* and *AUCSIA2* are highly expressed in flower buds, although these expression levels were substantially reduced after pollination (Molesini et al. 2009). The role of *AUCSIA* during fruit set is still unclear, but it might be involved in either auxin synthesis or transport.

PIN-FORMED (PIN) auxin efflux transporters play important roles in fruit set by controlling polar auxin transport between ovules and nearby tissues. Of the ten *PIN* genes (*SlPIN1*–*SlPIN10*) identified in tomato (Pattison and Catalá 2012), *SlPIN4* has been shown to participate in fruit set (Mounet et al. 2012). *SlPIN4* is predominantly expressed in flower buds and young developing fruits, where the expression level is higher in the placenta than in the locular tissue and pericarp. Specific silencing of *SlPIN4* using an RNAi strategy resulted in parthenocarpy, suggesting a negative role in fruit set (Mounet et al. 2012).

7.2.2 Modifications and Mutations of Genes Associated with Auxin Signal Transduction

Auxin signaling is mediated by both transcription-dependent and -independent pathways, but only a few molecular components underlying the latter pathway have been identified (Mockaitis and Estelle 2008; Hayashi 2012). In the transcription-dependent pathway, protein–protein interactions among several key components lead to auxin responses. At low auxin levels, auxin/IAA (Aux/IAA) transcriptional repressors interact with auxin response factor (ARF) transcription

factors, which repress the transcriptional expression of auxin-responsive genes (Tiwari et al. 2004; Guilfoyle and Hagen 2007). At high auxin levels, auxin promotes the degradation of the Aux/IAA proteins via a ubiquitin–proteasome system (Gray et al. 1999; Dharmasiri and Estelle 2002). Auxin is perceived by the TIR1/AFB family of F-box proteins acting as auxin receptors, which form SCF E3 ubiquitin ligase complexes, leading to the ubiquitination of Aux/IAA (Gray et al. 2001; Kepinski and Leyser 2005; Dharmasiri et al. 2005; Maraschin et al. 2009). Auxin-dependent proteolysis of Aux/IAA leads to the induction of auxin-responsive gene expression via activation of ARF transcription factors.

A total of 26 Aux/IAA repressor family genes (*SlIAA1*–*SlIAA26*) have been found in tomato (Wu et al. 2012). *SlIAA9*, which is highly expressed throughout the plant, has been show to play a regulatory role in fruit development, and antisense lines of *SlIAA9* show a wide range of auxin-related growth alterations, including reduced leaf complexity and the production of parthenocarpic fruit with size, color, and flesh consistency that are similar to those of wild-type fruits (Wang et al. 2005). The accumulation of *SlIAA9* transcripts at anthesis forms a gradient, where the transcript levels are higher in the ovule, sporogenous tissue, placenta, and funiculus but lower in the ovary wall and columella (Wang et al. 2009). Rapid dissipation of the signal gradient occurs approximately one day after pollination, suggesting an important role for *SlIAA9* in the early stages of fertilization-induced fruit set. Three independent mutants of *SlIAA9* (*iaa9-3*, *iaa9-4*, and *iaa9-5*) exhibiting altered vegetative phenotypes and parthenocarpy have been identified in ethyl methyl sulfonate (EMS)-mutagenized or γ-ray-irradiated populations (Saito et al. 2011). The rates of parthenocarpy and seedless fruit expansion vary among the mutants, suggesting that the functional activity and extent to which parthenocarpy is conferred vary for the different *SlIAA9* alleles.

The tomato ARF family comprises at least 17 members (*SlARF1*–*SlARF17*; Kumar et al. 2011). *SlARF7* is predominantly expressed in unpollinated tomato ovaries and its expression rapidly decreases after pollination (de Jong et al. 2009b). RNAi transgenic tomato lines with reduced *SlARF7* mRNA levels produce parthenocarpic fruits, suggesting that this ARF gene acts as a negative regulator of fruit set. Furthermore, the parthenocarpic fruits showed GA-related phenotypes, such as a thick pericarp due to extensive cell expansion, in addition to auxin-related phenotypes, specifically a heart-shaped fruit and the formation of seedlike structures resembling pseudoembryos. These findings suggest that SlARF7 could be involved in the cross talk between auxin and GA during fruit set, and one model suggests that SlARF7 activates auxin response-attenuating genes (such as *Aux/IAA*s) in unpollinated ovaries, while downregulation of *SlARF7* after pollination results in an activation of both auxin and GA signaling that is required for fruit set (de Jong et al. 2011).

In *A. thaliana*, *atarf8* mutants produce parthenocarpic fruits (siliques) without fertilization, suggesting that *AtARF8* is a negative regulator of fruit set (Goetz et al. 2006). Goetz et al. (2007) further showed that the introduction of aberrant forms of *AtARF8* led to parthenocarpy in *A. thaliana* and tomato. Since the expression of *AtARF8* was not reduced in the transgenic plants, the mutated form

of the AtARF8 protein may have functionally competed with endogenous AtARF8 protein and its tomato homolog. A model was proposed by the authors in which ARF8 forms a regulatory complex with Aux/IAA, and this complex directly or indirectly represses transcription of fruit set-regulating genes (Goetz et al. 2006, 2007).

A putative tomato auxin receptor, SlTIR1, plays an important role in the early stage of fruit set (Ren et al. 2011). *SlTIR1* is highly expressed in the ovary and sepal at anthesis, but its expression decreases after pollination. Overexpression of *SlTIR1* results in an auxin-responsive phenotype, including altered vegetative morphology, sterility, and parthenocarpy, and SlTIR1 has been suggested to positively regulate the auxin response via the 26S proteasome-mediated signaling pathway (Ren et al. 2011).

7.2.3 Altered Expression and Mutations of GA-Related Genes

The regulatory effect of GAs on fruit development has been well documented (Wittwer et al. 1957; Sastry and Muir 1963; Serrani et al. 2007a). Endogenous GAs in plants are synthesized in two parallel pathways, the non-13-hydroxylation and early 13-hydroxylation pathways, and in tomato fruit set, the early 13-hydroxylation pathway appears to predominate (Bohner et al. 1988; Fos et al. 2000). Expression of genes in the tomato GA20ox family (*SlGA20ox1*–*SlGA20ox3*) that mediate bioactive GA synthesis increases in the ovaries after pollination, suggesting a central role for *GA20ox* genes in GA synthesis during fruit set (Serrani et al. 2007b). Additionally, overexpression of a citrus *GA20ox* gene (*CcGA20ox1*) in tomato resulted in pleiotropic phenotypes similar to those of GA-treated plants, including parthenocarpy (García-Hurtado et al. 2012).

DELLA family proteins act as key repressors of GA signaling. GA induces the degradation of DELLA proteins via the ubiquitin–proteasome system, leading to GA responses (Dill et al. 2001; Mcginnis et al. 2003; Sun 2010). Tomato has a single DELLA gene (*SlDELLA*), and antisense-mediated silencing of this gene results in constitutive GA-responsive phenotypes, such as elongated plant shape and parthenocarpy (Martí et al. 2007). In addition to being smaller and elongated, the parthenocarpic fruit had a reduced number of cells, which were elongated in the pericarp. These features are similar to those seen in GA-induced fruits (Serrani et al. 2007a), suggesting that parthenocarpic fruit development in *SlDELLA* antisense bypasses auxin-regulated cell division (Martí et al. 2007). A loss-of-function mutant of *SlDELLA*, *procera* (*pro*), also exhibits a constitutive GA-responsive phenotype, including parthenocarpy (Bassel et al. 2008). The *pro* mutation influences auxin signaling with a reduction of *SlARF7* expression during fruit set (Carrera et al. 2012), suggesting a role of SlARF7 in the cross talk between GA and auxin signaling during fruit set.

7.2.4 Modification of the Expression of Ethylene-Related Genes

Ethylene plays a critical role in many developmental processes, such as senescence and abscission of leaves (Lim et al. 2007) and flowers (van Doorn and Woltering 2008), and fruit ripening (Barry and Giovannoni 2007). Llop-Tous et al. (2000) showed that pollination induces transient increases in the production of ethylene in tomato pistils for several hours, although this apparently does not induce ovary senescence (Vriezen et al. 2008) and ethylene production decreases after 12 h of pollination (Llop-Tous et al. 2000). The expression of various genes related to the biosynthesis and signaling of ethylene has been observed to change during the early development of both pollinated and parthenocarpic tomato fruits (Vriezen et al. 2008; Pascual et al. 2009; Wang et al. 2009), all suggesting that ethylene also plays a regulatory role in tomato fruit set; however, further studies are required to elucidate the exact mechanisms.

Overexpression of a tomato signaling component of ethylene (*S. lycopersicum TETRATRICOPEPTIDE REPEAT PROTEIN 1, SlTPR1*) that interacts with the ethylene receptors NEVER RIPE (NR) and *S. lycopersicum* ETHYLENE RECEPTOR 1 (SlETR1/LeETR1) results in ethylene-related pleiotropic effects and parthenocarpic fruit set (Lin et al. 2008). The upregulation of an auxin-responsive gene in the buds of SlTPR1-overexpressing plants suggests that SlTPR1 is directly or indirectly involved in auxin signaling, while downregulation of *SlIAA9* in the ovaries of *SlTPR1* transgenic plants may contribute to parthenocarpic fruit set. Further studies are required to clarify the role of SlTPR in ethylene signaling and fruit set.

The application of ethylene biosynthesis or action inhibitors has been found to induce parthenocarpy in zucchini (*Cucurbita pepo*), indicating that ethylene negatively regulates its fruit set (Martínez et al. 2013). In *A. thaliana*, ethylene is involved in ovule senescence and negatively regulates parthenocarpic fruit set induced by GA (Carbonell-Bejerano et al. 2011). Recently, either the treatment of 1-methylcyclopropene (1-MCP), an ethylene action inhibitor, or ethylene-insensitive *Sletr1* mutation was found to induce parthenocarpic fruit set from emasculated flowers, most likely due to the accumulation of bioactive GAs (Shinozaki et al. 2015). The efficiency of the pollination-independent fruit set induced by *Sletr1* mutation was different depending on the genetic background, and unidentified locus/loci in a dwarf cultivar Micro-Tom, other than *dwarf*, can enhance the parthenocarpic efficiency. Ethylene may play a role in tomato fruit set by suppressing GA metabolism before pollination, while the molecular mechanism of the suppression has not yet been uncovered.

7.2.5 Altered Expression and Mutations of MADS-Box Transcription Factor Genes

MADS-box proteins are multifunctional transcription factors found in a wide range of eukaryotic organisms, and plant MADS-box proteins function in the regulation of organ and cell differentiation in flower development (Theissen and Saedler 2001). Tomato has at least 36 MADS-box genes (Hileman et al. 2006), and several studies have indicated a relationship between tomato parthenocarpic fruit development and MADS-box proteins. In *A. thaliana*, three MADS-box genes *SEPALLATA1–SEPALLATA3* (*SEP1–SEP3*) are required for normal floral organ development (Pelaz et al. 2000). Studies with tomato have shown that *Tomato MADS-box 29* (*TM29*), a homolog of *A. thaliana SEP1*, is continuously expressed in developing flowers and preferentially in the peripheral region of well-differentiated ovaries and fruits (Ampomah-Dwamena et al. 2002). Transgenic tomato plants constitutively expressing an antisense construct of *TM29* produce morphologically altered flowers and parthenocarpic fruits, suggesting that TM29 not only is required for normal flower development but may also function as a negative regulator of fruit set. In apple (*Malus pumila*), antisense suppression of *MADS8* and *MADS9* (homologs of *SEP1* and *SEP2*, respectively) resulted in an increased expression of auxin biosynthetic genes, a reduced expression of the GH3 auxin-conjugating enzyme genes, and a high accumulation of free auxin in the fruits during the early ripening stage (Schaffer et al. 2013). Although auxin concentration in the *TM29*-suppressed ovaries had not been measured, production of parthenocarpic fruits by silencing of these *SEP* family genes might be a consequence of the elevated auxin concentration.

TM5, a tomato ortholog of *A. thaliana SEP3*, is known to function in both flower and fruit development (Pnueli et al. 1994). *TM5* is continuously expressed in the central apical zone of the floral meristem throughout differentiation in the tissues of petals, stamens, and pistils. Antisense suppression of *TM5* expression resulted in parthenocarpy and altered identities of floral organs, manifested by sepaloid green petals and abnormal sterile anthers, but with no obvious change in vegetative organs (Pnueli et al. 1994). In addition, parthenocarpic fruit development resulted from a mutation in, or silencing of, the duplicated MADS-box genes, *Tomato APETALLA3* (*TAP3*)/*SlDEF* and *TM6* (de Martino et al. 2006). These genes belong to the *AP3* group, a subfamily of class B MADS-box genes that are required for specification of petals and stamens. *TAP3* is expressed predominantly in developing petals and stamen primordia until the late stage of flower differentiation, when the expression is restricted to several floral tissues. A *tap3* null mutant was shown to have sepaloid petals and carpel-like anthers, and occasionally exhibited parthenocarpy. Recently, two *stamenless* (*sl*) mutants (*slPr* and *sl-LA0269*), which exhibit floral homeotic conversion to different degrees, were found to develop parthenocarpic fruits, and their phenotypes most likely resulted from mutation(s) in the coding sequence and promoter region, respectively, of *TAP3* (Quinet et al. 2014). *TAP3*-antisense plants exhibited similar floral homeotic conversion to *tap3*

mutants, developing parthenocarpic fruits to which carpel-like stamens remained attached, and the severity was found to correlate with the extent of gene silencing. Similarly, *TM6* is weakly but constitutively expressed in the primordia of petals, stamens, and carpels (de Martino et al. 2006), and *TM6*-RNAi transgenic plants are defective in stamen development and exhibit occasional parthenocarpy.

Even with efficient parthenocarpy and normal vegetative development, the floral reversion-like phenotype in the expression of downregulated or loss-of-function mutants of MADS-box genes described above leads to sterility, which limits their use for breeding. More spatially and temporally specific and/or more finely tuned regulation may be needed to develop practical parthenocarpic varieties using these genes. Both parthenocarpy and anther ablation were observed in the mutants and MADS-box gene transgenic plants described above. In the following section, we provide another example of a transgenic tomato line that supports the idea of a relationship between parthenocarpy and anther ablation.

7.2.6 Early Anther Ablation

It has been suggested that the stamens of *A. thaliana* play a regulatory role in preventing initiation of fruit in the absence of fertilization (Vivian-Smith et al. 2001). Parthenocarpy with male sterility has been reported in tomato *parthenocarpic fruit* (*pat*), a mutant derived from natural parthenocarpic variation (Mazzucato et al. 1998), implying that dysfunction of male organ, stamen, is involved in the parthenocarpic fruit development. Roque et al. (2007) showed that the induction of a *Bacillus amyloliquefaciens* ribonuclease gene (*barnase*) under the control of an anther-specific promoter from the *Pisum sativum ENDOTHECIUM 1* (*PsEND1*) gene of pea resulted in specific ablation of the anther at early stages of the development and male sterility in *A. thaliana*, tobacco, oilseed rape, and tomato. Interestingly, tomato plants transformed with the *PsEND1::barnase* construct showed not only early anther ablation but also highly efficient parthenocarpy, while the other species completely impaired fruit development (Roque et al. 2007; Medina et al. 2013). The fruit productivity of the transgenic tomato plants was not significantly changed compared to wild-type plants, since although several transgenic lines produced smaller fruits, a significant increase in the number of fruits per plants was observed in all of the transgenic lines. Furthermore, *PsEND1::barnase* tomato plants produced high-quality fruits with respect to nutritional components such as γ-aminobutyric acid (GABA), glutamic acid, neoxanthin, and tocopherols. This approach in tomato crop production is attractive because it confers effective parthenocarpy without adverse effects on vegetative tissues. Further studies are required to understand the mechanisms underlying the link between early anther ablation accompanied by male sterility and parthenocarpic fruit development.

7.2.7 Modifications of Flavonoid Synthesis-Related Pathways

Genetic engineering of the flavonoid biosynthesis pathway represents yet another method to obtain parthenocarpic fruit. RNAi-mediated suppression of *chalcone synthase* (*CHS*), which encodes an important enzyme in flavonoid biosynthesis, has been reported to reduce total flavonoid levels and induce parthenocarpy (Schijlen et al. 2007). Transgenic plants showed normal vegetative growth, but their fruits were seedless and smaller than those of the seeded control fruits. Flavonoids play an essential role in reproductive processes such as pollen development and pollen tube growth. The parthenocarpic fruit development in the *CHS* RNAi plants appeared to be pollination associated, as pollen tube growth was impaired, and so fertilization was prevented. These findings suggest that pollination is required and sufficient to trigger fruit set and that fertilization leads to subsequent normal fruit development and expansion.

Seedless fruits with reduced flavonoid levels were also generated by introduction of the grape (*Vitis vinifera*) *stilbene synthase* (*StSy*), a key enzyme in the synthesis of the antioxidant resveratrol (Giovinazzo et al. 2005; Ingrosso et al. 2011). It was suggested that the altered flavonoid metabolism in *StSy*-transformed plants was caused by the competition between the biosynthetic pathways of resveratrol and chalcone and the parthenocarpic fruits contained high concentrations of soluble antioxidants, ascorbate and glutathione (Giovinazzo et al. 2005). Flowers of *StSy*-transformed plants displayed an open anther structure and were disturbed in pollen development, resulting in reduced seed set. The possibility that male sterility in the transgenic plants may be associated with its parthenocarpy was also suggested (Ingrosso et al. 2011; Medina et al. 2013).

7.3 Natural Variants and Introgression Lines Associated with Parthenocarpy

Several parthenocarpic variants have been developed for efficient fruit production, even under unfavorable conditions (Ho and Hewitt 1986), and three major natural variants for facultative parthenocarpy, *pat*, *pat-2*, and *pat-3*/*pat-4,* have been reported. In addition, two different lines exhibiting parthenocarpy have been described and used for quantitative trait locus (QTL) analysis (Gorguet et al. 2008). Identification of the associated genes will provide not only completely linked (so-called perfect) markers for parthenocarpy but also the identification of important pathways that confer parthenocarpy.

The *pat* mutant, harboring a recessive mutation in a single gene associated with parthenocarpy, was independently found in the tomato cultivars "*Soressi*" and "*Montfavet 191*." The *pat* mutation results in several growth defects, including abnormal anthers, female gametes with lower viability and fewer seeds, and

reduced fruit size and weight (Mazzucato et al. 1998). Altered development of stamens and parthenocarpy in the *pat* mutants suggests homeotic functions of *PAT*; however, while the *pat* locus has been mapped to the long arm of chromosome 3 (Beraldi et al. 2004), the candidate gene has not yet been identified.

The tomato cultivar "*Severianin*" exhibits strong parthenocarpy and its fruits reach almost normal size even under unfavorable conditions (Philouze and Maisonneuve 1978). "*Severianin*" was developed from crosses between several cultivars of tomato (*S. lycopersicum*) and the wild tomato species *S. habrochaites*. A genetic analysis suggested that the resulting parthenocarpy is controlled by two recessive loci, with major (*pat-2*) and minor (*mp*) effects (Vardy et al. 1989). Difference in yield and vigor has been observed in plants in which *pat-2* was introduced, depending on the genetic background (Gorguet et al. 2008). Another strong parthenocarpic cultivar, "*PR75/59*," was developed from a cross between the two weaker parthenocarpic cultivars, "*Atom*" and "*Bubjekosoko*," and so two loci are thought to be responsible for the parthenocarpic phenotype, which have been designated *pat-3* and *pat-4* (Philouze 1989). However, the considerable variation in fruit size in *pat-3*/*pat-4* makes this a less attractive cultivar for commercial production and hence for breeding.

The high accumulation of GAs in *pat*, *pat-2*, and *pat-3*/*pat-4* ovaries may be associated with their parthenocarpic fruit set (Fos et al. 2000, 2001; Olimpieri et al. 2007). Olimpieri et al. (2007) showed that *GA20ox1* is constitutively expressed in the ovaries of the *pat* mutant, while *GA20ox3* has been shown by others to be highly expressed in *pat-3*/*pat-4* ovaries (Pascual et al. 2009). In addition, the early growth of parthenocarpic *pat-2* fruits is associated with polyamine metabolism. Polyamines are required for the parthenocarpy in *pat-2*, and that treatment of wild-type unpollinated tomato ovaries with exogenous polyamines induces partial parthenocarpy, proposing a model that the elevated GA content in the *pat-2* ovaries induces polyamine synthesis (Fos et al. 2003).

In a QTL analysis using two tomato introgression lines (IL5-1 and IVT-line 1) carrying *S. habrochaites* chromosome segments and exhibiting highly stable parthenocarpy (Gorguet et al. 2008), four QTLs associated with parthenocarpy on chromosomes 4 (*pat4.1*, *pat4.2*), 5 (*pat5.1*), and 9 (*pat9.1*) were found. The two QTLs, *pat4.1* from IL5-1 and *pat4.2* from IVT-line 1, are allelic and closely linked to *SlARF8*, indicating that this gene is potentially responsible for parthenocarpy in these ILs, providing new target loci for breeding.

7.4 Perspectives

Parthenocarpic tomato fruits can be artificially produced using chemicals or via genetic modification. Although parthenocarpic varieties may contribute to efficient fruit production, as well as reductions in labor and cost for many crops, they have not yet been widely introduced in tomato cultivation, mainly because of a lack of genetic resources. The parthenocarpic plants described above provide possible resources for the development of commercially viable tomato varieties; however,

several parthenocarpic genotypes exhibit undesirable traits, such as small-sized fruit and morphological defects. Techniques targeting spatial and temporal regulation of gene expression at adequate levels, for instance, using promoters that function specifically in ovarian tissues such as ovule, pericarp, and placenta during early stages of fruit development, may be helpful in eliminating such undesirable traits. In addition, the examples of varied phenotypes among mutant alleles of *SlIAA9* (Saito et al. 2011) and *TAP3* (Quinet et al. 2014) indicate that screening or generating various mutant alleles of genes associated with fruit set, via techniques such as targeting-induced local lesions in genomes (TILLING) (Gady et al. 2009; Okabe et al. 2011) and genome editing (Osakabe and Osakabe 2015), may contribute to increasing genetic variation and obtaining better breeding resources for parthenocarpic cultivars. Until now, only a few parthenocarpic genotypes have been evaluated for productivity performance, such as fruit quality, vegetative phenotypes, and yield, in commercial field environments that include exposure to low and high temperatures. To identify appropriate breeding resources, further practical trait evaluation is necessary. Parthenocarpic *pro* plants often show severe fruit malformation under greenhouse conditions (Carrera et al. 2012), but we have found that a different mutated allele of *DELLA* results in fewer defects in morphology of the parthenocarpic fruits under both moderate- and high-temperature condition compared with the *pro* mutant (unpublished data). This illustrates the necessity and benefit of trait evaluation in practice. Genes and loci with potential for improving tomato fruit set are now emerging (Table 9.1), but genetic resources and understanding of the fruit set mechanisms are still incomplete. Although molecular pathways and genes that are required for fruit set, including those downstream of hormone signaling, are poorly understood, continuing advances in genetic, genomic, and molecular tools and associated bioinformatic platforms are likely to accelerate their discovery.

References

Ampomah-Dwamena C, Morris BA, Sutherland P, Veit B, Yao JL (2002) Down-regulation of *TM29*, a tomato *SEPALLATA* homolog, causes parthenocarpic fruit development and floral reversion. Plant Physiol 130:605–617

Ariizumi T, Shinozaki Y, Ezura H (2013) Genes that influence yield in tomato. Breed Sci 63:3–13

Barry CS, Giovannoni JJ (2007) Ethylene and fruit ripening. J Plant Growth Regul 26:143–159

Bassel GW, Mullen RT, Bewley JD (2008) *procera* is a putative DELLA mutant in tomato (*Solanum lycopersicum*): effects on the seed and vegetative plant. J Exp Bot 59:585–593

Beraldi D, Picarella ME, Soressi GP, Mazzucato A (2004) Fine mapping of the *parthenocarpic fruit* (*pat*) mutation in tomato. Theor Appl Genet 108:209–216

Bohner J, Hedden P, Bora-Haber E, Bangerth F (1988) Identification and quantitation of gibberellins in fruits of *Lycopersicon esculentum*, and their relationship to fruit size in *L. esculentum* and *L. pimpinellfolium*. Physiol Plant 73:348–353

Bünger-Kibler S, Bangerth F (1982) Relationship between cell number, cell size and fruit size of seeded fruits of tomato (*Lycopersicon esculentum* Mill.), and those induced parthenocarpically by the application of plant growth regulators. Plant Growth Regul 154:143–154

Carbonell-Bejerano P, Urbez C, Granell A, Carbonell J, Perez-Amador MA (2011) Ethylene is involved in pistil fate by modulating the onset of ovule senescence and the GA-mediated fruit set in *Arabidopsis*. BMC Plant Biol 11:84

Carmi N, Salts Y, Dedicova B, Shabtai S, Barg R (2003) Induction of parthenocarpy in tomato via specific expression of the *rolB* gene in the ovary. Planta 217:726–735

Carrera E, Ruiz-Rivero O, Peres LEP, Atares A, García-Martínez JL (2012) Characterization of the *procera* tomato mutant shows novel functions of SlDELLA protein in the control of flower morphology, cell division and expansion and auxin-signaling pathway during fruit-set and development. Plant Physiol 160:1581–1596

Charles WB, Harris RE (1972) Tomato fruit-set at high and low temperatures. Can J Plant Sci 506: 497–506

de Jong M, Mariani C, Vriezen WH (2009a) The role of auxin and gibberellin in tomato fruit set. J Exp Bot 60:1523–1532

de Jong M, Wolters-Arts M, Feron R, Mariani C, Vriezen WH (2009b) The *Solanum lycopersicum* auxin response factor 7 (*Sl*ARF7) regulates auxin signaling during tomato fruit set and development. Plant J 57:160–170

de Jong M, Wolters-Arts M, García-Martínez JL, Mariani C, Vriezen WH (2011) The *Solanum lycopersicum* AUXIN RESPONSE FACTOR 7 (SlARF7) mediates cross-talk between auxin and gibberellin signalling during tomato fruit set and development. J Exp Bot 62:617–626

de Martino G, Pan I, Emmanuel E, Levy A, Irish VF (2006) Functional analyses of two tomato *APETALA3* genes demonstrate diversification in their roles in regulating floral development. Plant Cell 18:1833–1845

Dharmasiri S, Estelle M (2002) The role of regulated protein degradation in auxin response. Plant Mol Biol 49:401–409

Dharmasiri N, Dharmasiri S, Weijers D, Lechner E, Yamada M, Hobbie L, Ehrismann JS, Jürgens G, Estelle M (2005) Plant development is regulated by a family of auxin receptor F box proteins. Dev Cell 9:109–119

Dill A, Jung HS, Sun T (2001) The DELLA motif is essential for gibberellin-induced degradation of RGA. Proc Natl Acad Sci USA 98:14162–14167

Ding J, Chen B, Xia X, Mao W, Shi K, Zhou Y, Yu J (2013) Cytokinin-induced parthenocarpic fruit development in tomato is partly dependent on enhanced gibberellin and auxin biosynthesis. PLoS One 8, e70080

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

Ficcadenti N, Sestili S, Pandolfini T, Cirillo C, Rotino GL, Spena A (1999) Genetic engineering of parthenocarpic fruit development in tomato. Mol Breed 5:463–470

Fos M, Nuez F, García-Martínez JL (2000) The gene *pat-2*, which induces natural parthenocarpy, alters the gibberellin content in unpollinated tomato ovaries. Plant Physiol 122:471–480

Fos M, Proaño K, Nuez F, García-Martínez JL (2001) Role of gibberellins in parthenocarpic fruit development induced by the genetic system *pat-3/pat-4* in tomato. Physiol Plant 111:545–550

Fos M, Proan K, Alabadı D, Nuez F, Carbonell J, García-Martínez JL (2003) Polyamine metabolism is altered in unpollinated parthenocarpic *pat-2* tomato ovaries. Plant Physiol 131: 359–366

Gady AL, Hermans FW, Van de Wal MH, van Loo EN, Visser RG, Bachem CW (2009) Implementation of two high through-put techniques in a novel application: detecting point mutations in large EMS mutated plant populations. Plant Methods 5:13

García-Hurtado N, Carrera E, Ruiz-Rivero O, López-Gresa MP, Hedden P, Gong F, García-Martínez JL (2012) The characterization of transgenic tomato overexpressing *gibberellin 20-oxidase* reveals induction of parthenocarpic fruit growth, higher yield, and alteration of the gibberellin biosynthetic pathway. J Exp Bot 63:5803–5813

Gillaspy G, Ben-David H, Gruissem W (1993) Fruits: a developmental perspective. Plant Cell 5: 1439–1451

Giovinazzo G, D'Amico L, Paradiso A, Bollini R, Sparvoli F, DeGara L (2005) Antioxidant metabolite profiles in tomato fruit constitutively expressing the grapevine stilbene synthase gene. Plant Biotechnol J 3:57–69

Goetz M, Vivian-smith A, Johnson SD, Koltunow AM (2006) *AUXIN RESPONSE FACTOR8* is a negative regulator of fruit initiation in *Arabidopsis*. Plant Cell 18:1873–1886

Goetz M, Hooper LC, Johnson SD, Rodrigues JCM, Vivian-Smith A, Koltunow AM (2007) Expression of aberrant forms of *AUXIN RESPONSE FACTOR8* stimulates parthenocarpy in Arabidopsis and tomato. Plant Physiol 145:351–366

Gorguet B, Eggink PM, Ocaña J, Tiwari A, Schipper D, Finkers R, Visser RGF, van Heusden AW (2008) Mapping and characterization of novel parthenocarpy QTLs in tomato. Theor Appl Genet 116:755–767

Gray WM, Pozo JC, Walker L, Hobbie L, Risseeuw E, Banks T, Crosby WL, Yang M, Ma H, Estelle M (1999) Identification of an SCF ubiquitin–ligase complex required for auxin response in *Arabidopsis thaliana*. Genes Dev 53:1678–1691

Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M (2001) Auxin regulates SCF TIR1-dependent degradation of AUX/IAA proteins. Nature 414:271–276

Guilfoyle TJ, Hagen G (2007) Auxin response factors. Curr Opin Plant Biol 10:453–460

Gustafson FG (1936) Inducement of fruit development by growth-promoting chemicals. Proc Natl Acad Sci USA 22:628–636

Gustafson FG (1937) Parthenocarpy induced by pollen extracts. Am J Bot 24:102–107

Gustafson FG (1939) The cause of natural parthenocarpy. Am J Bot 26:135–138

Hayashi K (2012) The interaction and integration of auxin signaling components. Plant Cell Physiol 53:965–975

Hileman LC, Sundstrom JF, Litt A, Chen M, Shumba T, Irish VF (2006) Molecular and phylogenetic analyses of the MADS-box gene family in tomato. Mol Biol Evol 23:2245–2258

Ho LC, Hewitt JD (1986) Fruit development. In: Atherton JG, Rudich J (eds) The tomato crop. Chapman and Hall, New York, pp 201–239

Ingrosso I, Bonsegna S, De Domenico S, Laddomada B, Blando F, Santino A, Giovinazzo G (2011) Over-expression of a grape stilbene synthase gene in tomato induces parthenocarpy and causes abnormal pollen development. Plant Physiol Biochem 49:1092–1099

Iwahori S, Takahashi K (1963) High temperature injuries in tomato. II. Effect of duration of high temperature on fruit setting and yield. J Jpn Soc Hortic Sci 33:67–74

Kepinski S, Leyser O (2005) The *Arabidopsis* F-box protein TIR1 is an auxin receptor. Nature 435:446–451

Kumar R, Tyagi AK, Sharma AK (2011) Genome-wide analysis of auxin response factor (ARF) gene family from tomato and analysis of their role in flower and fruit development. Mol Genet Genomics 285:245–260

Lim PO, Kim HJ, Nam HG (2007) Leaf senescence. Annu Rev Plant Biol 58:115–136

Lin Z, Arciga-Reyes L, Zhong S, Alexander L, Hackett R, Wilson I, Grierson D (2008) SlTPR1, a tomato tetratricopeptide repeat protein, interacts with the ethylene receptors NR and LeETR1, modulating ethylene and auxin responses and development. J Exp Bot 59:4271–4287

Llop-Tous I, Barry C, Grierson D (2000) Regulation of ethylene biosynthesis in response to pollination in tomato flowers. Plant Physiol 123:971–978

Maraschin FS, Memelink J, Offringa R (2009) Auxin-induced, SCF(TIR1)-mediated polyubiquitination marks AUX/IAA proteins for degradation. Plant J 59:100–109

Martí C, Orzáez D, Ellul P, Moreno V, Carbonell J, Granell A (2007) Silencing of *DELLA* induces facultative parthenocarpy in tomato fruits. Plant J 52:865–876

Martinelli F, Uratsu SL, Reagan RL, Chen Y, Tricoli D, Fiehn O, Rocke DM, Gasser CS, Dandekar AM (2009) Gene regulation in parthenocarpic tomato fruit. J Exp Bot 60:3873–3890

Martínez C, Manzano S, Megías Z, Garrido D, Picó B, Jamilena M (2013) Involvement of ethylene biosynthesis and signalling in fruit set and early fruit development in zucchini squash (*Cucurbita pepo* L.). BMC Plant Biol 13:139

Matsuo S, Kikuchi K, Fukuda M, Honda I, Imanishi S (2012) Roles and regulation of cytokinins in tomato fruit development. J Exp Bot 63:5569–5579

Mazzucato A, Taddei AR, Soressi GP (1998) The *parthenocarpic fruit* (*pat*) mutant of tomato (*Lycopersicon esculentum* Mill.) sets seedless fruits and has aberrant anther and ovule development. Development 125:107–114

McAtee P, Karim S, Schaffer R, David K (2013) A dynamic interplay between phytohormones is required for fruit development, maturation, and ripening. Front Plant Sci 4:79

Mcginnis KM, Thomas SG, Soule JD, Strader LC, Zale JM, Sun T, Steber CM (2003) The Arabidopsis *SLEEPY1* gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell 15:1120–1130

Medina M, Roque E, Pineda B, Cañas L, Rodriguez-Concepción M, Beltrán JP, Gómez-Mena C (2013) Early anther ablation triggers parthenocarpic fruit development in tomato. Plant Biotechnol J 11:770–779

Mockaitis K, Estelle M (2008) Auxin receptors and plant development: a new signaling paradigm. Annu Rev Cell Dev Biol 24:55–80

Molesini B, Pandolfini T, Rotino GL, Dani V, Spena A (2009) *Aucsia* gene silencing causes parthenocarpic fruit development in tomato. Plant Physiol 149:534–548

Mounet F, Moing A, Kowalczyk M, Rohrmann J, Petit J, Garcia V, Maucourt M, Yano K, Deborde C, Aoki K et al (2012) Down-regulation of a single auxin efflux transport protein in tomato induces precocious fruit development. J Exp Bot 63:4901–4917

Okabe Y, Asamizu E, Saito T, Matsukura C, Ariizumi T, Brès C, Rothan C, Mizoguchi T, Ezura H (2011) Tomato TILLING technology: development of a reverse genetics tool for the efficient isolation of mutants from Micro-Tom mutant libraries. Plant Cell Physiol 52:1994–2005

Olimpieri I, Siligato F, Caccia R, Mariotti L, Ceccarelli N, Soressi GP, Mazzucato A (2007) Tomato fruit set driven by pollination or by the *parthenocarpic fruit* allele are mediated by transcriptionally regulated gibberellin biosynthesis. Planta 226:877–888

Osakabe Y, Osakabe K (2015) Genome editing in higher plants. In: Yamamoto T (ed) Targeted genome editing using site-specific nucleases. Springer, Tokyo, pp 197–205

Pandolfini T, Rotino G, Camerini S, Defez R, Spena A (2002) Optimisation of transgene action at the post-transcriptional level: high quality parthenocarpic fruits in industrial tomatoes. BMC Biotechnol 2:1

Pascual L, Blanca JM, Cañizares J, Nuez F (2009) Transcriptomic analysis of tomato carpel development reveals alterations in ethylene and gibberellin synthesis during *pat3*/*pat4* parthenocarpic fruit set. BMC Plant Biol 9:67

Pattison RJ, Catalá C (2012) Evaluating auxin distribution in tomato (*Solanum lycopersicum*) through an analysis of the *PIN* and *AUX*/*LAX* gene families. Plant J 70:585–598

Pelaz S, Ditta GS, Baumann E, Wisman E, Yanofsky MF (2000) B and C floral organ identity functions require *SEPALLATA* MADS-box genes. Nature 405:200–203

Philouze J (1989) Natural parthenocarpy in tomato. IV. A study of the polygenic control of parthenocarpy in line 75/59. Agronomie 9:63–75 (in French with English abstract)

Philouze J, Maisonneuve B (1978) Heredity of the natural ability to set parthenocarpic fruits in the Soviet variety Severianin. Tomato Genet Coop Rep 28:12–13

Philouze J, Buret M, Duprat F, Nicolas-Grotte M, Nicolas J (1988) Caractéristiques agronomiques et physico-chimiques de lignées de tomate isogéniques, sauf pour le gène *pat-2* de parthénocarpie, dans trois types variétaux, en culture de printemps, sous serre plastique trés peu chauffée. Agronomie 8:817–828 (in French with English abstract)

Pnueli L, Hareven D, Broday L, Hurwitz C, Lifschitz E (1994) The TM5 MADS Box gene mediates organ differentiation in the three inner whorls of tomato flowers. Plant Cell 6: 175–186

Quinet M, Bataille G, Dobrev PI, Capel C, Gómez P, Capel J, Lutts S, Motyka V, Angosto T, Lozano R (2014) Transcriptional and hormonal regulation of petal and stamen development by *STAMENLESS*, the tomato (*Solanum lycopersicum* L.) orthologue to the B-class *APETALA3* gene. J Exp Bot 65:2243–2256

Ren Z, Li Z, Miao Q, Yang Y, Deng W, Hao Y (2011) The auxin receptor homologue in S*olanum lycopersicum* stimulates tomato fruit set and leaf morphogenesis. J Exp Bot 62: 2815–2826

Roque E, Gómez MD, Ellul P, Wallbraun M, Madueño F, Beltrán JP, Cañas LA (2007) The *PsEND1* promoter: a novel tool to produce genetically engineered male-sterile plants by early anther ablation. Plant Cell Rep 26:313–325

Rotino GL, Acciarri N, Sabatini E, Mennella G, Lo Scalzo R, Maestrelli A, Molesini B, Pandolfini T, Scalzo J, Mezzetti B et al (2005) Open field trial of genetically modified parthenocarpic tomato: seedlessness and fruit quality. BMC Biotechnol 5:32

Ruan Y-L, Patrick JW, Bouzayen M, Osorio S, Fernie AR (2012) Molecular regulation of seed and fruit set. Trends Plant Sci 17:656–665

Saito T, Ariizumi T, Okabe Y, Asamizu E, Hiwasa-Tanase K, Fukuda N, Mizoguchi T, Yamazaki Y, Aoki K, Ezura H (2011) TOMATOMA: a novel tomato mutant database distributing Micro-Tom mutant collections. Plant Cell Physiol 52:283–296

Sastry KKS, Muir RM (1963) Gibberellin: effect on diffusible auxin in fruit development. Science 140:494–495

Schaffer RJ, Ireland HS, Ross JJ, Ling TJ, David KM (2013) *SEPALLATA1/2*-suppressed mature apples have low ethylene, high auxin and reduced transcription of ripening-related genes. AoB Plants 5:pls047

Schijlen EG, de Vos CH, Martens S, Jonker HH, Rosin FM, Molthoff JW, Tikunov YM, Angenent GC, van Tunen AJ, Bovy AG (2007) RNA interference silencing of chalcone synthase, the first step in the flavonoid biosynthesis pathway, leads to parthenocarpic tomato fruits. Plant Physiol 144:1520–1530

Serrani JC, Fos M, Atarés A, García-Martínez JL (2007a) Effect of gibberellin and auxin on parthenocarpic fruit growth induction in the cv micro-tom of tomato. J Plant Growth Regul 26:211–221

Serrani JC, Sanjuán R, Ruiz-Rivero O, Fos M, García-Martínez JL (2007b) Gibberellin regulation of fruit set and growth in tomato. Plant Physiol 145:246–257

Shinozaki Y, Hao S, Kojima M, Sakakibara H, Ozeki-Iida Y, Zheng Y, Fei Z, Zhong S, Giovannoni JJ, Rose JKC et al. (2015) Ethylene suppresses tomato (*Solanum lycopersicum*) fruit set through modification of gibberellin metabolism. Plant J 83:237–251

Sun T (2010) Gibberellin-GID1-DELLA: a pivotal regulatory module for plant growth and development. Plant Physiol 154:567–570

Theissen G, Saedler H (2001) Plant biology. Floral quartets. Nature 409:469–471

Tiwari SB, Hagen G, Guilfoyle TJ (2004) Aux/IAA proteins contain a potent transcriptional repression domain. Plant Cell 16:533–543

van Doorn WG, Woltering EJ (2008) Physiology and molecular biology of petal senescence. J Exp Bot 59:453–480

Vardy E, Lapushner D, Genizi A, Hewitt J (1989) Genetics of parthenocarpy in tomato under a low temperature regime: II. Cultivar 'Severianin'. Euphytica 41:9–15

Varoquaux F, Blanvillain R, Delseny M, Gallois P (2000) Less is better: new approaches for seedless fruit production. Trends Biotechnol 18:233–242

Vivian-Smith A, Luo M, Chaudhury A, Koltunow A (2001) Fruit development is actively restricted in the absence of fertilization in *Arabidopsis*. Development 128:2321–2331

Vriezen WH, Feron R, Maretto F, Keijman J, Mariani C (2008) Changes in tomato ovary transcriptome demonstrate complex hormonal regulation of fruit set. New Phytol 177:60–76

Wang H, Jones B, Li Z, Frasse P, Delalande C, Regad F, Chaabouni S, Latché A, Pech J-C, Bouzayen M (2005) The tomato *Aux/IAA* transcription factor *IAA9* is involved in fruit development and leaf morphogenesis. Plant Cell 17:2676–2692

Wang H, Schauer N, Usadel B, Frasse P, Zouine M, Hernould M, Latché A, Pech J-C, Fernie AR, Bouzayen M (2009) Regulatory features underlying pollination-dependent and -independent tomato fruit set revealed by transcript and primary metabolite profiling. Plant Cell 21:1428–1452

Wittwer SH, Bukovac MJ, Sell HM, Weller LE (1957) Some effects of gibberellin on flowering and fruit setting. Plant Physiol 32:39–41

Wu J, Peng Z, Liu S, He Y, Cheng L, Kong F, Wang J, Lu G (2012) Genome-wide analysis of *Aux/IAA* gene family in Solanaceae species using tomato as a model. Mol Genet Genomics 287:295–311

Chapter 8
Fruit Growth in Tomato and Its Modification by Molecular Breeding Techniques

Lamia Azzi, Frédéric Gévaudant, Frédéric Delmas, Michel Hernould, and Christian Chevalier

8.1 Introduction

In the current worldwide socio-economical context and to cope with its foreseen evolution, the use of plant products becomes more and more relevant to face challenges related to food and human nutrition supply and exhaustion of fossil resources. In this changing context, plant biologists are thus expected to produce fundamental knowledge to be transferred to the societal and economical demands.

Managing plant growth and development requires studies addressing fundamental cellular processes such as cell division, cell expansion and cell differentiation. These cellular processes in close interaction with genotype and environmental cues are thus of prime importance in contributing to plant growth and consequently in impacting plant organ size, plant yield and consequently the quality of plant products (Beemster et al. 2003). The cell division activity provides the building blocks, setting the number of cells that will compose an organ, whereas the cell expansion activity then determines its final size. However, how these fundamental developmental processes control organ size and shape is still largely unknown in plants.

The use of molecular markers in plant breeding has greatly facilitated the investigation of the genetic basis of complex quantitative traits, such as those governing grain and fruit sizes. As a result, many quantitative trait loci (QTLs) were identified in the last decade in plant species of great agronomical importance

L. Azzi • F. Gévaudant • F. Delmas • M. Hernould
University of Bordeaux, UMR 1332 Biologie du Fruit et Pathologie, INRA Bordeaux Aquitaine, CS20032, 33882 Villenave d'Ornon, France

C. Chevalier (✉)
INRA, Unité Mixte de Recherche, 1332 Biologie du Fruit et Pathologie, INRA Bordeaux Aquitaine, CS20032, 33882 Villenave d'Ornon, France
e-mail: chevalie@bordeaux.inra.fr

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_8

such as rice, maize or tomato (Tanksley 2004; Thévenot et al. 2005; Xing and Zhang 2010). Genetics and molecular approaches provide the means towards the identification of genes underlying these QTLs which may ultimately allow their use in selection and breeding schemes. Therefore, unravelling the regulatory networks affecting plant and organ growth became a major issue for plant biologists in the last decades.

Tomato (*Solanum lycopersicum* Mill.) belongs to the Solanaceae family which is the third most important plant taxon at the economic level and the most valuable in terms of vegetable crops. It includes also the tuber-bearing potato, a number of fruit-bearing vegetables (e.g. eggplant and pepper) and ornamental flowers (*Petunias*, *Nicotiana*). Tomato is ranked 1st in world fruit production and represents the main income for major vegetable seed companies. In addition, the organization of genomes within the Solanaceae presents an exceptionally high degree of conservation, thus rendering this a unique subject to explore the basis of phenotypic diversity and adaptation to natural and agricultural environments. Besides these agronomical advantages, tomato has been imposed as the model species for all fleshy fruits. Tomato displays a highly favourable biology with short life cycle, high multiplication rate, easy crosses and self-pollination. Because of the large morphological diversity encountered among domesticated tomato varieties, tomato represents a model of choice to unveil the genetic basis of fruit size and shape determination. The marker-assisted mapping using crosses between small and round wild tomatoes with domesticated varieties of various sizes and shapes contributed to enrich our knowledge on the genetic control of fruit development (Grandillo et al. 1999; Causse et al. 2007; Muños et al. 2011; Rodríguez et al. 2011). This approach contributed to highlight the links between the quantitatively determined fruit size trait and fruit growth (by increasing cell number and/or cell size) and importantly fruit composition (Causse et al. 2002; Prudent et al. 2009).

This chapter aims at addressing fruit growth in tomato and at describing the various processes involved in, namely, cell division and cell cycle regulation and cell expansion, with a particular attention to the influence of the endopolyploidy process named endoreduplication. We here provide an overview of the various biotechnological attempts to modify fruit growth in tomato.

8.2 Cell Cycle Control

Cell division or mitosis is the ultimate step in the cell cycle that leads to the transmission of the genetic information from one mother cell to two daughter cells. The mitotic cycle in eukaryotic cells is composed of four distinct phases: an undifferentiated DNA pre-synthetic phase with a 2C nuclear DNA content, termed the G1 phase; the S phase during which DNA is synthesized, with a nuclear DNA content intermediate between 2C and 4C (where C is the DNA content of the haploid genome); a second undifferentiated phase (DNA post-synthetic phase) with a 4C nuclear DNA content, termed the G2 phase; and the ultimate M phase

or mitosis. The classical cell cycle thus involves the accurate duplication of the chromosomal DNA stock during the S phase and its subsequent equal segregation in the nascent daughter cells following cytokinesis at the end of the M phase.

The progression within the cell cycle is regulated by a class of conserved heterodimeric protein complexes consisting in a catalytic subunit referred to as cyclin-dependent kinase (CDK) and a regulatory cyclin (CYC) subunit whose association determines the activity of the complex, its stability, its localization and substrate specificity (Inzé and De Veylder 2006). Specific checkpoints restrict the passage from one cell cycle phase to the other, requiring the cell to sense external and internal cues favourable to commit to the following step of the cell cycle. These commitments, namely, overriding the checkpoints, are under the control of the CDK/CYC complex activity. Specific CDK/CYC complexes operate at the boundaries between the G1 and S phases and between the G2 and M phases, to phosphorylate target proteins whose inhibitory or activatory post-translational modifications are essential for passing these cell cycle checkpoints (Joubès et al. 2000; De Veylder et al. 2003) (Fig. 8.1). The commitment to the S phase is dependent upon CDKA/CYCD complex activities which phosphorylate the retinoblastoma-related 1 (RBR1) protein (Gutierrez et al. 2002). The hyperphosphorylation of RBR1 leads to the release of sequestered E2F transcription factors required to drive the expression of S phase genes. Then CDK/CYCA complexes control the progression through the S phase and the commitment to mitosis whose proper completion depends on CDKA/CYCB complex activities.

The kinase activity of the CDK/CYC complexes is subjected to various levels of regulation. Obviously the availability of each of the partner is fundamental and regulated by transcription, translation and proteolytic degradation. The CDK activity is not only dependent on the presence of a regulatory CYC subunit but is also finely tuned by the phosphorylation/dephosphorylation status of the catalytic (kinase) subunit itself. To be active, the CDK moiety requires an activatory phosphorylation by a CDK-activating kinase (CAK) on a conserved threonine residue (e.g. Thr161 in plant CDKAs) (Harashima et al. 2007). Prior to its timely programmed activity at the onset of mitosis, the activated CDK/CYC complex is likely to be kept silent by inhibitory phosphorylation of Thr14 and Tyr15 residues of the CDK mediated by the WEE1 kinase, until a yet unknown phosphatase (in plant) dephosphorylates these residues to activate the CDK/CYC complex. The existing WEE1 function is restricted to the S phase to block the CDK/CYC activity unless DNA damages resulting from DNA replication defects or genotoxic stress have been corrected (De Schutter et al. 2007; Gonzalez et al. 2007; Cools et al. 2011). The completion of mitosis and exit from mitosis back to interphase require the loss of CDK/CYC complex activity. Since without its cyclin partner the CDK does not display anymore an activity, the proteolytic destruction of the cyclin moiety via the ubiquitin proteasome system (UPS) is sufficient to extinct the kinase activity. This process involves a specific E3-type ubiquitin ligase named the anaphase-promoting complex/cyclosome (APC/C) which is activated through its association with the CCS52 protein (Heyman and De Veylder 2012). Additionally the CDK/CYC complexes are inactivated by the specific binding of CDK inhibitors

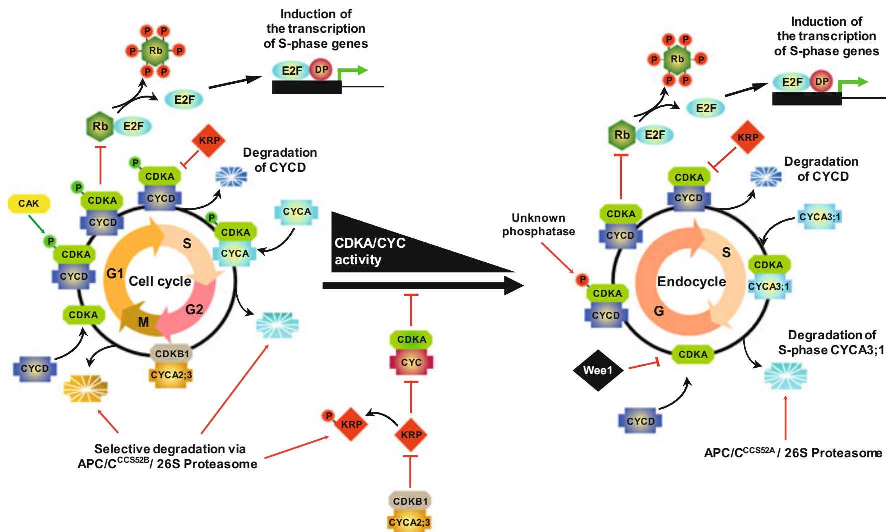


Fig. 8.1 Cell cycle and endoreduplication cycle control in plants. The progression within the four phases of the plant cell cycle is regulated by a class of conserved heterodimeric protein complexes, consisting of a catalytic subunit referred to as cyclin-dependent kinase (CDK) and a regulatory cyclin (CYC). The kinase activity of the complexes is dependent on the availability and binding of the CYC regulatory subunit, on the binding of specific CDK inhibitors (KRP), and on the phosphorylation/dephosphorylation status of the CDK itself (please refer to the text for the detailed control of the cell cycle actors). A high CDKA;1-associated kinase activity is required to maintain a cell cycling activity and to prevent the commitment to endoreduplication. This is ensured by the mitotic CDKB1/CYCA2;3 complex (proposed as being the mitosis-inducing factor, MIF) whose activity prevents a CDK inhibitor (KRP) to bind to and inactivate the CDKA/CYC complex. The commitment to the endoreduplication cycle (lacking mitosis) is triggered by the reduction in CDKA/CYC activity below a certain threshold, as a consequence of the inactivation of the mitotic CDKB1/CYCA2;3 complex in endoreduplicating tissues. The endoreduplication cycle or endocycle shares with the canonical cell cycle the same control mechanisms for the progression within its two phases. In early G, CDKA is maintained in an inactive state by the inhibitory phosphorylation mediated by WEE1 as to allow a proper cell growth prior to commit to another round of DNA synthesis. At the G-to-S transition, the CDKA/CYCD complex is likely inhibited by a KRP. In early S, the induced CYCA3;1 associates with CDKA in a complex that is necessary for the progression throughout the S phase. At the end of the S phase, CYCA3;1 is degraded by the APC^{CCS52A}/26S proteasome pathway; CDKA is then released and available for a new round of the endoreduplication cycle

of the Kip-related protein (KRP) (Torres-Acosta et al. 2011) and SIAMESE-related (SMR) type (Churchman et al. 2006; Van Leene et al. 2010). The CDK inhibitors are also subject to specific degradation mechanisms involving UPS (Verkest et al. 2005; Ren et al. 2008; Marrocco et al. 2010).

8.3 The Endoreduplication Process

Plant cells are able to modify the typical cell cycle into the endoreduplication cycle or endocycle where mitosis is lacking (Joubès and Chevalier 2000; Edgar and Orr-Weaver 2001; De Veylder et al. 2011). Endoreduplication is widespread in angiosperms (Nagl 1976; D'Amato 1984), and it has been evolutionary selected to determine developmental programmes leading to cell and organ differentiation or in response to environmental constraints (Hülskamp et al. 1999; Barow 2006; Ceccarelli et al. 2006; Cookson et al. 2006; Hase et al. 2006; Bramsiepe et al. 2010; Roeder et al. 2010; Adachi et al. 2011).

Arabidopsis, maize, *Medicago* and tomato as reviewed herein were studied as the most representative plant models to decipher the functional role of endoreduplication during development (Kondorosi and Kondorosi 2004; Chevalier et al. 2011; De Veylder et al. 2011; Sabelli et al. 2013). At the level of molecular control, the proper unfolding of the cell cycle and the commitment to endoreduplication are a matter of CDK/CYC activity levels (De Veylder et al. 2011). The progression through the G2-M transition requires the activity of a mitosis-inducing factor (MIF) that allows the CDKA/CYC complex activity to maintain above a certain threshold. The absence or reduced activity of this MIF is sufficient to drive cells into the endoreduplication cycle (Inzé and De Veylder 2006; Vlieghe et al. 2007) (Fig. 8.1). The G2/M-specific CDKB1;1 when bound to the A-type cyclin CYCA2;3 is able to inhibit endoreduplication (Yu et al. 2003; Boudolf et al. 2004, 2009), as the likely candidate CDK/CYC complex to be MIF. Then the selective degradation of CYCA2;3 achieved by the CCS52A-mediated activation of APC is the key process in the regulation of CDKB1;1 activity, as this provokes the commitment to endoreduplication by reducing or suppressing the MIF activity.

Since mitosis is bypassed, the endoreduplication cycle is thus made of successive rounds of DNA duplication occurring in iterative S phases separated by an undifferentiated G phase. As a consequence, polytenic chromosomes with multivalent (2, 4, 8, 16, etc.) chromatids attached to the centromere without any change in chromosome number are produced (Edgar and Orr-Weaver 2001; Bourdon et al. 2012). Hence, endoreduplication leads to high nuclear DNA contents (one speaks of endopolyploidy; Barow and Meister 2003) impacting the morphology of both nucleus and cell (Bourdon et al. 2012).

Endoreduplication and cell size in many different plant species, organs and cell types are naturally and intimately positively correlated (Joubès and Chevalier 2000; Sugimoto-Shirasu and Roberts 2003; Chevalier et al. 2011). The frequent observations that the size of the nucleus is related to that of the cell led to a theory commonly referred to as the "karyoplasmic ratio theory", firstly formulated by Theodor Boveri and reported by Wilson (1925), stating that there is a causal relationship between nuclear and cytoplasmic growth allowing a merely constant nuclear-to-cell volume ratio (Sugimoto-Shirasu and Roberts 2003; Chevalier et al. 2013). Recent advances towards the elucidation of the functional role of

endoreduplication were obtained in tomato as discussed as follows and confirmed that endoreduplication is a likely driver for cell expansion (but not exclusive) in fruit growth, according to the karyoplasmic ratio theory (Bourdon et al. 2012).

8.4 Tomato Fruit Development

The fruit is a specialized organ which results from the development of the ovary after successful flower pollination and fertilization. Like in many other species, fruit organogenesis in tomato results from the interplay of cell division and cell expansion activities which determines the cell number and the relative cell size inside the fruit, respectively (Bohner and Bangerth 1988). The cell division and cell expansion developmental processes are under the control of complex interactions between internal signals (due to plant hormones) and external factors (carbon partitioning, environmental influences) and represent ultimately determinants of essential criteria for quality traits such as the final size, weight and shape of fruits (Tanksley 2004). Beside these morphological traits, organoleptic and nutritional quality traits of tomato ripe fruit are also determined early during fruit development.

In a reference review, Gillaspy et al. (1993) described the development of tomato fruit as proceeding in four distinct phases: fruit set, two phases of intense cell divisions and of cell expansion both contributing to fruit growth and finally ripening. This classical developmental scheme for tomato fruit has to be more or less revised, since the distribution of mitotic activities (Joubès et al. 1999) and occurrence of cell expansion (Cheniclet et al. 2005) are spatially and temporally regulated according to the various fruit tissues. Cell division and cell expansion even coexist as early as in the developing ovary (Fig. 8.2).

Upon completion of pollination, the presence of fertilized ovules triggers very rapidly (within 2 days) the development of the ovary into a fleshy pericarp, encompassing the placental tissue and the seeds. The tomato berry then grows very rapidly due to high mitotic activity within the pericarp which lasts for about 7–10 days. Thereafter and until ripening, fruit growth to almost full size is principally obtained through cell expansion. Cell size in tomato fruit can reach spectacular levels, e.g. more than 0.5 mm in diameter inside the pericarp according to the variety (Cheniclet et al. 2005). These very large cells are found within the central part of the fleshy pericarp and the locular (jelly-like) tissue and are characterized by a spectacular increase in nuclear DNA content up to 512C and a consequent hypertrophy of the nucleus originating from endoreduplication (Bergervoet et al. 1996; Cheniclet et al. 2005; Joubès et al. 1999; Bourdon et al. 2011, 2012).

Interestingly, a remarkable conservation of pericarp pattern, including cell layer number and cell size, is observed at anthesis in tomato genotypes varying in a large range of fruit weight at maturation (Cheniclet et al. 2005). Thereafter during

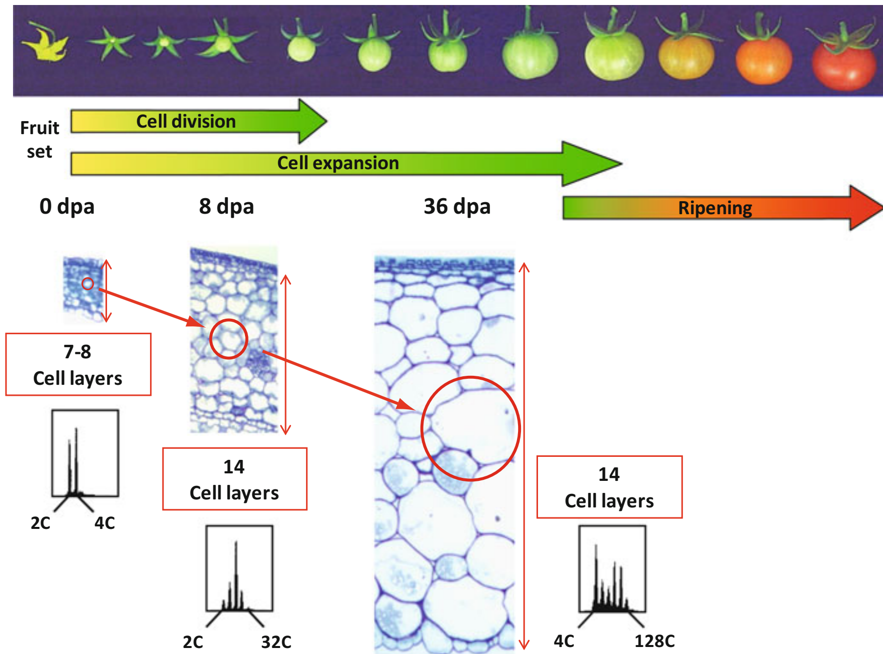


Fig. 8.2 Developmental characteristics of tomato fruit growth. Following ovary fertilization and the decision to set fruit, tomato fruit growth results first from intense cell division activities which amplify very rapidly the number of cell layers across the pericarp. Cell enlargement occurs concomitantly to cell division and starts as early as 2 to 3 dpa, thus contributing to increase the pericarp thickness. In less than 8–10 days, the number of cell layers is fixed within the pericarp: cell divisions then cease and cell expansion only account for fruit growth afterwards. During tomato fruit development, cell expansion is accompanied by the process of endoreduplication which amplifies the nuclear DNA content. As a result, very high endopolyploid DNA levels can be reached within pericarp cells: up to 128C and sometimes more (512C)

development, the large genotypic variation in fruit weight correlates with the mean ploidy level achieved in pericarp cells which itself correlates with the mean cell size (Cheniclet et al. 2005). Using an in situ and direct determination of the DNA content of individual nuclei, Bourdon et al. (2011) were able to establish a DNA ploidy map across the tomato pericarp at mature green stage. This study did confirm the positive correlation between cell size and DNA ploidy levels but revealed additionally that cell size is not only dependent on ploidy levels but also upon the position of cells within the pericarp, since a precise cell size may be associated to various nuclear DNA contents.

It was concluded from these cytological analyses of tomato pericarp structural characteristics that endoreduplication would favour a larger range of cell sizes rather than any particular class; furthermore, endoreduplication would precede cell expansion, providing the potential to support a range of cell size which ultimately participates in modulating the rate of fruit growth (Bourdon et al. 2012).

8.5 Modifying Tomato Fruit Growth with Cell Cycle and Endocycle Regulatory Genes

In planta functional analyses of key cell cycle and endocycle regulatory genes have been conducted in tomato to investigate their putative contribution in fruit growth and fruit size. The above-described functional analyses all represented attempts to modulate the CDK/CYC complex activity during fruit development, either through direct targeting of CDK gene expression or through post-translational regulation of the complex itself.

As the key player in cell cycle progression, modifying the expression of the canonical CDKA;1 was expected to alter fruit development. When CDKA;1 is down-regulated using an artificial microRNA (*amiCDKA*) in a fruit-specific manner, smaller fruits were produced; pericarp thickness was reduced because of an overall decreased number of cell layers within the exocarp (displaying the smallest cells), while the mesocarp displayed normally enlarged cells without any significant difference in ploidy levels (Czerednik et al. 2012). Such a phenotype was equally obtained with the fruit-specific overexpression of both mitosis-associated CDKB1 and CDKB2 (Czerednik et al. 2012). Again fruits were smaller, with a thinner pericarp sharing the same modified structure. Interestingly the expression of CDKA;1 was greatly repressed in these overexpressing CDKB1 and CDKB2 lines, in accordance with the phenotype *of amiCDKA* line. The modification of CDKA or CDKB1/B2 expression in tomato fruits produced phenotypes highlighting differential effects on cell division, cell expansion and endoreduplication. Czerednik et al. (2012) did not provide any clear interpretation of these phenotypes. The complexity of the observed data came most probably from the choice of the TPRP promoter to drive the CDK gene misexpression. The TPRP promoter is a fruit-specific promoter whose maximum of expression occurs during the cell expansion phase of fruit development (Fernandez et al. 2009). As a consequence, *CDKB1* and *CDKB2* were overexpressed outside their natural timing of expression (Joubès et al. 1999, 2001), which may have greatly impaired the availability of regulatory cyclins for the proper composition of CDK/CYC complexes, and/or created an artificial competition for regulatory cyclins among the misexpressed CDKs during tomato fruit development. Remarkably, the common phenotype between down-regulated *CDKA* and up-regulated *CDKB* transgenic fruits was a smaller fruit size because of smaller cells in the outer pericarp, as a result of *CDKA* repression. It is likely to originate from the prevention of youngest cells generated in the outermost layers of pericarp (namely, the exocarp) to enter into endoreduplication-driven cell expansion because of the lack of endocycle-specific CDKA/CYC complex activity. Hence, the phenotypes described by Czerednik et al. (2012) provided evidence for the importance of CDKA for the onset of endoreduplication and consequent cell growth and fruit growth.

CCS52A as an activator of the APC^{CCS52A} E3 ubiquitin ligase was found to be involved in the commitment towards endoreduplication, since the UPS-mediated selective proteolytic destruction of the cyclin subunits provokes the loss of mitotic

CDK/CYC complex activity. An ectopic loss of function of *CCS52A* in transgenic tomato plants led to the production of smaller fruits than in wild-type plants (Mathieu-Rivet et al. 2010). The DNA ploidy levels in these fruits were shifted towards lower levels, in correlation with a decrease in mean cell size and an increase in cell number. A second APC/C activator is encoded by the tomato genome, namely, SlCCS52B. The *SlCCS52B* gene is preferentially expressed during cell division, while *SlCCS52A* is involved in the endoreduplication-driven cell expansion (Mathieu-Rivet et al. 2010). Surprisingly, the ectopic down-expression *SlCCS52B* gave an opposite phenotype to that of down-regulated *SlCCS52A* plants, as fruits were larger (personal unpublished data). The cytological and molecular characterization of antisense *SlCCS52B* fruits revealed that this phenotype originates from higher ploidy levels and larger cell sizes induced by the compensatory overexpression of the endogenous *SlCCS52A* gene, thus indicating that *SlCCS52A* regulates the commitment towards endoreduplication. This was further confirmed in gain of function of *SlCCS52A*. When *SlCCS52A* was ectopically overexpressed, fruits grew much slower than wild-type fruits but resumed growth and were able to reach almost the size of wild-type fruit at the end of the growing period (~35 days post-anthesis, dpa). Kinetic analysis of the appearance of polyploid nuclei during fruit growth revealed that endoreduplication was increased at 20 dpa and afterwards due to a burst in the appearance of 32C and 64C nuclei. This production of highly polyploid nuclei generated cells of an increased size inside the pericarp of *SlCCS52A* overexpressing fruits, which ended up with an accelerated fruit growth (Mathieu-Rivet et al. 2010). Thus, the regulatory role of *SlCCS52A* in triggering endoreduplication is deeply associated with the cell expansion process driving fruit growth in tomato.

When *WEE1* was ectopically repressed in tomato transgenic plants, smaller fruits were produced; the thickness of the pericarp was altered because of a significant reduction in cell size, together with a strong reduction in ploidy levels (Gonzalez et al. 2007). At the molecular level, the impairment of WEE1 kinase activity due to the gene repression resulted in an enhanced CDK/CYC complex activity, because the overall inhibitory phosphorylation of the Tyr15 residue in CDKA was impaired. The WEE1 phosphorylation activity on its CDK targets appears to be an important mode of regulation for the promotion of endoreduplication during tomato fruit development, thus contributing to cell size determination and consequently influencing final fruit size (Chevalier et al. 2011). Interestingly, it was recently reported that the expression of *WEE1* in the context of cell size determination (putatively associated to endoreduplication) and fruit size determination may be subjected to epigenetic control (Liu et al. 2012).

Transgenic fruits were generated with the aim to overexpress a tomato CDK-specific inhibitor protein, termed SlKRP1 (Nafati et al. 2011). Since the overexpression of such an inhibitor could be highly detrimental for the whole plant regeneration process if expressed ectopically, the *SlKRP1* gene was placed under the control of the *PEPC2* promoter which allows not only a fruit-specific expression but more importantly a cell expansion-specific expression (Fernandez et al. 2009) in mesocarp endoreduplicated cells. In the *SlKRP1* overexpressing

fruits, DNA ploidy levels were significantly lowered in the mesocarp, as the inhibitory effect of SlKRP1 was exerted on endoreduplication-specific CDK/CYC complexes (Nafati et al. 2011). However, the *PEPC2*-driven overexpression of Sl*KRP1* during the phase of cell expansion uncoupled cell size from DNA ploidy levels since the mean pericarp cell size was neither affected nor final fruit size despite the significant negative impact on endoreduplication. Though being reduced, endoreduplication was not totally impaired in these transgenic fruits, suggesting that enough DNA ploidy still occurred to support cell growth. Altogether these functional analyses of cell cycle and endocycle regulatory genes indicate that endoreduplication per se would not determine the exact size of a cell but would rather act as a limiting factor for cell growth, setting up a potential for cell and organ growth (Schnittger et al. 2003; Nafati et al. 2011).

8.6 Genetic Control of Fruit Shape

As a result of domestication and extensive selection, cultivated tomato varieties display a tremendous diversity in fruit morphology affecting both fruit shape and size (Tanksley 2004; Paran and van der Knaap 2007).

As far as fruit shape is concerned, mutations in only four genes have been so far directly associated to this diversity, namely, *OVATE*, *SUN*, *FASCIATED* (*FAS*) and *LOCULE NUMBER* (*LC*) (Rodríguez et al. 2011). Both *OVATE* and *SUN* control fruit elongation: *OVATE* is a negative regulator of growth, reducing fruit length, whereas *SUN* is a positive regulator of growth resulting in elongated fruit. *FASCIATED* (*FAS*) and *LOCULE NUMBER* (*LC*) control the number of fruit locules which ultimately influences fruit size.

OVATE was the first fruit shape gene identified by positional cloning (Liu et al. 2002). It belongs to the Ovate family protein (OFP) family of a yet-unclear function (Liu et al. 2002; Wang et al. 2010, 2011). The effects of the *ovate* mutation on fruit shape vary from elongated fruits to pear- or round-shaped fruits, depending on the genetic background carrying the *ovate* mutation (Gonzalo and van der Knaap 2008). This suggests that *OVATE* alone may not be solely responsible for the observed phenotype and putatively interacts with other genes in an epistatic manner. The *ovate* pear-shaped fruit phenotype was complemented by a genomic DNA fragment covering the *OVATE* gene or by its ectopic overexpression, thus leading to round-shaped fruits (Liu et al. 2002). As a result, the *ovate* mutation is likely to be a loss-of-function mutation of a plant growth negative regulator whose function remains to be elucidated. Interestingly, OVATE-like genes from *Arabidopsis* were shown to act as transcriptional repressors, affecting in particular the expression of AtGA20ox1, a key player in the GA biosynthetic pathway, and consequently reducing cell elongation (Hackbusch et al. 2005; Wang et al. 2007, 2011). Whether OVATE controls fruit shape via the regulation of GA biosynthesis in tomato is still unknown.

The *sun* mutation resulting in the elongated fruit phenotype is caused by a gene duplication event mediated by a retrotransposon that caused *SUN* to be placed under the control of a defensin gene (*DEFL1*) promoter, thus leading to high expression in the fruit (Xiao et al. 2008; Jiang et al. 2009). *SUN* encodes a member of the IQ67 domain-containing plant protein family (Xiao et al. 2008). The induced overexpression of *SUN* leads to fruit elongation by increased cell number in the longitudinal direction and reduced cell number in the transverse direction of the fruit (Wu et al. 2011). The phenotypic effects resulting from high expression of *SUN* indicated that the gene is involved in several plant developmental processes in addition to fruit morphology, but the precise function exerted by the encoded protein remains unknown.

The number of fruit locules is determined by the number of carpels within the flower. Wild species of tomato produce fruits with 2–4 locules, while cultivated varieties can develop more than 15 locules. As a result, not only the shape of such fruits can be greatly impacted, but also this can account for a tremendous increase in fruit size by as much as 50 % (Tanksley 2004). The QTL *fasciated* was identified as governing an extreme fruit size trait that increases the number of locules from two to more than seven, while the QTL *locule number* has a weaker effect (Lippman and Tanksley 2001; Barrero et al. 2006). *FASCIATED* codes for a YABBY-like transcription factor (Cong et al. 2008), while *LOCULE NUMBER* seems to be associated with the post-transcriptional regulation of *WUSCHEL*, a WD40 repeat homeobox transcription factor (Muños et al. 2011), member of the plant-specific WUS homeobox (WOX) protein family. Most of the *WOX* genes studied so far can be related to either promotion of cell division and/or prevention of premature differentiation (van der Graaff et al. 2009), and WUS is more specifically involved in the control of stem cell fate and meristem size. *FAS* and *LC* can interact epistatically to produce fruits with an extremely high locule number (Barrero and Tanksley 2004), since the YABBY-like transcription factor encoded by *FAS* as well as the *LC*-associated WUSCHEL homeodomain protein both controls the floral meristem size and ultimate development of supernumerary carpels (locules) leading to larger fruits (Cong et al. 2008; Muños et al. 2011). Thus, the functional analyses of FAS and LC illustrate the link between the determination of fruit size and genes involved in developmental processes such as cell division and meristem size.

8.7 Genetic Control of Fruit Size

In tomato 28 quantitative trait loci (QTLs) controlling the fruit size/weight were detected (Grandillo et al. 1999). However, not all these loci are equal in the magnitude of their effects. Based on all of the genetic/mapping studies performed so far, it is estimated that less than ten loci, mapping to seven of the 12 chromosomes in tomato, account for the majority of the changes in size and shape associated with tomato domestication/agriculture (Grandillo et al. 1999).

Among these QTLs, *fw2.2* (for fruit weight QTL of chromosome 2, number 2) accounts for as much as a 30 % difference in fruit fresh weight between the domesticated tomato and its wild relatives (Alpert et al. 1995). The gene underlying the *fw2.2* QTL was not only identified but cloned and characterized (Alpert and Tanksley 1996; Frary et al. 2000). Genetics and molecular approaches showed the fundamental implication of FW2.2 in the control of fruit size in tomato, as a negative regulator of cell divisions. Several groups have addressed the contribution of FW2.2 homologues in different plant species (avocado, maize, soybean, cherry) (Dahan et al. 2010; Guo et al. 2010; Libault et al. 2010; De Franceschi et al. 2013), demonstrating the important role of this type of proteins in the determination of organ size. However, none of these studies including that in tomato allowed any clear assignment of a definitive biochemical, physiological and developmental function to FW2.2 and its orthologues. The effect on fruit size variations by controlling cell proliferation associated to the FW2.2-related loci in Solanaceae (tomato, eggplant and pepper) suggests that members of the FW2.2 family act as regulators of the cell number and thus as a regulator of fruit size. In addition, the variation of organ size in natural populations is mainly due to changes in cell number rather than cell size (Mizukami 2001; Bertin et al. 2009). Hence, the conservation of FW2.2 gene function in various plant species suggests the existence of similar genetic mechanisms controlling organ size.

FW2.2 belongs to a multigene family encompassing 17 homologous proteins to FW2.2 (hereafter referred to as *FW2.2-like*—*FWL*—genes). FW2.2 and FWL proteins all contain the uncharacterized PLAC8 motif which was originally identified in proteins from mammalian placenta. So far, up to 136 protein sequences resembling to FW2.2 have been identified in 25 animal, fungal and plant species (Guo et al. 2010). The family PLAC8 signature motif contains two conserved cysteine-rich domains (domains 1 and 2) separated by a variable region that are predicted to be transmembrane segments. As a member of the PLAC8-containing protein family, the original tomato FW2.2 protein possesses two transmembrane-spanning domains (Tanksley 2004) and is indeed addressed to the plasmalemma (Cong and Tanksley 2006). Within the PLAC8 motif, the closest transmembrane segment to the N-terminal end displays the CCXXXXCPC or CLXXXXCPC sequence. The CCXXXXCPC domain seems to confer an important role in transport of heavy metals such as cadmium and zinc, as identified in the PCR (for plant cadmium resistance) proteins in *Arabidopsis* (Song et al. 2010). The structural modelling studies indicated that AtPCR1 could multimerize into a homopentamer to form a transmembrane pore, with a hydrophilic side inward, facing the lumen, and the cysteine residues lining the lumen-facing side, suggesting that metal cations, including Cd^{2+}, could migrate through or interact with the channel (Guo et al. 2010). Interestingly the leucine residue in the CLXXXXCPC motif could affect the substrate recognition of the cation transporter when compared to the cysteine residue of the CCXXXXCPC motif. The original FW2.2 from tomato and its close counterpart in *Zea mays* ZmCNR1 (for *Zea mays* cell number regulator) display the CLXXXXCPC motif. Whether the biological function of FW2.2 and its

orthologues is related to ion transport stays unknown. In addition what kind of link is there between the FW2.2 protein function and cell cycle regulation is still elusive.

So far *fw3.2* is the only second major QTL for fruit size/weight to be fine mapped and cloned in tomato (Chakrabarti et al. 2013). The gene underlying this QTL encodes the orthologue of KLUH, SlKLUH, a P450 enzyme of the CYP78A subfamily. The phenotypical traits associated to the *SlKLUH* expression relate to fruit mass increase, due to enlarged pericarp and septum tissues, as the result of increased cell number in the large fruited lines. Using an RNAi strategy, the down-regulation of *SlKLUH* in tomato led not only to decreased fruit and seed size/mass but also precocious ripening time; in addition, plant architecture was also modified with a higher number and length of side shoots (Chakrabarti et al. 2013).

fw11.3 is another important locus governing fruit weight which overlaps with *fas* on tomato chromosome 11 (Grandillo et al. 1999; Van der Knaap and Tanksley 2003). The fine mapping of this locus demonstrated that *fw11.3* and *fas* were not allelic and narrowed down the genomic region to 22 candidate genes on chromosome 11 (Huang and van der Knaap 2011). Unlike *fw2.2* and *fas*, the large fruit allele of *fw11.3* is partially dominant. Therefore, the future cloning of *FW11.3* is expected to provide another player regulating the molecular mechanisms of increased fruit weight.

Tomato fruit size can be modulated by modifying the cell number or cell size. Among the transcription factors that are likely candidates to affect this latter process, the ectopic expression of *SlFSM1*, a small protein harbouring a non-canonical SANT/MYB-like domain (Barg et al. 2005), was shown to reduce fruit size, via a significant reduction in cell size, particularly in cells that normally have the greatest cell expansion potential during tomato fruit growth (Machemer et al. 2011). Tomato AGAMOUS-LIKE1 (TAGL1), the tomato orthologue of the duplicated SHATTERPROOF (SHP) MADS-box proteins of *Arabidopsis thaliana*, is worth being cited as another transcription factor involved in the regulation of fruit development fruit size determination (Vrebalov et al. 2009). Tomato plants repressing *TAGL1* by RNA interference produced smaller fruits with a thinner pericarp with much less cell layers and altered ripening-related fruit pigmentation, indicating that TAGL1 is important for regulating both fruit growth and the ripening process.

8.8 Modifying Tomato Fruit Growth with Genes Involved in Hormonal Signalling Pathways

After successful flower pollination and ovule fertilization, the fruit and seed initiation (during the so-called fruit set) and subsequent development occur concomitantly according to a genetically tight-controlled process operated by phytohormones (Gillaspy et al. 1993).

The role of plant hormones in the control of early fruit development has been recently reviewed, especially in light of seed and fruit set (Ruan et al. 2012), as well as their influence on yield in tomato (Ariizumi et al. 2013). In this paragraph, we aim at focusing on the most significant genetically engineered tomato plants whose fruit development was altered via the manipulation of genes involved in hormonal signalling pathways. We shall essentially pay attention to auxin and gibberellin (GA) that appear to be the most prominent hormones required for fruit initiation in response to fertilization, since exogenous applications of both hormone lead to fruit initiation and parthenocarpic development (de Jong et al. 2009). Cytokinin, ethylene and abscisic acid (ABA) also seem to be involved in fruit formation; however, apart from transcriptomic data of related gene expression and specific mutants, their respective functional role is less documented.

Upstream of signal transduction, the cellular and tissular availability of auxin, via its allocation, can be the first level to affect early fruit development. Recent evidence indicated that the PIN-FORMED (PIN) auxin efflux transport proteins are involved in the fruit set process and early fruit development in tomato (Pattison and Catalá 2012). Transgenic plants aimed at silencing specifically the tomato *SlPIN4* gene, which is predominantly expressed in tomato flower bud and young developing fruit, produced precociously developing seedless (parthenocarpic) fruits of reduced size (Mounet et al. 2012). The auxin signalling pathway involves the auxin receptor, the TRANSPORT INHIBITOR RESPONSE1 (TIR1) protein, which participates in the presence of auxin, in the recruitment of AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) transcriptional repressors to the proteolytic degradation machinery. Degradation of Aux/IAA proteins reduces the proportion of Aux/IAA-bound AUXIN RESPONSE FACTORS (ARFs), thereby allowing auxin-responsive element-mediated gene transcription to elicit an auxin response. Manipulating these transcriptional regulators revealed that the misexpression of the auxin receptor *TIR1* gene, as well as specific members of the tomato *Aux/IAA* and *ARF* gene family, alters normal flower-to-fruit transition and results in uncoupling fruit set from pollination and fertilization, giving rise to parthenocarpic fruits (Wang et al. 2005; de Jong et al. 2009; Ren et al. 2011).

In tomato, the auxin-induced fruit set is in part mediated by GAs according to a complex hormonal crosstalk (Serrani et al. 2008). Auxin synthesized both in the ovary and in the apical shoot prevents unpollinated ovaries to develop by reducing transcript levels of genes encoding GA biosynthetic enzymes, in particular GA 20-oxidases (Serrani et al. 2007). Upon pollination, transcripts for GA 20-oxidases are up-regulated leading to the production of active GA_1 and GA_4, suggesting that the GA 20-oxidase activity is the limiting factor for active GA biosynthesis and consequently for fruit set. Functional analyses were thus conducted to investigate the effects of a mis-regulation of *GA20ox* genes on tomato fruit initiation and growth. The constitutive co-suppression of *SlGA20ox1* resulted in severely affected plants for vegetative development and reduced pollen viability, in accordance with the pleiotropic developmental role of GAs (Olimpieri et al. 2011). Ovaries from these *SlGA20ox1*-silenced plants remain fertile and can develop normally after cross-pollination with wild-type pollen, except they are parthenocarpic. The

individual silencing of *SlGA20ox1*, *SlGA20ox2* and *SlGA20ox3* genes confirmed the expected effects on vegetative development, but again no effects on fruit set could be observed (Xiao et al. 2006). Altogether these data suggest that the expression of more than one *GA20ox* gene may be required to control fruit set in tomato. Nevertheless, the overexpression of the *CgGA20ox1* gene from citrus in tomato provided reconciling data with the expected influence of GA and GA20ox activity on fruit set and development (Garcia-Hurtado et al. 2012). The vegetative development was boosted with longer hypocotyls and roots and taller plants; transgenic flowers exhibited protruding stigma due to a longer style; overexpressing *GA20ox* fruits displayed parthenocarpic development. All these phenotypes were associated to an elevated GA_4 content which led to two agronomically interesting traits: a higher fruit yield due to an increased number of fruits per plant and a higher Brix index due to a higher content in citric acid (Garcia-Hurtado et al. 2012).

The GA signal transduction pathway requires the recognition of GA by its receptor called GA INSENSITIVE DWARF1 (GID1). The GID1-GA complex interacts with the nuclear repressor DELLA and targets its ubiquitin-dependent proteasomal degradation, thereby allowing the GA response. Silencing the *SlDELLA1* gene in tomato produced very similar vegetative and reproductive phenotypes than described before for *GA20ox1* overexpressor (Martí et al. 2007). Antisense *SlDELLA*-engineered fruits were facultative parthenocarpic, smaller in size and elongated in shape compared with wild type.

The INHIBITOR OF MERISTEM ACTIVITY (IMA) protein is a Mini Zinc Finger (MIF) protein harbouring an unusual zinc finger domain that was identified as an important effector involved in a multiple hormonal signalling pathway that links cell division, cell differentiation and hormonal control of development in tomato (Sicard et al. 2008a, b). IMA was shown to be involved in the regulatory pathway controlling meristem activity and the processes of flower and ovule development. The carpel primordia within the floral meristem were strongly reduced in IMA overexpressing plants, while they were conversely enlarged in RNAi-silenced plants compared to wild-type plants. These modifications in the carpel size originated from an alteration in the cell number, thus suggesting that *IMA* encodes an inhibitor of cell divisions. As a consequence, plants overexpressing *IMA* produced smaller flowers and fruits, while RNAi-silenced plants produced fruits composed of supernumerary ovaries. At the molecular level, it was thus shown that IMA inhibits cell proliferation and acts as a repressor of *WUSCHEL* expression which controls the meristem organizing centre and the determinacy of the nucellus during ovule development (Sicard et al. 2008a).

8.9 Metabolic Control of Fruit Development

The fruit acquires its organoleptic quality traits during the early stages of development. During the growth phase mainly associated to cell expansion, water, organic acids (primarily citric and malic acids) and minerals are accumulated inside the

vacuole of fruit cells (Coombe 1976), while starch accumulates transiently and is converted later on to reducing sugars (Wang et al. 1993). Fruit softening, colouring and sweetening then occur during the ripening phase (Giovannoni 2001, 2004). As a result, the link between fruit composition in primary and secondary metabolites and fruit weight is intimate during fruit development, and this has been the subject of recent authorative reviews (Carrari and Fernie 2006; Tohge et al. 2014).

Several processes and underlying genes may be responsible for the variation in fruit composition and fruit size. Genes involved in carbon metabolism or partitioning or any gene specifically expressed during synthesis and accumulation of carbohydrate reserves may contribute to the elaboration of the fruit composition trait. Quantitative genetic analyses were performed as to identify putative candidate genes linked to fruit weight and composition. Among these, the *Lin5* QTL that controls fruit sugar content was recovered (Fridman et al. 2000) and identified as encoding the apoplastic invertase (Fridman et al. 2004). These genetic analyses confirmed the previous work from Klann et al. (1996) who reported that tomato fruits harbouring an antisense-silenced acid invertase gene had increased sucrose and decreased hexose sugar concentrations. The sucrose-accumulating fruits were approximately 30 % smaller than control fruits, and this differential growth correlated with high rates of sucrose accumulation during the last stage of development. These data suggest that soluble acid invertase controls sugar composition in tomato fruit and that this change in composition contributes to alterations in fruit size (Klann et al. 1996).

The role of sucrose synthase (SuSy) in tomato fruit development was then assessed by silencing a fruit-specific isoform (D'Aoust et al. 1999). The inhibition of SuSy activity affected fruit set and very early fruit development, in relation to a reduced unloading capacity of sucrose. This led the authors to propose that SuSy participates in the control of sucrose import capacity of young tomato fruit and consequently influences fruit set and development. However, these conclusions are still questioned since independent transgenic plants failed to reproduce these data and no co-localization of any SuSy isoform with QTL for fruit weight or sugar content has been reported so far (Carrari and Fernie 2006).

Anyway, the development of fruit as a sink organ is highly dependent upon the partitioning of photoassimilates, which, when modified, significantly affect fruit development and size through the modulation of cell number and cell size (Bohner and Bangerth 1988; Bertin et al. 2002). This was observed in tomato plants submitted to extended darkness where a strong repression of cell cycle genes inside fruit tissues accounts for the growth impairment (Baldet et al. 2002). Conversely, tomato plants grown under a low fruit load (one fruit per truss) displayed an increased photoassimilate availability in the plant, and an increased flower and fruit growth rates correlated with higher cell number due to an enhancement of mitotic activities inside the carpel (Baldet et al. 2006).

Modification of photoassimilate partitioning was studied by manipulating the activity of key enzymes involved in primary carbon metabolism and photosynthesis. Tomato plants were generated with the aim at overexpressing the *Arabidopsis* hexokinase 1 (*AtHXK1*) gene (Menu et al. 2003). The constitutive overexpression

of *AtHXK1* in tomato plants resulted in marked phenotypic and biochemical changes in developing fruits: fruits were indeed of reduced size as the cell expansion was greatly decreased; the carbon supply in transgenic fruits was lower throughout development, most probably due to decreased photosynthesis and because sucrose unloaded into these fruits was used to fuel cell metabolism at the expense of starch storage; fruit extracts displayed lower respiratory rates accompanied by lower ATP levels and ATP/ADP ratios indicating profound metabolic perturbations.

As to establish a link between glycolysis, synthesis of hexose phosphates and their conversion into organic acids, transgenic tomato plants silenced for the mitochondrial TCA cycle-associated malate dehydrogenase (mMDH) gene were generated (Nunes-Nesi et al. 2005). These RNAi-mMDH plants showed not only enhanced chloroplastic-electron transport rate and photosynthetic activity but also increased fruit biomass (expressed as dry matter), indicating that the repression of mMDH improves carbon assimilation. Interestingly, these transgenic fruits accumulated the redox-related compound ascorbate, with an increased capacity to use L-galactonolactone, the terminal precursor of ascorbate biosynthesis, as a respiratory substrate. Relevant to this former observation, silencing the L-galactono-1,4-lactone dehydrogenase activity which catalyzes the last step in ascorbate biosynthesis modified significantly the mitochondrial function and altered ascorbate redox state, as well as primary metabolism, like the tricarboxylic acid cycle in tomato (Alhagdow et al. 2007). As a consequence, plant and fruit growth were deeply reduced, as cell expansion was affected. Additionally fruits from tomato plants silenced for the GDP-D-mannose 3,5-epimerase (GME), the central enzyme in ascorbate biosynthesis, exhibited growth defects affecting cell division, cell expansion and non-cellulosic cell-wall polysaccharide biosynthesis (Gilbert et al. 2009). Taken together, these findings indicate an ascorbate-mediated link between the energy-generating processes of respiration and photosynthesis, primary metabolism and developmental processes, so important for fruit growth.

Obiadalla-Ali et al. (2004) generated tomato plants where the activity of the chloroplastic isoform of fructose 1,6-bisphosphatase (cp-FBPase), an important enzyme in control of the Calvin cycle, was fruit-specifically repressed. Although the overall carbohydrate metabolism was only slightly altered, fruit growth and final fruit size were significantly reduced, suggesting that cp-FBPase contributes to fruit photosynthesis in providing carbon for the growth of the fruits.

The search for genomic regions spanning QTL connected to yield-associated traits identified nine candidate genes located to tomato chromosome 4 (Bermúdez et al. 2008). Among these genes, a DnaJ chaperone-like encoding gene was isolated and seemed to be associated with changes in primary metabolites across tomato fruit development. The *in planta* functional analysis of this gene, subsequently named *SPA* for *sugar partitioning affecting*, demonstrated that SPA participates in determining the harvest index in tomato by affecting source-sink carbon distribution (Bermúdez et al. 2014). SPA as a putative chaperone protein was shown to act through a mechanism involving the regulation of phosphoglucomutase, sugar kinase and invertase enzyme activities during tomato fruit growth.

8.10 Conclusion

Tomato has been imposed as the model species for all fleshy fruits, since it presents both agronomical and scientific advantages. Indeed tomato, as a representative member of the Solanaceae family which is the third most important plant taxon at the economic level and the most valuable in terms of vegetable crops, is ranked first in world fruit production and represents the main income for major vegetable seed companies. Within the Solanaceae, the organization of genomes presents an exceptionally high degree of conservation, thus rendering this a unique subject to explore the basis of phenotypic diversity and adaptation to natural and agricultural environments. Besides these agronomical advantages, tomato has become the research model species for all fleshy fruits. Indeed, tomato displays a highly favourable biology with short life cycle, high multiplication rate, easy crosses and self-pollination. Over the last 15 years, the wide range of genetic resources (cultivars, mutants, segregating populations) covered by the large morphological diversity encountered in cultivated tomato varieties has been exploited, together with the development of molecular tools (marker-assisted mapping of quantitative traits; positional cloning of genes; reverse genetic tools such as *Agrobacterium*-mediated stable and transient transformation; VIGS, virus-induced gene silencing; TILLING technology, targeting induced local lesions in genomes) to unveil the genetic basis of important traits such as fruit yield, fruit size and shape determination. The release of the full tomato genome sequence (Tomato Genome Consortium 2012) now represents an extraordinary benefit to rely on not only for developmental studies on fleshy fruits but also for breeding programmes aimed at improving fruit production and fruit quality traits.

Tomato was the first species for which a genetically engineered crop product has been commercialized, namely, the FLAVR SAVR™ tomato that was brought to the market in 1994 (Kramer and Redenbaugh 1994). This genetically engineered tomato was designed as producing fruits with longer shelf-life by repressing the cell-wall pectin-degrading enzyme polygalacturonase. Since then many biotechnological approaches via transgenesis (without further marketing) were proven efficient and useful to modify essential quality traits such as fruit size, shape and composition. In this review, we decided to focus mainly on developmental and cellular processes that relate to the determination of the above-mentioned traits in fleshy fruits and described various functional analyses leading to fruit size modifications using transgenic tomato plants, summarized in Table 8.1. Although the perception of transgenic use is far from being accepted, these functional analyses provide wealthy informations about genes as likely candidate for genetic engineering and biotechnological improvement of fruit quality.

Table 8.1 Genes with strong effect on fruit size

Gene coding for	Biochemical function	Modification	Trait affected	References
Cell cycle control				
SlCDKA;1	Cyclin-dependent kinase (CDK)	Antisense (fruit specific)	Slightly smaller fruits; reduced nb of cell layers in exocarp	Czerednik et al. (2012)
SlCDKB1	Cyclin-dependent kinase (CDK)	Overexpression (fruit specific)	Slightly smaller fruits; reduced nb of cell layers in exocarp	Czerednik et al. (2012)
SlCDKB2	Cyclin-dependent kinase (CDK)	Overexpression (fruit specific)	Slightly smaller fruits; reduced nb of cell layers in exocarp	Czerednik et al. (2012)
SlCSS52A	APC/C activator	Antisense (ectopic)	Smaller fruits, smaller cells, decreased ploidy	Mathieu-Rivet et al. (2010)
SlCSS52A	APC/C activator	Overexpression (ectopic)	Slightly smaller fruits; larger cells; increased ploidy	Mathieu-Rivet et al. (2010)
SlCSS52B	APC/C activator	Antisense (ectopic)	Larger fruits, larger cells; increased ploidy	Gévaudant et al. (unpublished data)
SlWEE1	Inhibitory phosphorylation of CDK	Antisense (ectopic)	Smaller fruits, smaller cells, decreased ploidy	Gonzalez et al. (2007)
SlKRP1	CDK inhibitor	Overexpression (fruit specific)	Fruit size unchanged; cell size unchanged; decreased ploidy	Nafati et al. (2011)
DDB1	Component of CUL4-based E3 ligase, epigenetic regulation of cell cycle genes	Overexpression (ectopic)	Smaller fruits; reduced nb of cell layers in exocarp	Liu et al. (2012)
Fruit shape and fruit size/weight				
OVATE	Ovate family protein (transcriptional repressors)	Overexpression (ectopic)	Reduces the size of floral organs; complements the pear-shaped phenotype	Liu et al. (2002)
SUN	IQ domain family protein	Overexpression (ectopic)	Modifies the direction of cell division in septum and pericarp; elongated fruits	Wu et al. (2011)

(continued)

Table 8.1 (continued)

Gene coding for	Biochemical function	Modification	Trait affected	References
SlFASCIATED	YABBY transcription factor	Loss-of-function mutation	Larger fruits with high locule number	Cong et al. (2008)
LOCULE NUMBER (LC)	Post-transcriptional regulation of WUSCHEL	Single-nucleotide polymorphism	Larger fruits with increased number of locules	Muños et al. (2011)
FW2.2	Unknown	Overexpression (ectopic)	Smaller fruits with reduced cell divisions	Liu et al. (2003)
FW3.2/KLUH	Cytochrome P450 (CYP78A subfamily)	RNAi (ectopic)	Smaller fruits with reduced nb of cell layers in pericarp; reduced nb of seeds	Chakrabarti et al. (2013)
FW11.3	Unknown	Not determined	Larger fruits	Huang and Van der Knaap (2011)
SlFSM1	MYB-like transcription factor	Overexpression (ectopic)	Smaller fruits; smaller cells	Machemer et al. (2011)
TAGL1	AGAMOUS-like, MADS-box protein	RNAi (ectopic)	Smaller fruits; reduced nb of cell layers in pericarp	Vrebalov et al. (2009)
Hormone signalling				
SlARF7	Auxin signal transduction	RNAi (ectopic)	Parthenocarpic fruits; heart-like shape; thick pericarp with increased cell expansion	De Jong et al. (2009)
SlIAA9	Auxin signal transduction	Antisense (ectopic)	Parthenocarpic fruits; enlarged ovary; fused sepals	Wang et al. (2005)
SlPIN4	Auxin efflux transporter	RNAi (ectopic)	Parthenocarpic and smaller fruits; enlarged ovary in obligate parthenocarpic fruits	Mounet et al. (2012)
SlPIN4	Auxin efflux transporter	RNAi (ectopic)	No alterations in fruit development	Pattison and Catalá (2012)
SlTIR1	Auxin receptor	Overexpression (ectopic)	Parthenocarpic fruits; enlarged ovary	Ren et al. (2011)
SlGA20ox1	GA biosynthesis	Co-suppression (ectopic)	Parthenocarpic fruits	Olimpieri et al. (2011)

(continued)

Table 8.1 (continued)

Gene coding for	Biochemical function	Modification	Trait affected	References
SlGA20ox1, SlGA20ox2, SlGA20ox3	GA biosynthesis	RNAi (ectopic)	No effects on fruit set or fruit development	Xiao et al. (2006)
GA20 oxidase (citrus)	GA biosynthesis	Overexpression (ectopic)	Higher fruit yield; higher fruit weight; reduced nb of seeds	Garcia-Hurtado et al. (2012)
SlDELLA	GA signal transduction	Loss-of-function mutation	Parthenocarpic and smaller fruits; thicker pericarp; additional fruit-like structures	Martí et al. (2007)
SlIMA	Signal transduction	RNAi (ectopic)	Larger fruits; increased nb of carpels; larger floral meristem	Sicard et al. (2008a)
SlIMA	Signal transduction	Overexpression (ectopic)	Smaller fruits; smaller floral meristem; increased nb of ovules	Sicard et al. (2008b)
Primary metabolism				
LIN5	Cell-wall invertase	Single-nucleotide polymorphism	Increased fruit yield; increased total soluble sugar content	Fridman et al. (2004)
SlTIV1	Acid invertase, b-fructosidase	Antisense (ectopic)	Reduced fruit growth; increased sucrose and decreased hexose sugar contents	Klann et al. (1996)
SuSY	Sucrose synthase	Antisense (ectopic)	Reduced fruit set and early development; reduced sucrose unloading	D'Aoust et al. (1999)
AtHXK1	Hexokinase	Overexpression (ectopic)	Smaller fruits; reduced cell expansion, reduced carbon supply	Menu et al. (2003)
mMDH	Mitochondrial malate dehydrogenase	RNAi (ectopic)	Increased fruit biomass; improved carbon assimilation	Nunes-Nesi et al. (2005)
cp-FBPase	Chloroplastic 1,6-fructose bisphosphatase	Antisense (patatin B33 fruit specific)	Smaller fruits; impaired carbon allocation	Obiadalla-Ali et al. (2004)

(continued)

Table 8.1 (continued)

Gene coding for	Biochemical function	Modification	Trait affected	References
SPA (sugar partitioning affecting)	DNAJ chaperone-like	RNAi (ectopic)	Increased fruit weight; increased harvest index; modified source-sink relationships	Bermúdez et al. (2014)
Secondary metabolism				
L-Galactono-1,4-lactone dehydrogenase	Ascorbate (vitamin C) synthesis	RNAi (ectopic)	Reduced fruit growth; reduced cell expansion	Alhagdow et al. (2007)
GDP-D-mannose 3,5-epimerase	Ascorbate (vitamin C) synthesis	RNAi (ectopic)	Reduced fruit growth; reduced cell division and expansion; loss of firmness	Gilbert et al. (2009)

References

Adachi S, Minamisawaa K, Okushima Y et al (2011) Programmed induction of endoreduplication by DNA double-strand breaks in Arabidopsis. Proc Natl Acad Sci USA 108:10004–10009

Alhagdow M, Mounet F, Gilbert L et al (2007) Silencing of the mitochondrial ascorbate synthesizing enzyme L-galactono-1,4-lactone dehydrogenase affects plant and fruit development in tomato. Plant Physiol 145:1408–1422

Alpert KB, Tanksley SD (1996) High-resolution mapping and isolation of a yeast artificial chromosome contig containing fw2.2: a major fruit weight quantitative trait locus in tomato. Proc Natl Acad Sci USA 93:15503–15507

Alpert KB, Grandillo S, Tanksley SD (1995) *fw2.2*: a major QTL controlling fruit weight is common to both red- and green-fruited tomato species. Theor Appl Genet 91:994–1000

Ariizumi T, Shinozaki Y, Ezura H (2013) Genes that influence yield in tomato. Breed Sci 63:3–13

Baldet P, Devaux C, Chevalier C et al (2002) Contrasted responses to carbohydrate limitation in tomato fruit at two stages of development. Plant Cell Environ 25:1639–1649

Baldet P, Hernould M, Laporte F et al (2006) The expression of cell proliferation-related genes in early developing flower is affected by fruit load reduction in tomato plants. J Exp Bot 57:961–970

Barg R, Sobolev I, Eilon T et al (2005) The tomato early fruit specific gene *Lefsm1* defines a novel class of plant-specific SANT/MYB domain proteins. Planta 221:197–211

Barow M (2006) Endopolyploidy in seed plants. BioEssays 28:271–281

Barow M, Meister A (2003) Endopolyploidy in seed plants is differently correlated to systematics, organ, life strategy and genome size. Plant Cell Environ 26:571–584

Barrero LS, Tanksley SD (2004) Evaluating the genetic basis of multiple-locule fruit in a broad cross section of tomato cultivars. Theor Appl Genet 109:669–679

Barrero LS, Cong B, Wu F, Tanksley SD (2006) Developmental characterization of the fasciated locus and mapping of Arabidopsis candidate genes involved in the control of floral meristem size and carpel number in tomato. Genome 49:991–1006

Beemster G, Fiorani F, Inzé D (2003) Cell cycle: the key to plant growth control? Trends Plant Sci 8:154–158

Bergervoet JHW, Verhoeven HA, Gilissen LJW, Bino RJ (1996) High amounts of nuclear DNA in tomato (*Lycopersicon esculentum* Mill.) pericarp. Plant Sci 116:141–145

Bermúdez L, Urias U, Milstein D et al (2008) A candidate gene survey of quantitative trait loci affecting chemical composition in tomato fruit. J Exp Bot 59:2875–2890
Bermúdez L, de Godoy F, Baldet P et al (2014) Silencing of the tomato Sugar Partitioning Affecting protein (SPA), modifies sink strength through a shift in leaf sugar metabolism. Plant J 77:676–687
Bertin N, Gautier H, Roche C (2002) Number of cells in tomato fruit depending on fruit position and source-sink balance during plant development. Plant Growth Regul 36:105–112
Bertin N, Causse M, Brunel B et al (2009) Ecophysiological processes involved in genetic variation of in tomato fruit size and composition. J Exp Bot 60:237–248
Bohner J, Bangerth F (1988) Cell number, cell size and hormone levels in semi-isogenic mutants of *Lycopersicon pimpinellifolium* differing in fruit size. Physiol Plant 72:316–320
Boudolf V, Vlieghe K, Beemster GTS et al (2004) The plant-specific Cyclin-Dependent Kinase CDKB1;1 and transcription factor E2Fa-DPa control the balance of mitotically dividing and endoreduplicating cells in Arabidopsis. Plant Cell 16:2683–2692
Boudolf V, Lammens T, Boruc J et al (2009) CDKB1;1 forms a functional complex with CYCA2;3 to suppress endocycle onset. Plant Physiol 150:1482–1493
Bourdon M, Coriton O, Pirrello J et al (2011) In planta quantification of endoreduplication using fluorescent in situ hybridization (FISH). Plant J 66:1089–1099
Bourdon M, Pirrello J, Cheniclet C et al (2012) Evidence for karyoplasmic homeostasis during endoreduplication and a ploidy-dependent increase in gene transcription during tomato fruit growth. Development 139:3817–3826
Bramsiepe J, Wester K, Weinl C et al (2010) Endoreplication controls cell fate maintenance. PLoS Genet 6, e1000996
Carrari F, Fernie AR (2006) Metabolic regulation underlying tomato fruit development. J Exp Bot 57:1883–1897
Causse M, Saliba-Colombani V, Lecomte L et al (2002) Genetic analysis of fruit quality attributes in fresh market tomato. J Exp Bot 53:2089–2098
Causse M, Chaïb J, Lecomte L et al (2007) Both additivity and epistasis control the genetic variation for fruit quality traits in tomato. Theor Appl Genet 115:429–442
Ceccarelli M, Sanantonio E, Marmottini F et al (2006) Chromosome endoreduplication as a factor of salt adaptation in *Sorghum bicolor*. Protoplasma 227:113–118
Chakrabarti M, Zhang N, Sauvage C et al (2013) A cytochrome P450 regulates a domestication trait in cultivated tomato. Proc Natl Acad Sci USA 110:17125–17130
Cheniclet C, Rong WY, Causse M et al (2005) Cell expansion and endoreduplication show a large genetic variability in pericarp and contribute strongly to tomato fruit growth. Plant Physiol 139:1984–1994
Chevalier C, Nafati M, Mathieu-Rivet E et al (2011) Elucidating the functional role of endoreduplication in tomato fruit development. Ann Bot 107:1159–1169
Chevalier C, Bourdon M, Pirrello J et al (2013) Endoreduplication and fruit growth in tomato: evidence in favour of the karyoplasmic ratio theory. J Exp Bot 65:2731–2746
Churchman ML, Brown ML, Kato N et al (2006) SIAMESE, a plant-specific cell cycle regulator, controls endoreplication onset in Arabidopsis thaliana. Plant Cell 18:3145–3157
Cong B, Tanksley SD (2006) FW2.2 and cell cycle control in developing tomato fruit: a possible example of gene co-option in the evolution of a novel organ. Plant Mol Biol 62:867–880
Cong B, Barrero LS, Tanksley SD (2008) Regulatory change in YABBY-like transcription factor led to evolution of extreme fruit size during tomato domestication. Nat Genet 40:800–804
Cookson SJ, Radziejwoski A, Granier C (2006) Cell and leaf size plasticity in Arabidopsis: what is the role of endoreduplication? Plant Cell Environ 29:1273–1283
Cools T, Iantcheva A, Weimer AK et al (2011) The Arabidopsis thaliana checkpoint kinase WEE1 protects against premature vascular differentiation during replication stress. Plant Cell 23:1435–1448
Coombe B (1976) The development of fleshy fruits. Annu Rev Plant Physiol 27:507–528
Czerednik A, Busscher M, Bielen BAM et al (2012) Regulation of tomato fruit pericarp development by an interplay between CDKB and CDKA1 cell cycle genes. J Exp Bot 63:2605–2617

Dahan Y, Rosenfeld R, Zadiranov V, Irihimovitch V (2010) A proposed conserved role for an avocado FW2.2-like gene as a negative regulator of fruit cell division. Planta 232:663–676
D'Amato F (1984) Role of polyploidy in reproductive organs and tissues. In: Johri BM (ed) Embryology of angiosperms. Springer, New York, pp 519–566
D'Aoust MA, Yelle S, Nguyen-Quoc B (1999) Antisense inhibition of tomato fruit synthase decreases fruit setting and the sucrose unloading capacity of young fruit. Plant Cell 11:2407–2418
De Franceschi P, Stegmeir T, Cabrera A et al (2013) Cell number regulator genes in Prunus provide candidate genes for the control of fruit size in sweet and sour cherry. Mol Breed 32:311–326
de Jong M, Wolters-Arts M, Feron R et al (2009) The Solanum lycopersicum auxin response factor 7 (SlARF7) regulates auxin signaling during tomato fruit set and development. Plant J 57:160–170
De Schutter K, Joubès J, Cools T et al (2007) Arabidopsis WEE1 kinase controls cell cycle arrest in response to activation of the DNA integrity checkpoint. Plant Cell 19:211–225
De Veylder L, Joubès J, Inzé D (2003) Plant cell cycle transitions. Curr Opin Plant Biol 6:536–543
De Veylder L, Larkin JC, Schnittger A (2011) Molecular control and function of endoreduplication in development and physiology. Trends Plant Sci 16:624–634
Edgar BA, Orr-Weaver TL (2001) Endoreplication cell cycles: more for less. Cell 105:297–306
Fernandez AI, Viron N, Alhagdow M et al (2009) Flexible tools for gene expression and silencing in tomato. Plant Physiol 151:1729–1740
Frary A, Nesbitt TC, Frary A et al (2000) *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit size. Science 289:85–88
Fridman E, Pleban T, Zamir D (2000) A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. Proc Natl Acad Sci USA 97:4718–4723
Fridman E, Carrari F, Liu YS et al (2004) Zooming in on a quantitative trait for tomato yield using interspecific introgressions. Science 305:1786–1789
Garcia-Hurtado N, Carrera E, Ruiz-Rivero O et al (2012) The characterization of transgenic tomato overexpressing *gibberellin 20-oxidase* reveals induction of parthenocarpic fruit growth, higher yield, and alteration of the gibberellin biosynthetic pathway. J Exp Bot 63:5803–5813
Gilbert L, Alhagdow M, Nunes-Nesi A et al (2009) GDP-D-mannose 3,5-epimerase (GME) plays a key role at the intersection of ascorbate and non-cellulosic cell-wall biosynthesis in tomato. Plant J 60:499–508
Gillaspy G, Ben-David H, Gruissem W (1993) Fruits: a developmental perspective. Plant Cell 5:1439–1451
Giovannoni J (2001) Molecular biology of fruit maturation and ripening. Annu Rev Plant Physiol Plant Mol Biol 52:725–749
Giovannoni J (2004) Genetic regulation of fruit development and ripening. Plant Cell 16:S170–S180
Gonzalez N, Gévaudant F, Hernould M et al (2007) The cell cycle-associated protein kinase WEE1 regulates cell size in relation to endoreduplication in developing tomato fruit. Plant J 51:642–655
Gonzalo MJ, van der Knaap E (2008) A comparative analysis into the genetic bases of morphology in tomato varieties exhibiting elongated fruit shape. Theor Appl Genet 116:647–656
Grandillo S, Ku HM, Tanksley SD (1999) Identifying loci responsible for natural variation in fruit size and shape in tomato. Theor Appl Genet 99:978–987
Guo M, Rupe MA, Dieter JA et al (2010) Cell Number Regulator1 affects plant and organ size in maize: implications for crop yield enhancement and heterosis. Plant Cell 22:1057–1073
Gutierrez C, Ramirez-Parra E, Castellano MM, Del Pozo JC (2002) G1 to S transition: more than a cell cycle engine switch. Curr Opin Plant Biol 5:480–486
Hackbusch J, Richter K, Müller J et al (2005) A central role of Arabidopsis thaliana ovate family proteins in networking and subcellular localization of 3-aa loop extension homeodomain proteins. Proc Natl Acad Sci USA 12:4908–4912
Harashima H, Shinmyo A, Sekine M (2007) Phosphorylation of threonine 161 in plant cyclin-dependent kinase A is required for cell division by activation of its associated kinase. Plant J 52:435–448

Hase Y, Trung KH, Matsunaga T, Tanaka A (2006) A mutation in the uvi4 gene promotes progression of endoreduplication and confers increased tolerance towards ultraviolet B light. Plant J 46:317–326

Heyman J, De Veylder L (2012) The anaphase-promoting complex/cyclosome in control of plant development. Mol Plant 5:1182–1194

Huang Z, Van der Knaap E (2011) Tomato fruit weight 11.3 maps close to fasciated on the bottom of chromosome 11. Theor Appl Genet 123:465–474

Hülskamp M, Schnittger A, Folkers U (1999) Pattern formation and cell differentiation: trichomes in Arabidopsis as a genetic model system. Int Rev Cytol 186:147–178

Inzé D, De Veylder L (2006) Cell cycle regulation in plant development. Annu Rev Genet 40:77–105

Jiang N, Gao D, Xiao H, van der Knaap E (2009) Genome organization of the tomato sun locus and characterization of the unusual retrotransposon Rider. Plant J 60:181–193

Joubès J, Chevalier C (2000) Endoreduplication in higher plants. Plant Mol Biol 43:737–747

Joubès J, Phan T-H, Just D et al (1999) Molecular and biochemical characterization of the involvement of Cyclin-Dependent Kinase CDKA during the early development of tomato fruit. Plant Physiol 121:857–869

Joubès J, Chevalier C, Dudits D et al (2000) Cyclin-dependent kinases related protein kinases in plants. Plant Mol Biol 43:607–621

Joubès J, Lemaire-Chamley M, Delmas F et al (2001) A new C-type Cyclin-Dependent Kinase from tomato expressed in dividing tissues does not interact with mitotic and G1 cyclins. Plant Physiol 126:1403–1415

Klann EM, Hall B, Bennett AB (1996) Antisense acid invertase (TIV1) gene alters soluble sugar composition and size in transgenic tomato fruit. Plant Physiol 112:1321–1330

Kondorosi E, Kondorosi A (2004) Endoreduplication and activation of the anaphase-promoting complex during symbiotic cell development. FEBS Lett 567:152–157

Kramer MG, Redenbaugh K (1994) Commercialization of a tomato with an antisense polygalacturonase gene: the FLAVR SAVR^{TM} story. Euphytica 79:293–297

Libault M, Zhang XC, Govindarajulu M et al (2010) A member of the highly conserved FWL (tomato FW2.2-like) gene family is essential for soybean nodule organogenesis. Plant J 62:852–864

Lippman Z, Tanksley SD (2001) Dissecting the genetic pathway to extreme fruit size in tomato using a cross between the small-fruited wild species Lycopersicon pimpinellifolium and L. esculentum var. Giant Heirloom. Genetics 158:413–422

Liu J, Van Eck J, Cong B, Tanksley SD (2002) A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. Proc Natl Acad Sci USA 99:13302–13306

Liu J, Cong B, Tanksley S (2003) Generation and analysis of an artificial gene dosage series in tomato to study the mechanisms by which the cloned quantitative trait locus fw2.2 controls fruit size. Plant Physiol 132:292–299

Liu J, Tang X, Gao L, Gao Y, Li Y, Huang S, Sun X, Miao M, Zeng H, Tian X, Niu X, Zheng L, Giovannoni J, Xiao F, Liu Y (2012) A role of tomato UV-Damaged DNA Binding Protein 1 (DDB1) in organ size control via an epigenetic manner. PLoS ONE 7:e42621

Machemer K, Shaiman O, Salts Y et al (2011) Interplay of MYB factors in differential cell expansion, and consequences for tomato fruit development. Plant J 68:337–350

Marrocco K, Bergdoll M, Achard P et al (2010) Selective proteolysis sets the tempo of the cell cycle. Curr Opin Plant Biol 13:631–639

Martí C, Orzáez D, Ellul P et al (2007) Silencing of *DELLA* induces facultative parthenocarpy in tomato fruits. Plant J 52:865–876

Mathieu-Rivet E, Gevaudant F, Sicard A et al (2010) The functional analysis of the Anaphase Promoting Complex activator CCS52A highlights the crucial role of endoreduplication for fruit growth in tomato. Plant J 62:727–741

Menu T, Saglio P, Granot D et al (2003) High hexokinase activity in tomato fruit perturbs carbon and energy metabolism and reduces fruit and seed size. Plant Cell Environ 27:89–98

Mizukami Y (2001) A matter of size: developmental control of organ size in plants. Curr Opin Plant Biol 4:533–539

Mounet F, Moing A, Kowalczyk M et al (2012) Down-regulation of a single auxin efflux transport protein in tomato induces precocious fruit development. J Exp Bot 63:4901–4917

Muños S, Ranc N, Botton E et al (2011) Increase in tomato locule number is controlled by two key SNP located near Wuschel. Plant Physiol 156:2244–2254

Nafati M, Cheniclet C, Hernould M et al (2011) The specific overexpression of a Cyclin Dependent Kinase Inhibitor in tomato fruit mesocarp cells uncouples endoreduplication and cell growth. Plant J 65:543–556

Nagl W (1976) DNA endoreduplication and polyteny understood as evolutionary strategies. Nature 261:614–615

Nunes-Nesi A, Carrari F, Lytovchenko A et al (2005) Enhanced photosynthetic performance and growth as a consequence of decreasing mitochondrial malate dehydrogenase activity in transgenic tomato plants. Plant Physiol 137:611–622

Obiadalla-Ali H, Fernie AR, Lytovchenko A et al (2004) Inhibition of chloroplastic fructose 1,6-bisphosphatase in tomato fruits leads to surprisingly small changes in carbohydrate metabolism and decreases fruit size. Planta 219:533–540

Olimpieri I, Caccia R, Picarella ME et al (2011) Constitutive co-suppression of the *GA 20-oxidase1* gene in tomato leads to severe defects in vegetative and reproductive development. Plant Sci 180:496–503

Paran I, van der Knaap E (2007) Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. J Exp Bot 58:3841–3852

Pattison RJ, Catalá C (2012) Evaluating auxin distribution in tomato (Solanum lycopersicum) through an analysis of the PIN and AUX/LAX gene families. Plant J 70:585–598

Prudent M, Causse M, Génard M et al (2009) Genetic and ecophysiological analysis of tomato fruit weight and composition – influence of carbon availability on QTL detection. J Exp Bot 60:923–937

Ren H, Santner A, Del Pozo JC et al (2008) Degradation of the cyclin-dependent kinase inhibitor KRP1 is regulated by two different ubiquitin E3 ligases. Plant J 53:705–716

Ren Z, Li Z, Miao Q, Yang Y et al (2011) The auxin receptor homologue in *Solanum lycopersicum* stimulates tomato fruit set and leaf morphogenesis. J Exp Bot 62:2815–2826

Rodríguez GR, Muños S, Anderson C et al (2011) Distribution of SUN, OVATE, LC, and FAS alleles in tomato germplasm and their effect on fruit morphology. Plant Physiol 156:275–285

Roeder AHK, Chickarmane V, Cunha A et al (2010) Variability in the control of cell division underlies sepal epidermal patterning in *Arabidopsis thaliana*. PLoS Biol 8, e1000367

Ruan YL, Patrick JW, Bouzayen M et al (2012) Molecular regulation of seed and fruit set. Trends Plant Sci 17:656–665

Sabelli PA, Liu Y, Dante RA et al (2013) Control of cell proliferation, endoreduplication, cell size, and cell death by the retinoblastoma-related pathway in maize endosperm. Proc Natl Acad Sci USA 110:E1827–E1836

Schnittger A, Weinl C, Bouyer D et al (2003) Misexpression of the cyclin-dependent kinase inhibitor ICK1/KRP1 in single-celled Arabidopsis trichomes reduces endoreduplication and cell size and induces cell death. Plant Cell 15:303–315

Serrani JC, Sanjuan R, Ruiz-Rivero O, Fos M, Garcia-Martinez JL (2007) Gibberellin regulation of fruit set and growth in tomato. Plant Physiol 145:246–257

Serrani JC, Ruiz-Rivero O, Fos M, Garcia-Martinez JL (2008) Auxin induced fruit-set in tomato is mediated in part by gibberellins. Plant J 56:922–934

Sicard A, Petit J, Mouras A et al (2008a) Meristem activity during flower and ovule development in tomato is controlled by the mini zinc finger gene *INHIBITOR OF MERISTEM ACTIVITY*. Plant J 55:415–427

Sicard A, Hernould M, Chevalier C (2008b) The INHIBITOR OF MERISTEM ACTIVITY (IMA) protein. The nexus between cell division, differentiation and hormonal control of development. Plant Signal Behav 3:908–910

Song WY, Choi KS, Kim DY et al (2010) Arabidopsis PCR2 is a zinc exporter involved in both zinc extrusion and long-distance zinc transport. Plant Cell 22:2237–2252

Sugimoto-Shirasu K, Roberts K (2003) "Big it up": endoreduplication and cell-size control in plants. Curr Opin Plant Biol 6:544–553

Tanksley SD (2004) The genetic, developmental, and molecular bases of fruit size and shape variation in tomato. Plant Cell 16:S181–S189

The Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635–641

Thévenot C, Simond-Côte E, Reyss A et al (2005) QTLs for enzyme activities and soluble carbohydrates involved in starch accumulation during grain filling in maize. J Exp Bot 56:945–958

Tohge T, Alseekh S, Fernie AR (2014) On the regulation and function of secondary metabolism during fruit development and ripening. J Exp Bot 65(16):4599–4611. doi:10.1093/jxb/ert443

Torres-Acosta JA, Fowke LC, Wang H (2011) Analyses of phylogeny, evolution, conserved sequences and genome-wide expression of the ICK/KRP family of plant CDK inhibitors. Ann Bot 107:1141–1157

van der Graaff E, Laux T, Rensing SA (2009) The WUS homeobox-containing (WOX) protein family. Genome Biol 10:248

Van der Knaap E, Tanksley SD (2003) The making of a bell pepper-shaped tomato fruit: identification of loci controlling fruit morphology in Yellow Stuffer tomato. Theor Appl Genet 107:139–147

Van Leene J, Hollunder J, Eeckhout D et al (2010) Targeted interactomics reveals a complex core cell cycle machinery in Arabidopsis thaliana. Mol Syst Biol 6:397

Verkest A, de O Manes CL, Vercruysse S et al (2005) The Cyclin-Dependent Kinase inhibitor KRP2 controls the onset of endoreduplication cycle during Arabidopsis leaf development through inhibition of mitotic CDKA;1 kinase complexes. Plant Cell 17:1723–1736

Vlieghe K, Inzé D, De Veylder L (2007) Physiological relevance and molecular control of the endocycle in plants. In: Inzé D (ed) Cell cycle control and plant development, annual plant reviews, vol 32. Blackwell, Oxford, pp 227–248

Vrebalov J, Pan IL, Arroyo AJ et al (2009) Fleshy fruit expansion and ripening are regulated by the Tomato SHATTERPROOF gene TAGL1. Plant Cell 21:3041–3062

Wang F, Sanz A, Brenner ML, Smith A (1993) Sucrose synthase, starch accumulation, and tomato fruit sink strength. Plant Physiol 101:321–327

Wang H, Jones B, Li Z et al (2005) The tomato *Aux*/*IAA* transcription factor *IAA9* is involved in fruit development and leaf morphogenesis. Plant Cell 17:2676–2692

Wang S, Chang Y, Guo J, Chen JG (2007) Arabidopsis Ovate Family Protein 1 is a transcriptional repressor that suppresses cell elongation. Plant J 50:858–872

Wang YK, Chang WC, Liu PF et al (2010) Ovate family protein 1 as a plant Ku70 interacting protein involving in DNA double-strand break repair. Plant Mol Biol 74:453–466

Wang S, Chang Y, Guo J et al (2011) Arabidopsis ovate family proteins, a novel transcriptional repressor family, control multiple aspects of plant growth and development. PLoS One 6: e23896

Wilson EB (1925) The cell in development and heredity. Macmillan, New York

Wu S, Xiao H, Cabrera A et al (2011) SUN regulates vegetative and reproductive organ shape by changing cell division patterns. Plant Physiol 157:1175–1186

Xiao J, Li H, Zhang J et al (2006) Dissection of GA 20-oxidase members affecting tomato morphology by RNAi-mediated silencing. Plant Growth Regul 50:179–189

Xiao H, Jiang N, Schaffner E et al (2008) A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. Science 319:1527–1530

Xing Y, Zhang Q (2010) Genetic and molecular bases of rice yield. Annu Rev Plant Biol 61:421–442

Yu Y, Steinmetz A, Meyer D et al (2003) The tobacco A-type cyclin, Nicta;CycA2;3, at the nexus of cell division and differentiation. Plant Cell 15:2763–2777

Chapter 9
Sugar Accumulation in Tomato Fruit and Its Modification Using Molecular Breeding Techniques

Chiaki Matsukura

9.1 Introduction

Fruit sweetness is one of the most important properties for determining the market value of both fresh and processed tomatoes (*Solanum lycopersicum* L.). Fruit sweetness is generally represented as the total soluble solids content (TSS, measured as °Brix). In fact, fruit sweetness primarily depends on the soluble sugar content and its composition. The soluble sugar content and composition largely influence not only the organoleptic quality of fruit but also its processing efficiency; fruits with higher soluble sugar content (i.e., less water content) provide economic advantages such as fewer concentration steps and saving transportation and raw material costs (Stark et al. 1996). Therefore, high fruit sugar content is profitable from a commercial point of view and has been one of the major targets of breeding programs, together with yield and disease resistance, in most berry fruit crops, including tomato. However, the development of new fruit varieties with high sugar content has depended primarily on organoleptic assessments by breeders because of insufficient information regarding the biological mechanism(s) controlling fruit sweetness. Over the last two decades, research on fruit metabolic physiology and functional genomics has produced substantial progress for quality breeding in tomato. In this chapter, recent research progress on the regulation mechanisms of sugar accumulation is introduced from a physiological point of view, and the applications of this research to organoleptic quality breeding in tomato are discussed.

Fruit development in tomato is generally classified into three stages (Ho and Hewitt 1986; Ho 1996). In the first stage, the cell number increases as a result of active cell division, which affects the potential mature fruit size. In the second

C. Matsukura (✉)
Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan
e-mail: matsukura.chiaki.fw@u.tsukuba.ac.jp

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_9

stage, the fruit size enlarges due to rapid cell expansion, and in the third stage, fruit ripening occurs and is associated with ethylene production and an increase in cell respiration. Generally, sugar accumulation in the fruit is thought to proceed by the following steps: (1) active assimilation (mainly sucrose) and water influx into the fruit via the vascular system, (2) sugar metabolism and the biosynthesis of starch as a transient storage form of carbohydrate, and (3) the breakdown of starch and intensive increases in the levels of hexose sugars, such as glucose and fructose (Dinar and Stevens 1981; Schaffer and Petreikov 1997). Steps (1) and (2) occur in the late first and second stages of tomato growth. Rapid sucrose hydrolysis and starch accumulation cause intensive sink strength in the immature fruit (Ho 1996). Step (3) occurs during ripening, which is accompanied by rapid fruit softening and drastic metabolic shifts in the major fruit components, such as organic acids, carotenoids, hexose sugars, and cell wall components (Carrari et al. 2006). Of course, these processes are affected by cultivation management and environmental conditions, such as temperature, rainfall level, humidity, and insolation conditions (Prudent et al. 2011). Our understanding of fruit carbohydrate status, including sugar content and composition, has generally been based on the concept that the futile cycles of sucrose/hexose interchange, which are governed by sucrose synthase, sucrose phosphatase synthase, and invertase, regulate the sink strength and sugar level and composition (Nguyen-Quoc and Foyer 2001). These enzymes are thought to be involved in the intra- and intercellular transport of sugars among vacuoles and the cytosol and apoplasts involved in futile cycles in cooperation with sucrose/hexose transporters. In this chapter, the basic mechanisms regulating sugar level and composition are first described, and several new findings reported by recent studies are introduced. Finally, the possibility of molecular breeding for the modification of sugar content and composition in tomato fruit is discussed.

9.2 Invertase Plays a Key Role in Determining Fruit Sugar Content and Composition

It is now well known that sugars function as signal molecules as well as carbon energy sources in the various stages of the plant life cycle. The amount and balance of sucrose and its cleavage products are particularly important in regulating both metabolism and development. There are two notable enzymes involved in the cleavage of disaccharide sucrose to monosaccharides: sucrose synthase and invertase. Because sugars are important regulators of gene expression in plants, these enzymes are thought to participate in the control of various developmental processes. Sucrose synthase (EC2.4.1.13) converts sucrose into fructose and UDP-glucose. By contrast, invertase (EC 3.2.1.26) irreversibly catalyzes the hydrolytic cleavage of sucrose into glucose and fructose. In tomato, sucrose synthase was considered to be a major factor determining the fruit sink strength because of the following reasons: (1) there is a strong correlation among sucrose synthase activity,

ADP-glucose pyrophosphorylase, and starch accumulation in early developing fruit (Robinson et al. 1988; Yelle et al. 1988); (2) sucrose synthase activity increases in parallel with fruit growth, and there is a linear correlation between its activity and the final fruit size in both wild species and commercial varieties (Sun et al. 1992); and (3) antisense transgenic plants in which the sucrose synthase activity was inhibited displayed reduced fruit setting and sucrose import capacity in young fruit (D'Aoust et al. 1999). Meanwhile, the suppression of the sucrose synthase gene in tomato did not lead to remarkable alterations in starch and sugar accumulation in the fruit (Chengappa et al. 1999). Although a clear correlation between sink strength and sucrose synthase activity has been observed, there is poor evidence that sucrose synthase is directly involved in the control of fruit sugar content and composition in tomato.

On the other hand, increasing evidence in the last two decades has indicated that invertase is an essential factor for regulating sugar content in tomato fruit. In plants, invertases are classified into three isozyme types—cell wall invertase (CWIN) (also often referred to as apoplastic or extracellular invertase), vacuolar invertase (VIN), and cytoplasmic invertase (CIN) (also referred to as neutral invertase)—according to their solubility, subcellular localization, isoelectric point (pI), and optimal pH (Sturm 1999). Among these isozyme types, CWIN and VIN are characterized as acid invertases because of their acidic optimal pH, whereas CIN is characterized as a neutral invertase due to its neutral optimal pH. Several studies have revealed the diverse roles of invertase in the plant life cycle, including its participation in various responses to abiotic and biotic stresses such as drought, hypoxia, high temperature, wounding, and pathogen infection. In addition, invertase regulates seed and pollen development, sugar composition in fruit, and sugar storage in sink organs, among other effects (see Roitsch and González 2004, for a review). Most of these processes are considered to result from modified gene expression and changes in carbohydrate partitioning, which are regulated by sucrose and its cleavage products as signal molecules. In plants, CWIN and VIN have similar properties except for their cellular localization and pI; that is, both enzymes are β-fructofuranosidases with acidic optimum pH values, are glycoproteins, and share high sequence homology (Unger et al. 1994). In contrast to CWIN and VIN, little information is available on CIN in tomato, and its physiological function has yet to be elucidated.

In tomato, genetic and biochemical analyses investigating differences in sugar composition between wild species and *S. lycopersicum*, from which most cultivars are derived, have proven that the two acid invertases CWIN and VIN are involved in the determination of fruit sugar content and composition in different stages (Yelle et al. 1988; Klann et al. 1993; Fridman et al. 2000; Husain et al. 2001; Miron et al. 2002). Utilizing progeny lines derived from interspecific crossing between *S. lycopersicum* and a wild species, *S. chmielewskii*, which mainly accumulates sucrose instead of reducing hexoses in its fruit, Klann et al. (1993) revealed a lack of VIN activity during fruit maturation, which resulted in the sucrose-accumulation property of *S. chmielewskii*. In addition, *S. pimpinellifolium* showed higher VIN activity and hexose contents in red fruit compared with *S. lycopersicum* (Husain et al. 2001). These results indicate that VIN changes the fruit sugar balance

to high hexose and low sucrose during maturation in tomato. This observation was also supported by a transgenic approach utilizing antisense transgenic tomato plants in which VIN gene (*TIV1*) expression was suppressed, resulting in a marked increase in sucrose and a decrease in hexose content in the fruit (Ohyama et al. 1995). Interestingly, although the invertase activity was largely suppressed in the transgenic tomato, remarkable differences in fruit development and total soluble solids were not observed compared with a non-transgenic plant. These results suggest that vacuole-localizing acid invertase expression in the later fruit developmental stage is not essential for determining the total sugar level and fruit sink strength.

During the last decade, a cell wall invertase has attracted attention for its influence on the total soluble solids in tomato. CWIN is thought to be an essential enzyme for supplying carbohydrates into sink organs through the hydrolytic cleavage of phloem-unloaded sucrose to hexose. To date, four genes encoding CWIN—*LIN5*, *LIN6*, *LIN7*, and *LIN8*—have been identified in tomato, and tissue-specific expression patterns have been reported (Godt and Roitsch 1997; Ohyama et al. 1998; Fridman and Zamir 2003). Transcriptional analyses have indicated that *LIN5*, *LIN6*, and *LIN7* are mainly expressed in the floral reproductive organs, whereas *LIN8* is expressed in the vegetative organs, such as the roots and leaves. Among the floral-expressing *LIN*s, whereas *LIN7* showed very limited expression in stamens and pollen, *LIN5* was strongly expressed in ovaries and immature green fruit in addition to in the floral organs. By contrast, *LIN6* showed a broader expression pattern, including expression in the roots, stems, leaves, and young fruit (Fridman and Zamir 2003; Ohyama et al. 2006). From a physiological point of view, the most interesting isozyme is most likely *LIN6*, as this gene responds to various biotic and abiotic stimuli, including wounding, pathogen infection, and sugars (Godt and Roitsch 1997; Ohyama et al. 1998; Sinha et al. 2002). *LIN6* expression is also induced in response to cytokines and brassinosteroids, a group of phytohormones known to promote cell division and active growth in plants (Godt and Roitsch 1997; Goetz et al. 2000). The responses of *LIN6* are considered essential in the process of supplying carbohydrates to damaged tissues and to growing organs to accommodate the increasing demand for metabolic energy. Among the other CWINs, *LIN7*, which is expressed specifically in stamens and pollen, was suggested to be involved in heat stress tolerance because the expression level of *LIN7* was significantly higher in a heat stress-tolerant variety than in a sensitive variety, and its expression was specifically promoted by heat stress in the heat-tolerant variety (Li et al. 2012). Li et al. (2012) also suggested that the high import ability of sucrose into young fruits contributes to the heat tolerance of the variety and should be provided by *LIN7* expression.

For fruit sugar content, the most important CWIN should be LIN5 because the encoding gene is the most abundantly expressed in the fruit in the early development stages (Godt and Roitsch 1997; Ohyama et al. 1998; Fridman and Zamir 2003). This assumption is further supported by a QTL mapping study utilizing an introgression line developed from an interspecific cross between *S. pennellii* and *S. lycopersicum* (Eshed and Zamir 1995). A field trial revealed that 23 QTLs were

related to a high total soluble solid content, and one of those was mapped on chromosome 9 as *Brix9-2-5* (Eshed and Zamir 1995, 1996). By fine mapping utilizing an F_2 hybrid population generated from near isogenic lines (NILs), *Brix9-2-5* was finally mapped to a 484 bp region ranging from exon 3 to exon 4 of the *LIN5* gene originating from *S. pennellii* (Fridman et al. 2000). Comparing the specific region of *LIN5*, there are several differences between the two species. For instance, three SNPs with amino acid substitutions, 18 bp and 7 bp repeat sequences, and a hypothetical ORF of 30 amino acids were found in the *S. pennellii*-derived sequence. Subsequent analyses revealed that one of the SNPs near the catalytic site facilitates the enzyme kinetics of *LIN5*, facilitates the uptake of assimilate, and results in increased TSS in the fruit of plants bearing the *Brix9-2-5* allele (Fridman et al. 2002, 2004). An RNAi-based approach confirmed the role of *LIN5* in the regulation of the fruit Brix content. In addition, the suppression of *LIN5* revealed its multiple functions in normal floral and fruit development, including functions related to morphology, size, number, and pollen development. Furthermore, reductions in phytohormone levels, such as ABA, JA, and GA, and the expression of genes associated with biosynthesis and/or with the response of those phytohormones were also observed in the *LIN5* RNAi plant, suggesting its role in hormone metabolism (Zanor et al. 2009).

These studies have led to substantial progress in understanding the genetic and transcriptional regulation processes governing the roles of invertase in plants. Additionally, during the last decade, increasing evidence has indicated that invertase activity is regulated by posttranscriptional suppression through its inhibitory protein (Hothorn et al. 2004; Jin et al. 2009). In tomato, the suppression of the invertase inhibitor (*INVINH1*) leads to posttranscriptional increases in CWIN activity, seed weight, and hexose levels in fruit without any alteration in VIN and CIN activities (Jin et al. 2009). The cellular and subcellular localization of *INVINH1* occur in the apoplast vasculature (phloem parenchyma) of young fruit; this expression pattern is consistent with that of *LIN5*. Other types of invertase inhibitors have also been reported in tomato, and one of these, SolyCIF, showed specific localization to the cell wall compartment (Reca et al. 2008). Invertase inhibitors localizing to both apoplasts and vacuoles have been identified in other crops (Rausch and Greiner 2004), indicating that *INVINH* comprises a gene family and functions as a modulator for invertase activity in an intracellular-specific manner.

As described above, regarding the physiological function of cytoplasmic invertase (CIN), there is little information available for plants because of its low and unstable enzymatic activity, and few genes have been isolated. In tomato continuous sucrose import during fruit ripening is important for a wild relative *Lycopersicum* species, *cheesmanii*, and there is a positive correlation between CIN activity and hexose levels in the fruit of plants exposed to salinity stress (Balibrea et al. 1996, 2006). These results indicate that CIN functions in a specific genetic background, such as wild *Lycopersicum* germplasm, or under specific environmental conditions, such as salinity stress.

9.3 Sucrose Unloading and Starch Accumulation in Immature Fruit Partially Determines the Fruit Sugar Level

Alongside invertase, early studies have related the starch level in the immature and mature green fruit stages to the level of soluble solids in ripe tomato fruit (Davies and Cocking 1965; Dinar and Stevens 1981; Robinson et al. 1988; Schaffer and Petreikov 1997). ADP-glucose pyrophosphorylase (AGPase, EC 2.7.7.27) is proposed to regulate starch biosynthesis during the early stages of fruit development (Schaffer and Petreikov 1997; Schaffer et al. 2000). AGPase catalyzes the synthesis of ADP-glucose from glucose-1-phosphate and ATP (Preiss 1988), which is the first regulatory step in starch biosynthesis in plants (Tsai and Nelson 1966; Lin et al. 1988; Müller-Röber et al. 1992; Stark et al. 1992). Plant AGPase is a hetero-tetrameric enzyme composed of two small and two large subunits (Morell et al. 1987). The former subunits function as the catalytic molecule, and the latter subunits function as allosteric modulators (Okita et al. 1990). In tomato AGPase, there are two isoforms of the small subunit and three isoforms of the large subunit (Chen and Janes 1997). One gene encoding the small subunit (*AgpS1*) and three genes encoding the large subunit (*AgpL1*, *AgL2*, and *AgL3*) have thus far been isolated as cDNAs (Chen et al. 1998; Park and Chung 1998). The predominant transcripts in developing fruit are *AgpL1* and *AgpS1*, and the expression of these two genes peaks during the early development stages, which are responsible for starch accumulation (Park and Chung 1998; Petreikov et al. 2006; Yin et al. 2010). Plant AGPase genes are regulated at the transcriptional level by phosphate, nitrate, and sugars (Müller-Röber et al. 1990; Scheible et al. 1997; Nielsen et al. 1998; Sokolov et al. 1998; Li et al. 2002). Additionally, our previous work revealed that *AgpS1* and *AgpL1* were specifically upregulated at the transcriptional level by salinity stress in early developing fruits in an ABA- and osmotic-stress-independent manner (Yin et al. 2010). In fact, the *AgpL1* response to salinity was found to be a sugar-mediated response, as evidenced by the elevated carbohydrate influx into the fruit under salinity stress (Yin et al. 2010). The observation that starch synthesis in fruit is dependent on the sugar supply is consistent with the results of N'tchobo et al. (1999). Auxin has also been demonstrated to be involved in starch biosynthesis and sugar accumulation. Amyloplast development and the gene expression of starch biosynthetic enzymes, including AGPase, were suppressed by auxin in tobacco-cultured cells (Miyazawa et al. 1999). In tomato, the downregulation of *auxin response factor 4* (*SlARF4*), a member of the transcription factor family regulating auxin-responsive genes, led to increased chlorophyll content with excessive numbers of chloroplasts in the fruit (Jones et al. 2002). Further analyses revealed that the *SlARF4*-suppressed line showed enhanced starch accumulation in early developing fruit and increased sugar contents in mature fruit, suggesting a negative role of *SlARF4* and auxin on starch and sugar accumulation in tomato fruit (Sagar et al. 2013).

Enhanced starch accumulation in young fruit was also observed in different germplasms with higher invertase activity and the total soluble solids content than

those of normal tomato cultivars, such as in the *S. pennellii*-derived introgression line possessing the *Brix9-2-5* allele (Robinson et al. 1988; Baxter et al. 2005). Expression analyses with the promoter-GUS transgenic plants driven by the *AgpL1* and *AgpS1* promoters revealed a high expression of both genes in the vascular tissue of young fruit, stems, and roots at the transcriptional level (Xing et al. 2005; Goto et al. 2013). Vasculature-specific expression was also observed for CWIN and for the invertase inhibitor gene in tomato (Jin et al. 2009). A CWIN has been thought to play a role in supplying carbohydrate energy to sink organs in the hexose form as well as in the apoplastic hydrolysis of sucrose. The associated expression pattern of these genes suggests the functional collaboration of CWIN and AGPase in vascular tissue. In contrast to invertase, sucrose synthase seems less likely to be related to starch synthesis in tomato fruit because a transgenic plant with suppressed sucrose synthase expression exhibited unaltered starch accumulation and sugar content in its fruits (Chengappa et al. 1999). However, Baroja-Fernández et al. (2012) reported the involvement of sucrose synthase in starch biosynthesis through cytoplasmic ADP-glucose production in *Arabidopsis*. The contribution of sucrose synthase to starch synthesis is still unclear in tomato.

9.4 Inhibition of the Sucrose Transporter Affects Fruit Sugar Content as well as Seed Development and Yield in Fruit

In plants, photoassimilates are mainly translocated as nonreducing disaccharides, such as sucrose, from the source leaves to the sink tissues/organs. The sucrose transporter (SUT, also known as SUC) is an essential membrane protein for the long-distance transport of sucrose, in particular at phloem loading/unloading in source/sink organs in higher plants. In tomato, three SUT genes—*LeSUT1*, *LeSUT2*, and *LeSUT4*—were isolated, and it was demonstrated that all proteins localize in the phloem sieve element (Barker et al. 2000; Weise et al. 2000). *LeSUT1* is specifically expressed in phloem companion cells in source leaves and is suggested to play a crucial role in phloem loading. *LeSUT2* is mainly expressed in sink organs, such as stems and fruits, and in anthers, whereas *LeSUT4* is expressed in ovaries and immature fruit (Barker et al. 2000; Hackel et al. 2006; Weise et al. 2000). Among these SUTs, the antisense inhibition of *LeSUT2* led to significant reductions in the soluble sugar (glucose, fructose, and sucrose) and starch contents in young fruits, which was accompanied by reductions in yield due to decreased fertility and fruit size (Hackel et al. 2006). Because there were no significant changes in the carbohydrate composition in the leaves of the antisense *SUT* transgenic lines, the results observed in the fruit were not due to a reduction in the sucrose supply from the source leaves. Although LeSUT2 has been suggested to act as a sucrose sensor (Barker et al. 2000), it has bilateral functions as a physiologically functional transporter of fruit sugar content. These results also showed

that the mode of phloem unloading is mediated by an apoplastic step in young fruit. Although early studies in the 1980s suggested that a symplastic pathway functions in young fruit, it is not likely to be associated with phloem unloading in sink organs. However, the function of LeSUT4 has still not been elucidated, although phylogenetic analyses have indicated that this transporter can be categorized as a type III SUT, which are associated with vacuolar membranes (Endler et al. 2006; Reinders et al. 2012). Therefore, LeSUT4 may function in the control of sugar composition in cooperation with vacuolar acid invertase. Taken together, these findings show that apoplastic invertase, AGPase, and sucrose transporters play essential roles in vascular apoplasts in a coordinated manner and the carbohydrate dynamism in early developing fruit is very important for determining the sugar level in red ripe fruit.

9.5 Novel Vacuolar Processing Enzyme Is Involved in the Control of Fruit Sugar Composition in Mature Fruit

Recently, newly isolated vacuolar processing enzymes (VPE) were demonstrated to participate in the modification of sugar content and composition in tomato fruit. VPE proteins are members of the cysteine proteinase family, which is well conserved among various organisms, including plants. VPE was originally identified as a cysteine proteinase involved in the processing of seed storage proteins (Hara-Nishimura et al. 1991). In tomato, five genes (*SlVPE1*–*SlVPE5*) coding a VPE protein were isolated and characterized: SlVPE1 and SlVPE2 as seed coat types, SlVPE4 as a seed type, and SlVPE3 and SlVPE5 as vegetative types (Ariizumi et al. 2011). Based on histochemical analyses of the promoter-GUS plants, both *SlVPE3* and *SlVPE5* exhibited specific expression in the vascular bundles from the seeds to the placenta and around the endocarp tissue in the fruit during all developmental stages. A transgenic approach revealed increased sugar accumulation in mature fruits of the RNAi tomato lines with decreased VPE expression. Among the SlVPEs, the suppression of *SlVPE5* had the greatest effect on the fruit sugar content. These results indicate that SlVPEs participate in the regulation of fruit sugar content as a negative regulator in tomato. The target proteins of SlVPEs have yet to be identified. In *Arabidopsis*, γVPE, an ortholog of SlVPE, targets various types of hydrolases, such β-glycosidase, α-galactosidase, and α-mannosidase (Rojo et al. 2003). Because γVPE is also involved in the proteolysis of a vacuolar invertase, β-FRUCTOSIDASE4, it is likely to display a similar mechanism to that of SlSVPs; that is, reduced VPE activity caused by the suppression of *SlVPE5* produces increased invertase activity in vacuoles, resulting in enhanced sugar accumulation in transgenic RNAi fruits (Ariizumi et al. 2011).

9.6 Molecular Breeding for the Modification of Fruit Sugar Content and Composition in Tomato

As described above, several experimental trials have successfully modified fruit sugar contents or composition in tomato by modifying the expression of target genes using transgenic approaches. Stark et al. (1996) developed transgenic tomato lines overexpressing a small subunit gene of a bacterial ADP-glucose pyrophosphorylase (*glgC16*) that displayed higher starch accumulation in young fruit and total soluble solids increased by approximately 20 % in mature fruit. No remarkable changes in the phenotype or growth of the plant or in the size and yield of fruits were observed. Indeed, an overexpression approach for sugar/starch biosynthetic enzyme genes may be a simple and effective way to generate new tomato varieties with high sugar contents. However, considering the recent consumer attitude against genetically modified crops (GMO), transgenic approaches would be undesirable for the development of a commercial variety. From this point of view, a knockdown strategy, such as mutant screening based on a reverse genetic approach, would be a valid option. In tomato, ethyl methanesulfonate (EMS)-mutagenized populations assisted by TILLING (Targeting Induced Local Lesions in Genomes; Till et al. 2004) have been developed (Okabe et al. 2011; Just et al. 2013). For a new variety with high sugar contents, negative regulators, such as invertase inhibitor (*INVINH1*), auxin response factor (*SlARF4*), and vacuolar processing protein (*SlVPE5*), are potential candidate genes for mutant screening.

During the last decade, the availability of genomic resources has rapidly expanded in tomato, including high-resolution linkage maps, DNA markers, ESTs, and the tomato genomic sequence (Tomato Genome Consortium 2012). In combination with these genomic resources, the availability of existing genetic resources, including wild species-derived introgression/recombinant inbred lines (Eshed and Zamir 1995; Fulton et al. 2002; Causse et al. 2004; Prudent et al. 2009), has also improved. With the accumulation of QTLs and/or mutation alleles for high sugar contents into an appropriate germplasm (so-called pyramiding), a more efficient and rapid breeding system will be established for a new cultivar with high organoleptic quality in the near future.

References

Ariizumi T, Higuchi K, Arakaki S, Sano T, Asamizu E, Ezura H (2011) Genetic suppression analysis in novel vacuolar processing enzymes reveals their roles in controlling sugar accumulation in tomato fruits. J Exp Bot 62:2773–2786

Balibrea ME, Santa Cruz AM, Bolarín M, Pérez-Alfocea F (1996) Sucrolytic activities in relation to sink strength and carbohydrate composition in tomato fruit growing under salinity. Plant Sci 118:47–55

Balibrea ME, Martinez-Andújar C, Cuartero J, Bolarín M, Pérez-Alfocea F (2006) The high fruit soluble sugar content in wild *Lycopersicon* species and their hybrids with cultivars depends on

sucrose import during ripening rather than on sucrose metabolism. Funct Plant Biol 33:279–288

Barker L, Kühn C, Weise A, Schulz A, Gebhardt C, Hirner B, Hellmann H, Schulze W, Ward JM, Frommer WB (2000) SUT2, a putative sucrose sensor in sieve elements. Plant Cell 12:1153–1164

Baroja-Fernández E, Muñoza FJ, Lia J, Bahajia A, Almagroa G, Monteroa M, Etxeberriac E, Hidalgoa M, Sesmaa MT, Pozueta-Romero J (2012) Sucrose synthase activity in the *sus1*/*sus2*/*sus3*/*sus4* Arabidopsis mutant is sufficient to support normal cellulose and starch production. Proc Natl Acad Sci USA 109:321–326

Baxter CJ, Carrari F, Bauke A, Overy S, Hill SA, Quick PW, Fernie AR, Sweetlove LJ (2005) Fruit carbohydrate metabolism in an introgression line of tomato with increased fruit soluble solids. Plant Cell Physiol 46:425–437

Carrari F, Baxter C, Usadel B, Urbanczyk-Wochniak E, Zanor MI, Nunes-Nesi A, Nikiforova V, Centero D, Ratzka A, Pauly M, Sweetlove LJ, Fernie AR (2006) Integrated analysis of metabolite and transcript levels reveals the metabolic shifts that underlie tomato fruit development and highlight regulatory aspects of metabolic network behavior. Plant Physiol 142:1380–1396

Causse M, Duffe P, Gomez MC, Buret M, Damidaux R, Zamir D, Gur A, Chevalier C, Lemaire-Chamley M, Rothan C (2004) A genetic map of candidate genes and QTLs involved in tomato fruit size and composition. J Exp Bot 55:1671–1685

Chen BY, Janes HW (1997) Multiple forms of ADP-glucose pyrophosphorylase from tomato fruit. Plant Physiol 113:235–241

Chen BY, Janes HW, Gianfagna T (1998) PCR cloning and characterization of multiple ADP-glucose pyrophosphorylase cDNA from tomato. Plant Sci 6:59–67

Chengappa S, Guilleroux M, Wendy P, Shields R (1999) Transgenic tomato plants with decreased sucrose synthase are unaltered in starch and sugar accumulation in the fruit. Plant Mol Biol 40:213–221

D'Aoust MA, Yelle S, Nguyen-Quoc B (1999) Antisense inhibition of tomato fruit sucrose synthase decrease fruit setting and the sucrose unloading capacity of young fruit. Plant Cell 11:2407–2418

Davies JN, Cocking EC (1965) Changes in carbohydrates, proteins and nucleic acids during cellular development in tomato fruit locule tissue. Planta 67:242–253

Dinar M, Stevens MA (1981) The relationship between starch accumulation and soluble solids content of tomato fruits. J Am Soc Hortic Sci 106:415–418

Endler A, Meyer S, Schelbert S, Schneider T, Weschke W, Peters SW, Keller F, Baginsky S, Martinoia E, Schmidt UG (2006) Identification of a vacuolar sucrose transporter in barley and arabidopsis mesophyll cells by a tonoplast proteomic approach. Plant Physiol 141:196–207

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141:1147–1162

Eshed Y, Zamir D (1996) Less-than-additive epistatic interactions of quantitative trait loci in tomato. Genetics 143:1807–1817

Fridman E, Zamir D (2003) Functional divergence of a syntenic invertase gene family in tomato, potato and Arabidopsis. Plant Physiol 131:603–609

Fridman E, Pleban T, Zamir D (2000) A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. Proc Natl Acad Sci USA 97:4718–4723

Fridman E, Liu YS, Carmel-Goren L, Shoresh AGM, Pleban T, Eshed Y, Zamir D (2002) Two tightly linked QTLs modify tomato sugar content via different physiological pathways. Mol Gen Genomics 266:821–826

Fridman E, Carrari F, Liu YS, Fernie AR, Zamir D (2004) Zooming in on a quantitative trait for tomato yield using interspecific introgressions. Science 305:1786–1789

Fulton TM, Bucheli P, Voirol E, López J, Pétiard V, Tanksley SD (2002) Quantitative trait loci (QTL) affecting sugars, organic acids, and other biochemical properties possibly contributing to flavor, identified in four advanced backcross populations of tomato. Euphytica 127:163–177

Godt D, Roitsch T (1997) Regulation and tissue-specific distribution of mRNAs for three extracellular invertase isoenzymes of tomato suggests an important function in establishing and maintaining sink metabolism. Plant Physiol 115:273–282

Goetz M, Godt D, Roitsch T (2000) Tissue-specific induction of the mRNA for an extracellular invertase isoenzyme of tomato by brassinosteroids suggests a role for steroid hormones in assimilate partitioning. Plant J 22:515–522

Goto Y, Nonaka S, Yin YG, Koiwa T, Asamizu E, EzuraH MC (2013) Isolation and characterisation of the ADP-glucose pyrophosphorylase small subunit gene (*AgpS1*) promoter in tomato (*Solanum lycopersicum* L.). Plant Biotechnol 30:279–286

Hackel A, Schauer N, Carrari F, Fernie AR, Grimm B, Kühn C (2006) Sucrose transporter LeSUT1 and LeSUT2 inhibition affects tomato fruit development in different ways. Plant J 45:180–192

Hara-Nishimura I, Inoue K, Nishimura M (1991) A unique vacuolar processing enzyme responsible for conversion of several proprotein precursors into the mature forms. FEBS Lett 294:89–93

Ho LC (1996) The mechanism of assimilate partitioning and carbohydrate compartmentation in fruit in relation to the quality and yield of tomato. J Exp Bot 47:1239–1243

Ho LC, Hewitt JD (1986) Fruit development. In: Atherton JG, Rudich J (eds) The tomato crop. Chapman and Hall, London, pp 201–240

Hothorn M, Wolf S, Aloy P, Greiner S, Scheffzek K (2004) Structural insights into the target specificity of plant invertase and pectin methylesterase inhibitory proteins. Plant Cell 16:3437–3447

Husain SE, James C, Shields R, Foyer CH (2001) Manipulation of fruit sugar composition but not content in *Lycopersicon esculentum* fruit by introgression of an acid invertase gene from *Lycopersicon pimpinellifolium*. New Phytol 150:65–72

Jin Y, Ni DA, Ruan YL (2009) Posttranslational elevation of cell wall invertase activity by silencing its inhibitor in tomato delays leaf senescence and increases seed weight and fruit hexose level. Plant Cell 21:2072–2089

Jones B, Frasse P, Olmos E, Zegzouti H, Li ZG, Latché A, Pech JC, Bouzayen M (2002) Down-regulation of DR12, an auxin-response-factor homolog, in the tomato results in a pleiotropic phenotype including dark green and blotchy ripening fruit. Plant J 32:603–613

Just D, Garcia V, Fernandez L, Bres C, Mauxion JP, Petit J, Jorly J, Assali J, Bournonville C, Ferrand C, Baldet P, Lemaire-Chamley M, Mori K, Okabe Y, Ariizumi T, Asamizu E, Ezura H, Rothan C (2013) Micro-Tom mutants for functional analysis of target genes and discovery of new alleles in tomato. Plant Biotechnol 30:225–231

Klann EM, Chetelat RT, Bennett AB (1993) Expression of acid invertase gene controls sugar composition in tomato *(Lycopersicon)* fruit. Plant Physiol 103:863–870

Li XY, Xing JP, Thomas JG, Harry JW (2002) Sucrose regulation of ADP-glucose pyrophosphorylase subunit genes transcript levels in leaves and fruits. Plant Sci 162:239–244

Li Z, Palmer WM, Martin AP, Wang R, Rainsford F, Jin Y, Patrick JW, Yang Y, Ruan YL (2012) High invertase activity in tomato reproductive organs correlates with enhanced sucrose import into, and heat tolerance of, young fruit. J Exp Bot 63:1155–1166

Lin TP, Caspar T, Somerville C, Preiss J (1988) A starch-deficient mutant of *Arabidopsis thaliana* with low ADP-glucose pyrophosphorylase activity lacks one of the two subunits of the enzyme. Plant Physiol 88:1175–1181

Miron D, Petreikov M, Carmi N, Shen S, Levin I, Granot D, Zamski E, Schaffer AA (2002) Sucrose uptake, invertase localization and gene expression in developing fruit of Lycopersicon esculentum and the sucrose-accumulating Lycopersicon hirsutum. Physiol Plant 115:35–47

Miyazawa Y, Sakai A, Miyagishima S, Takano H, Kawano S, Kuroiwa T (1999) Auxin and cytokinin have opposite effects on amyloplast development and the expression of starch synthesis genes in cultured bright yellow-2 tobacco cells. Plant Physiol 121:461–469

Morell MK, Bloom M, Knowles V, Preiss J (1987) Subunit structure of Spinach leaf ADP glucose pyrophosphorylase. Plant Physiol 85:182–187

Müller-Röber B, Kossamann J, Hannah LC, Willmitzer L, Sonnewald U (1990) One of two different ADP-glucose pyrophosphorylase genes from potato responds strongly to elevated levels of sucrose. Mol Gen Genomics 224:136–146

Müller-Röber B, Sonnewald U, Willmitzer L (1992) Inhibition of ADP-glucose pyrophosphorylase leads to sugar-storing tubers and influences tuber formation and expression of tuber storage protein genes. EMBO J 11:1229–1238

N'tchobo H, Dali N, Nguyen-Quoc B, Foyer CH, Yelle S (1999) Starch synthesis in tomato remains constant throughout fruit development and is dependent on sucrose supply and sucrose synthase activity. J Exp Bot 50:1457–1463

Nguyen-Quoc B, Foyer CH (2001) A role for 'futile cycles' involving invertase and sucrose synthase in sucrose metabolism of tomato fruit. J Exp Bot 52:881–889

Nielsen TH, Krapp A, Röper-Schwarz U, Stitt M (1998) The sugar-mediated regulation of genes encoding the small subunit of Rubisco and the regulatory subunit of ADP-glucose pyrophosphorylase is modified by nitrogen and phosphate. Plant Cell Environ 21:443–455

Ohyama A, Ito H, Sato T, Nishimura S, Imai T, Hirai M (1995) Suppression of acid invertase activity by antisense RNA modifies the sugar composition of tomato fruit. Plant Cell Physiol 36:369–376

Ohyama A, Nishimura S, Hirai M (1998) Cloning of cDNA for a cell wall-bound acid invertase from tomato (*Lycopersicon esculentum*) and expression of soluble and cell wall-bound invertases in plants and wounded leaves of *L. esculentum* and *L. peruvianum*. Genes Genet Syst 73:149–157

Ohyama A, Suwabe K, Nunome T, Fukuoka H (2006) Characterization of the promoter of the *Wiv-1*(*Lin6*) gene encoding a wound-inducible cell wall-bound acid invertase in tomato. Plant Biotechnol 23:365–371

Okabe Y, Asamizu E, Saito T, Matsukura C, Ariizumi T, Bres C, Rothan C, Mizoguchi T, Ezura H (2011) Tomato TILLING technology: development of a reverse genetics tool for the efficient isolation of mutants from Micro-Tom mutant libraries. Plant Cell Physiol 52:1994–2005

Okita TW, Nakata PA, Anderson JM, Sowokinos J, Morell M, Preiss J (1990) The subunit structure of potato tuber ADP-glucose pyrophosphorylase. Plant Physiol 93:785–790

Park SW, Chung WI (1998) Molecular cloning and organ-specific expression of three isoforms of tomato ADP-glucose pyrophosphorylase gene. Gene 206:215–221

Petreikov M, Shen S, Yeselson Y, Levin I, Bar M, Schaffer AA (2006) Temporally extended gene expression of the ADP-Glc pyrophosphorylase large subunit *(AgpL1)* leads to increased enzyme activity in developing tomato fruit. Planta 224:1465–1479

Preiss J (1988) Biosynthesis of starch and its regulation. In: Preiss J (ed) The biochemistry of plants, vol 14. Academic, San Diego, CA, pp 181–254

Prudent M, Causse M, Génard M, Tripodi P, Grandillo S, Bertin N (2009) Genetic and physiological analysis of tomato fruit weight and composition: influence of carbon availability on QTL detection. J Exp Bot 60:923–937

Prudent M, Lecomte A, Bouchet JP, Bertin N, Causse M, Genard M (2011) Combining ecophysiological modelling and quantitative trait locus analysis to identify key elementary processes underlying tomato fruit sugar concentration. J Exp Bot 62:907–919

Rausch T, Greiner S (2004) Plant protein inhibitors of invertases. Biochim Biophys Acta 16962:253–261

Reca IB, Brutus A, D'Avino R, Villard C, Bellincampi D, Giardina T (2008) Molecular cloning, expression and characterization of a novel apoplastic invertase inhibitor from tomato (*Solanum lycopersicum*) and its use to purify a vacuolar invertase. Biochimie 90:1611–1623

Reinders A, Sivitz AB, Ward JM (2012) Evolution of plant sucrose uptake transporters. Front Plant Sci 3:2–12
Robinson NL, Hewitt JD, Bennett AB (1988) Sink metabolism in tomato fruit. I. Developmental changes in carbohydrate metabolizing enzymes. Plant Physiol 87:727–730
Roitsch T, González MC (2004) Function and regulation of plant invertases: sweet sensations. Trends Plant Sci 9:606–613
Rojo E, Zouhar J, Carter C, Kovaleva V, Raikhel NV (2003) A unique mechanism for protein processing and degradation in Arabidopsis thaliana. Proc Natl Acad Sci USA 100:7389–7394
Sagar M, Chervin C, Mila I, Hao Y, Roustan JP, Benichou M, Gibon Y, Biais B, Maury P, Latché A, Pech JC, Bouzayen M, Zouine M (2013) SlARF4, an auxin response factor involved in the control of sugar metabolism during tomato fruit development. Plant Physiol 161:1362–1374
Schaffer AA, Petreikov M (1997) Sucrose-to-starch metabolism in tomato fruit undergoing transient starch accumulation. Plant Physiol 113:739–746
Schaffer AA, Levin I, Ogus I, Petreikov M, Cincarevsky F, Yeselson E, Shen S, Gilboa N, Bar M (2000) ADP-glucose pyrophosphorylase activity and starch accumulation in immature tomato fruit: the effect of a *Lycopersicon hirsutum*-derived introgression encoding for the large subunit. Plant Sci 152:135–144
Scheible WR, Gonzàlez-Fontes A, Lauerer M, Müller-Röber B, Caboche M, Stitt M (1997) Nitrate acts as a signal to induce organic acid metabolism and repress starch metabolism in tobacco. Plant Cell 9:783–798
Sinha AK, Hofmann MG, Römer U, Köckenberger W, Elling L, Roitsch T (2002) Metabolizable and non-metabolizable sugars activate different signal transduction pathways in tomato. Plant Physiol 128:1480–1489
Sokolov LN, Dejardin A, Kleczkowski LA (1998) Sugars and light/dark exposure trigger differential regulation of ADP-glucose pyrophosphorylase genes in *Arabidopsis thaliana* (thale cress). Biochem J 336:681–687
Stark DM, Timmerman KP, Barry GF, Preiss J, Kishore GM (1992) Regulation of the amount of starch in plant tissues by ADP-glucose pyrophosphorylase. Science 258:287–292
Stark DM, Barry GF, Kishore GM (1996) Improvement of fruit quality traits through enhancement of starch biosynthesis. Ann N Y Acad Sci 792:26–36
Sturm A (1999) Invertases. primary structures, functions, and roles in plant development and sucrose partitioning. Plant Physiol 121:1–7
Sun J, Loboda T, Sung S-JS, Black CC (1992) Sucrose synthase in wild tomato, *Lycopersicon chmielewskii*, and tomato fruit sink strength. Plant Physiol 98:1163–1169
The Tomato Genome Consortium (TGC) (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635–641
Till BJ, Reynolds SH, Weil C, Springer N, Burtner C, Kim Y, Bowers E, Codomo CA, Enns LC, Odden AR, Greene EA, Comai L, Henikoff S (2004) Discovery of induced point mutations in maize genes by TILLING. BMC Plant Biol 4:12
Tsai CY, Nelson OE (1966) Starch-deficient maize mutant lacking adenosine diphosphate glucose pyrophosphorylase activity. Science 151:341–343
Unger C, Hardegger M, Lienhard S, Sturm A (1994) cDNA cloning of carrot (*Daucus carota*) soluble acid, 6-fructofuranosidases and comparison with the cell wall isoenzyme. Plant Physiol 104:1351–1357
Weise A, Barker L, Kühn C, Lalonde S, Buschmann H, Frommer WB, Ward JM (2000) A new subfamily of sucrose transporters, SUT4, with low affinity/high capacity localized in enucleate sieve elements of plants. Plant Cell 12:1345–1355
Xing J, Li X, Luo Y, Gianfagna TJ, Janes HW (2005) Isolation and expression analysis of two tomato ADP-glucose pyrophosphorylase S(large)subunit gene promoters. Plant Sci 169:882–893

Yelle S, Hewitt JD, Nieder M, Robinson NL, Damon S, Bennett AB (1988) Sink metabolism in tomato fruit. III. Analysis of carbohydrate assimilation in a wild species. Plant Physiol 87:731–736

Yin YG, Kobayashi Y, Sanuki A, Kondo S, Fukuda N, Ezura H, Sugaya S, Matsukura C (2010) Salinity induces carbohydrate accumulation and sugar-regulated starch biosynthetic genes in tomato (*Solanum lycopersicum* L. cv. 'Micro-Tom') fruits in an ABA- and osmotic stress-independent manner. J Exp Bot 61:563–574

Zanor MI, Osorio S, Nunes-Nesi A, Carrari F, Lohse M, Usadel B, Kühn C, Bleiss W, Giavalisco P, Willmitzer L, Sulpice R, Zhou YH, Fernie AR (2009) RNA interference of LIN5 in tomato confirms its role in controlling brix content, uncovers the influence of sugars on the levels of fruit hormones, and demonstrates the importance of sucrose cleavage for normal fruit development and fertility. Plant Physiol 150:1204–1218

Chapter 10
Fruit Ripening in Tomato and Its Modification by Molecular Breeding Techniques

Kyoko Hiwasa-Tanase

10.1 Introduction

Fruits are indispensable in the human diet because plants make their fruit attractive as a strategy to spread their seeds. Humans have been fascinated by the nutritional quality and taste of fruits, and to stably supply fruit, we have increased and spread accidentally found plants that had desirable properties in premodern times. Systematic crossbreeding started after "rediscovering" Mendelian inheritance in the 1900s. However, crossing and selection must be repeated millions of times to create a cultivated variety of fruit with desirable traits because the selection must be performed based on visible and measurable traits, which may vary with the environment. Molecular biological techniques have progressed greatly in recent years. These techniques have been made available and developed for breeding. Plant molecular breeding uses molecular biology techniques and genetic information to select desirable plants or genetic engineering techniques to introduce desirable traits into plants. The former tool is called marker-assisted selection, and systematic and effective methods make plant selection easy and fast. Moreover, breeding using transgenic techniques makes it possible to use the gene of a plant that cannot be crossed. The variety of traits that can be added via these methods has opened up the possibility of creating new types of fruit.

Tomato (*Solanum lycopersicum*) is a primary horticultural crop that is mass-produced and widely consumed throughout the world (Klee and Giovannoni 2011), and it is the most studied model system for fruit ripening. Its ripening is characteristic of the climacteric type of fruits, which includes apple, avocado, banana, pear, peach, and melon. In these fruits, including tomato, ethylene is a critical phytohor-

K. Hiwasa-Tanase (✉)
Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan
e-mail: kytanase@gene.tsukuba.ac.jp

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_10

mone for normal ripening. Its production and perception trigger various aspects of the ripening process, including fruit softening, distinct color changes (pigment accumulation), texture conversion, aromatic compound production, and other biochemical and physiological events. Therefore, in addition to having economic importance, studies on tomatoes have focused on understanding ripening in light of its role as a climacteric model. A variety of genetic resources, well-characterized ripening mutant collections, and transgenic techniques in addition to extensive physiological and biochemical investigations have accumulated during the tomato's long history and have contributed to the facilitation of molecular genetics and molecular biological studies. Currently, genetic information from high-density genetic maps, QTL (quantitative trait locus) information, expressed sequence tag (EST) resources, microarrays, full-genome sequencing data, abundant and organized DNA markers, and omics data collections is extensively and continuously accumulating (Giovannoni 2007). These resources can all be utilized for novel fruit creation as breeding tools and breeding materials, particularly through the identification of useful candidate genes and loci.

What types of traits do producers and customers request in ripening fruit? For producers, the desirable attributes are likely the ease of harvesting and transporting the fruit, the shortening of the fruit maturation and ripening periods, the ease of evaluating the proper harvest time and excellent shelf life, the ability to sell for a high price, and the ability to be sold widely. Consumers prefer traits such as freshness, attractive color, preferred flavor, palatability, and high nutritional value. Some traits are common to producers and consumers. For example, excellent shelf life is important for freshness, and a favorable quality of fruit is necessary to generate many sales at high prices. Based on these demands, the major objectives of breeding concerning the ripening process of fleshy fruit are to improve taste (sweetness, umami, sourness, texture, flavor, and aroma), appearance (color), shelf life, and value-added compounds such as nutritional and bioactive substances that promote human health. Moreover, conducting a preference survey of the mass market is important for defining a direction for breeding because taste preferences vary depending on the country or area. There is more than one acceptable taste. The desired traits differ depending on whether the fruit is grown for eating or cooking.

This chapter describes recent progress on tomato ripening research and the possibility of using breeding materials, especially those pertaining to controlling the global ripening process. Molecular breeding as it relates to texture, sugar accumulation, and functional materials is the focus of other chapters.

10.2 Regulation of Tomato Fruit Ripening

Ethylene is necessary for tomato fruit ripening. Tomato fruits ripen to a red or pink color through "breaker," which is the onset of ripening and the starting point of a burst of ethylene production after the green stage. If ethylene production does not occur or ethylene is not recognized due to treatment with inhibitors of ethylene

synthesis and perception or the manipulation of these processes by transgenic or mutant approaches, the fruit fails to ripen (Barry and Giovannoni 2007). These demonstrations have established the role of ethylene in tomato ripening. Despite the absolute requirement for ethylene, some ripening processes occur independently of direct ethylene regulation. The control mechanism during ripening is called developmental or ethylene-independent regulation. Some transcription factors that are involved in ethylene regulation and function upstream of ethylene were recently discovered. The ripening phenomenon of fleshy fruits is an elaborate and highly orchestrated event that consists of many biochemical changes that occur simultaneously upon the shift in the expression levels of hundreds to thousands of genes. To precisely control ripening through molecular breeding, understanding the role of ethylene in the ripening process, integrating the information concerning ethylene biosynthesis and perception, and expanding our knowledge of ethylene-independent regulation are important.

10.3 The Potential to Control Ripening by Controlling the Ethylene Biosynthetic Process

Ethylene is the only gaseous plant hormone and has the lowest molecular weight of the plant hormones. In higher plants, the ethylene biosynthesis pathway is well characterized and has been sufficiently reviewed (Barry and Giovannoni 2007; Argueso et al. 2007). Ethylene is synthesized from methionine via three steps. First, the amino acid methionine is converted to S-adenosylmethionine (SAM) by SAM synthase. Second, the conversion of SAM to 1-aminocyclopropane-1-carboxylic acid (ACC) is catalyzed by the enzyme 1-aminocyclopropane-1-carboxylase synthase (ACS). Then, ACC oxidase (ACO) converts ACC into ethylene. Three enzymes are involved in this pathway. ACS and ACO are crucial enzymes for ethylene biosynthesis because SAM is involved in not only ethylene biosynthesis but also multiple aspects of cellular metabolism (Barry and Giovannoni 2007).

As in most plant species, a number of genes encoding ACS and ACO have been identified and characterized in tomato plants, and the expression of each gene is temporally and spatially regulated in response to environmental stimuli and developmental signals. A previous expression analysis revealed that at least four *ACS* (*LeACS1A*, *LeACS2*, *LeACS4*, and *LeACS6*) and four *ACO* (*LeACO1*, *LeACO3*, *LeACO4*, and *LeACO5*) genes are expressed during tomato fruit ripening among the twelve *ACS* and seven *ACO* genes that have been found in the tomato genome (Barry et al. 1996, 2000; Nakatsuka et al. 1998; Rottmann et al. 1991; Yokotani et al. 2009; Barry and Giovannoni 2007; Seymour et al. 2013; Van de Poel et al. 2012). There are two systems of ethylene production in tomatoes and other climacteric fruit. The low level of ethylene production before breaker is system 1, and the increased ethylene production after breaker is called system 2. Autocatalytic ethylene production in system 2 is attributable to the accumulation of *LeACS2* and *LeACS4*

transcripts, which are significantly upregulated during and after the onset of ripening. Conversely, *LeACS1A* and *LeACS6* are involved in the basal level of ethylene production from system 1 onward and before the transition to ripening; their expression decreases after the breaker stage.

The mechanism of the switch from system 1 to system 2 at the onset of ripening remains unknown (Yokotani et al. 2009; Barry and Giovannoni 2007; Cara and Giovannoni 2008; Klee and Giovannoni 2011). Although immature fruit does not ripen upon a short exogenous ethylene treatment, this treatment reduces the time required for the onset of ripening (Yang 1987). This result suggests that it is necessary to consider both the developmental and ethylene-regulated control of fruit ripening in tomato (Klee 2004). Klee (2004) also proposed that when the cumulative effects of system 1 ethylene through development reach a certain threshold, ripening is initiated. This hypothesis is supported by the observation that the ripening onset is delayed by the inhibition of system 1 ethylene production in immature fruit. Barry et al. (2000) suggested that the transition phase is initiated by changes in ethylene sensitivity due to continual system 1 ethylene production. Kevany et al. (2007) demonstrated that the shortened period to ripening upon the application of exogenous ethylene is closely related to the level of the ethylene receptor protein, a negative regulator of the ethylene signal, as ethylene exposure causes a reduction in the level of the ethylene receptor protein. Conversely, system 2 ethylene production due to *LeACS2* and *LeACS4* expression is induced even if the effect of system 1 ethylene is eliminated in a suppressed ethylene response (Yokotani et al. 2009). Therefore, the authors proposed that the transition between system 1 and system 2 occurs in association with the limited expression of *LeACS2* and *LeACS4*, which are regulated by developmental factor(s) such as the LeMADS-RIN transcription factor (Vrebalov et al. 2002) rather than accumulated ethylene. Revealing the relationship between cumulative ethylene effects, changes in ethylene sensitivity, and transcriptional and posttranscriptional control of transcription factors will be conductive to understanding the ripening transition process.

Despite the complexity of the transition pathway, the level of ACS transcription clearly plays a major role in the transition phase, and many data support a model in which ACS is the rate-limiting enzyme, suggesting that it functions as a point of ripening regulation (Barry et al. 2000; Nakatsuka et al. 1998; Klee and Giovannoni 2011; Van de Poel et al. 2012). The activities of ACS enzymes are tightly regulated by both transcriptional and posttranscriptional control (Nakatsuka et al. 1998; Barry et al. 2000; Chae and Kieber 2005; Argueso et al. 2007; Kamiyoshihara et al. 2010). Tomatoes expressing antisense *LeACS2* showed reductions in not only *LeACS2* transcripts but also *LeACS4* transcripts; consequently, fruit ripening was sufficiently inhibited (Oeller et al. 1991). Additionally, the inhibitory effect was recovered by exogenous ethylene. These data suggest a means for artificially controlling ripening. At the posttranscriptional level, LeACS2 is stabilized by phosphorylation and degraded in the ubiquitin/26S-proteasome pathway after dephosphorylation (Kamiyoshihara et al. 2010). The destabilization of LeACS2 by the modification of the phosphorylation site might suppress the ripening process by disrupting ACS activity.

ACO is not a limiting factor in the ripening transition phase because its activity is sufficiently high prior to ripening initiation, although its expression is induced at the onset of ripening. In contrast, Van de Poel et al. (2012) indicated that ACO is the rate-limiting enzyme of post-climacteric ethylene biosynthesis. Tomatoes with reduced levels of *LeACO1*, which is the most highly expressed *ACO* gene during fruit ripening, were generated using antisense technology. These tomatoes exhibited reduced ethylene production, delayed ripening progression, and greater resistance to over-ripening and shriveling than the control tomatoes (Hamilton et al. 1990; Picton et al. 1993). The resistance to over-ripening is attributed to the role of ACO as a rate-limiting enzyme in the post-climacteric phase. Although the ripening inhibitory effect is not stronger than that of the *LeACS2*-antisense tomato, the moderate phenotype which does not require exogenous ethylene treatment for ripening initiation may result in improved shelf life.

The expression of a bacterial *ACC deaminase* gene, which is capable of degrading ACC to α-ketoglutaric acid, also resulted in significant ripening suppression of tomato fruit (Klee et al. 1991). In these transgenic tomatoes, the vegetative phenotype was normal, but the mature fruit remained firm for more than 6 weeks.

10.4 Ethylene Perception and the Signaling Pathway

10.4.1 Ethylene Receptor

Ethylene perception starts with the binding between ethylene and the ethylene receptor. The ethylene receptor is an endoplasmic reticulum (ER)-associated integral membrane protein with protein kinase activity (Chen et al. 2002; Gamble et al. 1998; Moussatche and Klee 2004) that acts as a negative regulator of the ethylene response pathway via disulfide-linked dimers (Schaller et al. 1995; Hua and Meyerowitz 1998; Tieman et al. 2000). The receptors actively suppress ethylene responses in the absence of ethylene, and ethylene binding relieves the suppression in the presence of ethylene, consequently transferring the ethylene signal to a downstream pathway.

Ethylene receptors are classified into subfamily-1 and subfamily-2 based on their amino acid sequences. However, the general structures resemble each other in that they have a transmembrane domain at the N-terminus, a GAF domain, and a histidine (His) kinase domain at the C-terminus. Subfamily-1 members are characterized by three membrane-spanning domains and a functional His kinase domain with five subdomains that define the catalytic core. Subfamily-2 members possess four membrane-spanning domains, lack the necessary amino acid residues for His kinase activity, and are thought to function as Ser/Thr kinases (Klee 2004; Moussatche and Klee 2004). In addition, some receptors possess a receiver domain irrespective of the subfamily.

The transmembrane domain regions contain ethylene-binding sites (Hall et al. 1999; Rodríguez et al. 1999; Schaller and Bleecker 1995) and are engaged in the localization to the ER (Chen et al. 2002; Dong et al. 2008; Grefen et al. 2008). Additionally, the His kinase activity is involved in ethylene signaling modulation, although the enzymatic activity is not absolutely required for signaling (Hall et al. 2012).

Some receptors are quickly degraded, likely through the proteasome-dependent pathway, after binding to ethylene. As a consequence of the reduction in the number of receptor proteins that are actively suppressed, an ethylene response inevitably occurs (Kevany et al. 2007; Chen et al. 2007).

Seven ethylene receptor genes (*LeETR1*, *LeETR2*, *NR*, *LeETR4*, *LeETR5*, *LeETR6*, and *LeETR7*) were discovered in tomatoes and tomato genome sequences (Klee and Giovannoni 2011; Seymour et al. 2013). *NR*, *LeETR4*, and *LeETR6* are the major genes that are expressed during fruit ripening (Kevany et al. 2007). Their expression levels increase at the breaker stage. In contrast, their protein levels decrease due to the degradation of the ethylene-binding receptor. Kevany et al. (2007) indicated that modifications in ethylene sensitivity depending on the timing of ripening initiation are caused mainly by reductions in the LeETR4 and LeETR6 proteins at the breaker stage. Antisense tomatoes targeting the *LeETR4* or *LeETR6* genes by the 35S promoter showed an early ripening phenotype with increased ethylene sensitivity as a consequence of the reduction in the gene expression and protein levels (Kevany et al. 2007; Tieman et al. 2000). Early maturation has some advantages to growers because shortening the cultivation time is conducive to increases in production and because the first fruit available in a market in a given season can fetch a high price. However, the transgenic tomatoes generated using this constitutive promoter also displayed undesirable traits, including severe epinasty, enhanced flower senescence, and increased ethylene sensitivity throughout the plant. Kevany et al. (2008) created new transgenic tomatoes with suppressed *LeETR4* expression in a fruit-specific manner and showed that the fruit size, yield, and flavor-related chemical composition were largely unchanged from those of wild type, although the plants had an early ripening phenotype. In most research, efforts have been focused on delaying fruit ripening, which is associated with a prolonged shelf life. Therefore, this approach is unique, and the fruit-specific suppression of the ethylene receptor LeETR4 is an interesting concept with practical applications.

The *Never-ripe* (*Nr*) mutant is a non-ripening mutant with reduced ethylene sensitivity, and the phenotype is attributed to a single amino acid substitution within the N-terminal ethylene-binding domain of the NR protein (Wilkinson et al. 1995). Although the *Nr* fruit is superior in terms of shelf life, it has shown an incomplete ripening phenotype, including insufficient red coloration even in the heterozygous state, which indicates that this allele functions in a semidominant manner (Lanahan et al. 1994; Okabe et al. 2011). Moreover, there are some undesirable traits, such as increased susceptibility to some pathogens that are associated with Nr (Cantu et al. 2009; Francia et al. 2007; Kavroulakis et al. 2007).

An antisense gene targeting NR does not affect the plant and fruit ripening phenotypes as a consequence of the functional compensation by increased *LeETR4* mRNA (Tieman et al. 2000). The ethylene receptor is a negative regulator, and the ethylene response occurs as a result of the lack of a negative signal for the degradation of the receptor protein-binding ethylene through the ubiquitin/26S proteasome-dependent pathway (Kevany et al. 2007). Therefore, the decreased receptor protein due to the antisense gene or protein degradation due to ethylene binding caused increased ethylene sensitivity. In contrast, a receptor protein that impairs ethylene-binding capability, such as Nr, is hard to degrade because it is unable to bind with ethylene; consequently, the mutant displays ethylene insensitivity through the remaining negative signal. Therefore, ripening control by the ethylene receptor, in contrast to control by ethylene biosynthesis, is complicated because it is a negative regulator. Additionally, the functional compensation makes it more complicated.

Similar to *Nr*, two ripening mutants (*Sletr1-1* and *Sletr1-2*) in which missense mutations in *LeETR1* (*SlETR1*) are located in the ethylene-binding domains were discovered from an ethyl methanesulfonate (EMS)-mutagenized tomato library by TILLING (Targeting Induced Local Lesions In Genomes, please see Chapter 6) (Okabe et al. 2011). The allelic mutants displayed different levels of impaired fruit ripening likely due to the different mutation positions. Similar to *Nr*, the *Sletr1-1* fruits did not turn red, even at late stages of ripening. Although *Sletr1-2* did not show a visible difference from the wild type regarding ripening, the fruit shelf life was extremely prolonged, and the surface remained intact even at 60 days after harvest. This result suggested that the subtle difference in the degree of ethylene sensitivity affected fruit ripening. Furthermore, the mutation in *SlETR1*, which is not a major receptor gene during fruit ripening, conferred practical traits for tomato breeding. These data indicated that a modification of a receptor gene or protein, even if it is not a main player in ripening, has the potential to affect ripening.

10.4.2 Regulators of Ethylene Receptor Function

The mutants carrying the dominant *Green-ripe* (*Gr*) allele or another allele, *Never-ripe 2* (*Nr-2*), also displayed reduced ethylene responsiveness in a subset of tissues, resulting in impaired fruit ripening (Barry et al. 2005). The non-ripening phenotype resulted from a deletion at the junction between the 5′-UTR and the promoter and the ectopically expressed *SlGR* gene in the fruit (Barry and Giovannoni 2006). *Reversion to ethylene sensitivity 1* (*RTE1*), identified as a positive regulator of the ethylene receptor in *Arabidopsis*, is a homologue of *SlGR* (Resnick et al. 2006). The RTE1 protein is required for the function of ETR1 and is thought to facilitate the conformational change in the receptor following ethylene binding (Resnick et al. 2008). RTE1 loss of function leads to enhanced ethylene responsiveness, whereas its overexpression results in reduced sensitivity, as observed in the *Gr*

mutant (Resnick et al. 2006). Therefore, the SlGR protein may act at the level of the ethylene receptor.

The tomato genome contains two additional genes, designated as *Green-ripe Like 1* (*SlGRL1*) and *SlGRL2* (Barry and Giovannoni 2006). However, the *SlGRL1* and *SlGRL2* overexpression lines did not exhibit the typical inhibition of fruit ripening observed in the *Gr* mutant, indicating that the functions of these genes diverged from each other (Ma et al. 2012). The *GR* overexpression transformant generated using a constitutive promoter exhibited the non-ripening fruit phenotype observed in the *Gr* mutant but did not exhibit a whole-plant reduction in ethylene responsiveness, suggesting a tissue-specific modulation of the ethylene response in tomatoes (Barry and Giovannoni 2006). The tissue-specific and lack of functionally redundant characteristics of the protein are interesting because they are conducive to strongly affecting ripening without causing undesirable growth effects in other tissues. However, this strong effect may make it difficult to use this plant as breeding material for ripening control.

10.4.3 CTR1

Constitutive triple response 1 (CTR1), a Raf-like protein kinase that acts as a negative regulator of the ethylene signaling pathway, is located downstream of the receptors (Clark et al. 1998; Gao et al. 2003; Huang et al. 2003). The receptors actively suppress downstream responses through direct interactions with CTR1 in the absence of ethylene (Zhong et al. 2008). The loss-of-function *ctr1* mutants exhibit the constitutive activation of ethylene signaling (Kieber et al. 1993). In contrast to *Arabidopsis*, which only contains one constitutively expressed *CTR1* gene, four *CTR* genes (*LeCTR1*, *LeCTR2*, *LeCTR3*, and *LeCTR4*) have been characterized in tomato (Lin et al. 1998; Leclercq et al. 2002; Adams-Phillips et al. 2004). *LeCTR1*, *LeCTR3*, and *LeCTR4* functionally complement the *Arabidopsis ctr1* mutant, suggesting that they are functional *CTR1* homologues (Adams-Phillips et al. 2004). Expression analysis also showed that the expression of *LeCTR1* is high in tomato fruit and that it increases during ripening and upon ethylene treatment, whereas the *LeCTR3* and *LeCTR4* mRNAs do not change in this manner (Adams-Phillips et al. 2004).

10.4.4 EIN2 and Its Regulation

Ethylene insensitive 2 (EIN2), which has homology to the Nramp family of metal ion transporters, acts downstream of the receptors and CTR1 as an essential positive regulator of ethylene signaling (Alonso et al. 1999; Roman et al. 1995; Hall and Bleecker 2003). The loss-of-function mutants display ethylene insensitivity (Alonso et al. 1999). EIN2 is localized to the ER where it interacts with the ethylene

receptor depending on the phosphorylation status of the kinase domain in an ethylene-dependent manner (Alonso et al. 1999; Bisson et al. 2009; Bisson and Groth 2010). In the absence of ethylene, EIN2 is phosphorylated by activated CTR1 within the ER, but dephosphorylated EIN2 in the presence of ethylene leads to its proteolytic cleavage, resulting in the translocation of the cleaved C-terminus into the nucleus, where it promotes *EIN3* and ethylene-dependent transcription (Qiao et al. 2012). *LeEIN2* is likely a single-copy gene in tomatoes, as it is in *Arabidopsis*, based on a DNA gel-blot analysis (Zhu et al. 2006). Its expression is largely unchanged throughout the different stages of fruit development and is unaffected by ethylene (Zhu et al. 2006). Furthermore, *LeEIN2*-silenced fruit generated using a virus-induced gene silencing system exhibited a delay in fruit development and ripening, suggesting that LeEIN2 positively mediates the ethylene response.

Although there is still no report concerning the LeEIN2 degradation system, *Arabidopsis* EIN2 is regulated at the protein accumulation level through the ubiquitin/26S proteasome pathway by two F-box proteins, ETP1 and ETP2 (EIN2 targeting proteins 1 and 2), which mediate protein degradation in the absence of ethylene (Qiao et al. 2009). The ETP1 and ETP2 proteins are downregulated in the presence of ethylene, and accumulated EIN2 actively functions in the ethylene signaling pathway. Therefore, the overexpression of *ETP1* or *ETP2* disrupts EIN2 protein accumulation, and the transgenic plants exhibit a strong ethylene-insensitive phenotype. Conversely, knocking down both ETP1 and ETP2 induces constitutive ethylene response phenotypes.

10.4.5 Transcription Factors Downstream of the Ethylene Signaling Pathway

At the bottom of the signaling cascade, downstream of EIN2, are two families of transcription factors termed ethylene insensitive 3 (EIN3) and ethylene response factor (ERF) in *Arabidopsis*. EIN3, a nuclear-localized protein that exhibits DNA-binding activity, has been identified as a positive regulator of the ethylene signal transduction pathway, with its loss of function resulting in ethylene insensitivity (Chao et al. 1997). In addition to two related EIN3-like proteins (EIL1 and EIL2), EIN3 has been shown to recognize conserved motifs known as primary ethylene responsive elements that are present within the promoters of ERF1 and several senescence- and ripening-related genes (Solano et al. 1998). ERF1 binds to the GCC-box promoter elements of ethylene-regulated genes, initiating a transcriptional cascade in ethylene signaling. The overexpression of ERF1 results in the activation of ethylene response genes and their associated phenotypes (Solano et al. 1998). Conversely, ERF1 and related genes belong to the large APETALA2 (AP2)/ERF family of DNA-binding proteins, acting as activators or repressors on the basis of differences in their amino acid sequences (Fujimoto et al. 2000; Ohta et al. 2001).

In tomato, four genes termed *LeEIL1–4* are homologous to *EIN3-like* (Tieman et al. 2001; Yokotani et al. 2003). Each of the *LeEIL1–3* genes complemented the *ein3* phenotype in *Arabidopsis* (Tieman et al. 2001). Moreover, transgenic tomato plants with the reduced expression of a single *LeEIL* gene did not exhibit significant changes in the ethylene response, whereas the reduced expression of multiple *LeEIL* genes resulted in the loss of ethylene responses, including leaf epinasty, flower abscission, flower senescence, and fruit ripening. These results indicate that the *LeEILs* are functionally redundant and regulate multiple ethylene responses throughout development (Tieman et al. 2001). However, the overexpression of *LeEIL1* in the *Nr* background did not restore the upregulation of all ripening-related genes and all ethylene responses, such as the seedling triple response, suggesting that the functions of these genes may not be exactly the same (Chen et al. 2004). Recently, the phosphorylation of a conserved region in LeEIL1 has been shown to be essential for transcription through dimerization of the LeEIL1 proteins (Li et al. 2012). In fact, a mutation in the phosphorylation site of LeEIL1 is able to abolish the constitutive ethylene responses displayed by the LeEIL1-overexpressing lines.

Studies using *Arabidopsis* also revealed that the ubiquitin/26S proteasome pathway negatively regulates ethylene responses by targeting EIN3 for degradation (Gagne et al. 2004; Guo and Ecker 2003; Potuschak et al. 2003). In the degradation process, two F-box proteins, EBF1 and EBF2 (EIN3-binding factors 1 and 2, respectively), interact physically with EIN3/EIL and mediate its proteolysis via ubiquitination in the absence of ethylene. The overexpression of EBF1 or EBF2 results in plants that are insensitive to ethylene through the destabilization of EIN3, whereas mutations in either or both genes result in enhanced or constitutive ethylene responses by stabilizing EIN3 (Guo and Ecker 2003; Potuschak et al. 2003). In tomato, two F-box genes, *SlEBF1* and *SlEBF2*, have been identified (Yang et al. 2010). Dynamic changes in their expression levels have been observed in flowers during the bud to post-anthesis stages and at the onset of fruit ripening. Although the silencing of a single *SlEBF* gene did not influence the plant growth phenotype due to compensation by the other gene, these plants displayed accelerated fruit ripening and moderate fertility defects. In contrast, co-silencing of both *SlEBF* genes resulted in severely dwarf plants with reduced fertility and accelerated plant senescence and fruit ripening.

Eighty-five AP2/ERF genes have been described in tomato using raw EST data from various public repositories, with 57 being differentially expressed during fruit development (Sharma et al. 2010). Because this family is so large, information on individual genes, except for *SlAP2a* and *SlERF6*, concerning fruit ripening is limited. The RNAi-induced repression of *SlAP2a* results in fruits that overproduce ethylene, ripen early, and have modified carotenoid accumulation profiles due to altered gene expression related to the carotenoid pathway (Chung et al. 2010; Karlova et al. 2011). The transcriptome and metabolite profiling analyses showed that *SlAP2a* positively regulates the synthesis of several carotenoids and tocopherol during fruit ripening. At the same time, upregulated ethylene production in *SlAP2a* RNAi fruit suggests that SlAP2a is a negative regulator of ethylene biosynthesis in

ripening fruit. *SlERF6* suppression also increases carotenoid and ethylene accumulation during ripening as a result of the upregulation of carotenoid-related genes and ethylene biosynthesis-related genes. This observation suggests the role of this gene as a negative regulator in the carotenoid and ethylene synthesis pathways (Lee et al. 2012).

10.5 Transcriptional Ripening Control Upstream of Ethylene

Fruit ripening is orchestrated by both ethylene-dependent and -independent processes. In tomato, three transcription factors involved in ethylene-independent ripening regulation have been identified by characterizing pleiotropic non-ripening mutants: *ripening-inhibitor* (*rin*), *non-ripening* (*nor*), and *colorless non-ripening* (*Cnr*). In all these mutants, the fruits fail to undergo measurable ripening phenomena including ethylene synthesis, increased respiration, carotenoid accumulation, softening, and production of flavor compounds, whereas they are able to develop normally to the mature green stage, with a normal fruit size and mature seeds (Cara and Giovannoni 2008; Klee and Giovannoni 2011; Vrebalov et al. 2002). The impaired ripening is not restored even upon the application of exogenous ethylene, although some ethylene-regulated gene expression is partially induced, indicating the retention of ethylene sensitivity. Together, these characteristics suggest that *rin*, *nor*, and *Cnr* act as central ripening regulators upstream of ethylene.

The *rin* locus encodes a MADS-box transcription factor termed LeMADS-RIN (RIN), which is a member of the SEPALLATA clade (Vrebalov et al. 2002). Genetic complementation and antisense experiments demonstrated that mutations in the *RIN* gene were responsible for the non-ripening phenotype (Vrebalov et al. 2002). NOR is a NAC domain transcription factor, although more comprehensive data are not yet available (Giovannoni 2004). The *Cnr* mutation resulted from a dominant epigenetic mutation that causes the hypermethylation of a SBP-box (SQUAMOSA promoter binding protein-like) gene promoter region and reduces its expression (Manning et al. 2006).

A chromatin immunoprecipitation (ChIP) approach revealed that RIN interacts with the promoters of numerous genes including *RIN* itself and other transcription factors related to ripening regulation (*NOR*, *HB1*, *CNR*, *TDR4*, and *AP2a*), ethylene biosynthesis (*LeACS2* and *LeACS4*), ethylene perception (*NR*), downstream ethylene responses (*E4* and *E8*), cell wall metabolism (*polygalacturonase2a, expansin1, endo-(1,4)-β-mannanase 4*, and *β-galactosidase 4*), and carotenoid biosynthesis (*phytoene synthase1*) (Martel et al. 2011; Fujisawa et al. 2011). In a more recent study, a combined ChIP-chip and transcriptome analysis identified 241 direct RIN target genes that contained a RIN-binding site (Fujisawa et al. 2013). This result indicates that RIN participates in the regulation of many physiological processes

including lycopene accumulation, ethylene production, and chlorophyll degradation during ripening as both a transcriptional activator and repressor. Additionally, the interactions of RIN with its target loci depend on the presence of a functional CNR because RIN binding is greatly diminished in the *Cnr* mutant background (Martel et al. 2011). MADS-box genes act together in multimeric complexes. Indeed, some MADS-box genes, including *TDR4*, *TAGL1*, *TAG1*, *and MBP7*, are able to directly interact with RIN in a two-hybrid screen (Martel et al. 2011). Although CNR proteins do not directly interact with RIN, the expression of TDR4, which is able to bind RIN, is significantly reduced in the *Cnr* mutant, suggesting that TDR4 may be a CNR target and that the ability of RIN to bind its target loci may be influenced by CNR through the regulation of *TDR4* expression (Eriksson et al. 2004; Martel et al. 2011).

Tomato orthologs of the *Arabidopsis FRUITFUL* (*FUL*) MADS-box genes *TDR4*/*FUL1* and *MBP7*/*FUL2* have redundant functions in fruit ripening. They are involved in cell wall modification, lycopene accumulation, cuticle component production, volatile compound production, and glutamic acid accumulation (Bemer et al. 2012). In particular, the *FUL1*/*2*-silenced fruits exhibited reduced lycopene and glutamic acid accumulation, increased GABA content, and increased water loss. FUL1 and FUL2 do not regulate ethylene biosynthesis, and the mutant phenotype is not restored by ethylene treatment despite normal ethylene perception, indicating that FUL1/2 acts downstream or independently of climacteric ethylene responses. Additionally, knockdown analysis showed that the expression levels of *RIN* and *TAGL1* are repressed by FUL1/2, but that the expression of *CNR* is unchanged.

Tomato AGAMOUS-LIKE1 (*TAGL1*) is a member of the *AGAMOUS* clade of MADS-box genes in tomato. TAGL1 is transcription factor that is necessary for tomato fruit ripening. *TAGL1* RNAi tomatoes showed impaired ripening phenotypes, including reduced carotenoid content, suppressed chlorophyll degradation, and decreased ethylene production through reduced *LeACS2* expression, which is the major enzyme involved in the ethylene production required for fruit ripening (Giménez et al. 2010; Itkin et al. 2009; Vrebalov et al. 2009). In addition to having alterations in other agronomically important traits, the transgenic plants displayed reductions in fruit pericarp thickness, leading to the loss of fleshiness, decreased fruit weight, decreased sugar content, and increased water loss. In contrast, the overexpression of *TAGL1* caused increases in the lycopene and sugar contents of the fruit (Giménez et al. 2010). Moreover the sepals were converted to fleshy organ and accumulated lycopene and sugars such as in the fruit. These data indicate a role for TAGL1 in both the fruit ripening stage and in the carpel expansion prior to ripening. Furthermore, edible sepals due to the ectopic expression of TAGL1 may be marketable due to their shape and increased edible area.

Tomato LeHB1, which is a HD-Zip homeobox transcription factor, directly interacts with the promoter of *LeACO1*, which is expressed during ripening, and upregulates the *LeACO1* expression level, resulting in ripening inhibition in *LeHB1*-silenced fruit using virus-induced gene silencing (Lin et al. 2008). Putative LeHB1-binding sites are also found in the promoters of a number of ripening-

related genes, including *LeACO2*, *PG1*, *RIN*, and *NOR*. However, LeHB1 is involved in floral organogenesis, and its systematic overexpression and silencing could not be analyzed in tomatoes because of deleterious or lethal effects (Lin et al. 2008), suggesting a role for LeHB1 throughout growth.

Recently, a new tomato MADS-box transcription factor belonging to the SEPALLATA subfamily, SlMADS1, was identified as a repressive modulator of fruit ripening (Dong et al. 2013). SlMADS1-silenced tomatoes exhibited shorter fruit ripening times and increased carotenoid accumulation and ethylene production as well as upregulation of related genes including *phytoene synthase1*, *LeACS1A*, *LeACS6*, *LeACO1*, and *LeACO3*. In addition, the interaction between SlMADS1 and RIN was revealed by a yeast two-hybrid assay, and the *SlMADS1* transcript levels decrease significantly in accordance with fruit ripening. This evidence suggests an important role for SlMADS1 in fruit ripening as a negative regulator.

An attempt to generate a practical variety from naturally occurring mutants was performed with the *rin* mutant. Some fruits of F1 hybrid lines (*RIN*/*rin*) created from a cross between the wild type and a *rin* mutant exhibited an extremely long shelf life and acceptable quality (Kitagawa et al. 2005). However, other fruits did not display the favorable phenotypes, and the heterozygosity effect of *rin* on coloring and shelf life depended on the parental lines. Appropriate selection of parental lines is important to develop a marketable tomato that includes *rin* traits.

10.6 Variation in Ripening Control

10.6.1 Epigenetic Diversity

Recently, the developmental trigger of fruit ripening in tomato was reported to be an epigenetic switch involving changes in the DNA methylation level (Zhong et al. 2013). In this report, changes in the location-specific methylation status that occur in RIN target promoters during fruit ripening were revealed using whole-genome bisulfite sequencing, and the treatment with a methylation inhibitor induced early ripening. This result indicates that the transcriptional activation of ripening-related genes is controlled by at least two factors: the binding of functional transcription factors to the target loci and the promoter methylation status. The authors also proposed considering not only DNA sequence variation among plant lines but also the information encoded in the epigenome for trait improvement in crops. Although *Cnr* is an epigenetic mutant related to fruit ripening derived from a natural variant (Manning et al. 2006), most epigenetic mutants might be overlooked because breeding programs have depended on DNA-based molecular markers until now. In future crop-improvement strategies, the identification of epigenetic variation in genes that encode economically important plant traits might be an important new resource for creating improved crop varieties (Ecker 2013).

Short RNAs (sRNAs) are also engaged in epigenetic variation and posttranscriptionally regulate gene expression via an RNA-induced silencing system. They guide the DNA methylation and heterochromatin formation that lead to transcriptional gene silencing (Dalmay 2010). The differential expression of thousands of sRNAs during tomato fruit development and ripening has been revealed (Moxon et al. 2008; Mohorianu et al. 2011). Among these genes, some of the validated targets of sRNAs include *CNR* and a member of the *CTR* family involved in fruit ripening (Moxon et al. 2008). A recent genome-wide analysis showed the dynamic differential expression of sRNAs at the transition of the ripening phases and that these sRNAs map to specific sites in fruit-related promoter regions (Tomato Genome Consortium 2012). Although these results suggest a role for sRNAs in ripening, the implications of these changes remain to be elucidated (Seymour et al. 2013).

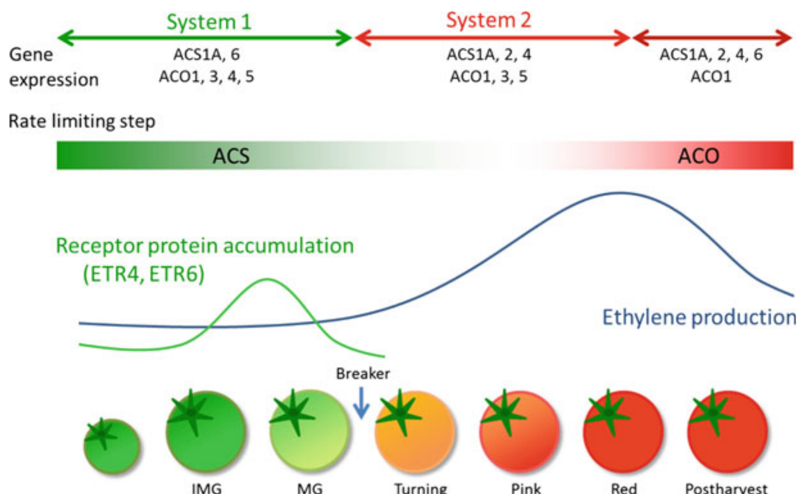
10.6.2 Application of Quantitative Trait Locus Approaches

In tomato, hundreds of quality-related quantitative trait loci (QTLs), such as those controlling fruit size, sugar accumulation, fruit texture, ascorbic acid content, and carotenoid content, have been positioned on the genetic map (Chapman et al. 2012; Frary et al. 2000; Lippman et al. 2007). With respect to ethylene biosynthesis during ripening, a number of QTLs were discovered from *S. habrochaites* introgression lines in which portions of the genome of this wild species were introgressed into the cultivated tomato background (Dal Cin et al. 2009). Although several QTLs were known ethylene-related genes, many were novel genes that were likely to be involved in the control of ethylene biosynthesis and ripening. Identifying the candidate novel genes underlying these QTLs might lead to the discovery of new factors that control ripening, and this information could lead to crop improvements.

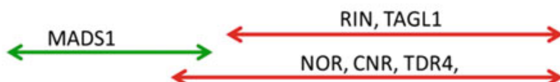
10.7 Conclusions

Identifying candidate genes for important traits and understanding the exact function of each nucleotide polymorphism within each of these genes are important to achieve the desired traits in breeding programs. This chapter has shown that many steps regulate ripening (Fig. 10.1). Understanding of the fruit ripening process has increasingly expanded with respect to the knowledge of ethylene biosynthesis, the response processes, and the transcriptional and posttranscriptional regulation of related proteins. Many genes with the potential for ripening control have been nominated as candidates for breeding selection. Upstream of ethylene, transcription factors that systematically control ripening have been identified, and their implications and roles are being discovered. Moreover, the epigenetic control of ripening

Ethylene biosynthesis and perception control



Transcriptional control (Gene expression of transcription factors)



Epigenetic control

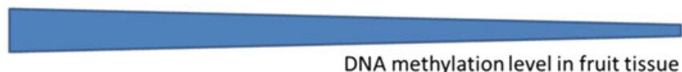


Fig. 10.1 A schematic representation of the ripening process in tomato fruit. Basal level of ethylene production in system 1 is mediated by expression of *ACS1A* and *ACS6* and *ACO1*, *ACO3*, *ACO4*, and *ACO5*. Climacteric ethylene production after the breaker stage in system 2 is mediated by the expression of *ACS1A*, *ACS2*, and *ACS4* and *ACO1*, *ACO3*, and *ACO5*. ACS is the rate-limiting step of system 1 and system 2 ethylene biosynthesis, whereas ACO is the rate-limiting step of post-climacteric ethylene biosynthesis. Protein accumulation of ETR4 and ETR6 increases during fruit development and then decreases before the breaker stage, which is accompanied by increased ethylene sensitivity. Gene expression of ripening-related transcription factors changes near the ripening transition phase. *MADS1*mRNA accumulates in immature green (IMG) and mature green (MG) fruit and then decreases at and before the breaker stage, whereas *RIN* and *TAGL1* mRNA accumulations increase after breaker stage. *NOR*, *CNR*, and *TDR4* mRNA accumulations begin to increase before breaker stage. Ripening-gene promoters are gradually demethylated during fruit development. The decrease in promoter methylation leads to the increased binding of ripening-related transcription factor to their promoters and a corresponding transcriptional activation of the ripening genes during fruit ripening

has attracted much attention as a crop-improvement strategy. Further discovery of the role of epigenetics in fruit ripening might contribute to finding a use for not only known epigenetic variants but also other epigenetic information.

With the advances in the tomato genome sequence, genomics and epigenetics, the possibility of designing ripening is increasing. Moreover, metabolomic data and analysis tools will support the selection of candidate gene or QTLs for various purposes in breeding by surveying the effects of the gene or QTL on metabolites. Using this information, new marketable tomatoes that have improved traits, such as long-term freshness, palatability, and the ease of transport, are expected to appear in worldwide markets in the near future.

References

Adams-Phillips L, Barry C, Kannan P et al (2004) Evidence that CTR1-mediated ethylene signal transduction in tomato is encoded by a multigene family whose members display distinct regulatory features. Plant Mol Biol 54:387–404

Alonso JM, Hirayama T, Roman G et al (1999) EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. Science 284:2148–2152

Argueso GT, Hansen M, Kieber JJ (2007) Regulation of ethylene biosynthesis. J Plant Growth Regul 26:92–105

Barry CS, Giovannoni JJ (2006) Ripening in the tomato *Green-ripe* mutant is inhibited by ectopic expression of a protein that disrupts ethylene signaling. Proc Natl Acad Sci USA 103:7923–7928

Barry CS, Giovannoni JJ (2007) Ethylene and fruit ripening. J Plant Growth Regul 26:143–159

Barry CS, Blume B, Bouzayen M et al (1996) Differential expression of the 1-aminocyclopropane-1-carboxylate oxidase gene family of tomato. Plant J 9:525–535

Barry CS, Llop-Tous MI, Grierson D (2000) The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. Plant Physiol 123:979–986

Barry CS, McQuinn RP, Thompson AJ et al (2005) Ethylene insensitivity conferred by the *Green-ripe* and *Never-ripe 2* ripening mutants of tomato. Plant Physiol 138:267–275

Bemer M, Karlova R, Ballester AR et al (2012) The tomato FRUITFULL homologs TDR4/FUL1 and MBP7/FUL2 regulate ethylene-independent aspects of fruit ripening. Plant Cell 24:4437–4451

Bisson MM, Groth G (2010) New insight in ethylene signaling: autokinase activity of ETR1 modulates the interaction of receptors and EIN2. Mol Plant 3:882–889

Bisson MM, Bleckmann A, Allekotte S et al (2009) EIN2, the central regulator of ethylene signaling, is localized at the ER membrane where it interacts with the ethylene receptor ETR1. Biochem J 424:1–6

Cantu D, Blanco-Ulate B, Yang L et al (2009) Ripening-regulated susceptibility of tomato fruit to *Botrytis cinerea* requires *NOR* but not *RIN* or ethylene. Plant Physiol 150:1434–1449

Cara B, Giovannoni JJ (2008) Molecular biology of ethylene during tomato fruit development and maturation. Plant Sci 175:106–113

Chae HS, Kieber JJ (2005) Eto Brute? Role of ACS turnover in regulating ethylene biosynthesis. Trends Plant Sci 10:291–296

Chao Q, Rothenberg M, Solano R et al (1997) Activation of the ethylene gas response pathway in Arabidopsis by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. Cell 89:1133–1144

Chapman NH, Bonnet J, Grivet L et al (2012) High-resolution mapping of a fruit firmness-related quantitative trait locus in tomato reveals epistatic interactions associated with a complex combinatorial locus. Plant Physiol 159:1644–1657

Chen YF, Randlett MD, Findell JL et al (2002) Localization of the ethylene receptor ETR1 to the endoplasmic reticulum of *Arabidopsis*. J Biol Chem 277:19861–19866

Chen G, Alexander L, Grierson D (2004) Constitutive expression of EIL-like transcription factor partially restores ripening in the ethylene-insensitive *Nr* tomato mutant. J Exp Bot 55:1491–1497

Chen YF, Shakeel SN, Bowers J et al (2007) Ligand-induced degradation of the ethylene receptor ETR2 through a proteasome-dependent pathway in *Arabidopsis*. J Biol Chem 282:24752–24758

Chung M, Vrebalov J, Alba R et al (2010) A tomato (*Solanum lycopersicum*) *APETALA2/ERF* gene, *SlAP2a*, is a negative regulator of fruit ripening. Plant J 64:936–947

Clark KL, Larsen PB, Wang X et al (1998) Association of the *Arabidopsis* CTR1 Raf-like kinase with the ETR1 and ERS ethylene receptors. Proc Natl Acad Sci USA 95:5401–5406

Dal Cin V, Kevany B, Fei Z et al (2009) Identification of *Solanum habrochaites* loci that quantitatively influence tomato fruit ripening-associated ethylene emissions. Theor Appl Genet 119:1183–1192

Dalmay T (2010) Short RNAs in tomato. J Integr Plant Biol 52:388–392

Dong CH, Rivarola M, Resnick JS et al (2008) Subcellular co-localization of Arabidopsis RTE1 and ETR1 supports a regulatory role for RTE1 in ETR1 ethylene signaling. Plant J 53:275–286

Dong T, Hu Z, Deng L et al (2013) A tomato MADS-box transcription factor, SlMADS1, acts as a negative regulator of fruit ripening. Plant Physiol 163:1026–1036

Ecker JR (2013) Epigenetic trigger for tomato ripening. Nat Biotechnol 31:119–120

Eriksson EM, Bovy A, Manning K et al (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

Francia D, Demaria D, Calderini O et al (2007) Wounding induces resistance to pathogens with different lifestyles in tomato: role of ethylene in cross-protection. Plant Cell Environ 30:1357–1365

Frary A, Nesbitt TC, Frary A et al (2000) fw2.2: a quantitative trait locus key to the evolution of tomato fruit size. Science 289:85–88

Fujimoto SY, Ohta M, Usui A et al (2000) Arabidopsis ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box-mediated gene expression. Plant Cell 12:393–404

Fujisawa M, Nakano T, Ito Y (2011) Identification of potential target genes for the tomato fruit-ripening regulator RIN by chromatin immunoprecipitation. BMC Plant Biol 11:26

Fujisawa M, Nakano T, Shima Y et al (2013) A large-scale identification of direct targets of the tomato MADS box transcription factor RIPENING INHIBITOR reveals the regulation of fruit ripening. Plant Cell 25:371–386

Gagne JM, Smalle J, Gingerich DJ et al (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

Gamble RL, Coonfield M, Schaller GE (1998) Histidine kinase activity of the ETR1 ethylene receptor from *Arabidopsis*. Proc Natl Acad Sci USA 95:7825–7829

Gao Z, Chen YF, Randlett MD et al (2003) Localization of the Raf-like kinase CTR1 to the endoplasmic reticulum of *Arabidopsis* through participation in ethylene receptor signaling complexes. J Biol Chem 278:34725–34732

Giménez E, Pineda B, Capel J et al (2010) Functional analysis of the *Arlequin* mutant corroborates the essential role of the *ARLEQUIN/TAGL1* gene during reproductive development of tomato. PLoS One 5, e14427

Giovannoni JJ (2004) Genetic regulation of fruit development and ripening. Plant Cell 16:170–180

Giovannoni JJ (2007) Fruit ripening mutants yield insights into ripening control. Curr Opin Plant Boil 10:283–289

Grefen C, Städele K, Ruzicka K et al (2008) Subcellular localization and in vivo interactions of the *Arabidopsis thaliana* ethylene receptor family members. Mol Plant 1:308–320

Guo H, Ecker JR (2003) Plant responses to ethylene gas are mediated by SCFEBF1/EBF2-dependent proteolysis of EIN3 transcription factor. Cell 115:667–677

Hall AE, Bleecker AB (2003) Analysis of combinatorial loss-of-function mutants in the Arabidopsis ethylene receptors reveals that the *ers1 etr1* double mutant has severe developmental defects that are EIN2 dependent. Plant Cell 15:2032–2041

Hall AE, Chen QG, Findell JL et al (1999) The relationship between ethylene binding and dominant insensitivity conferred by mutant forms of the ETR1 ethylene receptor. Plant Physiol 121:291–300

Hall BP, Shakeel SN, Amir M et al (2012) Histidine kinase activity of the ethylene receptor ETR1 facilitates the ethylene response in Arabidopsis. Plant Physiol 159:682–695

Hamilton AJ, Lycett GW, Grierson D (1990) Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. Nature 346:284–287

Hua J, Meyerowitz EM (1998) Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. Cell 94:261–271

Huang Y, Li H, Hutchison CE et al (2003) Biochemical and functional analysis of CTR1, a protein kinase that negatively regulates ethylene signaling in Arabidopsis. Plant J 33:221–233

Itkin M, Seybold H, Breitel D et al (2009) TOMATO AGAMOUS-LIKE 1 is a component of the fruit ripening regulatory network. Plant J 60:1081–1095

Kamiyoshihara Y, Iwata M, Fukaya T et al (2010) Turnover of LeACS2, a wound-inducible 1-aminocyclopropane-1-carboxylic acid synthase in tomato, is regulated by phosphorylation/dephosphorylation. Plant J 64:140–150

Karlova R, Rosin FM, Busscher-Lange J et al (2011) Transcriptome and metabolite profiling show that APETALA2a is a major regulator of tomato fruit ripening. Plant Cell 23:923–941

Kavroulakis N, Ntougias S, Zervakis GI et al (2007) Role of ethylene in the protection of tomato plants against soil-borne fungal pathogens conferred by an endophytic *Fusarium solani* strain. J Exp Bot 58:3853–3864

Kevany BM, Tieman DM, Taylor MG et al (2007) Ethylene receptor degradation controls the timing of ripening in tomato fruit. Plant J 51:458–467

Kevany BM, Taylor MG, Klee HJ (2008) Fruit-specific suppression of the ethylene receptor *LeETR4* results in early-ripening tomato fruit. Plant Biotechnol J 6:295–300

Kieber JJ, Rothenberg M, Roman G et al (1993) *CTR1*, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the raf family of protein kinases. Cell 72:427–441

Kitagawa M, Ito H, Shiina T et al (2005) Characterization of tomato fruit ripening and analysis of gene expression in F1 hybrids of the *ripening inhibitor* (*rin*) mutant. Physiol Plant 123:331–338

Klee HJ (2004) Ethylene signal transduction. Moving beyond *Arabidopsis*. Plant Physiol 135:660–667

Klee HJ, Giovannoni JJ (2011) Genetics and control of tomato fruit ripening and quality attributes. Annu Rev Genet 45:41–59

Klee HJ, Hayford MB, Kretzmer KA et al (1991) Control of ethylene synthesis by expression of a bacterial enzyme in transgenic tomato plants. Plant Cell 3:1187–1193

Lanahan MB, Yen HC, Giovannoni JJ et al (1994) The *never ripe* mutation blocks ethylene perception in tomato. Plant Cell 6:521–530

Leclercq J, Adams-Phillips LC, Zegzouti H et al (2002) *LeCTR1*, a tomato *CTR1*-like gene, demonstrates ethylene signaling ability in Arabidopsis and novel expression patterns in tomato. Plant Physiol 130:1132–1142

Lee JM, Joung JG, McQuinn R et al (2012) Combined transcriptome, genetic diversity and metabolite profiling in tomato fruit reveals that the ethylene response factor *SlERF6* plays an important role in ripening and carotenoid accumulation. Plant J 70:191–204

Li J, Li Z, Tang L et al (2012) A conserved phosphorylation site regulates the transcriptional function of ETHYLENE-INSENSITIVE3-like1 in tomato. J Exp Bot 63:427–439

Lin Z, Hackett R, Payton S et al (1998) A tomato sequence, *TCTR2* (Accession No. AJ005077), encoding an *Arabidopsis CTR1* homolog. Plant Physiol 117:1126

Lin Z, Hong Y, Yin M et al (2008) A tomato HD-Zip homeobox protein, LeHB-1, plays an important role in floral organogenesis and ripening. Plant J 55:301–310

Lippman ZB, Semel Y, Zamir D (2007) An integrated view of quantitative trait variation using tomato interspecific introgression lines. Curr Opin Genet Dev 17:545–552

Ma Q, Du W, Brandizzi F et al (2012) Differential control of ethylene responses by *GREEN-RIPE* and *GREEN-RIPE LIKE1* provides evidence for distinct ethylene signaling modules in tomato. Plant Physiol 160:1968–1984

Manning K, Tör M, Poole M et al (2006) A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. Nat Genet 38:948–952

Martel C, Vrebalov J, Giovannoni JJ (2011) The tomato (*Solanum lycopersicum*) MADS-box transcription factor RIN interacts with promoters involved in numerous ripening processes in a CNR dependent manner. Plant Physiol 157:1568–1579

Mohorianu I, Schwach F, Jing R et al (2011) Profiling of short RNAs during fleshy fruit development reveals stage-specific sRNAome expression patterns. Plant J 67:232–246

Moussatche P, Klee HJ (2004) Autophosphorylation activity of the Arabidopsis ethylene receptor multigene family. J Biol Chem 279:48734–48741

Moxon S, Jing R, Szittya G et al (2008) Deep sequencing of tomato short RNAs identifies microRNAs targeting genes involved in fruit ripening. Genome Res 18:1602–1609

Nakatsuka A, Murachi S, Okunishi H et al (1998) Differential expression and internal feedback regulation of 1-aminocyclopropane-1-carboxylate synthase, 1-aminocyclopropane-1-carboxylate oxidase, and ethylene receptor genes in tomato fruit during development and ripening. Plant Physiol 118:1295–1305

Oeller PW, Min-Wong L, Taylor LP et al (1991) Reversible inhibition of tomato fruit senescence by antisense RNA. Science 254:437–439

Ohta M, Matsui K, Hiratsu K et al (2001) Repression domains of class II ERF transcriptional repressors share an essential motif for active repression. Plant Cell 13:1959–1968

Okabe Y, Asamizu E, Saito T et al (2011) Tomato TILLING technology: development of a reverse genetics tool for the efficient isolation of mutant from Micro-Tom mutant libraries. Plant Cell Physiol 52:1994–2005

Picton S, Barton SL, Hamilton AJ et al (1993) Altered fruit ripening and leaf senescence in tomatoes expressing an antisense ethylene-forming enzyme transgene. Plant J 3:469–481

Potuschak T, Lechner E, Parmentier Y et al (2003) EIN3-dependent regulation of plant ethylene hormone signalling by two Arabidopsis F-box proteins: EBF1 and EBF2. Cell 115:679–689

Qiao H, Chang KN, Yazaki J et al (2009) Interplay between ethylene, ETP1/ETP2 F-box proteins, and degradation of EIN2 triggers ethylene responses in Arabidopsis. Genes Dev 23:512–521

Qiao H, Shen Z, Huang SS et al (2012) Processing and subcellular trafficking of ER-tethered EIN2 control response to ethylene gas. Science 338:390–393

Resnick JS, Wen C, Shockey JA et al (2006) *REVERSION-TO-ETHYLENE SENSITIVITY1*, a conserved gene that regulates ethylene receptor function in *Arabidopsis*. Proc Natl Acad Sci USA 103:7917–7922

Resnick JS, Rivarola M, Chang C (2008) Involvement of *RTE1* in conformational changes promoting ETR1 ethylene receptor signaling in Arabidopsis. Plant J 56:423–431

Rodríguez FI, Esch JJ, Hall AE et al (1999) A copper cofactor for the ethylene receptor ETR1 from *Arabidopsis*. Science 283:996–998

Roman G, Lubarsky B, Kieber JJ et al (1995) Genetic analysis of ethylene signal transduction in *Arabidopsis thaliana*: five novel mutant loci integrated into a stress response pathway. Genetics 139:1393–1409

Rottmann WH, Peter GF, Oeller PW et al (1991) 1-Aminocyclopropane-1-carboxylate synthase in tomato is encoded by a multigene family whose transcription is induced during fruit and floral senescence. J Mol Biol 22:937–961

Schaller GE, Bleecker AB (1995) Ethylene-binding sites generated in yeast expressing the Arabidopsis ETR1 gene. Science 270:1809–1811

Schaller GE, Ladd AN, Lanahan MB et al (1995) The ethylene response mediator ETR1 from *Arabidopsis* forms a disulfide-linked dimer. J Biol Chem 270:12526–12530

Seymour GB, Chapman NH, Chew BL et al (2013) Regulation of ripening and opportunities for control in tomato and other fruits. Plant Biotechnol J 11:269–278

Sharma MK, Kumar R, Solanke AU et al (2010) Identification, phylogeny, and transcript profiling of ERF family genes during development and abiotic stress treatments in tomato. Mol Genet Genomics 284:455–475

Solano R, Stepanova A, Chao Q et al (1998) Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev 12:3703–3714

Tieman DM, Taylor MG, Ciardi JA et al (2000) The tomato ethylene receptors NR and LeETR4 are negative regulators of ethylene response and exhibit functional compensation within a multigene family. Proc Natl Acad Sci USA 97:5663–5668

Tieman DM, Ciardi JA, Taylor MG et al (2001) Members of the tomato *LeEIL* (*EIN3-like*) gene family are functionally redundant and regulate ethylene responses throughout plant development. Plant J 26:47–58

Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635–641

Van de Poel B, Bulens I, Markoula A et al (2012) Targeted systems biology profiling of tomato fruit reveals coordination of the Yang cycle and a distinct regulation of ethylene biosynthesis during postclimacteric ripening. Plant Physiol 160:1498–1514

Vrebalov J, Ruezinsky D, Padmanabhan V et al (2002) A MADS-box gene necessary for fruit ripening at the tomato *ripening-inhibitor* (*rin*) locus. Science 296:343–346

Vrebalov J, Pan IL, Arroyo AJM et al (2009) Fleshy fruit expansion and ripening are regulated by the Tomato *SHATTERPROOF* gene *TAGL1*. Plant Cell 21:3041–3062

Wilkinson JQ, Lanahan MB, Yen HC et al (1995) An ethylene-inducible component of signal transduction encoded by Never-ripe. Science 270:1807–1809

Yang SF (1987) The role of ethylene and ethylene synthesis in fruit ripening. In: Thompson W, Nothnagel E, Huffaker R (eds) Plant senescence: its biochemistry and physiology. The American Society of Plant Physiologists, Rockville, MD, pp 156–165

Yang Y, Wu Y, Pirrello J et al (2010) Silencing *Sl-EBF1* and *Sl-EBF2* expression causes constitutive ethylene response phenotype, accelerated plant senescence, and fruit ripening in tomato. J Exp Bot 61:697–708

Yokotani N, Tamura S, Nakano R et al (2003) Characterization of a novel tomato *EIN3-like* gene (*LeEIL4*). J Exp Bot 54:2775–2776

Yokotani N, Nakano R, Imanishi S et al (2009) Ripening-associated ethylene biosynthesis in tomato fruit is autocatalytically and developmentally regulated. J Exp Bot 60:3433–3442

Zhong S, Lin Z, Grierson D (2008) Tomato ethylene receptor-CTR interactions: visualization of NEVER-RIPE interactions with multiple CTRs at the endoplasmic reticulum. J Exp Bot 59:965–972

Zhong S, Fei Z, Chen YR et al (2013) Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. Nat Biotechnol 31:154–159

Zhu HL, Zhu BZ, Shao Y et al (2006) Tomato fruit development and ripening are altered by the silencing of *LeEIN2* gene. J Integr Plant Biol 48:1478–1485

Chapter 11
Disease Resistance in Melon and Its Modification by Molecular Breeding Techniques

Michel Pitrat

11.1 Introduction

Many diseases can be observed on melons (*Cucumis melo* L.). The relative importance of these diseases varies according to the geographic locations, the type of culture (open field or protected) and the climatic conditions of the growing seasons. On a melon plant, it is quite common to observe four or five diseases, not including the pests, for instance, *Fusarium* wilt, powdery mildew and two or three viruses.

Control of the diseases includes prophylactic measures such as the use of healthy seeds, grafting and soil disinfection for soilborne pathogens, removing the weeds or the old contaminated crops which could be the pathogen reservoirs, spraying chemicals, cross protection with mild strains, etc. The use of resistant cultivars is for the farmer one of the easiest ways to control the diseases. Following the pioneer work of Jagger and Scott (1937) on powdery mildew, genetic resistances to viruses, bacteria or fungi/oomycetes have been found in the genetic resources and introduced in improved cultivars in the main cultigroups.

In this chapter, we will review the conventional resistances which have been described in the genetic resources of melon, the use of marker-assisted selection for breeding new resistant cultivars, the molecular knowledge on resistance genes and the use of transgenic plants, mainly through resistance derived from the pathogen. We will not develop some molecular tools which are very useful for breeding such as (RT-)PCR for the detection of viruses (or other pathogens) in resistant or susceptible plants.

M. Pitrat (✉)
INRA, UR1052, Génétique et amélioration des fruits et légumes, CS 60094, 84143 Montfavet cedex, France
e-mail: Michel.Pitrat@avignon.inra.fr

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_11

11.2 Conventional Disease Resistance in the Genetic Resources

Resistance can be characterised by several traits: level of resistance (immunity, partial resistance), genetic control (mono- or polygenic, dominant or recessive), stability or durability (stable resistance or overcome by some strains = races differing by their virulence).

Some resistance has been introduced in commercial cultivars and is available for farmers at least in some cultigroups; others have been described in genetic resources but not yet used in breeding programmes.

Up to now no character has been introduced in melon from related species, cultivated like cucumber (*Cucumis sativus*) or wild like *Cucumis metuliferus*, *C. anguria*, etc. Direct sexual interspecific crosses have not been successful, and regeneration from protoplast fusion is still a challenge. Only the intraspecific variability has been used.

11.2.1 Resistance to Viruses

More than 50 viruses have been described on melon, but resistance has been described only for some of them. A list of the sources of resistance and the genetic control is given in Table 11.1.

11.2.1.1 Aphid-Transmitted Viruses

Partial resistance to *Cucumber mosaic virus* (CMV), the virus type for the *Cucumovirus* genus, was found in several accessions from Eastern Asia. It is under oligogenic recessive control in Freeman's cucumber and PI 161375 (Table 11.1). A major recessive gene (*cmv1*) confers total resistance at least to some strains (Table 11.1). A monogenic dominant resistance has also been described in Yamatouri. The resistance in PI 161375 is overcome by some strains, but the frequency of these virulent strains seems not to increase even after the cultivation of resistant genotypes (Lecoq et al. 2004). Other sources of resistances have been described in the *conomon* and *makuwa* groups of melon (Daryono et al. 2003; Enzie 1943; Hirai and Amemiya 1989; Risser et al. 1977; Webb and Bohn 1962), but the genetic control is unknown. Resistance has also been described in accessions from Iran (Arzani and Ahoonmanesh 2000) and India (Dhillon et al. 2006).

Several viruses belonging to the genus *Potyvirus* can infect melon. Partial monogenic resistance to *Watermelon mosaic virus* (WMV) has been described in PI 414723 from India and PI 482420 from Zimbabwe (Table 11.1). Other sources have been described in accessions from Iran (Arzani and Ahoonmanesh 2000).

Table 11.1 Genetic control of virus resistance in melon

Virus[a]	Resistant accession	Genetic control	References
CMV	Freeman's cucumber	Oligo rec	Karchi et al. (1975)
	PI 161375	Oligo rec	Dogimont et al. (2000), Guiu-Aragonés et al. (2014), Risser et al. (1977)
		Mono rec (*cmv1*)	Essafi et al. (2009)
	Yamatouri	Mono dom (*Creb-2*)	Daryono et al. (2003)
WMV	PI 414723	Mono dom (*Wmr*)	Gilbert et al. (1994)
	PI 482420 (TGR 1551)	Mono rec (*wmr-2*)	Diaz-Pendon et al. (2005)
ZYMV	PI 414723	Mono dom (*Zym*)	Pitrat and Lecoq (1984)
		Oligo dom (*Zym-1*, *Zym-2*, *Zym-3*)	Danin-Poleg et al. (1997)
PRSV	PI 180280	Mono dom (Prv^{1})	Webb (1979)
	PI 180283	Mono dom (Prv^{2})	Kaan (1973)
	PI 124112	Mono dom (*Prv-2*)	McCreight and Fashing-Burdette (1996)
CABYV	PI 124112	Oligo rec (*cab-1* and *cab-2*)	Dogimont et al. (1997)
BPYV	Nagata Kin Makuwa	Mono partially dom (*My*)	Esteva and Nuez (1992)
	PI 161375	Mono partially rec	Esteva and Nuez (1992)
CYSDV	PI 482420 (TGR 1551)	Mono dom (*Cys*)	López-Sesé and Gómez-Guillamón (2000)
	PI 313970	Mono rec (*cys-2*)	McCreight and Wintermantel (2011)
LIYV	PI 313970	Mono dom (*Liy*)	McCreight (2000)
CuLCrV	PI 313970	Mono rec (*culcrv*)	McCreight et al. (2008)
CVYV	PI 164323	Mono dom ($Cvy\text{-}1^{1}$)	Pitrat et al. (2012)
	HSD 2458	Mono rec (*cvy-2*)	Pitrat et al. (2012)
SqMV	China 51	Mono rec (*sqmv*)	Provvidenti (1998)
MNSV	Gulfstream, Planters Jumbo	Mono rec (*nsv*)	Coudriet et al. (1981)
	Doublon	Oligo dom (*Mnr-1* and *Mnr-2*)	Mallor Gimenez et al. (2003)
CGMMV	Chang Bougi	Oligo rec (*cgmmv-1* and *cgmmv-2*)	Sugiyama et al. (2007)

Mono = monogenic; oligo = oligogenic; dom = dominant; rec = recessive
[a]See in the text (paragraph "resistance to viruses") for the acronyms of the viruses

Resistance to *Zucchini yellow mosaic virus* (ZYMV) has been identified in PI 414723. The genetic control in this accession has been described as monogenic or oligogenic dominant, probably according to the strain of ZYMV (Table 11.1). Other sources have been found in accessions from Iran (Arzani and Ahoonmanesh 2000) and two accessions from India (PI 179905, IC-274014) (Pitrat and Lecoq, unpublished). Strains of ZYMV able to overcome the resistance of PI 414723 have

been identified in the laboratory and isolated in the field. Two pathotypes of ZYMV have been defined according to their ability to induce wilting and necrotic symptoms on melon lines carrying the dominant allele *Flaccida necrosis* (symbol *Fn*). About 50 % of the melon accessions carry this allele.

Resistance to *Papaya ringspot virus* (PRSV) is controlled by dominant alleles (Table 11.1): Prv^1 from PI 180280 has been introduced in WMR 29; Prv^2 is present in PI 180283 or PI 414723. Resistance in PI 124112 has been assigned to another locus (*Prv*-2), but the allelism test with the locus *Prv* has not been made. These three accessions are from India. It seems that the allele Prv^1 has not been overcome by strains of PRSV.

Other viruses belonging to the PRSV cluster have been characterised: *Moroccan watermelon mosaic virus* (MWMV), *Algerian watermelon mosaic virus* (AWMV), *Zucchini yellow fleck virus* (ZYFV) and *Zucchini tigré mosaic virus* (ZTMV). Interestingly WMR 29 and PI 414723 which are resistant to PRSV are also resistant to these four viruses (Quiot-Douine et al. 1990; Romay et al. 2013; Yakoubi et al. 2008). The same locus is probably involved. MWMW and AWMV induce necrotic symptoms and death of the plants on many accessions carrying a dominant allele for incompatible reaction called *Necrosis with Moroccan strain of WMV* (symbol *Nm*) (Quiot-Douine et al. 1990).

Cucurbit aphid-borne yellows virus (CABYV) belonging to the *Polerovirus* is transmitted in a persistent manner, whereas *Cucumovirus* and *Potyvirus* are transmitted in a non-persistent manner. Several accessions from India, Eastern Asia or Africa are resistant (Dogimont et al. 1996). Two complementary recessive genes are involved in the genetic control of resistance in PI 124112 (Table 11.1).

Resistance to the colonisation of melon by *Aphis gossypii* is linked to the resistance of transmission of aphid-borne viruses by this vector (see Chap. 12 on Insect Resistance). This character is complementary of resistance to the viruses for the control of virus epidemics.

11.2.1.2 Whitefly-Transmitted Viruses

The first three viruses listed in this section belong to the *Crinivirus* genus. *Beet pseudo-yellows virus* (BPYV) is transmitted by the greenhouse whitefly *Trialeurodes vaporariorum*, while the two others are transmitted by *Bemisia tabaci*. Partial resistance to BPYV (under the name *Melon yellows virus*) has been found in Nagata Kin Makuwa and PI 161375 from Eastern Asia (Table 11.1).

Resistance to *Lettuce infectious yellows virus* (LIYV) is under a monogenic dominant control in PI 313970 from India (Table 11.1).

Cucurbit yellow stunting disorder virus (CYSDV) belongs also to the criniviruses. Resistance under a monogenic dominant control has been found in PI 482420 and under a monogenic recessive control in PI 313970 (Table 11.1). Other sources from India (Ames 20203, PI 614185, PI 614213) have been recently described, but the genetic control is unknown (McCreight and Wintermantel 2008).

By using *Agrobacterium*-mediated inoculation, resistance to *Watermelon chlorotic stunt virus* (WmCSV), belonging to the *Begomovirus* genus, has been described in several accessions from India (PI 124112, PI 414723, 90625) and Africa (HSD 2445-005, PI 282448), but the genetic control is unknown (Yousif et al. 2007).

PI 313970, resistant to LIYV, is also resistant to *Cucurbit leaf crumple virus* (CuLCrV), another *Begomovirus*, and the resistance is under a monogenic recessive control (Table 11.1).

Cucumber vein yellowing virus (CVYV) belongs to the *Ipomovirus* genus. Resistance has been found in PI 164323 from India and is controlled by one dominant allele $Cvy\text{-}1^1$ (Table 11.1). At the same locus, the allele $Cvy\text{-}1^2$, present, for instance, in HSD 93-20-A from Sudan, controls a systemic necrosis followed by the death of the plant. A partial resistance is present in HSD 2458 from Sudan and is controlled by a recessive allele (*cvy-2*). Another gene is also involved in the type of symptoms: severe stunting and yellowing of the plants are controlled by the dominant allele *Cvy-3*; this gene is present in Ouzbeque 2, for instance, but also in PI 164323.

After inoculation with SqVYV, another *Ipomovirus*, some melon accessions (Honey Yellow, Lily, Diplomat) were observed asymptomatic, but the virus was detected in the plants (Webster et al. 2013). Inheritance of this trait is unknown.

11.2.1.3 Viruses Transmitted by Other Vectors

A partial resistance to *Squash mosaic virus* (SqMV), belonging to the *Comovirus* genus, has been described in the accession China 51 (Table 11.1).

Immunity to *Melon necrotic spot virus* (MNSV), belonging to the *Carmovirus* genus, is present in accessions from Eastern Asia or from the USA and is controlled by the recessive allele *nsv* (Table 11.1). This gene which has been introduced in many cultivars is overcome by strains of MNSV isolated in Spain (Díaz et al. 2002). Another mechanism of resistance with typical symptoms on the inoculated parts of the plant but no systemic movement of the virus is controlled by two complementary dominant alleles (*Mnr-1* and *Mnr-2*) (Table 11.1), the locus *Mnr-1* being linked (19 cM) to the locus *nsv*.

Cucumber green mottle mosaic virus (CGGMV) belongs to the *Tobamovirus* genus, and a resistance controlled by two recessive genes has been described in a Korean accession (Table 11.1).

Resistance to the *Kyuri green mottle mosaic virus* (KGGMV), another *Tobamovirus*, has been identified in accessions from Eastern Asia (Mawatauri, PI 161375, Kohimeuri) or India (PI 371795), but the genetic control has not been published (Daryono et al. 2005).

11.2.1.4 General Comments on Resistance to Viruses

The most common symptoms induced by viruses on melon are (1) mosaic with or without leaf deformation (CMV, ZYMV, SqMV, etc.) or (2) yellowing often of the older leaves (CABYV, BPYV, CYSDV, etc.). Some viruses, for instance, MNSV, induce necrotic symptoms. Systemic necrosis leading to the death of the plants can also be observed in interactions between some melon accessions and viruses belonging to the *Potyvirus* or the *Ipomovirus* genus. This incompatible interaction is under a monogenic dominant control: allele *Fn* for ZYMV, allele *Nm* for MWMV or allele $Cvy\text{-}1^2$ for CVYV. Can these alleles or other alleles at the same locus be considered as resistance gene which could lead to an incompatible interaction without systemic symptoms with some specific strains of the corresponding viruses? This is the case with $Cvy1^1$ and $Cvy1^2$, but for *Fn* or *Nm*, up to now no other alleles controlling resistance have been identified.

Resistance under dominant genetic control has been observed for CMV, ZYMV, WMV, PRSV (and probably other potyviruses belonging to the PRSV cluster), BPYV, CYSDV, LIYV, CVYV and MNSV and under recessive genetic control for CMV, CABYV, BPYV, CYSDV, CuLCrV, CVYV, MNSV and CGMMV. There is no general rule for dominant *vs*. recessive or monogenic *vs*. oligo- or polygenic control.

Except for resistance to MNSV which has been found in several accessions from Eastern Asia and the USA, resistance to other viruses has been observed in a small number of accessions. Most of the sources of resistance belong to the *momordica* and *acidulus* groups from India and the *conomon*, *makuwa* and *chinensis* groups from Eastern Asia (China, Japan and Korea).

Very few virus-resistant cultivars have been released. Resistance to MNSV has been intensively introduced in the Galia type and also in some Piel de Sapo type. Resistance to CMV is quantitative, and a few cultivars with some level of resistance have been released in the Galia type and in Japan. Resistance to ZYMV and WMV from PI 414723 has been introduced in the cv. Hannah's Choice (Henning et al. 2005). WMR 29, Cinco and W-1 (all of them in the American cantaloupe type) with resistance to PRSV have been released.

11.2.2 Resistance to Bacteria

Resistance to bacterial fruit blotch (*Acidovorax avenae* subsp. *citrulli*) has been recently described, but the genetic control is unknown (Wechter et al. 2011): PI 536473 (from Maldives), PI 353814 (from Israel) and PI 614401 (from India).

Partial resistance to *Erwinia tracheiphila* has been observed in the cv. Burrell Gem and the F_1 Super Market (Owens and Peterson 1982).

11.2.3 Resistance to Oomycetes and Fungi

Many oomycetes and fungi have been described as pathogens of melon in different growing conditions. A list of the sources of resistance and the genetic control is given in Table 11.2.

11.2.3.1 Soilborne Oomycetes and Fungi

Four races of *Fusarium oxysporum* f.sp. *melonis* have been reported according to the interaction with major genes in melon accessions. Genes *Fom-1*, *Fom-3* or *fom-4* control resistance to races 0 and 2; gene *Fom-2* controls resistance to races 0 and 1 (Table 11.2). Race 1.2 overcomes all these four genes. Two complementary recessive genes (*fom 1.2a* and *fom 1.2b*) have been described as controlling resistance to race 1.2 in the line BIZ whose origin is unknown. In other accessions, resistance to race 1.2 has been described as polygenic and recessive (Table 11.2).

Sources of resistance to *Pythium ultimum* and *P. aphanidermatum* have been described in 61090 and 92393 (= F_1 PI 125861 × 61090) (McCreight 1983); the genetic control is unknown.

Vine decline has been reported as caused by *Monosporascus cannonballus* and/or *Acremonium species*. A semidominant allele (*Mvd*) controls a partial resistance to the complex in the accession Pat 81 (Table 11.2). Partial resistance to *M. cannonballus* has been described in several accessions: Deltex (an Ananas hybrid) (Wolff and Miller 1998); Nabijani, Sfidak khatdar, Sfidak bekhat, Ghandak, Mollamosai, Chappat, Hajmashallahi and Shadgan from Iran (Salari et al. 2012); and K134068, K133069, Wondae and PI 414723 (Park et al. 2012). Partial resistance to *M. cannonballus* has been found, in some cases, linked with root vigour (Crosby et al. 2000; Dias et al. 2004; Iglesias et al. 2000).

11.2.3.2 Foliar and Stem Oomycetes and Fungi

Resistance to downy mildew caused by *Pseudoperonospora cubensis* has been found mainly in accessions from India. Genetic control has been described as two dominant genes (*Pc-1* and *Pc*-2) in PI 124111 or its derivative MR-1 or oligogenic recessive (Table 11.2). Similarly in PI 124112 two dominant genes (*Pc-1* or *Pc-2* present in MR-1 and *Pc-4*) have been described and a polygenic recessive control (Table 11.2). Partial dominant resistance has also been described in PI 414723 and the line 5-4-2-1 (Table 11.2). Other sources of resistance have been described, but the genetic control has not been published (More et al. 2001; Pandey et al. 2008; Somkuwar and More 1993; Sowell and Corley 1974; Takada 1983; Whitner 1960).

There is a lot of confusion around resistance to powdery mildew incited by *Podosphaera xanthii* and *Golovinomyces cichoracearum*, partly because the races were not always accurately described and partly because the allelism tests between

Table 11.2 Genetic control of oomycetes and fungi resistance in melon

Oomycete or fungi	Resistant accession	Genetic control	References
Fusarium wilt	Doublon	Mono dom (*Fom-1*)	Risser (1973)
	CM 17187	Mono dom (*Fom-2*)	Risser (1973)
	Perlita FR	Mono dom (*Fom-3*)	Zink and Gubler (1985)
	Tortuga	Mono rec (*fom-4*)	Oumouloud et al. (2010)
	BIZ	Oligo rec (*fom 1.2a* and *fom 1.2b*)	Herman and Perl-Treves (2007)
	Ogon 9	Oligo rec	Risser and Rode (1973) Perchepied and Pitrat (2004)
	BG-5384	Oligo rec	Chikh-Rouhou et al. (2011)
Vine decline	Pat 81	Mono dom (*Mvd*)	Iglesias et al. (2000)
Downy mildew	PI 124111, MR-1	Oligo dom (*Pc-1* and *Pc-2*)	Cohen et al. (1985), Thomas et al. (1988)
		Oligo rec	Epinat and Pitrat (1994a, b)
	PI 414723	Mono dom (*Pc-3*)	Epinat and Pitrat (1989)
	PI 124112	Oligo dom (*Pc-4* and *Pc-1* or *Pc-2*)	Kenigsbuch and Cohen (1992)
		Oligo rec	Epinat and Pitrat (1994a, b), Perchepied et al. (2005b)
	Line 5-4-2-1	Oligo (*Pc-5*, *M-Pc-5*)	Angelov (1996), Angelov and Krasteva (2000)
Powdery mildew	PMR 45	Mono dom (*Pm-1*)	Jagger et al. (1938)
	PMR 5	Oligo dom (*Pm-1*, *Pm-2* and *Pm-E*)	Bohn and Whitaker (1964), Epinat et al. (1993)
	AR 5	Oligo dom	Fukino et al. (2008)
	PI 124111	Oligo dom (*Pm-3* and *Pm-6*)	Harwood and Markarian (1968b), Kenigsbuch and Cohen (1989)
	PI 124112	Oligo dom (*Pm-4*, *Pm-5*, *Pm-F*, *Pm-G*, *PmV.1*, *PmXII.1*)	Epinat et al. (1993), Harwood and Markarian (1968a, b), Perchepied et al. (2005b)
	PI 414723	Oligo dom (*Pm-7*, *Pm-x*)	Anagnostou et al. (2000), Pitrat (1991)
	PI 134198	Mono dom (*Pm-8*)	Liu et al. (2010)
	Nantais oblong	Mono dom (*Pm-H*)	Epinat et al. (1993)
	PI 482420 (TG 1551)	Mono dom (*Pm-R1-2*) Mono dom (*Pm-R5*)	Yuste-Lisbona et al. (2011a)
	PI 313970 (=90625)	Oligo dom and rec (*pm-s*, *Pm-z*, *A*, *B*, *c*, *d*, *e*)	McCreight (2003), McCreight and Coffey (2011), Pitrat and Besombes (2008)
	WMR 29	Mono dom (*Pm-w*)	Epinat et al. (1993), Pitrat (1991)
	VA 435	Mono dom (*Pm-y*)	Pitrat (1991)

(continued)

Table 11.2 (continued)

Oomycete or fungi	Resistant accession	Genetic control	References
Alternaria leaf blight	MR-1	Mono dom (*Ac*)	Thomas et al. (1990)
Gummy stem blight	PI 140471	Mono dom (*Gsb-1*)	Prasad and Norton (1967)
	PI 157082	Mono dom (*Gsb-2*)	Zuniga et al. (1999)
	PI 511890	Mono dom (*Gsb-3*)	Zuniga et al. (1999)
	PI 482398	Mono dom (*Gsb-4*)	Frantz and Jahn (2004)
	PI 482399	Mono rec (*gsb-5*)	Frantz and Jahn (2004)
	Lines C1 and C8	Mono dom (*Gsb-6*)	Prasad and Norton (1967)
	PI 420145	Mono dom (*Gsb-7*)	Wolukau et al. (2007)

Mono, monogenic; oligo, oligogenic; dom, dominant; rec, recessive

the different sources of resistance were not always conducted. Moreover, some of the genes are specific of *P. xanthii* (for instance, *Pm-1* in PMR 45) or of *G. cichoracearum* (for instance, *Pm-H* in Nantais oblong), while some are effective against both species. So the list presented in Table 11.2 should be read with caution. Most of the sources of resistance to *P. xanthii* are from India, while resistance to *G. cichoracearum* has been described in accessions from several geographical origins.

Sulphur is often used to control powdery mildew but many melon accessions are susceptible to sulphur exhibiting leaf necrosis. A recessive gene (symbol *sr*) for sulphur resistance has been described (Table 11.3).

Resistance to *Alternaria* leaf blight (*Alternaria cucumerina*) is quite common in many accessions belonging to different groups (Table 11.2).

Resistance to Gummy stem blight (*Didymella bryoniae*) has been described in accessions from different countries including China, Japan, Mexico and Zimbabwe. Several independent loci have been described even if no races have been formally recognised (Table 11.2). Other sources of resistance (PI 157076 and PI 323498 both from China) have been identified but the genetic control is unknown (Wolukau et al. 2007).

11.2.3.3 General Comments on Resistance to Oomycetes and Fungi

Some genes for fungi resistance are quite common in many independent melon accessions, for instance the genes *Fom-1* and *Fom-2* for *Fusarium* wilt or resistance to *G. cichoracearum* (Pitrat et al. 1996).

Resistance to *Fusarium* wilt (at least for races 1 and 2) and to powdery mildew (at least for the most common races) has been introduced in many commercial cultivars.

Table 11.3 Localisation of genes and QTLs involved in disease resistance on the melon linkage groups

Linkage group	Trait	Gene or QTL	References
I	Sulphur resistance	*sr*	Perchepied et al. (2004)
	CMV	QTL (*cmvqw1.1*)	Guiu-Aragonés et al. (2014)
II	CMV	2 QTLs	Dogimont et al. (2000)
	ZYMV	*Zym*	Pitrat (1991)
	WMV	*Wmr*	Anagnostou et al. (2000)
	Fusarium wilt	*fom1.2a*	Herman et al. (2008)
	Downy mildew	QTL (*pcII.1*)	Perchepied et al. (2005b)
	Powdery mildew	*Pm-x* + 1 gene in AR 5	Fukino et al. (2008), Pitrat (1991)
III	CMV	2 QTLs	Dogimont et al. (2000)
		QTL (*cmvqw3.1*)	Guiu-Aragonés et al. (2014)
	Fusarium wilt	3 QTLs (*fomIII.1*, *fomIII.2*, *fom III.3*)	Perchepied et al. (2005a)
IV	Downy mildew	QTL (*pcIV.1*)	Perchepied et al. (2005b)
V	ZYMV	*Fn*	Pitrat et al. (1982)
	Fusarium wilt	2 QTLs (*fomV.1*, *fomV.2*)	Perchepied et al. (2005a)
	Powdery mildew	*Pm-w*	Epinat et al. (1993), Pitrat (1991)
		PmV.1	Perchepied et al. (2005b)
		Pm-R1-2, *Pm-R5*	Yuste-Lisbona et al. (2011b)
VI	*Fusarium* wilt	2 QTLs (*fomVI.1*, *fomVI.2*)	Perchepied et al. (2005a)
	Downy mildew	QTL (*pcVI.1*)	Perchepied et al. (2005b)
VII	Powdery mildew	*Pm-8*	Liu et al. (2010)
VIII	CMV	QTL	Dogimont et al. (2000)
	Downy mildew	QTL (*pcVIII.1*)	Perchepied et al. (2005b)
	Powdery mildew	Minor QTL	Yuste-Lisbona et al. (2011b)
IX	CMV	QTL	Dogimont et al. (2000)
	PRSV	*Prv*	Périn et al. (2002), Pitrat (1991), Teixeira and Camargo (2006)
	Fusarium wilt	*Fom-1*	Périn et al. (2002), Pitrat (1991)
	Powdery mildew	*Pm-1*	Teixeira et al. (2008)

(continued)

Table 11.3 (continued)

Linkage group	Trait	Gene or QTL	References
X	CMV	QTL (*cmvqw10.1*)	Guiu-Aragonés et al. (2014)
XI	*Fusarium* wilt	*Fom-2* and 1 QTL	Perchepied et al. (2005a)
	Downy mildew	QTL (*pcXI.1*)	Perchepied et al. (2005b)
XII	CMV	Major QTL	Dogimont et al. (2000)
		cmv1 = *cmvqw12.1*	Essafi et al. (2009), Guiu-Aragonés et al. (2014)
	MNSV	*nsv*	Périn et al. (2002), Pitrat (1991)
		Mnr-1	Mallor Gimenez et al. (2003)
	Fusarium wilt	QTL (*fomXII.1*)	Perchepied et al. (2005a)
	Downy mildew	QTL (*pcXII.1*)	Perchepied et al. (2005b)
	Powdery mildew	*Pm-y*	Pitrat (1991)
		PmXII.1	Perchepied et al. (2005b)
		Gene from AR 5	Fukino et al. (2008)

11.2.4 Conclusions on Conventional Resistance in the Genetic Resources

Disease resistance has been identified mainly in cultivars (landraces) and very few in wild melon accessions. Maybe wild accessions are underrepresented in melon collections. But disease resistance could also represent a diversification trait resulting from human selection.

Some accessions cumulate several resistances. For instance, PI 313970 (90625) is resistant to CABYV, WmCSV, LIYV, CuLCrV, powdery mildew and downy mildew; PI 414723 to ZYMV, PRSV, powdery mildew, downy mildew and *Fusarium* wilt; PI 161375 to CMV, MNSV, BPYV, KGGMV and *Fusarium* wilt; and PI 482420 (TGR 1551) to CYSDV, WMV and powdery mildew.

Some cultigroups from India (*momordica*, *acidulus*) and Eastern Asia (*chinensis*, *conomon*, *makuwa*) are particularly rich in disease resistance genes.

11.3 Mapping Resistance Genes and Marker-Assisted Selection

The major genes and QTLs involved in disease resistance which have been localised on genetic maps are listed in Table 11.3 (Díaz et al. 2011).

From the melon genome sequence, 79 resistance genes have been identified in 19 clusters on the 12 linkage groups (LG) (Garcia-Mas et al. 2012). Some clusters of genes or QTLs have been clearly identified on LG II with resistance to CMV, ZYMV, WMV and downy and powdery mildew; on LG V with ZYMV, powdery mildew and *A. gossypii*; on LG IX with PRSV and *Fusarium* wilt; and on LG XII with CMV, MNSV and downy and powdery mildew. Many genes and QTLs have not yet been mapped.

Markers linked to other disease resistance genes have also been identified, but their localisation on the melon map is unknown, for instance, gene *Creb-2* for resistance to CMV (Daryono et al. 2010) and gene *fom-4* for resistance to *Fusarium* wilt (Oumouloud et al. 2012).

The usefulness or effectiveness of marker-assisted selection in melon is limited by the high recombination rate and the polymorphism between the donor resistant parent and the susceptible recurrent parent (see Chap. 5). Moreover, some resistance genes are present in different sources, for instance, *Fom-1* or *Fom-2*, which are present in 22 % of the genetic resources (Pitrat et al. 1996); according to the accession used as the donor parent, the linked markers could be different. There are many reports and observations from breeders that very few universal molecular markers can be used for breeding elite material in different melon types (Galia, Cantaloupes, Charentais, Piel de Sapo, etc.). Polymorphism in the gene sequence useful for MAS is available only for a few genes, for instance, the gene *nsv* for MNSV resistance (see following section).

11.4 Molecular Knowledge on Resistance Genes

Five genes involved in disease resistance have been cloned in melon by chromosome walking strategy: *nsv* for MNSV resistance, *Prv* for PRSV resistance, *Fom-1* and *Fom-2* for *Fusarium* wilt resistance and *Pm-w* for powdery mildew resistance.

The *nsv* gene on LG XII is effective against all strains of MNSV except to MNSV-264 (Díaz et al. 2002). Using a strategy combining positional cloning and candidate gene approach, the gene *nsv* was identified as a eukaryotic translation initiation factor 4E (*Cm-eIF4E*) (Nieto et al. 2006). A change of one amino acid in position 228 led to resistance (leucine) or susceptibility (histidine) to MNSV. The same point mutation was identified by Eco-TILLING in independent accessions suggesting a unique origin for resistance (Nieto et al. 2007). Using chimeric viruses between virulent and avirulent strains, the virulence determinant was identified at the 3′ untranslated region of the viral genome (Díaz et al. 2004; Truniger et al. 2008).

The genes *Prv* and *Fom-1* are closely linked in repulsion phase on LG IX (Pitrat 1991). By using a chromosome walking strategy on a BAC library followed by 454 sequencing, it was demonstrated that the area was a cluster of resistance gene

homologs (RGHs) (Brotman et al. 2012). Both *Prv* and *Fom-1* belonged to the TIR-NB-LRR family and were head to head oriented. The phenotype of the two alleles Prv^1 and Prv^2 could not be clearly related to molecular data.

The gene *Fom-2* on LG XI belongs also to a cluster of RGH. Two candidate genes were identified in this area, and a gene belonging to the CC NB-LRR family was identified as *Fom-2* (Joobeur et al. 2004). The validation of the function of this gene has been made on composite plants where transgenic roots were produced using *Agrobacterium rhizogenes* (Normantovich et al. 2012). On the entire sequence, the genes *Fom-1* and *Fom-2* exhibited only 13 % of identity.

The gene *Pm-w* on LG V which controls resistance to races 1, 2 and 3 of *P. xanthii* is very surprisingly an allele of *Vat* controlling *A. gossypii* resistance (see Chap. 12). Both belong to the CC NB-LRR family and are very similar in the CC and NB domains. In the D domain of the LRR region, while *Vat* gene encodes for four repeats of a conserved 65-amino acid sequence, *Pm-w* encodes for five repeats of the same 65-amino acid sequence (Dogimont et al. 2008).

A reverse genetic strategy has been used to identify genes involved in the resistance to downy mildew. A protein (P45) is present in the melon accession PI 124111 resistant to *P. cubensis* and barely detectable in susceptible accessions. By performing RT-PCR with primers derived from the sequence of the protein, two genes (*At1* and *At2*) were identified (Taler et al. 2004). They code for two very similar proteins (93 % amino acid homology) with a glyoxylate aminotransferase activity. Susceptible accessions have very similar genes, but the level of the protein is very low due to regulation at the transcriptional level. Genes *Pc-1* and *Pc-2* (Table 11.2) are probably involved in the recognition of downy mildew and regulation of the defence mechanisms and do not correspond to the genes *At1* and *At2*. Transgenic melon plants overexpressing the *At1* or *At2* genes exhibit a high level of glyoxylate aminotransferase activity and a high level of resistance to downy mildew (Taler et al. 2004).

A mutant plant obtained by *Agrobacterium*-mediated T-DNA insertion was found to have a good level of resistance to *D. bryoniae*, but the function of this gene named *edr2* is unknown (Ren et al. 2009).

More than 400 putative disease resistance genes have been described in the melon genome (Garcia-Mas et al. 2012). Among the 81 belonging to the cytoplasmic group (NB-LRR sensu lato), four have been identified and correspond to *Fom-1*, *Fom-2*, *Prv* and *Pm-w*. These R-genes are generally involved in the pathogen recognition which is followed in the case of an incompatible reaction by a cascade of defence mechanisms. But other genes such as *At1* and *At2* of the photorespiratory pathway are also involved in resistance by the level of enzyme expression. In fact, the total number of genes involved in resistance and defence mechanisms is unknown.

11.5 Resistance Derived from the Pathogen and Transgenic Plants

Transgenic resistance represents several advantages (for a review on virus resistance, see Gaba et al. 2004). Most of the applications have been made to control viruses using translatable genes, mainly the coat protein. More recently, gene silencing using untranslatable RNA has also been used in melon.

One of the limitations in the use of transgenic melon plants is the low efficiency of regeneration by organogenesis or somatic embryogenesis and transformation of melon. Nevertheless, several independent laboratories in the world have been able to obtain transgenic melon plants, not only for disease resistance (see Chap. 13).

11.5.1 Coat Protein

The viral coat protein (CP) has been used, first with CMV (Gonzalves et al. 1994; Yoshioka et al. 1991, 1992, 1993) and later with different potyviruses: ZYMV (Fang and Grumet 1993), PRSV (Krubphachaya et al. 2007), ZYMV and WMV (Clough and Hamm 1995). Cantaloupes with the CP of CMV, WMV and ZYMV were also produced (Fuchs et al. 1997).

In the case of ZYMV, the presence of the full-length CP gene induces a complete resistance to ZYMV, but the conserved core portion of the CP or an antisense construct induces only a delay in the symptoms or a reduction of the virus concentration (Fang and Grumet 1993).

It seems that the level of resistance is directly correlated with the concentration of CP in the transgenic plants (Fuchs et al. 1997).

11.5.2 Ribozyme

Different constructions with several active sites have been developed against CMV (Lenee et al. 1994; Plagès 1997) or potyviruses (ZYMV and WMV) (Huttner et al. 1999, 2001). Transgenic melon plants with a high level of resistance have been produced.

11.5.3 Post-transcriptional Gene Silencing

The post-transcriptional gene silencing (PTGS) can be effective for virus resistance if a virus-derived sequence is silenced in a transgenic plant. In opposition with the CP-mediated resistance, a low level of accumulation of transgene transcripts is

associated with a high level of resistance. Three cases of untranslatable constructs resulting in virus resistance have been published.

An untranslatable chimeric construct of partial CP genes of ZYMV and PRSV has been introduced in susceptible melon plants (Wu et al. 2010). Small interfering RNAs were detected in highly resistant and immune plants.

Transgenic melon plants with a hairpin construct of the helper-component (HC-Pro) gene of ZYMV exhibited high level of resistance to this virus. Interestingly, cucumber plants with the same construct showed a high level of resistance to ZYMV but also to two other potyviruses (WMV or PRSV), while melon had no resistance to the last two viruses (Leibman et al. 2011). Transgenic small interfering RNA of 21–22 nucleotides was present in sense and antisense orientations.

Melon plants expressing the direct repeat of the movement protein (MP) of CGMMV showed a high level of resistance, while plants with a single copy of the MP were susceptible (Emran et al. 2012).

11.5.4 Enzymes

Transgenic melon plants overexpressing genes coding for an "antifungal protein" and a chitinase were produced. A partial resistance against *Rhizoctonia solani* and *F. oxysporum* was observed (Bezirganoglu et al. 2013).

11.5.5 Field Experiment and Risk Assessment

In closed or semi-closed greenhouses, no differences were detected between transgenic and non-transgenic melon with the coat protein of CMV for plant and fruit morphology and gene flow (Tabei et al. 1994a). Using the same material, transgenic plants had no influence on the soil microflora (Tabei et al. 1994b).

Experiments were conducted to study the effect of the presence of the coat protein in transgenic plant on the aphid transmission of a virus. Transgenic melon plants producing the CP of a virus-transmissible strain of CMV were mechanically inoculated with the WL or the C strain of CMV which are respectively aphid transmissible and not aphid transmissible. Using these plants as virus sources for aphid transmission, the WL strain was transmitted, while the C strain was not transmitted to test plants (Fuchs et al. 1998).

Transgenic melon plants with coat protein gene constructs of CMV, WMV and ZYMV were observed in the field in natural inoculation conditions (Fuchs et al. 1997). Homozygous plants were well protected against the three viruses, while in hemizygous plants the virus contaminations were delayed by 2–3 weeks but systemic symptoms were observed 9–10 weeks after field planting. The frequency of mixed infections by two or three viruses was more reduced in

homozygous than in hemizygous plants and in both cases significantly lower than in the susceptible controls.

11.6 Conclusions

Disease resistance is one of the main objectives in melon breeding. The conventional resistance found in the genetic resources is limited to the intraspecific variability. Indeed no resistance observed in related species has been introduced in cultivated melons. Efforts in overcoming the reproductive barriers to interspecific crosses have to be done.

Nevertheless, many sources of resistance have been described in the melon genetic resources. This effort should continue in the future with the phenotypic evaluation of wild and cultivated accessions. Biotechnological tools such as *Agrobacterium*-mediated virus inoculation could be useful in some cases for the germplasm evaluation (Yousif et al. 2007). The development of mutant populations (see Chap. 6) is another source of variability which can be evaluated phenotypically or by reverse genetics using candidate genes.

The release of resistant elite material or improved open pollinated or F_1 hybrid cultivars is still low except for MNSV, *Fusarium* wilt or powdery mildew, even if the release by seed companies is not well documented in scientific publications. Of course cultivars should not be resistant to all diseases. According to the regions and countries, to the growing conditions (greenhouses or open field), resistance to only a few diseases is economically important. Marker-assisted selection efficiency could be increased with the availability of the sequence of the melon genome (Chap. 2) and with the molecular identification of more genes involved in disease resistance.

There are no some many resistance genes available in the genetic resources or in the mutant populations. The management of these genes for a better durability of the resistance could be improved by biotechnological tools. A better knowledge of the function of the resistance genes could help in the strategies of pyramiding different genes in a single cultivar or in the deployment of the genes in time and space.

References

Anagnostou K, Jahn M, Perl-Treves R (2000) Inheritance and linkage analysis of resistance to zucchini yellow mosaic virus, watermelon mosaic virus, papaya ringspot virus and powdery mildew in melon. Euphytica 116:265–270

Angelov D (1996) Inheritance of resistance to the downy mildew in the muskmelon line 5-1-2. In: Gómez-Guillamón ML, Soria C, Cuartero J, Torès JA, Fernandez-Munoz R (eds) Cucurbits toward 2000. VIth EUCARPIA meeting on Cucurbit Genetics and Breeding, Málaga (ES), pp 260–262

Angelov D, Krasteva L (2000) Dominant inheritance of downy mildew resistance in melons. In: Katzir N, Paris HS (eds) Proceedings of Cucurbitaceae 2000, vol 510. Acta Horticulturae (ISHS), Ma'ale Ha Hamisha (IL), pp 273–275

Arzani A, Ahoonmanesh A (2000) Study of resistance to cucumber mosaic virus, watermelon mosaic virus and zucchini yellow mosaic virus in melon cultivars. Iran Agric Res 19:129–144

Bezirganoglu I, Hwang SY, Fang TJ, Shaw JF (2013) Transgenic lines of melon (*Cucumis melo* L. var. *makuwa* cv. 'Silver Light') expressing antifungal protein and chitinase genes exhibit enhanced resistance to fungal pathogens. Plant Cell Tissue Organ Cult 112:227–237

Bohn GW, Whitaker TW (1964) Genetics of resistance to powdery mildew race 2 in muskmelon. Phytopathology 54:587–591

Brotman Y, Normantovich M, Goldenberg Z, Zvirin Z, Kovalski I, Stovbun N, Doniger T, Bolger AM, Troadec C, Bendahmane A, Cohen R, Katzir N, Pitrat M, Dogimont C, Perl-Treves R (2012) Dual resistance of melon to *Fusarium oxysporum* races 0 and 2 and to *Papaya ring-spot virus* is controlled by a pair of head-to-head oriented NB-LRR genes of unusual architecture. Mol Plant 6(1):235–8

Chikh-Rouhou H, Gonzalez-Torres R, Oumouloud A, Alvarez JM (2011) Inheritance of race 1.2 Fusarium wilt resistance in four melon cultivars. Euphytica 182:177–186

Clough GH, Hamm PB (1995) Coat protein transgenic resistance to watermelon mosaic and zucchini yellow mosaic virus in squash and cantaloupe. Plant Dis 79:1107–1109

Cohen Y, Cohen S, Eyal H, Thomas CE (1985) Inheritance of resistance to downy mildew in *Cucumis melo* PI 124111. Cucurbit Genet Coop Rep 8:36–38

Coudriet DL, Kishaba AN, Bohn GW (1981) Inheritance of resistance to muskmelon necrotic spot virus in a melon aphid resistant breeding lines of muskmelon. J Am Soc Hortic Sci 106:789–791

Crosby K, Wolff D, Miller M (2000) Comparisons of root morphology in susceptible and tolerant melon cultivars before and after infection by *Monosporascus cannonballus*. HortSci 35:681–683

Danin-Poleg Y, Paris HS, Cohen S, Rabinowitch HD, Karchi Z (1997) Oligogenic inheritance of resistance to zucchini yellow mosaic virus in melons. Euphytica 93:331–337

Daryono BS, Somowiyarjo S, Natsuaki KT (2003) New source of resistance to cucumber mosaic virus in melon. SABRAO J Breed Genet 35:19–26

Daryono BS, Somowiyarjo S, Natsuaki KT (2005) Screening for resistance to Kyuri green mottle mosaic virus in various melons. Plant Breed 124:487–490

Daryono BS, Wakui K, Natsuaki KT (2010) Linkage analysis and mapping of SCAR markers linked to CMV-B2 resistance gene in melon. SABRAO J Breed Genet 42:35–45

Dhillon NPS, Ranjana R, Singh K, Eduardo I, Monforte AJ, Pitrat M, Dhillon NK, Singh PP (2006) Diversity among landraces of Indian snapmelon (*Cucumis melo* var. *momordica*). Genet Resour Crop Evol 54:1267–1283

Dias RDS, Pico B, Espinos A, Nuez F (2004) Resistance to melon vine decline derived from *Cucumis melo* ssp *agrestis*: genetic analysis of root structure and root response. Plant Breed 123:66–72

Díaz JA, Nieto C, Moriones E, Aranda MA (2002) Spanish *Melon necrotic spot virus* isolate overcomes the resistance conferred by the recessive *nsv* gene of melon. Plant Dis 86:694

Díaz JA, Nieto C, Moriones E, Truniger V, Aranda MA (2004) Molecular characterization of a *Melon necrotic spot virus* strain that overcomes the resistance in melon and nonhost plants. Mol Plant-Microbe Interact 17:668–675

Díaz A, Fergani M, Formisano G, Ziarsolo P, Blanca J, Fei Z, Staub JE, Zalapa JE, Cuevas HE, Dace G, Oliver M, Boissot N, Dogimont C, Pitrat M, Hofstede R, van Koert P, Harel-Beja R, Tzuri G, Portnoy V, Cohen S, Schaffer A, Katzir N, Xu Y, Zhang H, Fukino N, Matsumoto S, Garcia-Mas J, Monforte AJ (2011) A consensus linkage map for molecular markers and Quantitative Trait Loci associated with economically important traits in melon (*Cucumis melo* L.). BMC Plant Biol 11:111

Diaz-Pendon JA, Fernandez-Munoz R, Gomez-Guillamon ML, Moriones E (2005) Inheritance of resistance to *Watermelon mosaic virus* in *Cucumis melo* that impairs virus accumulation, symptom expression, and aphid transmission. Phytopathology 95:840–846

Dogimont C, Slama S, Martin J, Lecoq H, Pitrat M (1996) Sources of resistance to *Cucurbit aphid borne yellows luteovirus* in a melon germ plasm collection. Plant Dis 80:1379–1382

Dogimont C, Bussemakers A, Martin J, Slama S, Lecoq H, Pitrat M (1997) Two complementary recessive genes conferring resistance to *Cucurbit aphid borne yellows Luteovirus* in an Indian melon line (*Cucumis melo* L.). Euphytica 96:391–395

Dogimont C, Lecomte L, Périn C, Thabuis A, Lecoq H, Pitrat M (2000) Identification of QTLs contributing to resistance to different strains of *Cucumber mosaic cucumovirus* in melon. In: Katzir N, Paris H (eds) Cucurbitaceae 2000, VIIth EUCARPIA meeting on cucurbit genetics and breeding, vol 510. Acta Horticulturae (ISHS), Ma'ale Hahamisha (IL), pp 391–398

Dogimont C, Chovelon V, Tual S, Boissot N, Rittener V, Giovinazzo N, Bendahmane A (2008) Molecular diversity at the *Vat/Pm-w* resistance locus in melon. In: Pitrat M (ed) Cucurbitaceae 2008, IXth EUCARPIA meeting on Genetics and Breeding of Cucurbitaceae. INRA, Avignon (FRA), pp 219–227

Emran AM, Tabei Y, Kobayashi K, Yamaoka N, Nishiguchi M (2012) Molecular analysis of transgenic melon plants showing virus resistance conferred by direct repeat of movement gene of *Cucumber green mottle mosaic virus*. Plant Cell Rep 31:1371–1377

Enzie WD (1943) A source of muskmelon mosaic resistance found in the oriental pickling melon, *Cucumis melo* var. *conomon*. Proc Am Soc Hortic Sci 43:195–198

Epinat C, Pitrat M (1989) Inheritance of resistance of three lines of muskmelon (*Cucumis melo*) to downy mildew (*Pseudoperonospora cubensis*). In: Thomas CE (ed) Cucurbitaceae 89. Charleston, SC, pp 133–135

Epinat C, Pitrat M (1994a) Inheritance of resistance to downy mildew (*Pseudoperonospora cubensis*) in muskmelon (*Cucumis melo*). II Generation means analysis of 5 genitors. Agronomie 14:249–257

Epinat C, Pitrat M (1994b) Inheritance of resistance to downy mildew (*Pseudoperonospora cubensis*) in muskmelon (*Cucumis melo*). I Analysis of a 8x8 diallel table. Agronomie 14:239–248

Epinat C, Pitrat M, Bertrand F (1993) Genetic analysis of resistance of five melon lines to powdery mildews. Euphytica 65:135–144

Essafi A, Díaz-Pendón JA, Moriones E, Monforte AJ, Garcia-Mas J, Martín-Hernández AM (2009) Dissection of the oligogenic resistance to *Cucumber mosaic virus* in the melon accession PI 161375. Theor Appl Genet 118:275–284

Esteva J, Nuez F (1992) Tolerance to a whitefly-transmitted virus causing muskmelon yellows disease in Spain. Theor Appl Genet 84:693–697

Fang G, Grumet R (1993) Genetic engineering of potyvirus resistance using constructs derived from the zucchini yellow mosaic virus coat protein gene. Mol Plant-Microbe Interact 6:358–367

Frantz JD, Jahn MM (2004) Five independent loci each control monogenic resistance to gummy stem blight in melon (*Cucumis melo* L.). Theor Appl Genet 108:1033–1038

Fuchs M, McFerson JR, Tricoli DM, McMaster JR, Deng RZ, Boeshore ML, Reynolds JF, Russell PF, Quemada HD, Gonzalves D (1997) Cantaloupe line CZW-30 containing coat protein genes of cucumber mosaic virus, zucchini yellow mosaic virus, and watermelon mosaic virus-2 is resistant to these three viruses in the field. Mol Breed 3:279–290

Fuchs M, Klas FE, McFerson JR, Gonsalves D (1998) Transgenic melon and squash expressing coat protein genes of aphid-borne viruses do not assist the spread of an aphid non-transmissible strain of cucumber mosaic virus in the field. Transgenic Res 7:449–462

Fukino N, Ohara T, Monforte AJ, Sugiyama M, Sakata Y, Kunihisa M, Matsumoto S (2008) Identification of QTLs for resistance to powdery mildew and SSR markers diagnostic for powdery mildew resistance genes in melon (*Cucumis melo* L.). Theor Appl Genet 118:165–175

Gaba V, Zelcer A, Gal-On A (2004) Cucurbit biotechnology - the importance of virus resistance. In Vitro Cell Dev Biol Plant 40:346–358

Garcia-Mas J, Benjak A, Sanseverino W, Bourgeois M, Mir G, González VM, Hénaff E, Câmara F, Cozzuto L, Lowy E, Alioto T, Capella-Gutiérrez S, Blanca J, Cañizares J, Ziarsolo P, Gonzalez-Ibeas D, Rodríguez-Moreno L, Droege M, Du L, Alvarez-Tejado M, Lorente-Galdos B, Melé M, Yang L, Weng Y, Navarro A, Marques-Bonet T, Aranda MA, Nuez F, Picó B, Gabaldón T, Roma G, Guigó R, Casacuberta JM, Arús P, Puigdomènech P (2012) The genome of melon (*Cucumis melo* L.). Proc Natl Acad Sci USA 109:11872–11877

Gilbert RZ, Kyle MM, Munger HM, Gray SM (1994) Inheritance of resistance to watermelon mosaic virus in *Cucumis melo* L. HortSci 29:107–110

Gonzalves C, Xue B, Yepes M, Fuchs M, Ling K, Namba S, Chee P, Slightom JL, Gonsalves D (1994) Transferring cucumber mosaic virus-white leaf strain coat protein gene into *Cucumis melo* L. and evaluating transgenic plants for protection against infections. J Am Soc Hortic Sci 119:345–355

Guiu-Aragonés C, Monforte AJ, Saladié M, Corrêa RX, Garcia-Mas J, Martín-Hernández AM (2014) The complex resistance to cucumber mosaic cucumovirus (CMV) in the melon accession PI 161375 is governed by one gene and at least two quantitative trait loci. Mol Breed 34:351–362

Harwood RR, Markarian D (1968a) The inheritance of resistance to powdery mildew in the cantaloupe variety Seminole. J Hered 59:126–130

Harwood RR, Markarian D (1968b) A genetic survey of resistance to powdery mildew in muskmelon. J Hered 59:213–217

Henning MJ, Munger HM, Jahn MM (2005) 'Hannah's Choice F1': a new muskmelon hybrid with resistance to powdery mildew, Fusarium race 2, and potyviruses. HortSci 40:492–493

Herman R, Perl-Treves R (2007) Characterization and inheritance of a new source of resistance to *Fusarium oxysporum* f. sp. *melonis* race 1.2 in *Cucumis melo*. Plant Dis 91:1180–1186

Herman R, Zvirin Z, Kovalski I, Freeman S, Denisov Y, Zuri G, Katzir N, Perl-Treves R (2008) Characterization of Fusarium race 1.2 resistance in melon and mapping of a major QTL for this trait near a fruit netting locus. In: Pitrat M (ed) Cucurbitaceae 2008, IXth EUCARPIA meeting on Genetics and Breeding of Cucurbitaceae. INRA, Avignon (FRA), pp 149–156

Hirai S, Amemiya Y (1989) Studies on the resistance of melon cultivars to cucumber mosaic virus. I. Virus multiplication in leaves or mesophyll protoplasts from susceptible and resistant cultivars. Ann Phytopathol Soc Jpn 55:458–465

Huttner E, Tucker W, Vermeulen A, Ignart F, Sawyer B, Birch R (1999) Ribozyme genes protecting transgenic melon plants against potyviruses. In: Rossi JJ, Couture L (eds) Intracellular ribozyme applications: principles and protocols. Horizon Scientific Press, Wymondham, GB, pp 271–283

Huttner E, Tucker W, Vermeulen A, Ignart F, Sawyer B, Birch R (2001) Ribozyme genes protecting transgenic melon plants against potyviruses. Curr Issues Mol Biol 3:27–34

Iglesias A, Picó B, Nuez F (2000) A temporal genetic analysis of disease resistance genes: resistance to melon vine decline derived from *Cucumis melo* var. *agrestis*. Plant Breed 119:329–334

Jagger IC, Scott GW (1937) Development of powdery mildew resistant cantaloupe No. 45. USDA Circul 441:1–5

Jagger IC, Whitaker TW, Porter DR (1938) Inheritance in *Cucumis melo* of resistance to powdery mildew (*Erysiphe cichoracearum*). Phytopathology 28:761

Joobeur T, King JJ, Nolin SJ, Thomas CE, Dean RA (2004) The fusarium wilt resistance locus *Fom-2* of melon contains a single resistance gene with complex features. Plant J 39:283–297

Kaan JF (1973) Recherches sur la résistance du melon aux maladies, notamment à la mosaïque de la pastèque et au *Pseudoperonospora*, appliquées au type variétal "Cantaloup Charentais", In: Risser G (ed) EUCARPIA meeting on melon, Avignon, FR, pp 41–49

Karchi Z, Cohen S, Govers A (1975) Inheritance of resistance to Cucumber Mosaic Virus in melons. Phytopathology 65:479–481

Kenigsbuch D, Cohen Y (1989) Independent inheritance of resistance to race 1 and race 2 of *Sphaerotheca fuliginea* in muskmelon. Plant Dis 73:206–208

Kenigsbuch D, Cohen Y (1992) Inheritance of resistance to downy mildew in *Cucumis melo* PI 124112 and commonality of resistance genes with PI 124111F. Plant Dis 76:615–617

Krubphachaya P, Juricek M, Kertbundit S (2007) Induction of RNA-mediated resistance to papaya ringspot virus type W. J Biochem Mol Biol 40:401–411

Lecoq H, Moury B, Desbiez C, Palloix A, Pitrat M (2004) Durable virus resistance in plants through conventional approaches: a challenge. Virus Res 100:31–39

Leibman D, Wolf D, Saharan V, Zelcer A, Arazi T, Yoel S, Gaba V, Gal-On A (2011) A high level of transgenic viral small RNA is associated with broad Potyvirus resistance in Cucurbits. Mol Plant-Microbe Interact 24:1220–1238

Lenee P, Perez P, Gruber V, Baudot G, Ollivo C (1994) Polyribozyme capable of conferring on plants resistance to cucumber mosaic virus and resistant plants producing this polyribozyme. US Patent 6,265,634

Liu L, Chen Y, Su Z, Zhang H, SZhu W (2010) A sequence-amplified characterized region marker for a single, dominant gene in melon PI 134198 that confers resistance to a unique race of *Podosphaera xanthii* in China. HortSci 45:1407–1410

López-Sesé AI, Gómez-Guillamón ML (2000) Resistance to *Cucurbit Yellowing Stunting Disorder Virus* (CYSDV) in *Cucumis melo* L. HortSci 35:110–113

Mallor Gimenez C, Álvarez JM, Luis Arteaga M (2003) Inheritance of resistance to systemic symptom expression of melon necrotic spot virus (MNSV) in *Cucumis melo* L. 'Doublon'. Euphytica 134:319–324

McCreight JD (1983) Potential sources of suddent wilt resistance in muskmelon. Cucurbit Genet Coop Rep 6:49–50

McCreight JD (2000) Inheritance of resistance to *Lettuce Infectious Yellows virus* in melon. HortSci 35:1118–1120

McCreight JD (2003) Genes for resistance to powdery mildew races 1 and 2U.S. in melon PI 313970. HortSci 38:591–594

McCreight JD, Coffey MD (2011) Inheritance of resistance in melon PI 313970 to cucurbit powdery mildew incited by *Podosphaera xanthii* race S. HortSci 46:838–840

McCreight JD, Fashing-Burdette P (1996) Resistance of PI 124112 and 'Eldorado-300' melons (*Cucumis melo* L.) to papaya ringspot virus watermelon strain. In: Gómez-Guillamón ML, Soria C, Cuartero J, Torès JA, Fernandez-Munoz R (eds) Cucurbits toward 2000. VIth EUCARPIA meeting on Cucurbit Genetics and Breeding, Málaga, ES, pp 298–301

McCreight JD, Wintermantel WM (2008) Potential new sources of genetic resistance in melon to *Cucurbit yellow stunting disorder virus*. In: Pitrat M (ed) Cucurbitaceae 2008, IXth EUCARPIA meeting on Genetics and Breeding of Cucurbitaceae. INRA, Avignon (FRA), pp 173–179

McCreight JD, Wintermantel WM (2011) Genetic resistance in melon PI 313970 to *Cucurbit yellow stunting disorder virus*. HortSci 46:1582–1587

McCreight JD, Liu H-Y, Turini TA (2008) Genetic resistance to *Cucurbit leaf crumple virus* in melon. HortSci 43:122–126

More TA, Dhakare BB, Sawant SV (2001) Identification of downy mildew resistant sources in muskmelon genotypes. In: Nishimura S, Ezura H, Matsuda T, Tazuke A (eds) Proceedings of the second international symposium on cucurbits, vol 588. Acta Horticulturae, Tsukuba, JP, pp 241–245

Nieto C, Morales M, Orjeda G, Clepet C, Monfort A, Truniger V, Sturbois B, Arus P, Caboche M, Puigdomenech P, Pitrat M, Dogimont C, Garcia-Mas J, Aranda M, Bendahmane A (2006) An *eIF4E* allele confers resistance to an uncapped and non-polyadenylated RNA virus in melon. Plant J 48:1–11

Nieto C, Piron F, Dalmais M, Marco CF, Moriones E, Gomez-Guillamon ML, Truniger V, Gomez P, Garcia-Mas J, Aranda MA, Bendahmane A (2007) EcoTILLING for the

identification of allelic variants of melon eIF4E, a factor that controls virus susceptibility. BMC Plant Biol 7:34

Normantovich M, Yogev O, Taylor CG, Perl-Treves R (2012) Study of the *Fom-2* resistance gene using composite melon plants. In: Sari N, Solmaz I, Aras V (eds) Cucurbitaceae 2012. Proceedings of the Xth EUCARPIA meeting on Genetics and Breeding of Cucurbitaceae. Çukurova University, Antalya, pp 240–246

Oumouloud A, Arnedo-Andres MS, Gonzalez-Torres R, Alvarez JM (2010) Inheritance of resistance to *Fusarium oxysporum* f. sp. *melonis* races 0 and 2 in melon accession Tortuga. Euphytica 176:183–189

Oumouloud A, El Otmani M, González Torres R, Garcés Claver A, Alvarez JM (2012) Toward the development of molecular markers linked to the *fom-4* gene in melon. In: Sari N, Solmaz I, Aras V (eds) Cucurbitaceae 2012. Proceedings of the Xth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae. Çukurova University, Antalya, pp 157–164

Owens KW, Peterson CE (1982) Response of muskmelon cultivars to bacterial wilt (*Erwinia tracheiphila* (E.F. Smith) Holland). Cucurbit Genet Coop Rep 5:26–27

Pandey S, Singh B, Rai M, Pandey KK (2008) B-159 (IC 396388, INGR07044) a snapmelon (*Cucumis melo* var. *momordica*) germplasm with downy mildew resistance. J Plant Genet Res 20:249–271

Park DK, Son SH, Huh YC, Lee WM, Lee HJ, Ko HC (2012) Germplasm selection on melon with resistance to Fusarium wilt and Monosporascus root rot for melon rootstocks. In: Sari N, Solmaz I, Aras V (eds) Cucurbitaceae 2012. Proceedings of the Xth EUCARPIA meeting on Genetics and Breeding of Cucurbitaceae. Çukurova University, Antalya, pp 434–440

Perchepied L, Pitrat M (2004) Polygenic inheritance of partial resistance to *Fusarium oxysporum* f. sp. *melonis* race 1.2 in melon. Phytopathology 94:1331–1336

Perchepied L, Périn C, Giovinazzo N, Besombes D, Dogimont C, Pitrat M (2004) Susceptibility to sulfur dusting and inheritance in melon. In: Lebeda A, Paris HS (eds) Progress in cucurbit genetics and breeding research. Proceedings of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding. Palacky University in Olomouc, Olomouc, pp 353–357

Perchepied L, Dogimont C, Pitrat M (2005a) Strain-specific and recessive QTLs involved in control of partial resistance to *Fusarium oxysporum* f. sp. *melonis* race 1.2 in a recombinant inbred line population of melon. Theor Appl Genet 111:65–74

Perchepied L, Bardin M, Dogimont C, Pitrat M (2005b) Relationship between loci conferring downy mildew and powdery mildew resistance in melon assessed by QTL mapping. Phytopathology 95:556–565

Périn C, Hagen LS, de Conto V, Katzir N, Danin-Poleg Y, Portnoy V, Baudracco-Arnas S, Chadoeuf J, Dogimont C, Pitrat M (2002) A reference map of *Cucumis melo* based on two recombinant inbred line populations. Theor Appl Genet 104:1017–1034

Pitrat M (1991) Linkage groups in *Cucumis melo* L. J Hered 82:406–411

Pitrat M, Besombes D (2008) Inheritance of *Podosphaera xanthii* resistance in melon line '90625'. In: Pitrat M (ed) Cucurbitaceae 2008, IXth EUCARPIA meeting on Genetics and Breeding of Cucurbitaceae. INRA, Avignon, pp 135–142

Pitrat M, Lecoq H (1984) Inheritance of *Zucchini yellow mosaic virus* resistance in *Cucumis melo* L. Euphytica 33:57–61

Pitrat M, Lecoq H, Risser G (1982) *Vat* and *Fn*, two linked genes in muskmelon. Cucurbit Genet Coop Rep 5:29–30

Pitrat M, Risser G, Bertrand F, Blancard D, Lecoq H (1996) Evaluation of a melon collection for diseases resistances. In: Gómez-Guillamón ML, Soria C, Cuartero J, Torès JA, Fernandez-Munoz R (eds) Cucurbits toward 2000. VIth EUCARPIA meeting on Cucurbit Genetics and Breeding, Málaga, pp 49–58

Pitrat M, Wipf-Scheibel C, Besombes D, Desbiez C, Lecoq H (2012) Resistance of melon to *Cucumber vein yellowing virus* (CVYV). In: Sari N, Solmaz I, Aras V (eds) Cucurbitaceae

2012. Proceedings of the Xth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae. Çukurova University, Antalya, pp 157–164

Plagès JN (1997) L'avenir des variétés génétiquement modifiées pour la résistance aux virus (un exemple développé par Limagrain). Comptes rendus de l'Académie d'Agriculture de France 83:161–164

Prasad K, Norton JD (1967) Inheritance of resistance to *Mycosphaerella citrullina* in muskmelon. Proc Am Soc Hortic Sci 91:396–400

Provvidenti R (1998) A source of high level of tolerance to squash mosaic virus in a melon from China. Cucurbit Genet Coop Rep 21:29–30

Quiot-Douine L, Lecoq H, Quiot JB, Pitrat M, Labonne G (1990) Serological and biological variability of virus isolates related to strains of *Papaya ringspot virus* (PRSV). Phytopathology 80:256–263

Ren HY, Fang L, Ru SJ, Wang HR (2009) A preliminary investigation of a mutant melon plant *edr2* on resistance to gummy stem blight. Sci Agric Sin 42:3131–3138

Risser G (1973) Étude de l'hérédité de la résistance du melon (*Cucumis melo*) aux races 1 et 2 de *Fusarium oxysporum* f.sp. *melonis*. Ann Amélior Pl 23:259–263

Risser G, Rode JC (1973) Breeding for resistance to *Fusarium oxysporum* f. *melonis*. In: Risser G (ed) EUCARPIA: La sélection du melon. Montfavet-Avignon, pp 37–39

Risser G, Pitrat M, Rode JC (1977) Etude de la résistance du melon (*Cucumis melo* L.) au virus de la mosaïque du concombre. Ann Amélior Pl 27:509–522

Romay G, Lecoq H, Desbiez C (2013) Zucchini tigré mosaic virus is a distinct potyvirus in the papaya ringspot virus cluster: molecular and biological insights. Arch Virol 159(2):277–289

Salari M, Panjehkeh N, Nasirpoor Z, Javad Abkhoo J (2012) Reaction of melon (*Cucumis melo* L.) cultivars to *Monosporascus cannonballus* (Pollack & Uecker) and their effect on total phenol, total protein and peroxidase activities. J Phytopathol 161:363–368

Somkuwar RG, More TA (1993) Downy mildew (*Pseudoperonospora cubensis* B & C) resistance in melon (*Cucumis melo* L.). Cucurbit Genet Coop Rep 16:40–41

Sowell G, Corley WL (1974) PI 321005 (Tainan #2), a high-quality source of resistance to three cantaloup diseases. Plant Disease Rep 58:899–902

Sugiyama M, Ohara T, Sakata Y (2007) Inheritance of resistance to *Cucumber green mottle mosaic virus* in *Cucumis melo* L. 'Chang Bougi'. J Jpn Soc Hortic Sci 76:316–318

Tabei Y, Oosawa K, Nishimura S, Hanada K, Yoshioka K, Fujisawa I, Nakajima K (1994a) Environmental risk evaluation of the transgenic melon with coat protein gene of cucumber mosaic virus in closed and semi-closed greenhouses (I). Jpn J Breed 44:101–105

Tabei Y, Oosawa K, Nishimura S, Watanabe S, Tsuchiya K, Yoshioka K, Fujisawa I, Nakajima K (1994b) Environmental risk evaluation of the transgenic melon with coat protein gene of cucumber mosaic virus in a closed and semi closed greenhouse (II). Jpn J Breed 44:207–211

Takada K (1983) Breeding and characteristics of disease-resistant melon varieties (lines 'Ano No. 1, No. 2 and No. 3'). Bull Veg Ornam Crops Res Stn 11:1–22

Taler D, Galperin M, Benjamin I, Cohen Y, Kenigsbuch D (2004) Plant eR genes that encode photorespiratory enzymes confer resistance against disease. Plant Cell 16:172–184

Teixeira APM, Camargo LEA (2006) A molecular marker linked to the *Prv*[1] gene that confers resistance to *Papaya ringspot virus*-type W in melon. Plant Breed 125:187–190

Teixeira APM, da Silva Barreto FA, Aranha Camargo LEA (2008) An AFLP marker linked to the *Pm-1* gene that confers resistance to *Podosphaera xanthii* race 1 in *Cucumis melo*. Genet Mol Biol 31:547–550

Thomas CE, Cohen Y, McCreight JD, Jourdain EL, Cohen S (1988) Inheritance of resistance to downy mildew in *Cucumis melo*. Plant Dis 72:33–35

Thomas CE, McCreight JD, Jourdain EL (1990) Inheritance of resistance to *Alternaria cucumerina* in *Cucumis melo* line MR-1. Plant Dis 74:868–870

Truniger V, Nieto C, Gonzalez-Ibeas D, Aranda M (2008) Mechanism of plant eIF4E-mediated resistance against a Carmovirus (Tombusviridae): cap-independent translation of a viral RNA controlled in cis by an (a)virulence determinant. Plant J 56:716–727

Webb RE (1979) Inheritance of resistance to watermelon mosaic virus in *Cucumis melo* L. HortSci 14:265–266

Webb RE, Bohn GW (1962) Resistance to cucurbit viruses in *Cucumis melo* L. Phytopathology 52:1221

Webster CG, Kousik CS, Turechek WW, Webb SE, Roberts PD, Adkins S (2013) *Squash vein yellowing virus* infection of vining Cucurbits and the vine decline response. Plant Dis 97:1149–1157

Wechter WP, Levi A, Ling KS, Kousik C, Block CC (2011) Identification of resistance to *Acidovorax avenae* subsp. *citrulli* among melon (*Cucumis* spp.) plant introductions. HortSci 46:207–212

Whitner BF (1960) Seminole. A high-yielding, good quality, downy and powdery mildew-resistant cantaloupe. University of Florida Agricultural Experiment Station, vol 122

Wolff DW, Miller ME (1998) Tolerance to monosporascus root rot and vine decline in melon (Cucumis melo L.) germplasm. HortSci 33:287–290

Wolukau JN, Zhou XH, Li Y, Zhang YB, Chen JF (2007) Resistance to gummy stem blight in melon (*Cucumis melo* L.) germplasm and inheritance of resistance from plant introductions 157076, 420145, and 323498. HortSci 42:215–221

Wu HW, Yu TA, Raja JAJ, Christopher SJ, Wang SL, Yeh SD (2010) Double-virus resistance of transgenic oriental melon conferred by untranslatable chimeric construct carrying partial coat protein genes of two viruses. Plant Dis 94:1341–1347

Yakoubi S, Lecoq H, Desbiez C (2008) *Algerian watermelon mosaic virus* (AWMV): a new potyvirus species in the PRSV cluster. Virus Genes 37:103–109

Yoshioka K, Hanada K, Minobe Y, Yakuwa T, Oosawa K (1991) Coat protein gene mediated resistance to Cucumber Mosaic Virus in transgenic melon and it's progeny. J Jpn Soc Hortic Sci 60(suppl 2):206–207

Yoshioka K, Hanada K, Nakazaki Y, Minobe Y, Yakuwa T, Oosawa K (1992) Successful transfert of the Cucumber Mosaic Virus coat protein gene in *Cucumis melo* L. Jpn J Breed 42:277–285

Yoshioka K, Hanada K, Harada T, Minobe Y, Oosawa K (1993) Virus resistance in transgenic melon plants that express the cucumber mosaic virus coat protein gene and their progeny. Jpn J Breed 43:629–634

Yousif MT, Kheyr-Pour A, Gronenborn B, Pitrat M, Dogimont C (2007) Sources of resistance to *Watermelon chlorotic stunt virus* in melon. Plant Breed 126:422–427

Yuste-Lisbona FJ, Capel C, Gomez-Guillamon ML, Capel J, Lopez-Sese AI, Lozano R (2011a) Codominant PCR-based markers and candidate genes for powdery mildew resistance in melon (*Cucumis melo* L.). Theor Appl Genet 122:747–758

Yuste-Lisbona FJ, Capel C, Sarria E, Torreblanca R, Gomez-Guillamon ML, Capel J, Lozano R, Lopez-Sese AI (2011b) Genetic linkage map of melon (*Cucumis melo* L.) and localization of a major QTL for powdery mildew resistance. Mol Breed 27:181–192

Zink FW, Gubler WD (1985) Inheritance of resistance in muskmelon to Fusarium wilt. J Am Soc Hortic Sci 110:600–604

Zuniga TL, Jantz JP, Zitter TA, Jahn MK (1999) Monogenic dominant resistance to gummy stem blight in two melon (*Cucumis melo*) accessions. Plant Dis 83:1105–1107

Chapter 12
Insect Resistance in Melon and Its Modification by Molecular Breeding

Catherine Dogimont and Nathalie Boissot

12.1 Introduction

Melon is grown on all continents, in tropical and temperate climates. Crop production systems vary considerably throughout the world. Melon is directly sown or transplanted, grown on dry and irrigated conditions, in open field, or under plastic tunnels. Throughout its range, melon is attacked by a large range of insect pests. Yield losses from insect feeding are difficult to estimate and depend greatly on location, but negative impacts on production systems are very high through an intensive use of pesticides. Among the chemicals, insecticides are more damaging to human health and to the environment because of their unintentional effects to the plant's natural enemies—predators and parasitoids—and to its pollinators, which are essential to the successful production of melon crops. Managing some insect pests, such as aphids and whiteflies, is challenging because of their short life span, high fecundity, and short generation times leading to multiple generations on a crop in a single season. Intensive use of certain families of insecticides selected insecticide resistance in these pests, which make the insect chemical control more difficult (Dennehy et al. 2010; Keil and Parrella 1990).

The development of resistant melon is widely considered to be the most effective and ecologically acceptable for managing pest insects. The first step of this approach is the identification, through screening of germplasm collections, of sources of resistance, which can be used as donors in breeding efforts. Screening may be difficult and screening methods, in field as well as in laboratory, must be developed. Despite these difficulties, resistance to several melon-damaging insects has been described. Often, such sources of resistance are in agronomic backgrounds

C. Dogimont (✉) • N. Boissot
INRA Avignon UR1052 Génétique et Amélioration des Fruits et Légumes, Avignon, France
e-mail: catherine.dogimont@avignon.inra.fr

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_12

far from the desired varieties. The second step is the characterization of the genetic basis of the resistance, which will guide the breeding process to transfer resistance to an improved background. In melon, the results of breeding programs have been mixed, with remarkable success in developing resistant varieties against the aphid *Aphis gossypii* but limited success against most other insects.

The development of molecular markers for mapping insect resistance components is an important step to assist the breeding process, but molecular markers linked to insect resistance genes are still scarce. Molecular isolation of an aphid resistance gene in melon, the *Vat* gene, has been a great step forward. The recent and rapid development of melon and insect genomic technologies, such as for aphids and whiteflies, will allow a better knowledge of insect/plant interaction and should lead to a larger use of insect genetic resistance in the perspective of a sustainable production system.

This paper gives a general overview of the literature, using previous reviews on insect resistance in the Cucurbitaceae published during the 1990s and compiling more recent studies (Elsey 1989; Dhillon and Wehner 1991; Robinson 1992; Webb 1998).

This article begins with a brief overview of the diversity and importance of insect pests that attack melon worldwide. Then we provide a review of insect resistances reported in melon and efforts done for the screening of melon genetic resources. Next, we review the genetic basis of resistance to various insects in melon and report our current understanding of the molecular bases of the *Vat*-mediated aphid resistance. Finally, we describe the current state of deployment of insect resistances and their integration into durable protection programs. We conclude by future research directions which may lead to a larger use of genetic resistance toward insect pests and may help for the delivery of new resistant varieties.

12.2 Major Melon Insect Pests

Major melon insect pests can be divided into two distinct groups based on mode of feeding, phloem-sucking insects and leaf-feeding insects (defoliators).

12.2.1 Phloem-Sucking Insects

Hemipteran insects such as aphids and whiteflies injure melon directly by removing xylem and phloem fluids with their piercing/sucking mouthparts. In large enough number, they can cause stunting, severe leaf curling, wilting, and death of plants. Aphids also secrete honeydew, which inhibits photosynthesis and also contaminates the fruit, favoring the development of sooty mold. *A. gossypii* (Sternorrhyncha: Aphididae) is the main aphid species colonizing melon crops. Two major species of

whiteflies *Bemisia tabaci* (Sternorrhyncha: Aleyrodidae) and *Trialeurodes vaporariorum* (Sternorrhyncha: Aleyrodidae) colonize melon. *B. tabaci* populations are highly variable and spread rapidly worldwide, almost displacing *T. vaporariorum* in the 1990s (Perring 2001).

These insects also injure melon indirectly by transmitting viruses, which are highly damaging. Aphids, such as *A. gossypii*, are efficient vectors of *Cucumber mosaic virus* (CMV) (*Cucumovirus*), *Zucchini yellow mosaic virus* (ZYMV) (*Potyvirus*), *Watermelon mosaic virus* (WMV) (*Potyvirus*), and *Papaya ringspot virus* (PRSV) (*Potyvirus*), which are transmitted on a nonpersistent manner. *Cucurbit aphid-borne yellow virus* (CABYV) (*Polerovirus*) is transmitted by aphids on a persistent manner.

Whiteflies transmit several yellowing RNA viruses to melon crops (Tzanetakis et al. 2013); they are members of the genus *Crinivirus* (family Closteroviridae) and transmitted in a semipersistent manner. The whitefly *T. vaporariorum* efficiently transmits *Beet pseudo-yellows virus* (Soria et al. 1991). *B. tabaci* is a vector of several other *Crinivirus*: *Lettuce infectious yellow virus*, which almost disappeared because it is transmitted specifically by the biotype A, which faded from prevalence by the emergence of the biotype B in the 1990s; *Cucurbit yellow stunting disorder virus*, transmitted by the biotype B and other biotypes, which, in contrast, has spread from the Middle East throughout the rest of the world; and the more recently characterized *Cucurbit chlorotic yellows virus*, which has been reported in various parts of the world. *B. tabaci* also transmit to melon several DNA viruses of the genus *Begomovirus* (family Geminiviridae) in a persistent and circulative manner: among them *Squash leaf curl virus* and *Watermelon chlorotic stunt virus* constitute new threats to melon crops in the Middle East and the Mediterranean regions (Abudy et al. 2010).

12.2.2 Leaf-Feeding Insects

Cucumber beetles (Coleoptera: Chrysomelidae) are a complex species, which includes the striped cucumber beetle *Acalymma vittatum* Fabricius, the spotted cucumber beetle *Diabrotica undecimpunctata howardi* Barber, the banded cucumber beetle *Diabrotica balteata* LeConte, and the red pumpkin beetle *Aulacophora foveicollis* Lucas (Clark et al. 2001). Adults and larvae extensively damage melon seedlings and fruits. They are the most damaging insects to cucurbits in North America. The striped cucumber beetle *A. vittatum* and the spotted cucumber beetle *D. undecimpunctata howardi* have the ability to carry and to transmit, as they feed, the bacterial wilt caused by *Erwinia tracheiphila* (Smith 1989), one of the most important diseases of cucurbits in the eastern United States (Rojas and Gleason 2012). The red pumpkin beetle *A. foveicollis* is one of the most important insect pests of cucurbits in India. It was shown to transmit a virus of the genus *Sobemovirus* to melons in Sudan (Lecoq et al. 2011).

In the tropical and subtropical areas of America, Lepidopteran larvae of the genus *Diaphania* (Lepidoptera: Crambidae) cause economic damage to cucurbits. The pickleworm *Diaphania nitidalis* Stoll can cause serious problems on melons by feeding on fruits, whereas the melonworm *Diaphania hyalinata* L., restricted to the Caribbean Basin, is mainly a leaf feeder.

Destructive populations of agromyzid leaf miners (Diptera: Agromyzidae) are common in melon crops. Females puncture the leaf for feeding and oviposition, and the larvae feed on leaf mesophyll tissue. Males are unable to create their own punctures, but they feed from punctures created by females. *Liriomyza sativae* Blanchard occurs in the New World, while American serpentine leaf miner *L. trifolii* occurs worldwide. *L. huidobrensis* Blanchard, endemic to South and Central America, has spread since the 1990s throughout the world and is now widespread in Europe, the Mediterranean region, the Middle East, and Asia. Unlike the other *Liriomyza* species, *L. huidobrensis* larvae do not feed on the upper mesophyll but feed within the lower mesophyll leaf layer and hence have a greater impact on leaf photosynthesis and are more difficult to control by chemical treatments.

The dipteran family Tephritidae consists of more than a hundred fruit fly species of economic importance. Two major fruit fly species (Diptera: Tephritidae) damage Cucurbitaceae fruits. The melon fly*Bactrocera cucurbitae* Coquillett (formerly *Dacus cucurbitae*) is distributed widely in temperate, tropical, and subtropical regions of the world and is one of the most serious pests on melon in Asia (Dhillon et al. 2005). Although it is present in Hawaii, it is absent from the continental United States. The Ethiopian fruit fly *Dacus ciliatus* Loew is a major pest in East Africa and on Reunion Island (Vayssieres et al. 2008). The fruit fly females puncture the pericarp and lay eggs 2–4 mm deep in the pulp of enlarged ovaries or young and tender fruits. The larvae feed inside and spoil the developing fruits, causing considerable damage.

12.3 Sources of Resistance and Mechanisms

Natural resistance variation present in melon germplasm has been described for most important insect pests (Table 12.1). Resistance to hemipteran insects in melon has been intensively explored. Screening germplasm for resistance to the aphid *A. gossypii* led to the discovery of several highly resistant accessions, whereas screening for resistance to the whiteflies *B. tabaci* and *T. vaporariorum* mainly highlighted partial resistance in a reduced number of accessions.

Table 12.1 Diversity of natural resistance to insects in melon

Insect order and family	Species (Latin name)	Species (English name)	Screening of genetic resources	Heredity	Resistance genes and QTLs
Coleoptera					
Chrysomelidae	*Aulacophora foveicollis* Lucas	Red pumpkin beetle	<50	Monogenic dominant	*Af* in a casaba cultivar
	Acalymma vittattum Mannerheim	Western spotted cucumber beetle		Monogenic recessive	cb_l in C922-174-B
	Diabrotica balteata LeConte	Banded cucumber beetle		Monogenic recessive	cb_l in C922-174-B
	Diabrotica undecimpunctata howardi Barber	Spotted cucumber beetle		Monogenic recessive	cb_l in C922-174-B
Diptera					
Agromyzidae	*Liriomyza trifolii* Burgess	American serpen-tine leaf miner	>100	Monogenic dominant	*Lt* in 'Nantais Oblong'
	Liriomyza sativae Blanchard		150		
	Liriomyza huidobrensis Blanchard	South American leaf miner	>100		
Tephrytidae	*Bactrocera cucurbitae* Coquillett = *Dacus cucurbitae*	Melon fly	>150		2 Complementary recessive genes *dc-1* and *dc-2*
	Dacus ciliatus Loew	Ethiopian melon fly	>100		
Hemiptera					
Sternorrhyncha					
Aleyrodidae	*Bemisia tabaci* Gennadius	Whitefly	>500	Polygenic, recessive	2 Mapped QTLs in the cross between 'Védrantais' x PI 161375
	Trialeurodes vaporariorum Westwood	Greenhouse whitefly	<50		

(continued)

Table 12.1 (continued)

Insect order and family	Species (Latin name)	Species (English name)	Screening of genetic resources	Heredity	Resistance genes and QTLs
Aphididae	*Aphis gossypii* Glover	Melon cotton aphid	>500	Monogenic dominant and polygenic	*Vat* cloned in PI 161375 4 QTLs and 2 pairs of epistatic QTLs
Lepidoptera					
Pyralidae	*Diaphania nitidalis* Stoll	Pickleworm	>1000		
Pyralidae	*Diaphania hyalinata* L.	Melonworm	15		

12.3.1 Resistance to the Aphid **Aphis gossypii**

About 5 % of the 500 melon accessions tested were found resistant to the melon-cotton aphid *A. gossypii* (Pitrat et al. 1996). Aphid resistance was reported in melon in Texas in the 1940s (Ivanoff 1944). A first screening was conducted in 1967 in California and identified the accession LJ 90234 (later designated PI 371795 and PI 414723, derived from PI 371795), from India, to be resistant to natural infestations of melon aphid (Kishaba et al. 1971, 1976). Aphid resistance in this accession results in modification of several aphid life traits. Under free-choice conditions in the field and in controlled conditions, alate and apterous aphids did not stay on resistant plants (Bohn et al. 1972; Kennedy and Kishaba 1977; Pitrat and Lecoq 1980). Under controlled no-choice tests, few insects survived on it and their fecundity was low (Kishaba et al. 1971, 1976). Leaves remained free of curling following massive aphid infestation (Bohn et al. 1973). Several other sources of aphid resistance have been reported from India (PI 164320, PI 180283, PI 313970, and the derived 90625) (MacCarter and Habeck 1974; Bohn et al. 1996). Recently, among fifty melon land races from southern India (Kerala, Tamil Nadu), two accessions (AM5 and AM2) were found resistant to *A. gossypii* (clone NM1) and three were segregating (AM7, AM78, AM87) (Fergany et al. 2011). Several Far East accessions were also reported to be resistant to *A. gossypii*. The leaves of the Korean accessions PI 161375 and PI 255478 did not curl when aphid infested (Bohn et al. 1973), and aphids got away from the plants after controlled infestation (Pitrat and Lecoq 1980). In the late 1970s, PI 161375 was also shown to have a very original feature: it is completely resistant to several nonpersistent viruses (CMV, WMV, and ZYMV) when the virus is transmitted by *A. gossypii* (Lecoq et al. 1979, 1980); it is partially resistant to CABYV, which is persistently transmitted by *A. gossypii* (Lecoq et al. 1992). The nonpersistent virus resistance when transmitted by *A. gossypii* was also shown to be present in several additional aphid-resistant accessions from the Far East (Chenggam, Ginsen Makuwa, K5442, Kanro Makuwa, Miel Blanc, PI 255478, PI 266935, Shiro Uri Okayama), in the Indian accessions 90625, PI 414723, PI 164320, and PI 164723, in the Chito accession Meloncillo from Colombia, in the African PI 224770 (Pitrat and Lecoq 1980; Romanow et al. 1986; Boissot et al. 2000, 2008), and in the accession TGR-1551 (=C-105) from Zimbabwe (Soria et al. 2003; Diaz et al. 2003). Nevertheless, several accessions were identified, which did not exhibit this double phenotype with certain *A. gossypii* clones, such as Meloncillo, Miel Blanc, and PI 164323 (Thomas et al. 2012b). Several other accessions from China and Central Asia were reported to be resistant to leaf curling after aphid infestation (Bohn et al. 1996). Additional sources of resistance were found in melon local lines from Spain: aphid and virus resistance was found in three accessions out of the 72 tested, Anso, Invernizo, and Escrito (Pitrat et al. 1988), and a moderate level of aphid resistance was reported in three accessions out of another set of 50 Spanish local lines, ANC-46, Baza, and Escrito-Z (Garzo et al. 2004).

The feeding behavior of *A. gossypii* was studied in several of these resistant accessions in comparison with susceptible ones, using the electrical penetration graph (EPG), which allows a detailed monitoring of aphid stylet penetration into plants. On susceptible plants, aphids can ingest phloem sap continuously for many hours. The aphid feeding behavior was shown to be highly modified on resistant accessions, with a drastic reduction of the phloem sap ingestion by the aphid. As a consequence, when aphids are forced to survive on the resistant host plant, they show reduced growth, retarded development, and decreased reproduction as compared to aphids maintained on susceptible plants. The brief intracellular punctures, which precede the feeding phase and during which nonpersistent viruses are transmitted to the plant, are maintained on resistant host plants (Kennedy et al. 1978b; Chen et al. 1997a, b; Klingler et al. 1998; Garzo et al. 2002; Tjallingii 2006).

12.3.2 Resistance to Whiteflies

Resistance to *B. tabaci* was studied prior to the emergence of *B. tabaci* biotype B in California (Kishaba et al. 1992). Fewer adults were counted on the cultivar 'WMR 29' as on the accession PI 414723 but stunting was similar on both genotypes. A large screening of more than 500 accessions was conducted in the 1990s under natural infestation by *B. tabaci* biotype B in California, with a limited success (Simmons and McCreight 1996; McCreight 1993). In southern Spain, the Far East accessions Kanro Makuwa and CUM190 exhibited less eggs and adults than cantaloupe accessions in a naturally infested greenhouse by *B. tabaci* biotype B (Moreno et al. 1993). Using no-choice and free-choice tests, TGR-1551 and the accession 'agrestis 87' (= CUM190) were shown to have a lower whitefly net reproduction in comparison with susceptible accessions (Soria et al. 1999; Sese et al. 1996). TGR-1551 was also reported to be resistant to the whitefly-transmitted *Cucurbit yellow stunting disorder virus* (Lopez-Sese and Gomez-Guillamon 2000). In the lesser Antilles where *B. tabaci* biotype B is present, a screening of 80 melon accessions conducted in field conditions allowed the identification of accessions with fewer larvae and adults, such as the Indian PI 414723, PI 164723, and 90625 and the Korean PI 161375 (Boissot et al. 2000, 2003); under controlled conditions, the net reproduction of the whitefly was shown to be significantly reduced in PI 161375, PI 414723, and PI 532841 (Sauvion et al. 2005). The glabrous melon 'SR-91' was shown to have a lower number of whitefly adults and nymphs in comparison with several pubescent varieties in Texas (Riley et al. 2001). In Brazil, 'AF-646' and 'Verreda' exhibited less adult nymphs and eggs of *B. tabaci* biotype B in the field than other varieties (Baldin et al. 2012). Under controlled conditions, only 'Hale's Best Jumbo' had less eggs and adults than controlled in free-choice and no-choice tests. This accession has been tested in field conditions in California under high pressure of whiteflies and was found as susceptible as most melon cultivars (McCreight and Simmons 1998).

Lower emergence rate (from egg to adult) of the greenhouse whitefly *T. vaporariorum* was reported in accessions from China and Uzbekistan (Laska and Lebeda 1989). Less oviposition, higher larval mortality, and shorter adult longevity were reported in an accession of the *agrestis* group (Soria et al. 1996).

12.3.3 Resistance to Chewing and Mining Insects

Sources of resistance to chewing and mining insects have been also investigated in melon.

In the 1970s, the screening of more than 1000 melon accessions and breeding lines in field conditions in the United States selected accessions showing significantly lower fruit-feeding damage by the pickleworm *D. nitidalis*: the Dudaim accession PI 273438 and the *agrestis* accessions PI 137843, PI 140471, PI 183311, and PI 296345 (Corley 1973; Day et al. 1978). Reduced oviposition of *D. nitidalis* in early stages of melon plants was also observed on PI 183311 (Nugent 1992). A field screening for resistance to the melonworm *D. hyalinata* was conducted in Guadeloupe, Lesser Antilles. Among the 15 melon accessions tested, the two Chito accessions Concombre Chien from Guadeloupe and Meloncillo from Colombia and the Indian accession 90625 showed less worm damages. In controlled conditions, newly hatched larvae fed with leaves of PI 140471 and Concombre Chien showed longer development and higher mortality than when fed on the susceptible control (Guillaume and Boissot 2001). These accessions are genetically related and resistance may have the same basis (Serres-Giardi and Dogimont 2012).

Screening of sources of resistance to the three major species of leaf miners has been conducted with variable success. A collection of 110 melon accessions were exposed to *L. trifolii* for oviposition in a no-choice test (Dogimont et al. 1995). Four types of resistance were observed: delay in larval development, many dead larvae in the mines, mines in chlorotic spots, and fewer larvae and mines. The most promising accession was the old French Charentais-type variety, ‘Nantais Oblong’, in which very few mines and a very low survival rate of the larvae were reported (Dogimont et al. 1995). Fifty melon accessions were exposed to *L. sativae* oviposition. The most resistant were PI 282448 from South Africa and PI 313970 from India, which exhibited fewer mines and the highest larval mortality (Kennedy et al. 1978a). More than 100 melon accessions were evaluated in field conditions in Sudan, where *L. sativae* is the most common species. The accession HDS 2445 was found resistant with an infestation rate of 16 % instead of 96 % on the susceptible MR1 (Gesmallah and Yousif 2004). Against *L. huidobrensis*, 100 accessions were screened under controlled conditions but none were found resistant. Resistance to *L. trifolii* identified in ‘Nantais Oblong’ was shown to be inefficient toward *L. huidobrensis* (unpublished data, Dogimont, Pitrat, and Bordat).

Source of resistance to the melon fly *B. cucurbitae* was reported in a wild melon (named *C. callosus*) (Sambandam and Chelliah 1969). A set of 168 melon cultivars, breeding lines, and wild accessions was evaluated in Sudan for resistance to the

Ethiopian fruit fly *D. ciliatus*, which was the predominant species there; two wild (HSD93-20A, Humaid 93-5-B) and two local (HSD1932, AR-1) accessions collected in Sudan showed a very low percentage of fruit infestation during three successive field trials (Gesmallah et al. 2002).

Resistance to the red pumpkin beetle *A. foveicollis* (Coleoptera: Chrysomelidae) was reported in a Casaba cultivar (Vashistha and Choudhury 1971), whereas all the Indian accessions tested were susceptible. The Indian accession PI 414723 was found to have a low level of resistance to seedling damage and a high level of resistance to fruit damage from feeding by a complex of cucumber beetles, in comparison with 22 accessions and breeding lines tested in natural infestation field conditions in California (Kishaba et al. 1998).

In field trials in Radjasthan, India, more than 90 *Epilachna* beetles (*Epilachna vigintioctopunctata* Fabr.) were counted in the Indian accession Durgapura Madhu and fewer than 30 on the variety 'Amco Sweet' (Pareek and Kavadia 1991).

Overall, insect resistance has been investigated for main insect pests in melon, but most of the studies suffer of the lack of knowledge and control of the genetic diversity of the pest. Therefore, some results appeared conflicting. Despite the efforts done, additional screenings are still required, and intraspecific variability of pests should be considered for having a comprehensive view of a pest resistance in melon. This will provide additional germplasm sources for insect resistance breeding in melon.

12.4 Genetic Control and Molecular Markers

Inheritance of insect resistance in melon has been reported to be simple as well as complex, controlled by dominant or recessive genes (Dogimont 2011) (Table 12.1).

A monogenic recessive resistance to cucumber beetles at the seedling stage was reported in C922-174-B in crosses among non-bitter melon accessions. The gene named cb_l *(=cb)* was shown to be efficient toward three species of Coleoptera: the banded beetle *D. balteata*, the spotted beetle *D. undecimpunctata howardi*, and the stripped beetle *Acalymma vittatum* (Nugent et al. 1984). In AR Top Mark, resistance to *D. undecimpunctata howardi* was also reported to be recessive and linked to the bitterness trait, controlled by the dominant gene *Bi* (Lee and Janick 1978) that makes the melon attractive to the spotted beetle (Nugent et al. 1984). Two complementary recessive genes (*dc-1* and *dc-2*) were reported to control resistance to the melon fly *B. cucurbitae* (Sambandam and Chelliah 1972). A single dominant gene, named *Af*, was reported to control resistance to the red pumpkin beetle (*A. foveicollis*) in a tolerant Casaba cultivar (Vashistha and Choudhury 1974). A single dominant gene, named *Lt*, was reported to control resistance to the leafminer *L. trifolii* in a cross between the resistant variety 'Nantais Oblong' and the susceptible one 'Védrantais'. These resistance genes have not been mapped and no linked markers are available.

In contrast, the genetic bases of resistance to the aphid *A. gossypii* and to the whitefly *B. tabaci* have been more extensively studied. Resistance to *A. gossypii* was reported to be oligogenic in the accession LJ 90234, later called PI 414723 (Kishaba et al. 1976; Bohn et al. 1973) and in PI 164320 (MacCarter and Habeck 1974). A major dominant gene that controlled this resistance and free leaf curling was named *Ag* (Bohn et al. 1973). Resistance to viruses when they are vectored specifically by *A. gossypii* is controlled by a single gene, named *Vat* (*Virus aphid transmission*); it was shown to cosegregate with resistance to *A. gossypii* (Pitrat and Lecoq 1980). The *Vat* locus was mapped to a subtelomeric position on the linkage group V (formerly 2) (Pitrat 1991; Baudracco-Arnas and Pitrat 1996; Brotman et al. 2002; Perin et al. 2002). Studying different parameters describing the behavior and the biotic potential of *A. gossypii*, four additive QTLs and two couples of epistatic QTLs affecting *A. gossypii* were mapped in recombinant inbred lines (RILs) derived from the cross between 'Védrantais' × PI 161375. Among them, a major QTL affects both the behavior and the biotic potential of *A. gossypii*; it colocalizes with and likely corresponds to the *Vat* gene (Boissot et al. 2010). These QTLs were reported on the consensus map of melon established in the frame of the International Cucurbit Genomics Initiative, providing access to a large number of markers (Díaz et al. 2011). Allelism tests and mapping data indicated that resistance identified in several sources was located either in the *Vat* locus (Pitrat et al. 1988; Perin et al. 2002; Sarria et al. 2008) or in loci close to *Vat* (Boissot et al. 2015).

Two QTLs affecting the biotic potential of the whitefly *B. tabaci* were mapped in the same 'Védrantais' × PI 161375 RIL population (Boissot et al. 2010). Both QTLs were independent from those affecting the behavior and the biotic potential of *A. gossypii*. Studying a RIL population obtained from a cross between the resistant accession TGR-1551 and the susceptible Bola de Oro and different resistance parameters, the low number of adult whiteflies and the low oviposition were reported to be controlled by recessive factors, whereas the higher number of empty pupal cases was shown to be controlled by dominant factors (Palomares-Rius et al. 2010). The mapping of these QTLs has not been reported yet.

At the end, inheritance of several melon pest resistances is known, but the mapping of these resistances is restricted to resistance to hemipterans. This is probably due to the fact that most other inheritance studies were conducted in the 1970s when mapping technologies were not available. Undoubtedly, this work has to be done, to make fully available insect pest resistance for melon breeding.

12.5 Molecular Basis and Functional Characterization of Aphid Resistance

Molecular basis for insect resistance has been scarcely explored in comparison with pathogen resistance (Dogimont et al. 2010; Smith and Clement 2012). Three plant resistance genes to insects have been cloned so far. The tomato *Mi-1* gene confers

resistance to root-knot nematodes *Meloidogyne* and to several insects, the potato aphid *Macrosiphum euphorbiae*, the whitefly *B. tabaci*, and the psyllid *Bactericerca cockerelli* (Rossi et al. 1998; Vos et al. 1998; Nombela et al. 2003; Casteel et al. 2006). The rice *Bph14* gene confers resistance to the phloem-feeding brown plant hopper *Nilaparvata lugens* Stal (Du et al. 2009). In melon, the *Vat* gene was isolated using a map-based cloning strategy. A single gene, which encodes a nucleotide-binding site leucine-rich repeat (NBS-LRR) protein, was shown to confer both resistance to *A. gossypii* colonization and virus resistance when vectored specifically by this aphid (Pauquet et al. 2004; Chovelon et al. 2012; Dogimont et al. 2014). Transforming the *Vat* gene into a susceptible melon variety was shown to result in the double-resistant phenotype. The *Vat* gene belongs to the coiled-coil (CC)-NBS-LRR subfamily, to which belong the insect resistance genes *Mi-1* and *Bph14* and a majority of the pathogen resistance genes isolated so far (Dangl and Jones 2001). CC-NBS-LRR genes are known to mediate resistance through direct or indirect recognition of pathogen-associated molecular patterns (PAMPs) or pathogen effectors in a specific gene-to-gene interaction (Jones and Dangl 2006; Takken and Goverse 2012). The C-terminal region of the *Vat* gene comprises four near-perfect repeats of 65 amino acids flanked by highly imperfect copies of an LRR motif, which may be involved in the specific recognition of aphid effector proteins. Plant aphid recognition is thought to trigger signaling cascades that rapidly activate plant defenses against aphids, in a similar scheme that was widely described for most plant-pathogen interactions (Dogimont et al. 2014).

Resistant plant recognition of a pathogen or an insect is usually associated to a hypersensitive response and programmed cell death at infection site. In melon, aphid-resistant plants exhibit no visible necrotic symptoms after *A. gossypii* infestation, such as in most aphid-resistant plant interactions. However, soon after aphid infestation, large callose deposits, lignin in the cell walls, an increased peroxidase activity, phenol synthesis, and a micro-oxidative burst were observed at aphid feeding sites of *Vat*-resistant plants (Shinoda 1993; Sarria Villada et al. 2009). These results are consistent with a fast programmed cell death induced by *A. gossypii* specifically in *Vat*-resistant plants, tightly restricted to the aphid-infested area.

The defense signaling pathways activated by aphid resistance genes were shown to partially overlap with those activated by pathogens and to depend on both salicylic acid and jasmonic acid signaling molecules (Kaloshian and Walling 2005). Ethylene-responsive genes, including genes associated with ethylene signaling pathway (*ETR2*, *EIN2*, *EIN3*, *EIL1*, and *ERF1*) and genes of the downstream response (*SAG-21*, *SSA-13*, *TypeI-PI*, *CAMTA1*), were shown to be highly activated in response to aphid feeding on *Vat*-resistant plants; they are highly induced within the first 6 h after infestation but the activation response disappears after 24 h (Anstead et al. 2010). MicroRNAs (miRNAs), a class of non-protein coding RNA that regulates gene expression of protein-coding genes at a posttranscriptional level, were also shown to be upregulated during the early stages of aphid infestation in the *Vat*-resistant plants and downregulated in the susceptible interaction (Sattar et al. 2012b). These data are a clear illustration of the massive transcriptional

reprogramming induced by *A. gossypii* infestation in *Vat*-resistant melon plants, leading to a wide range of plant defense responses. *A. gossypii* miRNAs were also shown to be differentially regulated during the resistant and susceptible interactions (Sattar et al. 2012a), consistently with the important reproductive, developmental, and morphological changes that occur in aphids in response to *Vat*-mediated resistance.

12.6 Insect-Resistant Varieties Release, Deployment, and Durability

The development and use of melon varieties with resistance to insect pests are still limited. However, the breeding and deployment of melon varieties bred for aphid resistance are a rare example of a large-scale deployment of insect resistance in vegetables (Dogimont et al. 2010). Programs for breeding for resistance to *A. gossypii* were started in the 1980s. Aphid resistance from PI 371795 was introgressed into susceptible American breeding lines ('Hale's Best Jumbo', 'PMR 5', and 'Topmark') by several successive backcrosses selected for aphid resistance for each generation, followed by several generations of selfing to produce nearly isogenic aphid-resistant lines (AR 'Hale's Best Jumbo', AR 5, and AR Topmark) (McCreight et al. 1984). These resistant breeding lines were provided to breeders but have had a limited commercial success. In France, *Vat*-mediated aphid resistance from PI 161375 was introgressed into Charentais-type melons. The INRA release 'Margot' and the near-isogenic lines Charentais VatR and Charentais VatS were obtained. Since 30 years, the *Vat*-resistant allele has been used extensively and incorporated into over 100 Charentais cultivars, representing over 40 % of the area of melon cultivation in France (Boissot et al. 2014). More recently, the *Vat* gene was introduced in Earl's-type melons from Japan (*C. melo* var. *reticulatus*) and a melon hybrid was released in 2006 (Sakata et al. 2006). In breeding programs, aphid resistance was usually selected using a single aphid clone, often not well identified.

According to McDonald and Linde (2002), the greatest risk for overcoming host resistance would occur in pathogens that have a mixed reproduction system, a high potential for genotype flow, a large effective population size, and high mutation rate. *A. gossypii*, like numerous aphid species, displays most of these features: alternative sexual versus asexual reproduction in areas with short-day length in winter and forms specialized for sedentary rapid reproduction versus dispersal. In the field, the genetic diversity of sedentary *A. gossypii* populations observed on melon crops is large (>250 multilocus genotypes identified using 8 SSR markers) and geographically structured (Thomas et al. 2012a). Biotypes and genotypes have a strong convergence in *A. gossypii* species in the frame of the interaction with the melon species (Thomas et al. 2012b). Thus, genotypic data, which can be obtained from a large number of individuals, can be used to infer the frequency of aphid

biotypes in field experiments. The *Vat* gene was shown to exert a selection pressure on aphid populations but the emergence of aphid biotypes overcoming the *Vat* resistance was shown to be very different in three agrosystems. *Vat* resistance was not expected durable in the Lesser Antilles, where an adapted clone existed before the resistance deployment. *Vat* resistance was expected durable in the South West France, where two sharp bottlenecks occur, one at the field level and the other one at the area level (no cucurbit crops grown for a long period in winter), preventing emergence of adapted clones. *Vat* resistance was expected to be less durable in the South East France, where only one sharp bottleneck occurs at the field level (Thomas et al. 2015). Moreover, it was observed that aphid clones, which develop well on aphid-resistant and aphid-susceptible genotypes, have a lower fitness than clones that are not able to develop on resistant plants (Lombaert et al. 2009). Thus, the *Vat*-mediated aphid resistance was shown to be an efficient means to reduce the use of insecticides in melon crops but its durability is questioned and sustainable systems have to be devised. The *Vat*-mediated virus resistance may be useful for reducing virus spread when *A. gossypii* is the primary vector species, but this effect may be very limited when other aphid vectors are more important (Gray et al. 1986; Schoeny et al. 2014).

One cultivar and eight parental lines bred for cucumber beetles resistance were released in the United States between 1973 and 1987 (Smith 1989). To our knowledge, the other insect resistances reported in melon have not been introduced into cultivated varieties.

12.7 Perspectives

Genetic resistance offers a very effective and promising way to control insects in melon, as in most cultivated crops. Sources of resistance to major insects were shown to be available in the natural melon diversity but much remains to be done to achieve a large deployment of these resistances. For instance, resistances to chewing insects described in the 1970s seem to have been neglected and deserve a renewed interest. Several points should be considered to promote the breeding of insect resistance varieties. In our opinion, phenotyping melon accessions with more than one insect strain or population is a key element to provide new sources of resistance and to predict the behavior of resistant genitors after deployment. This needs a better understanding of the natural diversity of the insect and of its ability to evolve more or less rapidly in presence of resistance genes.

The introduction into elite material, relatively easy when a single dominant gene is to be introduced, is more complicated when the resistance is recessively or polygenically inherited. A major effort should be made to map insect resistances in melon and provide molecular markers closely linked to insect resistance genes and QTLs. The development of molecular tools to assist the breeding transfer suffers the same difficulties as phenotyping. The isolation of insect resistance

genes, such as the *Vat* gene, and a good understanding of the diversity at the locus will provide molecular markers within the gene, specific to the resistant allele.

In the coming years, it is expected that genomics will become a key component of host plant resistance research and will lead to development of new and enhanced resistant varieties. The availability of the whole melon genome sequence and the development of various cutting-edge genomic tools (Clepet et al. 2011; Garcia-Mas et al. 2012) make it possible to widely investigate insect-melon plant interactions. Integrated genomic approaches will facilitate the molecular characterization of insect resistance and defense genes as well as genes underlying QTLs. Defense-related genes have been identified in several insect-plant interactions (Chen et al. 2012). They could be useful for developing novel insect-resistant melon, especially those genes that may provide broad-spectrum resistance. Novel strategies for insect resistance may be developed by interfering with the natural life cycle of the insect, using mutant plants in genes involved in important factors in the insect life cycle (Kater et al. 2003). In melon, these mutant plants in target genes can be obtained by TILLING as previously demonstrated (Dahmani-Mardas et al. 2010).

Finally, genetic resistance to insects should be included into a global integrated pest management (Dhillon et al. 2005). The wide use of aphid-resistant hybrids by French growers has reduced the number of insecticide applications and has allowed significant increases in the survival of parasitoids that reduce *A. gossypii* populations. However, at the farm level, a continued, often unnecessary, use of insecticides is still widespread. Melon genetic resistance should be used in conjunction with other management methods, such as biological control and cultural practices (HansPetersen et al. 2010; Simmons et al. 2010). Combining alternative protection methods should have additive effects on melon crop protection and should protect the resistance genes by preventing a rapid evolution of the insect pest populations.

References

Abudy A, Sufrin-Ringwald T, Dayan-Glick C, Guenoune-Gelbart D, Livneh O, Zaccai M, Lapidot M (2010) *Watermelon chlorotic stunt* and *Squash leaf curl begomoviruses*-New threats to cucurbit crops in the Middle East. Israel J Plant Sci 58(1):33–42. doi:10.1560/ijps.58.1.33

Anstead J, Samuel P, Song N, Wu CJ, Thompson GA, Goggin F (2010) Activation of ethylene-related genes in response to aphid feeding on resistant and susceptible melon and tomato plants. Entomol Exp Appl 134(2):170–181. doi:10.1111/j.1570-7458.2009.00945.x

Baldin ELL, da Silva J, Pannuti LER (2012) Resistance of melon cultivars to *Bemisia tabaci* biotype B. Hortic Bras 30(4):600–606

Baudracco-Arnas S, Pitrat M (1996) A genetic map of melon (*Cucumis melo* L.) with RFLP, RAPD, isozyme, disease resistance and morphological markers. Theor Appl Genet 93 (1–2):57–64

Bohn GW, Kishaba AN, Toba HH (1972) Mechanisms of resistance to melon aphid in a muskmelon line. Hortscience 7:281–282

Bohn GW, Kishaba AN, Principe JA, Toba HH (1973) Tolerance to melon aphid in *Cucumis melo* L. J Am Soc Hortic Sci 98:37–40

Bohn GW, Kishaba AN, McCreight JD (1996) A survey of tolerance to *Aphis gossypii* Glover in part of the world collection of *Cucumis melo* L. In: Gómez-Guillamón ML, Soria C, Cuartero J, Torès JA, Fernandez-Munoz R (eds) Cucurbits toward 2000. VIth EUCARPIA meeting on Cucurbit Genetics and Breeding, Málaga, ES, 28–30/05/1996, pp 334–339

Boissot N, Pavis C, Guillaume R, Lafortune D, Sauvion N (2000) Insect resistance in *Cucumis melo* accession 90625. In: Katzir N, Paris KS (eds) Proceedings of Cucurbitaceae 2000. Acta Horticulturae, pp 297–304

Boissot N, Lafortune D, Pavis C, Sauvion N (2003) Field resistance to *Bemisia tabaci* in *Cucumis melo*. Hortscience 38(1):77–80

Boissot N, Mistral P, Chareyron V, Dogimont C (2008) A new view on aphid resistance in melon: the role of *Aphis gossypii* variability. Cucurbitaceae 2008: Proceedings of the IXth Eucarpia meeting on Genetics and Breeding of Cucurbitaceae, pp 163–171

Boissot N, Thomas S, Sauvion N, Marchal C, Pavis C, Dogimont C (2010) Mapping and validation of QTLs for resistance to aphids and whiteflies in melon. Theor Appl Genet 121:9–20

Boissot N, Thomas S, Mistral P, Chareyron V (2014) Nouvelles sources de résistance au puceron *Aphis gossypii* chez le melon. Innov Agron 35:89–95

Boissot N, Thomas S, Chovelon V, Lecoq H (2015) NBS-LRR-mediated resistance triggered by aphids: viruses do not adapt; aphids adapt via different mechanisms. BMC Plant Biol in press

Brotman Y, Silberstein L, Kovalski I, Perin C, Dogimont C, Pitrat M, Klingler J, Thompson GA, Perl-Treves R (2002) Resistance gene homologues in melon are linked to genetic loci conferring disease and pest resistance. Theor Appl Genet 104(6-7):1055–1063

Casteel CL, Walling LL, Paine TD (2006) Behavior and biology of the tomato psyllid, *Bactericerca cockerelli*, in response to the *Mi-1.2* gene. Entomol Exp Appl 121(1):67–72

Chen JQ, Martin B, Rahbe Y, Fereres A (1997a) Early intracellular punctures by two aphid species on near-isogenic melon lines with and without the virus aphid transmission (*Vat*) resistance gene. Eur J Plant Pathol 103(6):521–536

Chen JQ, Rahbe Y, Delobel B, Sauvion N, Guillaud J, Febvay G (1997b) Melon resistance to the aphid *Aphis gossypii:* behavioural analysis and chemical correlations with nitrogenous compounds. Entomol Exp Appl 85(1):33–44

Chen H, Stout MJ, Qian Q, Chen F (2012) Genetic, molecular and genomic basis of rice defense against insects. Crit Rev Plant Sci 31(1):74–91. doi:10.1080/07352689.2011.616052

Chovelon V, Restier V, Giovinazzo N, Bendahmane A, Dogimont C (2012) Functional validation of the double phenotype triggered by the melon *Vat* gene by complementation experiments. In: Sari N, Solmaz I, Aras V (eds) Cucurbitaceae 2012, Proceedings of the Xth EUCARPIA meeting on Genetics and Breeding of Cucurbitaceae, Antalya, Turkey October 15–18th, 2012, pp 506–507

Clark TL, Meinke LJ, Foster JE (2001) Molecular phylogeny of *Diabrotica* beetles (Coleoptera : Chrysomelidae) inferred from analysis of combined mitochondrial and nuclear DNA sequences. Insect Mol Biol 10(4):303–314. doi:10.1046/j.0962-1075.2001.00269.x

Clepet C, Joobeur T, Zheng Y, Jublot D, Huang MY, Truniger V, Boualem A, Hernandez-Gonzalez ME, Dolcet-Sanjuan R, Portnoy V, Mascarell-Creus A, Cano-Delgado AI, Katzir N, Bendahmane A, Giovannoni JJ, Aranda MA, Garcia-Mas J, Fei ZJ (2011) Analysis of expressed sequence tags generated from full-length enriched cDNA libraries of melon. BMC Genomics 12:252. doi:10.1186/1471-2164-12-252

Corley WL (1973) Response of muskmelon botanical varieties to pickleworm infestation. Hortscience 8:326–328

Dahmani-Mardas F, Troadec C, Boualem A, Leveque S, Alsadon AA, Aldoss AA, Dogimont C, Bendahmane A (2010) Engineering melon plants with improved fruit shelf life using the TILLING approach. PLos One 5(12), e15776. doi:10.1371/journal.pone.0015776

Dangl JL, Jones JDG (2001) Plant pathogens and integrated defence responses to infection. Nature 411(6839):826–833

Day A, Nugent P, Robinson JF (1978) Variation of pickleworm feeding and oviposition on muskmelon and cucumbers. Hortscience 13:286–287

Dennehy TJ, Degain BA, Harpold VS, Zaborac M, Morin S, Fabrick JA, Nichols RL, Brown JK, Byrne FJ, Li XC (2010) Extraordinary resistance to insecticides reveals exotic Q biotype of *Bemisia tabaci* in the New World. J Econ Entomol 103(6):2174–2186. doi:10.1603/ec10239

Dhillon NPS, Wehner TC (1991) Host-plant resistance to insects in Cucurbit – germplasm resources, genetics and breeding. Trop Pest Manag 37(4):421–428

Dhillon MK, Singh R, Naresh JS, Sharma HC (2005) The melon fruit fly, *Bactrocera cucurbitae*: a review of its biology and management. J Insect Sci 5:40

Diaz JA, Mallor C, Soria C, Camero R, Garzo E, Fereres A, Alvarez JM, Gomez-Guillamon ML, Luis-Arteaga M, Moriones E (2003) Potential sources of resistance for melon to nonpersistently aphid-borne viruses. Plant Dis 87(8):960–964

Díaz A, Fergani M, Formisano G, Ziarsolo P, Blanca J, Fei Z, Staub JE, Zalapa JE, Cuevas HE, Dace G, Oliver M, Boissot N, Dogimont C, Pitrat M, Hofstede R, van Koert P, Harel-Beja R, Tzuri G, Portnoy V, Cohen S, Schaffer A, Katzir N, Xu Y, Zhang H, Fukino N, Matsumoto S, Garcia-Mas J, Monforte AJ (2011) A consensus linkage map for molecular markers and Quantitative Trait Loci associated with economically important traits in melon (*Cucumis melo* L.). BMC Plant Biol 11:111

Dogimont C (2011) Gene list for melon. Cucurbit Genet Coop Rep 33–34:104–133

Dogimont C, Bordat D, Pitrat M, Pages C (1995) Characterization of resistance to *Liriomyza trifolii* (Burgess) in melon (*Cucumis melo* L.). Fruits 50:449–452

Dogimont C, Bendahmane A, Chovelon V, Boissot N (2010) Host plant resistance to aphids in cultivated crops: Genetic and molecular bases, and interactions with aphid populations. C R Biol 333(6–7):566–573. doi:10.1016/j.crvi.2010.04.003

Dogimont C, Chovelon V, Pauquet J, Boualem A, Bendahmane A (2014) The *Vat* locus encodes for a CC-NBS-LRR protein that confers resistance to *Aphis gossypii* infestation and *A. gossypii*-mediated virus resistance. Plant J 80:993–1004

Du B, Zhang WL, Liu BF, Hu J, Wei Z, Shi ZY, He RF, Zhu LL, Chen RZ, Han B, He GC (2009) Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. Proc Natl Acad Sci USA 106(52):22163–22168. doi:10.1073/pnas.0912139106

Elsey KD (1989) Insect resistance in the cucurbits: status and potential. In: Thomas CE (ed) Evaluation and enhancement of Cucurbitaceae Proc Cucurbitaceae 1989 Conf Charleston, SC, pp 49–59

Fergany M, Kaur B, Monforte AJ, Pitrat M, Rys C, Lecoq H, Dhillon NPS, Dhaliwal SS (2011) Variation in melon (*Cucumis melo*) landraces adapted to the humid tropics of southern India. Genet Resour Crop Evol 58(2):225–243. doi:10.1007/s10722-010-9564-6

Garcia-Mas J, Benjak A, Sanseverino W, Bourgeois M, Mir G, Gonzalez VM, Henaff E, Camara F, Cozzuto L, Lowy E, Alioto T, Capella-Gutierrez S, Blanca J, Canizares J, Ziarsolo P, Gonzalez-Ibeas D, Rodriguez-Moreno L, Droege M, Du L, Alvarez-Tejado M, Lorente-Galdos B, Mele M, Yang LM, Weng YQ, Navarro A, Marques-Bonet T, Aranda MA, Nuez F, Pico B, Gabaldon T, Roma G, Guigo R, Casacuberta JM, Arus P, Puigdomenech P (2012) The genome of melon (*Cucumis melo* L.). Proc Natl Acad Sci USA 109 (29):11872–11877. doi:10.1073/pnas.1205415109

Garzo E, Soria C, Gomez-Guillamon ML, Fereres A (2002) Feeding behavior of *Aphis gossypii* on resistant accessions of different melon genotypes (*Cucumis melo*). Phytoparasitica 30 (2):129–140. doi:10.1007/bf02979695

Garzo E, Palacios I, Fereres A (2004) Characterization of melon germplasm resistant to *Aphis gossypii* Glover. Aphids in a new millennium, pp 441–447

Gesmallah AEE, Yousif MT (2004) Resistance in melons (*Cucumis melo* L.) to leafminers (*Liriomyza* spp.; Diptera:Agromyzidae). Gezira J Agric Sci 2004:125–130

Gesmallah AEE, Hassan NH, Omara SK (2002) Breeding melons *Cucumis melo* L. resistant to the lesser melon fruit fly *Dacus ciliatus* Lw. IIIrd Science Conference Al Assiout, Egypt

Gray SM, Moyer JW, Kennedy GG, Campbell CL (1986) Virus suppression and aphid resistance effects on spatial and temporal spread of *Watermelon mosaic virus* 2. Phytopathology 76 (11):1254–1259. doi:10.1094/Phyto-76-1254

Guillaume R, Boissot N (2001) Resistance to *Diaphania hyalinata* (Lepidoptera : Crambidae) in *Cucumis* species. J Econ Entomol 94(3):719–723. doi:10.1603/0022-0493-94.3.719

HansPetersen HN, McSorley R, Liburd OE (2010) The impact of intercropping squash with non-crop vegetation borders on the above-ground arthropod community. Fla Entomol 93 (4):590–608. doi:10.1653/024.093.0418

Ivanoff SS (1944) Resistance of cantaloupes to downy mildew and melon aphid. J Hered 35:34–39

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444(7117):323–329. doi:10.1038/nature05286

Kaloshian I, Walling LL (2005) Hemipterans as plant pathogens. Annu Rev Phytopathol 43:491–521. doi:10.1146/annurev.phyto.43.040204.135944

Kater MM, Franken J, Inggamer H, Gretenkort M, van Tunen AJ, Mollema C, Angenent GC (2003) The use of floral homeotic mutants as a novel way to obtain durable resistance to insect pests. Plant Biotechnol J 1(2):123–127. doi:10.1046/j.1467-7652.2003.00013.x

Keil CB, Parrella MP (1990) Characterization of insecticide resistance in 2 colonies of *Liriomyza trifolii* (Diptera, Agromyzidae). J Econ Entomol 83(1):18–26

Kennedy GG, Kishaba AN (1977) Response of alate melon aphids to resistant and susceptible muskmelon lines. J Econ Entomol 70(4):407–410

Kennedy GG, Bohn GW, Stoner KA, Webb RE (1978a) Leafminer resistance in muskmelon. J Am Soc Hortic Sci 103:571–574

Kennedy GG, McLean DL, Kinsey MG (1978b) Probing behavior of *Aphis gossypii* on resistant and susceptible muskmelon. J Am Soc Hortic Sci 71:13–16

Kishaba AN, Bohn GW, Toba HH (1971) Resistance to *Aphis gossypii* in muskmelon. J Econ Entomol 64:935–937

Kishaba AN, Bohn GW, Toba HH (1976) Genetic aspects of antibiosis to *Aphis gossypii* in *Cucumis melo* line from India. J Am Soc Hortic Sci 101:557–561

Kishaba AN, Castle S, McCreight JD, Desjardins PR (1992) Resistance of white-flowered gourd to sweet-potato whitefly. Hortscience 27(11):1217–1221

Kishaba AN, Castle SJ, Coudriet DL, McCreight JD, Bohn GW (1998) Resistance to western spotted and striped cucumber beetle in melon. In: McCreight JD (ed) Cucurbitaceae'98 Evaluation and enhancement of Cucurbits germplasm, Pacific Grove, CA, 30/11–04/12/1998. ASHS Press, Alexandria, VA, pp 101–105

Klingler J, Powell G, Thompson GA, Isaacs R (1998) Phloem specific aphid resistance in *Cucumis melo* line AR 5: effects on feeding behaviour and performance of *Aphis gossypii*. Entomol Exp Appl 86(1):79–88

Laska P, Lebeda A (1989) Resistance in wild *Cucumis* species to the glasshouse whitefly (*Trialeurodes vaporariorum*). Arch für Züchtungforshung 19:89–93

Lecoq H, Cohen S, Pitrat M, Labonne G (1979) Resistance to *Cucumber mosaic virus* transmission by aphids in *Cucumis melo*. Phytopathology 69(12):1223–1225

Lecoq H, Labonne G, Pitrat M (1980) Specificity of resistance to virus transmission by aphids in *Cucumis melo*. Ann Phytopathol 12(2):139–144

Lecoq H, Pitrat M, Bon M, Wipf Scheibel C, Bourdin D (1992) Resistance on melon to *Cucurbit aphid borne yellows virus*, a luteovirus infecting cucurbits. Proceedings of the Vth EUCARPIA Cucurbitaceae, Skierniewice, Warsaw, Poland, pp 191–196

Lecoq H, Dafalla G, Delecolle B, Wipf-Scheibel C, Desbiez C (2011) *Snake melon asteroid mosaic virus*, a tentative new member of the genus *Sobemovirus* infecting cucurbits. Plant Dis 95(2):153–157. doi:10.1094/pdis-06-10-0447

Lee CW, Janick J (1978) Inheritance of seedling bitterness in *Cucumis melo* L. Hortscience 13 (2):193–194

Lombaert E, Carletto J, Piotte C, Fauvergue X, Lecoq H, Vanlerberghe-Masutti F, Lapchin L (2009) Response of the melon aphid, *Aphis gossypii*, to host-plant resistance: evidence for high adaptive potential despite low genetic variability. Entomol Exp Appl 133(1):46–56. doi:10.1111/j.1570-7458.2009.00904.x

Lopez-Sese AI, Gomez-Guillamon ML (2000) Resistance to *Cucurbit yellowing stunting disorder virus* (CYSDV) in *Cucumis melo* L. Hortscience 35(1):110–113
MacCarter LE, Habeck DH (1974) Melon aphid resistance in *Cucumis* spp. Fla Entomol 57:195–204
McCreight JD (1993) Screening of melons for sweetpotato whitefly resistance: 1992. Cucurbit Genet Coop Rep 16:49–52
McCreight JD, Simmons AM (1998) Silverleaf whitefly resistance strategies in melon. In: McCreight JD (ed) Cucurbitaceae'98 Evaluation and enhancement of Cucurbits germplasm, Pacific Grove, CA. ASHS Press, Alexandria, VA, pp 113–117
McCreight JD, Kishaba AN, Bohn GW (1984) AR Hale's Best Jumbo, AR 5, and AR Topmark, melon aphid-resistant muskmelon breeding lines. Hortscience 19:309–310
McDonald BA, Linde C (2002) Pathogen population genetics, evolutionary potential, and durable resistance. Annu Rev Phytopathol 40:349–379
Moreno V, Gomez Aguilera JL, Guerau De Arellano C, Roig LA (1993) Preliminary screening of cucurbits species for *Bemisia tabaci* Genn. whitefly resistance. Cucurbit Genet Coop Rep 16:87–89
Nombela G, Williamson VM, Muniz M (2003) The root-knot nematode resistance gene *Mi-1.2* of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. Mol Plant-Microbe Interact 16(7):645–649
Nugent PE (1992) Pickleworm resistance studies in melon, *Cucumis melo*. In: Doruchowski RW, Kozik E, Niemirowicz-Szczytt K (eds) 5th EUCARPIA Cucurbitaceae Symposium, Skierniewice, PL, 27–31/07/1992, pp 163–165
Nugent PE, Cuthbert FP, Hoffman JC (1984) Two genes for cucumber beetle resistance in muskmelon. J Am Soc Hortic Sci 109(6):756–759
Palomares-Rius FJ, Lopez-Sese AI, Gomez-Guillamon ML (2010) Preliminary study of resistance against *Bemisia tabaci* Genn. in TGR-1551 melon genotype. In: Sun SX (ed) IV International Symposium on Cucurbits, vol 871. Acta Horticulturae, pp 245–250
Pareek BL, Kavadia VS (1991) Incidence of Epilachna beetle, *Epilachna vigintioctopunctata* Fabr on different varieties of muskmelon, *Cucumis melo* in the field. Ann Arid Zone 30(2):163–166
Pauquet J, Burget E, Hagen L, Chovelon V, Le Menn A, Valot N, Desloire S, Caboche M, Rousselle P, Pitrat M, Bendahmane A, Dogimont C (2004) Map-based cloning of the *Vat* gene from melon conferring resistance to both aphid colonization and aphid transmission of several viruses. In: Lebeda A, Paris H (eds) Cucurbitaceae 2004, the 8th EUCARPIA meeting on Cucurbit genetics and breeding, pp 325–329
Perin C, Hagen LS, De Conto V, Katzir N, Danin-Poleg Y, Portnoy V, Baudracco-Arnas S, Chadoeuf J, Dogimont C, Pitrat M (2002) A reference map of *Cucumis melo* based on two recombinant inbred line populations. Theor Appl Genet 104(6–7):1017–1034
Perring TM (2001) The *Bemisia tabaci* species complex. Crop Prot 20(9):725–737. doi:10.1016/s0261-2194(01)00109-0
Pitrat M (1991) Linkage groups in *Cucumis melo* L. J Hered 82(5):406–411
Pitrat M, Lecoq H (1980) Inheritance of resistance to *Cucumber mosaic virus* transmission by *Aphis gossypii* in *Cucumis melo*. Phytopathology 70(10):958–961
Pitrat M, Maestro-Tejada MC, Ferriere C, Ricard M (1988) Resistance to *Aphis gossypii* in Spanish melon (*Cucumis melo*). Cucurbit Genet Coop Rep 11:50–51
Pitrat M, Risser G, Bertrand F, Blancard D, Lecoq H (1996) Evaluation of a melon collection for disease resistances. In: Cucurbits Towards 2000, the proceedings of the 6th EUCARPIA meeting of Cucurbit Genetics and Breeding, Malaga, Spain:49–58
Riley D, Batal D, Wolff D (2001) Resistance in glabrous-type *Cucumis melo* L. to whiteflies (Homoptera : Aleyrodidae). J Entomol Sci 36(1):46–56
Robinson RW (1992) Genetic resistance in the Cucurbitaceae to insects and spider mites. Plant Breed Rev 10:309–360
Rojas ES, Gleason ML (2012) Epiphytic survival of *Erwinia tracheiphila* on muskmelon (*Cucumis melo* L.). Plant Dis 96(1):62–66. doi:10.1094/pdis-04-11-0277

Romanow LR, Moyer JW, Kennedy GG (1986) Alteration of efficiencies of acquisition and inoculation of *Watermelon mosaic virus-2* by plant resistance to the virus and to an aphid vector. Phytopathology 76(12):1276–1281. doi:10.1094/Phyto-76-1276

Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM (1998) The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. Proc Natl Acad Sci USA 95(17):9750–9754

Sakata Y, Oyabu T, Yabe K, Sugiyama M, Morishita M, Sugahara S, Saito T (2006) Development of an earl's-type melon, 'Earl's Kagayaki', with resistance to cotton-melon aphid, powdery mildew and fusarium wilt. Jarq-Japan Agric Res Q 40(2):177–181

Sambandam CN, Chelliah S (1969) *Cucumis callosus* (Rottl.) Cogn. (syn. *C. trigonus* Roxb.) a source of resistance to the fruit fly, *Dacus cucurbitae* Coquillett. Annamalai Univ Agric Res Ann 1:118–119

Sambandam CN, Chelliah S (1972) *Cucumis callosus* (Rottl.) Logn., a valuable material for resistance breeding in muskmelons. In: 3rd International Symposium Sub-tropical Horticulture, 1972, pp 63–68

Sarria E, Yuste-Lisbona FJ, Palomares FJ, Lopez-Sese AI, Gomez-Guillamon ML (2008) Inheritance of tolerance to *Aphis gossypii* in *C. melo* TGR-1551 and its relation with resistance to virus transmission. Cucurbitaceae 2008: Proceedings of the IXth Eucarpia Meeting on Genetics and Breeding of Cucurbitaceae

Sarria Villada E, Gonzalez EG, Lopez-Sese AI, Castiel AF, Gomez-Guillamon ML (2009) Hypersensitive response to *Aphis gossypii* Glover in melon genotypes carrying the *Vat* gene. J Exp Bot 60(11):3269–3277. doi:10.1093/jxb/erp163

Sattar S, Addo-Quaye C, Song Y, Anstead JA, Sunkar R, Thompson GA (2012a) Expression of small RNA in *Aphis gossypii* and its potential role in the resistance interaction with melon. PLos One 7(11), e48579. doi:10.1371/journal.pone.0048579

Sattar S, Song Y, Anstead JA, Sunkar R, Thompson GA (2012b) *Cucumis melo* microRNA expression profile during aphid herbivory in a resistant and susceptible interaction. Mol Plant-Microbe Interact 25(6):839–848. doi:10.1094/mpmi-09-11-0252

Sauvion N, Mauriello V, Renard B, Boissot N (2005) Impact of melon accessions resistant to aphids on the demographic potential of silverleaf whitefly. J Econ Entomol 98(2):557–567

Schoeny A, Boissot N, Lambion J, Wipf-Scheibel C, Mistral P, Gognalons P, Nozeran K, Lecoq H (2014) Conception d'associations en maraîchage de plein champ : exemple de production de melons associés à des bandes fleuries pour lutter contre les pucerons et les virus. Innov Agron 2014 (40):113–114

Serres-Giardi L, Dogimont C (2012) How microsatellite diversity helps to understand the domestication history of melon. In: Sari N, Solmaz I, Aras V (eds) Cucurbitaceae 2012, Proceedings of the Xth EUCARPIA meeting on Genetics and Breeding of Cucurbitaceae, Antalya, Turkey October 15–18th, 2012, pp 254–263

Sese AIL, Soria C, Gomez-Guillamon ML (1996) Resistance to *Bemisia tabaci* in *Cucumis melo*. Cucurbits towards 2000 Proceedings of the VIth EUCARPIA meeting on Cucurbit Genetics and Breeding, pp 344–350

Shinoda T (1993) Callose reaction induced in melon leaves by feeding of melon aphid, *Aphis gossypii* Glover as possible aphid resistant factor. Jpn J Appl Entomol Zool 37(3):145–152

Simmons AM, McCreight JD (1996) Evaluation of melon for resistance to *Bemisia argentifolii* (Homoptera: Aleyrodidae). J Econ Entomol 89(6):1663–1668

Simmons AM, Kousik CS, Levi A (2010) Combining reflective mulch and host plant resistance for sweetpotato whitefly (Hemiptera: Aleyrodidae) management in watermelon. Crop Prot 29 (8):898–902. doi:10.1016/j.cropro.2010.04.003

Smith CM (1989) Plant resistance to insects: a fundamental approach. Wiley, New York

Smith CM, Clement SL (2012) Molecular bases of plant resistance to arthropods. Annu Rev Entomol 57:309–328. doi:10.1146/annurev-ento-120710-100642

Soria C, Gomez Guillamon ML, Duffus JE (1991) Transmission of the agent causing a melon yellowing disease by the greenhouse whitefly *Trialeurodes vaporariorum* in Southeast Spain. Neth J Plant Pathol 97(5):289–296. doi:10.1007/bf01974224

Soria C, Sese AIL, Gomez-Guillamon ML (1996) Resistance mechanisms of *Cucumis melo* var *agrestis* against *Trialeurodes vaporariorum* and their use to control a closterovirus that causes a yellowing disease of melon. Plant Pathol 45(4):761–766. doi:10.1046/j.1365-3059.1996.d01-169.x

Soria C, Lopez-Sese AI, Gomez-Guillamon ML (1999) Resistance of *Cucumis melo* against *Bemisia tabaci* (Homoptera : Aleyrodidae). Environ Entomol 28(5):831–835

Soria C, Moriones E, Fereres A, Garzo E, Gomez-Guillamon ML (2003) New source of resistance to mosaic virus transmission by *Aphis gossypii* in melon. Euphytica 133(3):313–318. doi:10.1023/a:1025721329026

Takken FLW, Goverse A (2012) How to build a pathogen detector: structural basis of NB-LRR function. Curr Opin Plant Biol 15(4):375–384. doi:10.1016/j.pbi.2012.05.001

Thomas S, Boissot N, Vanlerberghe-Masutti F (2012a) What do spring migrants reveal about sex and host selection in the melon aphid? BMC Evol Biol 12:47. doi:10.1186/1471-2148-12-47

Thomas S, Dogimont C, Boissot N (2012b) Association between *Aphis gossypii* genotype and phenotype on melon accessions. Arthropod Plant Interact 6(1):93–101. doi:10.1007/s11829-011-9155-2

Thomas S, Mistral P, Loiseau A, Vanlerberghe-Masutti F, Boissot N (2015) Poor production of dispersal morphs and winter extinction in melon-aphid populations (*Aphis gossypii*) ensure the durability of *Vat*-based resistance in melon crops. Evol Appl (in press)

Tjallingii WF (2006) Salivary secretions by aphids interacting with proteins of phloem wound responses. J Exp Bot 57(4):739–745. doi:10.1093/jxb/erj088

Tzanetakis IE, Martin RR, Wintermantel WM (2013) Epidemiology of criniviruses: an emerging problem in world agriculture. Front Microbiol 4(119)

Vashistha RN, Choudhury B (1971) Studies on resistance to the red pumpkin beetle (*Aulacophora foveicollis* Lucas) in muskmelon, bottlegourd and watermelon. Progress Hortic 2:47–58

Vashistha RN, Choudhury B (1974) Inheritance of resistance to red pumpkin beetle in muskmelon. Sabrao J 6:95–97

Vayssieres JF, Carel Y, Coubes M, Duyck PF (2008) Development of immature stages and comparative demography of two cucurbit-attacking fruit flies in Reunion Island: *Bactrocera cucurbitae* and *Dacus ciliatus* (Diptera Tephritidae). Environ Entomol 37(2):307–314. doi:10.1603/0046-225x(2008)37[307:doisac]2.0.co;2

Vos P, Simons G, Jesse T, Wijbrandi J, Heinen L, Hogers R, Frijters A, Groenendijk J, Diergaarde P, Reijans M, Fierens-Onstenk J, de Both M, Peleman J, Liharska T, Hontelez J, Zabeau M (1998) The tomato *Mi-1* gene confers resistance to both root-knot nematodes and potato aphids. Nat Biotechnol 16(13):1365–1369

Webb SW (1998) Insect resistance in cucurbits: 1992-1998. In: McCreight JD (ed) Cucurbitaceae'98 Evaluation and enhancement of Cucurbits germplasm, Pacific Grove, CA, 30/11–04/12/1998. ASHS Press, Alexandria, VA, pp 79–83

Chapter 13
Genetic Engineering of Important Breeding Traits in Solanaceae and Cucurbitaceae

Satoko Nonaka and Hiroshi Ezura

13.1 Introduction

According to the United Nations, the global population reached 7 billion in 2011 and will reach 9.3 billion in 2050. This increase in the population will cause substantial food shortages, which must be quickly resolved. The breeding of new crops with improved yield performance and nutrition is a potential means to address this problem.

Solanaceae and Cucurbitaceae contain the important crops utilized by both the fresh vegetable and food processing industries. However, these crops have a variety of problems that must be overcome for the food shortage problems. To improve the food shortage problem, the traits which involved in yield and fruit quality have been noted. Recent studies have identified the genes involved in fruit setting, fruit size, shelf life, insect resistance, disease resistance, nutritional content, and sugar accumulation. Application of the information may improve yield and the nutritional value of fruit. The introduction of traits such as a higher fruit setting rate, larger fruit size, and longer shelf life would contribute to increases in yield. Subsequent reductions in crop damage caused by bacteria, fungi, and insects would also result in increases in yield. Metabolic engineering increases the nutritional value and sugar content of fruit, resulting in an improvement in fruit quality. For the application of genetic information to the crops, genetic engineering is efficacious.

Over time, natural mutations, hybridization, and selection have introduced favorable characteristics into the crops available today. However, traditional breeding of new crops with additional favorable traits would require at least decades.

S. Nonaka (✉) • H. Ezura
Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8572, Japan
e-mail: nonaka@gene.tsukuba.ac.jp; ezura.hiroshi.fa@u.tsukuba.ac.jp

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_13

Genetic engineering has the potential to accelerate the breeding process. Genetic engineering involves the following four techniques: transformation, gene targeting, random mutagenesis, and genome editing. In the Solanaceae and Cucurbitaceae families, the transformation technique is primarily used. This technique enables to reduce or increase the expression of genes of interest. This technique makes it possible to introduce the genes from different species into important crops for shorter period comparing to the conventional breeding. In this chapter, we described the crops of Solanaceae and Cucurbitaceae families which were given the traits involved in food productivity by genetic engineering.

13.2 Insect Resistance

Injury caused by insects affects crop production, and many types of chemicals have been developed to effectively rid crops of pests. However, chemical treatments have limitations. For example, damage to stalks caused by borers from within the stalks is difficult to control with externally applied pesticides. Chemical treatments are also losing their effectiveness due to the onset of pest resistance and have negative effects on the environment. Therefore, farmers are requesting new pesticides that better control pests. The environmental benefits of reducing our reliance on chemical pesticides are obvious. Through genetic engineering, a new pesticide system to generate insect-resistant crops can be achieved. Here, we introduce the pesticide systems that utilize Bt proteins, insect dsRNA, insect proteinase inhibitors, and chitinase.

13.2.1 Bt Toxins

Bacillus thuringiensis was identified as a silkworm killer in the 1900s. Because the production of silk was one of the most important industries at the time, scientists endeavored to clarify the mechanism underlying the effects of *B. thuringiensis*. The Bt crystal proteins (Cry) produced by *B. thuringiensis* were demonstrated to be insecticidal (δ-endotoxin). These Bt crystal proteins are toxic to lepidopterans, dipterans, and coleopterans and nontoxic for animals and humans. These proteins have been widely used as pesticides since 1961, and the first Cry gene was cloned and expressed in *Escherichia coli* in 1981 (Betz et al. 2000). Cry proteins are classified into 15 major groups that include approximately 70 subgroups (Schünemann et al. 2014). The Cry1 and Cry3 proteins have insecticidal activity against Solanaceae insect pests. The Cry1 and Cry3 groups have insecticidal activity against lepidopteran insects and the Colorado potato beetle (CPB), respectively. To improve their insecticidal activity, these proteins are modified (Zhou et al. 2012; Kou et al. 2014), or the most effective proteins are identified from within the subgroup (Li et al 2013). The first Bt-producing Solanaceae plants developed were tomato and tobacco (Fischhoff et al. 1987; Vaeck et al 1987).

Transgenic potato and eggplant were also developed to resist CPB and eggplant fruit and shoot borer (EFSB), respectively (Adang et al. 1993; Krattiger 2011). Many modifications have been carried out to achieve greater insect resistance for commercialization. Cry3A in potatoes confers resistance to CPB and has been available commercially in the United States since 1996 (James 1998, 1999). Transgenic eggplant and tomato including the *cry1Ac* gene were developed by the Indian biotech company Mahyco and commercialized in India (Krattiger 2011; James 2014). These attributes and the cost savings offered by these products have contributed to the rapid adoption of Bt-protected plants by farmers.

13.2.2 dsRNA

The RNA interference (RNAi) technique to knock down essential genes has been utilized for insect pests (Terenius et al. 2011). The first report on this technique was that the double-strand RNA (dsRNA) could trigger RNAi in *Caenorhabditis elegans* (Fire et al. 1998). dsRNA injection can knock down the targeted gene expression in many insects (Huvenne and Smagghe 2010; Bellés 2010). However, the injection of dsRNA into insects is not feasible for pest control in the field, and a simpler and more convenient method is required. Several reports have shown that oral feeding of dsRNA caused the RNAi effects in insects (Turner et al. 2006; Bautista et al. 2009). Transgenic crops that produce dsRNA targeting insect genes would make it possible to inhibit the gene expression in insect. Indeed, in transgenic tobacco engineered to express dsRNA of the ecdysone receptor (EcR) and ultraspiracle (USP) genes, which control insect molting and metamorphosis, the target genes were suppressed in insects (*Helicoverpa armigera*), and a significant reduction in insect feeding damage was achieved (Zhu et al. 2012). This dsRNA system has been applied to other Solanaceae crops. This trial was conducted in laboratory and greenhouse conditions; thus, such dsRNA systems must also be studied under field conditions.

13.2.3 Proteinase Inhibitors

Proteinase inhibitors, especially those related to the digestive system, affect the growth and development of many insect species (Bolter and Jongsma 1997; Murdock and Shade 2002). The following two major types of digestive enzyme inhibitors have been reported: the serine (trypsin) type, which is effective against Lepidoptera and Diptera (Matsumoto et al. 1995; Srinivasan et al. 2006), and alpha-amylase inhibitors (Morton et al. 2000; Nishizawa et al. 2007; Solleti et al. 2008). These proteinase inhibitors have been effective for bean storage and Solanaceae pest control. The expression of the alpha-amylase inhibitor gene in *Nicotiana tabacum* increased the mortality rate of the cotton-boll weevil (*Anthonomus grandis*) compared to non-transgenic tobacco (Dias et al. 2010). Transgenic

tobacco expressing sporamin (trypsin inhibitor) from sweet potato and CeCPI (phytocystatin) from taro showed resistance to both insects and phytopathogens. The larvae of *Helicoverpa armigera* that ingested tobacco leaves either died or showed delayed growth and development relative to the control larvae (Senthilkumar et al. 2010). The sugar beet serine-type proteinase inhibitor gene (*BvSTI*) was introduced into *N. benthamiana*, and the resulting plants showed resistance to five Lepidoptera insect pests (*Spodoptera frugiperda*, *S. exigua*, *Manduca sexta*, *Agrotis ipsilon*, *Heliothis virescens*) (Smigocki et al. 2013).

13.3 Disease Resistance

Fungi, bacteria, and viruses cause many types of diseases in crops, reducing yields. For example, potato virus Y (PVY) reduces crop yields by 20–80 % (James 2014). To improve disease resistance in crops, many studies have attempted to clarify these mechanisms. For the enhancement of crop disease resistance, the following two major types of technological trends have been introduced in Solanaceae and Cucurbitaceae: the transgenic deployment of resistance (*R*) genes and pattern-recognition receptor (*PRR*) genes for fungi and bacteria and the expression of plant-expressed siRNA directed against virus pathogens.

13.3.1 Deployment of Resistance (R) Genes and PRR Genes

Plants have two immune systems: one is the R gene system, and the other is made up of the transmembrane pattern-recognition receptors (PRRs). It is well known that both systems are induced by microbial infection. *R* proteins recognize pathogens either by direct binding to a pathogen molecule or indirectly through the action of a pathogen effector on its host cell target (Jones and Dang 2006). This interaction in turn initiates a conserved, downstream defense signal cascade terminating in a hypersensitive response (HR). Based on their structure and subcellular location, *R* genes encode nucleotide-binding leucine-rich repeat (NB-LRR) proteins with two domains. Extracellular membrane-anchored leucine-rich repeat (LRR) receptor-like proteins and receptor-like kinases monitor the exterior of the cell, and the nucleotide-binding domain oversees the intracellular environment. Genetically engineered tomato plants that express the pepper Bs2 gene, a member of the NBS-LRR class of R genes, can suppress the growth of *Xanthomonas campestris* pv. (Tai et al. 1999). Introducing two types of *NB-LRR* genes (*RPS4* and *RRS1*) into transgenic tomato and cucumber showed resistance to *Ralstonia solanacearum* (a bacterium) and *Colletotrichum orbiculare* (a fungus), respectively (Narusaka et al. 2013).

PRRs detect the pathogen-associated molecular patterns (PAMPs) that trigger basal immune responses and provide sufficient control of pathogen growth in the absence of additional pathogen effectors that function to subvert PAMP-triggered

immunity (Jones and Dang 2006; Segonzac and Zipfel 2011). The activity of a PRR is retained after its transfer between two plant families (Lacombe et al. 2010). Expression of EF-Tu reception gene (*EFR*), a PRR from *Arabidopsis thaliana*, confers responsiveness to the bacterial elongation factor Tu (EF-Tu) in the *Nicotiana benthamiana* and *Solanum lycopersicum*, making them more resistant to a range of phytopathogenic bacteria from different genera (Lacombe et al. 2010).

13.3.2 Expression of Plant-Expressed siRNA Directed Against Virus Pathogens

Effective ways to protect crops against viral infection include the eradication of infected plants to ensure the use of virus-free seeds and vegetative stocks, the propagation and maintenance of virus-free stocks, the modification of planting and harvesting systems, and the use of resistant varieties (Hull 2002). Another strategy for suppressing viral diseases is the utilization of posttranscriptional gene silencing (PTGS) or RNAi. These techniques introduce a partial viral gene sequence to dismantle a viral gene introduced into the plant cell. This method has been critical for effective and sustainable control against major viral diseases (Fuchs 2008; Waterhouse et al. 2001). PTGS and RNAi involve sequence-specific mRNA degradation that is triggered by dsRNA (Fire et al. 1998; Kennerdell and Carthew 1998). An endonuclease cleaves dsRNA into small interfering RNA (siRNA) (Bernstein et al. 2001). The siRNA is then integrated into the RNA-induced silencing complex (RISC) and works as a guide to recognize complementary RNA for its degradation (Hammond et al. 2000). In plants, this PTGS mechanism is induced by viral infection and acts as a defense against the virus (Waterhouse et al. 2001). The presence of siRNAs from a viral coat protein (CP) gene conferred resistance to the virus in transgenic watermelon (Yu et al. 2011; Lin et al. 2012). The downregulation of Cm-eIF4E, a eukaryotic translation initiation factor from the 4E family, via RNAi conferred broad virus resistance in melon (Rodríguez-Hernández et al. 2012).

13.3.3 Other Strategies

The overexpression of the wasabi defensin gene, which is a cysteine-rich polypeptide, in *Colocynthis citrullus* L. confers resistance to fusarium wilt and *Alternaria* leaf spot (Ntui et al. 2010). Transgenic potato plants expressing the wasabi defensin gene were also shown to have antifungal activity against *Botrytis cinerea* (Khan et al. 2006). *N. benthamiana* plants containing a balsam pear (*Momordica charantia* L.) chitinase (Mcchit1) displayed enhanced resistance to the plant pathogen *Phytophthora nicotianae* (Xiao et al. 2007). Transgenic tobacco plants

overexpressing the Magi6 peptide were resistant to *Spodoptera frugiperda* (Hernández-Campuzano et al. 2009). Tobacco plants containing a p35 gene from the baculovirus *Autographa californica* were resistant to TMV (Wang et al. 2009). The GbTLP1 and StPUB17 (UND/PUB/ARM) receptor-type genes were introduced into transgenic potato plants, and these genes conferred resistance to *Phytophthora infestans* and potato late blight, respectively (Ni et al. 2010; Liu et al. 2009).

13.4 Fruit Setting and Development

Fruit set and development are important traits that govern fruit yield. Several studies have described the molecular pathway that controls the fruit set system (Ariizumi et al. 2013). Successful pollination and fertilization induce fruit set, which is influenced by exogenous or endogenous phytohormones such as auxin, gibberellic acid (GA), cytokinins, ethylene, and abscisic acid (ABA). Genetic engineering projects related to auxin, GA, and ethylene have attempted to improve fruit setting and development.

13.4.1 Auxin

Auxin triggers fruit setting in tomato by activating cell division in the pericarp. Auxin accumulates in the vascular strands of the ovary and the micropyle pole of the embryo sac in the ovule. This hormone is then distributed to the integument of the ovule, finally localizing to the surface of the ovary wall (Pattison and Catalá 2012). Pattison and Catalá (2012) demonstrated that auxin plays an important role in the ovary during fruit setting and development. IAA9, which belongs to the Aux/IAA gene family, acts as a transcriptional repressor in the auxin signaling pathway. Therefore, the suppression of *SlIAA9* through RNA antisense increased the formation of parthenocarpic fruit (Wang et al. 2005). Auxin response factors (AFRs) specifically control auxin-dependent biological responses and negative regulators of fruit set. The downregulation of SlARF7 via RNAi results in a constitutive auxin response that induces parthenocarpic fruit formation (de Jong et al. 2009). In a transgenic tomato line that included a mutated allele of *ARF8* from *Arabidopsis*, parthenocarpic-like fruit formation was also stimulated (Goetz et al. 2007). The *transport inhibitor response protein 1* (*TIR1*) gene and the *auxin-binding f-box protein* (ABF) gene act as auxin receptors and F-box subunits of E3 ubiquitin ligase complexes, which target IAA/AUX proteins for degradation through the 26S proteasome pathway (Ariizumi et al. 2013). Therefore, these genes appear to be positive regulators of the auxin signaling pathway. The overexpression of *SlTIR* induced parthenocarpic fruit formation (Ren et al. 2011). By comparing auxin synthesis (*defH9-1aaM*) mutant parthenocarpic tomato flower buds with

wild-type flower buds via a cDNA-amplified fragment length polymorphism (cDNA-AFLP) analysis, the *auxin cum silencing action* (*AUSCIA*) gene was identified. The repression of *AUSCIA* gene expression via RNAi resulted in a 100-fold greater accumulation of IAA, resulting in a rise in the parthenocarpic fruit appearance rate (Molesini et al. 2009). Auxin responses are also influenced by flavonoids. The *chalcone synthase* (*CHS*) gene, which acts as the first step of the flavonoid biosynthesis pathway, negatively affects auxin responses. Indeed, the inhibition of *CHS* decreased the total flavonoid content. As a result, auxin responses were induced and the production of parthenocarpic fruits increased (Schijlen et al. 2007).

13.4.2 Gibberellin

Gibberellin is involved in fruit setting and development. The tomato GA biosynthesis mutants *gib1*, *gib2*, and *gib3* were dwarfed and failed during fruit setting and development. The application of exogenous GA allowed these mutant plants to overcome this phenotype (Bensen and Zeevaart 1990). However, the final size of the fruit is not determined by GA. Additionally, GA induction is induced by auxin, and GA biosynthesis seems to be regulated by auxin (Ariizumi et al. 2013).

DELLA acts as a repressor in the GA response pathway. Therefore, the removal of DELLA repressors results in an increase in the GA response. The silencing of *PROCERA/SlDELLA* resulted in an increase in parthenocarpic fruit formation. However, fruit size was smaller with elongated cells, and a reduced number of cells were observed in the pericarp. Pleiotropically, plant growth was abnormal (Martí et al. 2007).

13.4.3 Ethylene

The *tomato tetratricopeptide repeat protein 1* (*SlTPR1*) gene interacts with ethylene receptors such as NR and LeETR1. The overexpression of *SlTPR1* enhances the ethylene and auxin responses and results in an increase in the production of parthenocarpic fruit (Lin et al. 2008). However, overexpression of this gene also induces abnormal phenotypes in the plants, including dwarfism, reduced internode length, and a reduction in leaf complexity.

13.5 Fruit Size

Fruit size is important for crop yield. Therefore, fruit size has been increased through domestication and selection in crops such as tomato (Solanaceae) and melon (Cucurbitaceae). The morphology of Cucurbitaceae fruits may be the result

of orthologous genes also found in tomato in the same gene families. For tomato, some of the underlying quantitative trait loci (QTLs) for these genes have been cloned. For melon, the underlying genes have remained elusive (Monforte et al. 2014). Analyses have indicated that these QTLs include those responsible for fruit size in tomato. The factors that determine fruit size are cell division and locule number. The following six loci are involved in fruit weight: *fw1.1*, *fw2.2*, *fw2.3*, *fw3.1*, *fw3.2*, *fw4.1*, and *fw9.1* (Grandillo et al. 1999). Fruit weight is determined by enhanced cell division in the pericarp. The locule number is primarily affected by the following genes: *SUN*, *OVATE*, *LOCULE NUMBER* (*LC*), and *FASCIARED* (*FAS*) (Monforte et al. 2014). Crop breeding via genetic engineering has not been applied to commercialization. We describe the potential for applying these genes in genetic engineering below.

13.5.1 CNR/FW2.2

CNR/*FW2.2* is involved in the determination of fruit weight. QTL analysis demonstrated that approximately 30 % of the variation in fruit weight between the domesticated tomato and its relatives with smaller fruits is due to this locus (Frary et al. 2000). Comparing domesticated plants and their relatives reveals differences in this regulatory region; therefore, the mRNA expression level is more closely related to fruit size than protein function. In the relatives of tomato with smaller fruits, the mRNA expression level is greater and of longer duration during fruit development (Cong et al. 2002). This study revealed that greater gene expression levels result in smaller fruits with fewer cells and that *fw2.2* is a negative regulator of cell number. Therefore, the fruit weight of tomato or melon could be increased by suppressing the expression level of *FW2.2* via RNAi. Indeed, the downregulation of *FW2.2* increased the size of transgenic maize (Guo et al. 2010; Guo and Simmons 2011).

13.5.2 KLUH/FW3.2

A recent study achieved fine mapping of the *FW3.2* locus, which is the second major QTL related to fruit weight in tomato (Zhang et al. 2012). The *fw3.2* locus contained an ORF that is an ortholog of the *Arabidopsis KLUH* gene and a member of the P450 78A subfamily (*SlKLUH*) (Zhang et al. 2012). In an F_2 population derived from a cross between the heirloom tomato cultivar Yellow Stuffer (*ys*) and the wild-type species LA1589 (*wt*), 19 % of the variance in fruit mass could be attributed to this locus (van der Knaap et al. 2002). The *fw3.2*(*ys*) plants exhibited greater fruit weight than *fw3.2*(*wt*) plants. The expression of the *SlKLUH*/*FW3.2* gene in *fw3.2*(*ys*) plants was greater than that in *fw3.2*(*wt*) plants. The downregulation of *SlKLUH*/*FW3.2* resulted in reduced fruit weight (Chakrabarti

et al. 2013). These results demonstrate that the expression level of this gene is important for the determination of fruit weight. Therefore, the fruit weight of tomato or melons would be increased by the overexpression of *SlKLUH*. Indeed, *KLUH* overexpression results in large, seedless siliques in transgenic *Arabidopsis* (Ito and Meyerowitz 2000).

13.5.3 SUN

SUN is a member of the IQ domain family, which is characterized by a calmodulin-binding domain. A previous study indicated that SUN dramatically affects the early stages of fruit development (Wu et al. 2011). The overexpression of SUN increased the cell number in the proximal distal direction and decreased the cell number in the medial lateral direction of the fruit, leading to elongated parthenocarpic fruit (Wu et al. 2011).

13.5.4 **FAS *and* LC**

QTL analysis indicated that *FAS* and *LC* control the locule number. *FAS* is a member of the *YABBY* gene family, which regulates organ polarity; *LC* is considered to be an ortholog of the *A. thaliana* gene *WUSCHEL* (Cong et al. 2008; Muños et al. 2011). The overexpression of the *FAS* gene resulted in a reduction in fruit locule numbers. Conversely, a mutation in *FAS* increased locule numbers, resulting in a larger fruit (Cong et al. 2008). These results suggest that knocking down *FAS* or *LC* gene expression confers a larger fruit size.

13.5.5 **OVATE**

The tomato QTL analysis identified the *OVATE* gene, which is involved in fruit shape. *OVATE* encodes the ovate family protein (OFP) and is thought to negatively regulate the transcription of target genes (Liu et al. 2002). The *OVATE* gene was cloned from two pepper cultivars with different fruit shapes: cv. “Mytilini Round” and cv. “Piperaki Long” (Tsaballa et al. 2011). No significant structural differences were observed in the *OVATE* genes between the two cultivars. Comparisons of the transcriptional levels in these cultivars revealed that the expression level in the long cultivar was lower than that in the round cultivar; the developmental expression profile was also different. The downregulation of *OVATE* gene expression in the round cultivar pepper via virus-induced gene silencing (VIGS) changes the fruit to a larger and more oblong form (Tsaballa et al. 2011). The overexpression of this gene in tomato with oblong fruits resulted in fruits with a round shape and conferred

abnormal phenotypes (Liu et al. 2002). These results suggest that to breed larger fruits, the suppression of OVATE gene expression in round-shaped fruits may be effective.

13.5.6 HMGR

Although FuyuA and Natsu4 have nearly identical genetic backgrounds, fruit size in FuyuA is larger than that of Natsu4 (Higashi et al. 1999). Comparing cell size during development indicates that the cell proliferation period of FuyuA is longer than that of Natsu4, suggesting that the duration of the cell proliferation period determines fruit size. To investigate these differences, a cDNA library was screened. Cm-HMGR, which has a high sequence homology to 3-hydroxy-3-methylglutaryl coenzyme A reductase, was isolated (Kato-Emori et al. 2001). Gene expression and enzymatic analyses showed that the levels of Cm-HMGR expression and activity are increased after pollination in the early stages of fruit development. These results indicated that this gene is involved in the determination of fruit size (Ezura and Hiwasa-Tanase 2010). In a transgenic tomato line with Cm-HMGR driven by the 35S promoter, the fresh fruit weight increased by 20 % (Kobayashi et al. 2002; Omura et al. 2007), suggesting that the overexpression of Cm-HMGR via genetic engineering increases the fresh weight and size of melon fruits.

13.6 Shelf Life

Excessive softening is the main factor limiting fruit shelf life and storage. In developing countries, postharvest losses of fruits and vegetables reduce yields by 30–50 %. Therefore, a longer shelf life is important for the food supply. To reduce this loss, scientists have endeavored to reveal the mechanism behind ripening-related fruit softening. The critical factors appear to be cell wall components (Fry 2004), ethylene (Zheng and Wolff 2000; Bennett and Labavitch 2008), polyamines (PAs) (Nambeesan et al. 2010), and *N*-glycan (Priem et al. 1993). Genetic engineering technology may help create fruit with longer shelf lives. The downregulation of the cell wall-related genes polygalacturonase, pectin methylesterase, beta-glucanase, and beta-galactosidase results in fruit with a longer shelf life (Brummell and Harpster 2001). RNAi-mediated silencing of the 1-aminopropane-1-carboxylate oxidase gene 1 (ACO1) and the three homologs of the 1-aminopropane-1-carboxylate synthase (ACS) genes reduced ethylene evolution and delayed ripening in tomato fruits, resulting in a longer shelf life and enhanced processing quality (Ayub et al. 1996; Nuñez-Palenius et al. 2006; Behboodian et al. 2012; Gupta et al. 2013). The insertion of an ACO gene suppression cassette into melon via the pollen tube pathway using the

transformation of a marker-free and vector-free antisense construct resulted in a plant with fruit with a longer shelf life (Hao et al. 2011). The overexpression of a spermidine synthase gene also increased fruit shelf life (Nambeesan et al. 2010). RNAi technology succeeded in enhancing fruit shelf life by suppressing alpha-mannosidase (alpha-Man) and beta-D-*N*-acetylhexosaminidase (beta-Hex), both of which are *N*-glycan processing enzymes (Meli et al. 2010).

13.7 Nutrition and Sugar Accumulation

Solanaceae and Cucurbitaceae fruits contain many types of sugars and nutritional compounds, such as anthocyanins, flavonoids, carotenoids, vitamins, and essential amino acids. Fruit quality is determined by the concentration of these components. The higher the quality of the fruit, the more valuable it is. Therefore, cultivars with these traits have been developed through breeding. In tomato, QTL analyses have identified several genes involved in amino acid and sugar accumulation (in terms of Brix, the soluble solid content) (Toubiana et al. 2012; Fridman et al. 2002; Gilbert et al. 2009). Metabolome analyses have determined the key enzymes involved in anthocyanin, amino acid, carotenoid, and ascorbate metabolic pathways (Ronen et al. 2000; Römer et al. 2000). The utilization of these genes to improve fruit quality through genetic engineering has been achieved in many studies. We review some examples from recent studies. Gamma-aminobutyric acid (GABA), a four-carbon nonprotein amino acid, is a major inhibitory neurotransmitter and a functional component in the reduction of blood pressure in the human body. To increase the accumulation of GABA in tomato fruits, the GABA-metabolizing enzyme GABA transaminase was suppressed. However, dwarfism and infertility were observed in these plants (Koike et al. 2013). The overexpression of *MhSnRK1*, which plays an important role in plant carbon metabolism and development, from pingyitiancha (*Malus hupehensis* Rehd. var. *pinyiensis* Jiang) increased the accumulation and assimilation of both sugar and amino acids in the fruit (Wang et al. 2012). The constitutive expression of lycopene beta-cyclase (LYCB), which is a key enzyme for the synthesis of beta-carotene, increased the total carotenoid content of tomato fruits by 30 %; however, the sugar content was reduced in this transgenic line (Guo et al. 2012). To increase the ascorbate (vitamin C) content, RNAi technology was utilized to suppress the expression of GDP-D-mannose 3,5-epimerase, which is a central enzyme of the major ascorbate biosynthesis pathway (Gilbert et al. 2009). Introduced two transcription factors (Delila and Rosea1) from snapdragon into tomato dramatically increase anthocyanin levels, same as blackberries and blueberries, resulted in fruit with intense purple coloration in both peel and flesh (Butelli et al. 2008), and show longer shelf life and less disease (Klee 2013). The high-anthocyanin tomatoes have also the health-promoting effects. Indeed, in pilot test, feeding to the cancer-susceptible Trp53$^{-/-}$ mice showed a significant extension its life span (Butelli et al. 2008). This purple tomato has already been utilized for commercial as juice material. Canadian company, New Energy Farms (Ontario), is

now producing enough purple tomatoes in a 465 square meter greenhouse to make 2000 l of juice (Shukman 2014).

Not only metabolic engineering but also addition of function is a well-used strategy to increase nutrition. The taste-modifying protein, miraculin, functions to change the perception of a sour taste to a sweet one. This taste-modifying function can potentially be used as seasoning that could be the basis of a new dietary lifestyle. Sweet-tasting proteins and taste-modifying proteins have a great deal of potential in industry as substitutes for sugars and as artificial sweeteners. Transgenic miraculin tomato accumulates active miraculin protein; the accumulation and gene expression level was genetically stable from T1 to T5. For commercialization, the safety of this transgenic line is assessed (Hiwasa-Tanase et al. 2012).

13.8 Conclusions and Future Works

Transformation, which is one of the genetic engineering techniques, enables to improve important breeding traits in Solanaceae and Cucurbitaceae crops. Although the potential of transformation as a breeding tool has been experimentally demonstrated, there are few commercialized due to a lack of public acceptance. If these problems were solved, the number of commercialization lines would be increased.

Transformation is divided into the following two categories: the transformation of foreign genes and the control of endogenous gene expression. The introduction of foreign genes makes it possible to introduce new traits from different spices, such as insect resistance and changes to plant metabolism. The control of endogenous gene expression through the overexpression of endogenous genes or by RNA interference systems allows us to change metabolic pathways and dramatically alter the morphology of plants. These techniques confer valuable traits to crops. However, changes in endogenous gene expression, especially up regulation, can cause abnormal phenotypes. Overexpression of genes or RNA interference system disappears the effect on the progeny lines, through the gene silencing. The mechanism of the gene silencing is to be clarified.

New technologies such as gene targeting and genome editing are still developing to address these potential problems.

Gene targeting and genome editing are new genetic engineering technologies that enable a more precise engineering of crops than transformation. Gene targeting techniques allow for the integration of genes of interest into specific sites in the plant genome via homologous recombination, which is the DNA repair system. Transformation is a technique that results in the random insertion of genes into plant genomes. It is therefore difficult to regulate the level, location, and timing of gene expression. The gene inserted randomly in genome might be one of the reason for the gene silencing; therefore, control of the insert position is important via gene targeting technique.

Genome editing techniques introduce site-specific mutations using chimeric meganucleases such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas (CRISPR-associated) systems, which are based on RNA-guided DNA endonucleases. Utilization of this technique enables to make the knockout mutant. It is more effective than RNA interference in suppression of gene. Many studies using these techniques have been reported in animals, whereas few reports exist in model plants such as in maize, wheat, tobacco, and Arabidopsis. However, these techniques will also be applied to Solanaceae and Cucurbitaceae crops in near feature. To utilize these techniques, it is important to study about genome information and trait evaluation of Solanaceae and Cucurbitaceae crops, and it is also important to establish the genome editing methodology in Solanaceae and Cucurbitaceae. To breeding Solanaceae and Cucurbitaceae crops, to overcome the issue of food shortage, the genome information analysis of these crops will become more and more important in future.

References

Adang MJ, Brody MS, Cardineau G, Eagan N, Roush RT, Shewmaker CK, Jones A, Oakes JV, McBride KE (1993) The reconstruction and expression of a Bacillus thuringiensis cryIIIA gene in protoplasts and potato plants. Plant Mol Biol 21:1131–1145

Ariizumi T, Shinozaki Y, Ezura H (2013) Genes that influence yield in tomato. Breed Sci 63:3–13

Ayub R, Guis M, Ben Amor M, Gillot L, Roustan JP, Latché A, Bouzayen M, Pech JC (1996) Expression of ACC oxidase antisense gene inhibits ripening of cantaloupe melon fruits. Nat Biotechnol 14:862–866

Bautista L, Castro MJ, López-Barneo J, Castellano A (2009) Hypoxia inducible factor-2alpha stabilization and maxi-K+ channel beta1-subunit gene repression by hypoxia in cardiac myocytes: role in preconditioning. Circ Res 104:1364–1372

Behboodian B, Mohd Ali Z, Ismail I, Zainal Z (2012) Postharvest analysis of lowland transgenic tomato fruits harboring hpRNAi-ACO1 construct. ScientificWorldJournal 2012:439870

Bellés X (2010) Beyond Drosophila: RNAi in vivo and functional genomics in insects. Annu Rev Entomol 55:111–128

Bennett AB, Labavitch JM (2008) Ethylene and ripening-regulated expression and function of fruit cell wall modifying proteins. Plant Sci 175(1–2):130–136

Bensen RJ, Zeevaart JAD (1990) Comparison of Ent-kaurene synthetase A and B activities in cell-free extracts from young tomato fruits of wild-type and gib-1, gib-2, and gib-3 tomato plants. J Plant Growth Regul 9:237–242

Bernstein E, Caudy AA, Hammond SM, Hannon GJ (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 409:363–366

Betz FS, Hammond BG, Fucs RL (2000) Safety and Advantages of Bacillus thuringiensis Protected Plant to Control Insect Pests. Regul Toxicol Pharmacol 32:156–173

Bolter C, Jongsma MA (1997) The adaptation of insects to plant protease inhibitors. J Insect Physiol 43:885–895

Brummell DA, Harpster MH (2001) Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol Biol 47:311–340

Butelli E, Titta L, Giorgio M, Mock HP, Matros A, Peterek S, Schijlen EGWM, Hall RD, Bovy AG, Luo J, Martin C (2008) Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. Nat Biotechnol 26:1301–1308

Cong B, Liu J, Tanksley SD (2002) Natural alleles at a tomato fruit size quantitative trait locus differ by heterochronic regulatory mutations. Proc Natl Acad Sci USA 15:13606–13611

Cong B, Barrero LS, Tanksley SD (2008) Regulatory change in YABBY-like transcription factor led to evolution of extreme fruit size during tomato domestication. Nat Genet 40:800–804

Chakrabarti M, Zhang N, Sauvage C, Muños S, Blanca J, Cañizares J, Diez MJ, Schneider R, Mazourek M, McClead J, Causse M, van der Knaap E (2013) A cytochrome P450 regulates a domestication trait in cultivated tomato. Proc Natl Acad Sci USA 110:17125–17130

de Jong M, Wolters-Arts M, Feron R, Mariani C, Vriezen WH (2009) The Solanum lycopersicum auxin response factor 7 (SlARF7) regulates auxin signaling during tomato fruit set and development. Plant J 57:160–170

Dias SC, Maria da Silvaa CM, Teixeiraa FR, Figueiraa ELZ, de Oliveira-Netoa OB, de Limac LA, Francoc OL, Grossi-de-Sa MF (2010) Investigation of insecticidal activity of rye α-amylase inhibitor gene expressed in transgenic tobacco (*Nicotiana tabacum*) toward cotton boll weevil (*Anthonomus grandis*). Pestic Biochem Physiol 98:39–44

Ezura H, Hiwasa-Tanase K (2010) Fruit development. In: Pua EC, Davey MR (eds) Plant developmental biology – biotechnological perspectives, vol 1. Springer, Berlin, pp 301–318

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391:806–811

Fischhoff DA, Bowdish KS, Pealak FJ, Marrone PG, McCormic SM, Nidermeyer JG, Dean DA, Kusano-Kretzmer K, Mayer EJ, Rochester DE, Rogers SG, Fraley RT (1987) Insect tolerant transgenic tomato plants. Bio/Technology 5:807–813

Frary A, Nesbitt TC, Grandillo S, Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert KB, Tanksley SD (2000) fw2.2: a quantitative trait locus key to the evolution of tomato fruit size. Science 289:85–88

Fridman E, Liu YS, Carmel-Goren L, Gur A, Shoresh M, Pleban T, Eshed Y, Zamir D (2002) Two tightly linked QTLs modify tomato sugar content via different physiological pathways. Mol Genet Genomics 266:821–826

Fry SC (2004) Primary cell wall metabolism: tracking the careers of wall polymers in living plant cells. New Phytol 161:641–675

Fuchs M (2008) Pant resistance to viruses: engineered resistance. In: Mahy Brian WJ (ed) Desk encyclopedia of plant and fungal virology. Academic, New York, pp 45–52

Gilbert L, Alhagdow M, Nunes-Nesi A, Quemener B, Guillon F, Bouchet B, Faurobert M, Gouble B, Page D, Garcia V, Petit J, Stevens R, Causse M, Fernie AR, Lahaye M, Rothan C, Baldet P (2009) GDP-D-mannose 3,5-epimerase (GME) plays a key role at the intersection of ascorbate and non-cellulosic cell-wall biosynthesis in tomato. Plant J 60:499–508

Gupta A, Pal RK, Rajam MV (2013) Delayed ripening and improved fruit processing quality in tomato by RNAi-mediated silencing of three homologs of 1-aminopropane-1-carboxylate synthase gene. J Plant Physiol 170:987–995

Grandillo S, Ku HM, Tanksley SD (1999) Identifying the loci responsible for natural variation in fruit size and shape in tomato. Theor Appl Genet 99:978–987

Goetz M, Hooper LC, Johnson SD, Rodrigues JC, Vivian-Smith A, Koltunow AM (2007) Expression of aberrant forms of *AUXIN RESPONSE FACTOR8* stimulates parthenocarpy in Arabidopsis and tomato. Plant Physiol 145:351–366

Guo M, Rupe MA, Dieter JA, Zou J, Spielbauer D, Duncan KE, Howard RJ, Hou Z, Simmons CR (2010) Cell Number Regulator1 affects plant and organ size in maize: implications for crop yield enhancement and heterosis. Plant Cell 22:1057–1073

Guo M, Simmons CR (2011) Cell number counts--the fw2.2 and CNR genes and implications for controlling plant fruit and organ size. Plant Sci 181:1–7

Guo F, Zhou W, Zhang J, Xu Q, Deng X (2012) Effect of the citrus lycopene β-cyclase transgene on carotenoid metabolism in transgenic tomato fruits. PLoS One 7, e32221

Hammond SM, Bernstein E, Beach D, Hannon GJ (2000) An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature 404:293–296

Hao J, Niu Y, Yang B, Gao F, Zhang L, Wang J, Hasi A (2011) Transformation of a marker-free and vector-free antisense ACC oxidase gene cassette into melon via the pollen-tube pathway. Biotechnol Lett 33:55–61

Hernández-Campuzano B, Suárez R, Lina L, Hernández V, Villegas E, Corzo G, Iturriaga G (2009) Expression of a spider venom peptide in transgenic tobacco confers insect resistance. Toxicon 53:122–128

Higashi K, Hosoya K, Ezura H (1999) Histological analysis of fruit development between two melon (Cucumis melo L. reticulatus) genotypes setting a different size of fruit. J Exp Bot 50:1593–1597

Hull R (2002) Matthew's plant virology. Academic, New York

Huvenne H, Smagghe GJ (2010) Insect Physiol. Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: a review. J Insect Physiol 56(3):227–235

Ito T, Meyerowitz EM (2000) Overexpression of a gene encoding a cytochrome P450, CYP78A9, induces large and seedless fruit in arabidopsis. Plant Cell 12:1541–1550

James C (1998) Global review of commercialized transgenic crops: 1998, ISAAA Briefs No. 8. ISAAA. Ithaca, NY

James C (1999) Preview – global review of commercialized transgenic crops: 1999, ISAAA Briefs No. 12. ISAAA. Ithaca, NY

James C (2014) Global status of commercialized biotech/GM Crops: 2013, ISAAA Briefs No. 46. ISAAA. Ithaca, NY

Jones JDG, Dang JL (2006) The plant immune system. Nature 444:323–329

Kato-Emori S, Higashi K, Hosoya K, Kobayashi T, Ezura H (2001) Cloning and characterization of the gene encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase in melon (Cucumis melo L. reticulatus. Mol Genet Genomics 265:135–142

Kennerdell JR, Carthew RW (1998) Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. Cell 95:1017–1026

Khan RS, Nishihara M, Yamamura S, Nakamura I, Mii M (2006) Transgenic potatoes expressing wasabi defensin peptide confer partial resistance to gray mold (*Botrytis cinerea*). Plant Biotechnol 23:179–183

Klee HJ (2013) Purple tomatoes: longer lasting, less disease, and better for you. Curr Biol 23: R520–R521

Kobayashi T, Kato-Emori S, Tomita K, Ezura H (2002) Detection of 3-hydroxy-3-methylglutaryl coenzyme A reductase protein Cm-HMGR during fruit development in melon (Cucumis melo L.). Theor Appl Genet 104:779–785

Koike S, Matsukura C, Takayama M, Asamizu E, Ezura H (2013) Suppression of γ-aminobutyric acid (GABA) transaminases induces prominent GABA accumulation, dwarfism and infertility in the tomato (*Solanum lycopersicum* L.). Plant Cell Physiol 54:793–807

Kou B, Srivastava S, Sanyal I, Tripathi B, Sharma V, Amla DV (2014) Transgenic tomato line expressing modified Bacillus thuringiensis cry1Ab gene showing complete resistance to two lepidopteran pests. SpringerPlus 3:84–96

Krattiger A (2011) Intellectual property, commercial needs and humanitarian benefits: must there be a conflict? New Biotechnol 27:573–577

Lacombe S, Rougon-Cardoso A, Sherwood E, Peeters N, Dahlbeck D, van Esse HP, Smoker M, Rallapalli G, Thomma BP, Staskawicz B, Jones JD, Zipfel C (2010) Interfamily transfer of a plant pattern-recognition receptor confers broad-spectrum bacterial resistance. Nat Biotechnol 28:365–369

Li X, Li S, Lang Z, Zhang J, Zhu L, Huang D (2013) Chloroplast-targeted expression of the codon-optimized truncated cy1Ah gene in transgenic tobacco confers a high level of protection against insects. Plant Cell Rep 32:1299–1308

Lin CY, Ku HM, Chiang YH, Ho HY, Yu TA, Jan FJ (2012) Development of transgenic watermelon resistant to Cucumber mosaic virus and Watermelon mosaic virus by using a single chimeric transgene construct. Transgenic Res 21:983–993

Lin Z, Arciga-Reyes L, Zhong S, Alexander L, Hackett R, Wilson I, Grierson D (2008) SlTPR1, a tomato tetratricopeptide repeat protein, interacts with the ethylene receptors NR and LeETR1, modulating ethylene and auxin responses and development. J Exp Bot 59:4271–4287

Liu J, van Eck J, Cong B, Tanksley SD (2002) A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. Proc Natl Acad Sci USA 99:13302–13306

Liu X, Wang Z, Wang L, Wu R, Phillips J, Deng X (2009) EA4 group genes from the resurrection plant Boea hygrometrica confer dehydration tolerance in transgenic tobacco. Plant Sci 176:90–98

Martí C, Orzáez D, Ellu P, Moreno V, Carbonell J, Granell A (2007) Silencing of DELLA induces facultative parthenocarpy in tomato fruits. Plant J 52:865–876

Matsumoto I, Watanabe H, Abe K, Arai S, Emori Y (1995) A putative digestive cysteine proteinase from Drosophila melanogaster is predominantly expressed in the embryonic and larval midgut. Eur J Biochem 227:582–587

Meli VS, Ghosh S, Prabha TN, Chakraborty N, Chakraborty S, Datta A (2010) Enhancement of fruit shelf life by suppressing N-glycan processing enzymes. Proc Natl Acad Sci USA 9:2413–2418

Molesini B, Pandolfini T, Rotino GL, Dani V, Spena A (2009) Aucsia gene silencing causes parthenocarpic fruit development in tomato. Plant Physiol 149:534–548

Monforte AJ, Diaz AI, Caño-Delgado A, van der Knaap E (2014) The genetic basis of fruit morphology in horticultural crops: lessons from tomato and melon. J Exp Bot 65 (16):4625–4637

Morton RL, Schroeder HE, Bateman KS, Chrispeels MJ, Armstrong E, Higgins TJ (2000) Bean alpha-amylase inhibitor 1 in transgenic peas (Pisum sativum) provides complete protection from pea weevil (Bruchus pisorum) under field conditions. Proc Natl Acad Sci USA 97:3820–3825

Muños S, Ranc N, Botton E, Bérard A, Rolland S, Duffé P, Carretero Y, Le Paslier MC, Delalande C, Bouzayen M, Brunel D, Causse M (2011) Increase in tomato locule number is controlled by two single-nucleotide polymorphisms located near WUSCHEL. Plant Physiol 156:2244–2254

Murdock LL, Shade RE (2002) Lectins and protease inhibitors as plant defenses against insects. J Agric Food Chem 50:6605–6611

Nambeesan S, Datsenka T, Ferruzzi MG, Malladi A, Mattoo AK, Handa AK (2010) Overexpression of yeast spermidine synthase impacts ripening, senescence and decay symptoms in tomato. Plant J 63:836–847

Narusaka M, Kubo Y, Hatakeyama K, Imamura J, Ezura H, Nanasato Y, Tabei Y, Takano Y, Shirasu K, Narusaka Y (2013) Interfamily transfer of dual NB-LRR genes confers resistance to multiple pathogens. PLoS One 8, e55954

Ni X, Tian Z, Liu J, Song B, Li J, Shi X, Xie C (2010) StPUB17, a novel potato UND/PUB/ARM repeat type gene, is associated with late blight resistance and NaCl stress. Plant Sci 178:158–169

Nishizawa K, Teraishi M, Utsumi S, Ishimoto M (2007) Assessment of the importance of alpha-amylase inhibitor-2 in bruchid resistance of wild common bean. Theor Appl Genet 114:755–764

Ntui VO, Thirukkumaran G, Azadi P, Khan RS, Nakamura I, Mii M (2010) Stable integration and expression of wasabi defensin gene in "Egusi" melon (Colocynthis citrullus L.) confers resistance to Fusarium wilt and Alternaria leaf spot. Plant Cell Rep 29:943–954

Nuñez-Palenius HG, Cantliffe DJ, Huber DJ, Ciardi J, Klee HJ (2006) Transformation of a muskmelon 'Galia' hybrid parental line (Cucumis melo L. var. reticulatus Ser.) with an antisense ACC oxidase gene. Plant Cell Rep 25:198–205

Omura T, Watanabe S, Iijima Y, Aoki K, Shibata D, Ezura H (2007) Molecular and genetic characterization of transgenic tomato expressing 3-hydroxy-3-methylglutaryl coenzyme A reductase. Plant Biotechnol 24:107–116

Pattison RJ, Catalá C (2012) Evaluating auxin distribution in tomato (*Solanum lycopersicum*) through an analysis of the PIN and AUX/LAX gene families. Plant J 70:585–598
Priem B, Gitti R, Bush CA, Gross KC (1993) Structure of ten free N-glycans in ripening tomato fruit (Arabinose is a constituent of a plant N-glycan). Plant Physiol 102:445–458
Ren Z, Li Z, Miao Q, Yang Y, Deng W, Hao Y (2011) The auxin receptor homologue in Solanum lycopersicum stimulates tomato fruit set and leaf morphogenesis. J Exp Bot 62:2815–2826
Rodríguez-Hernández AM, Gosalvez B, Sempere RN, Burgos L, Aranda MA, Truniger V (2012) Melon RNA interference (RNAi) lines silenced for Cm-eIF4E show broad virus resistance. Mol Plant Pathol 13:755–763
Ronen G, Carmel-Goren L, Zamir D, Hirschberg J (2000) An alternative pathway to beta -carotene formation in plant chromoplasts discovered by map-based cloning of beta and old-gold color mutations in tomato. Proc Natl Acad Sci USA 97:11102–11107
Römer S, Fraser PD, Kiano JW, Shipton CA, Misawa N, Schuch W, Bramley PM (2000) Elevation of the provitamin A content of transgenic tomato plants. Nat Biotechnol 18:666–669
Schijlen EG, de Vos CH, Martens S, Jonker HH, Rosin FM, Molthoff JW, Tikunov YM, Angenent GC, van Tunen AJ, Bovy AG (2007) RNA interference silencing of chalcone synthase, the first step in the flavonoid biosynthesis pathway, leads to parthenocarpic tomato fruits. Plant Physiol 144:1520–1530
Schünemann R, Knaak N, Fiuza LM (2014) Mode of Action and Specificity of Bacillus thuringiensis Toxins in the Control of Caterpillars and Stink Bugs in Soybean Culture. ISRN Microbiol 2014:135675
Segonzac C, Zipfel C (2011) Activation of plant pattern-recognition receptors by bacteria. Curr Opin Microbiol 14:54–61
Senthilkumar R, Cheng CP, Yeh KW (2010) Genetically pyramiding protease-inhibitor genes for dual broad-spectrum resistance against insect and phytopathogens in transgenic tobacco. Plant Biotechnol J 8:65–75
Shukman D (2014) Genetically-modified purple tomatoes heading for shops. BBC News. http://www.bbc.com/news/science-environment-25885756
Solleti SK, Bakshi S, Purkayastha J, Panda SK, Sahoo L (2008) Transgenic cowpea (*Vigna unguiculata*) seeds expressing a bean alpha-amylase inhibitor 1 confer resistance to storage pests, bruchid beetles. Plant Cell Rep 27:1841–1850
Srinivasan A, Giri AP, Gupta VS (2006) Structural and functional diversities in lepidopteran serine proteases. Cell Mol Biol Lett 11:132–154
Smigocki AC, Ivic-Haymes S, Li H, Savić J (2013) Pest protection conferred by a Beta vulgaris serine proteinase inhibitor gene. PLoS One 8, e57303
Tai TH, Dahlbeck D, Clark ET, Gajiwala P, Pasion R, Whalen MC, Stall RE, Staskawicz BJ (1999) Expression of the Bs2 pepper gene confers resistance to bacterial spot disease in tomato. Proc Natl Acad Sci USA 96:14153–14158
Hiwasa-Tanase K, Hirai T, Kato K, Duhita N, Ezura H (2012) From miracle fruit to transgenic tomato: mass production of the taste-modifying protein miraculin in transgenic plants. Plant Cell Rep 31:513–525
Terenius O, Papanicolaou A, Garbutt JS, Eleftherianos I, Huvenne H, Kanginakudru S, Albrechtsen M, An C, Aymeric JL, Barthel A, Bebas P, Bitra K, Bravo A, Chevalier F, Collinge DP, Crava CM, de Maagd RA, Duvic B, Erlandson M, Faye I, Felföldi G, Fujiwara H, Futahashi R, Gandhe AS, Gatehouse HS, Gatehouse LN, Giebultowicz JM, Gómez I, Grimmelikhuijzen CJ, Groot AT, Hauser F, Heckel DG, Hegedus DD, Hrycaj S, Huang L, Hull JJ, Iatrou K, Iga M, Kanost MR, Kotwica J, Li C, Li J, Liu J, Lundmark M, Matsumoto S, Meyering-Vos M, Millichap PJ, Monteiro A, Mrinal N, Niimi T, Nowara D, Ohnishi A, Oostra V, Ozaki K, Papakonstantinou M, Popadic A, Rajam MV, Saenko S, Simpson RM, Soberón M, Strand MR, Tomita S, Toprak U, Wang P, Wee CW, Whyard S, Zhang W, Nagaraju J, Ffrench-Constant RH, Herrero S, Gordon K, Swevers L, Smagghe G (2011) RNA interference in Lepidoptera: an overview of successful and unsuccessful studies and implications for experimental design. J Insect Physiol 57:231–245

Toubiana D, Semel Y, Tohge T, Beleggia R, Cattivelli L, Rosental L, Nikoloski Z, Zamir D, Fernie AR, Fait A (2012) Metabolic profiling of a mapping population exposes new insights in the regulation of seed metabolism and seed, fruit, and plant relations. PLoS Genet 8, e1002612

Tsaballa A, Pasentsis K, Darzentas N, Tsaftaris AS (2011) Multiple evidence for the role of an Ovate-like gene in determining fruit shape in pepper. BMC Plant Biol 11:46

Turner CT, Davy MW, MacDiarmid RM, Plummer KM, Birch NP, Newcomb RD (2006) RNA interference in the light brown apple moth, Epiphyas postvittana (Walker) induced by double-stranded RNA feeding. Insect Mol Biol 15:383–391

Vaeck M, Reybnaerts A, Horte J, Jansens S, DeBeuckeleer M, Dean C, Zabeau M, van Montagu M, Lemans J (1987) Transgenic plants protected from insect attack. Nature 328:33–37

van Der Knaap E, Lippman ZB, Tanksley SD (2002) Extremely elongated tomato fruit controlled by four quantitative trait loci with epistatic interactions. Theor Appl Genet 104:241–247

Wang Z, Song J, Zhang Y, Yang B, Chen S (2009) Expression of baculovirus anti-apoptotic p35 gene in tobacco enhances tolerance to abiotic stress. Biotechnol Lett 31:585–589

Wang X, Peng F, Li M, Yang L, Li G (2012) Expression of a heterologous SnRK1 in tomato increases carbon assimilation, nitrogen uptake and modifies fruit development. J Plant Physiol 169:1173–1182

Wang H, Jones B, Li Z, Frasse P, Delalande C, Regad F, Chaabouni S, Latché A, Pech JC, Bouzayen M (2005) The tomato Aux/IAA transcription factor IAA9 is involved in fruit development and leaf morphogenesis. Plant Cell 17:2676–2692

Waterhouse PM, Wang MB, Lough T (2001) Gene silencing as an adaptive defence against viruses. Nature 411:834–842

Wu S, Xiao H, Cabrera A, Meulia T, van der Knaap E (2011) SUN regulates vegetative and reproductive organ shape by changing cell division patterns. Plant Physiol 157:1175–1186

Xiao YH, Li XB, Yang XY, Luo M, Hou L, Guo SH, Luo XY, Pei Y (2007) Cloning and characterization of a balsam pear class I chitinase gene (Mcchit1) and its ectopic expression enhances fungal resistance in transgenic plants. Biosci Biotechnol Biochem 71:1211–1219

Yu TA, Chiang CH, Wu HW, Li CM, Yang CF, Chen JH, Chen YW, Yeh SD (2011) Generation of transgenic watermelon resistant to Zucchini yellow mosaic virus and Papaya ringspot virus type W. Plant Cell Rep 30:359–371

Zhang N, Brewer MT, van der Knaap E (2012) Fine mapping of fw3.2 controlling fruit weight in tomato. Theor Appl Genet 125:273–284

Zheng XY, Wolff DW (2000) Ethylene production, shelf-life and evidence of RFLP polymorphisms linked to ethylene genes in melon (Cucumis melo L.) Theoretical and Applied. Genetics 101:613–624

Zhou Z, Pang J, Guo W, Zhong N, Tian Y, Xia G, Wu J (2012) Evaluation of the resistance of transgenic potato plants expressing various levels of Cry3A against the Colorado potato beetle (Leptinotarsa decemlineata Say) in the laboratory and field. Pest Manag Sci 68:1595–1604

Zhu JQ, Liu S, Ma Y, Zhang JQ, Qi HS, Wei ZJ, Yao Q, Zhang WQ, Li S (2012) Improvement of pest resistance in transgenic tobacco plants expressing dsRNA of an insect-associated gene EcR. PLoS One 7, e38572

Chapter 14
Genome-Editing Technologies and Their Use in Tomato

Jeong-Eun Lee and Hiroshi Ezura

14.1 Introduction

A variety of plant-breeding technologies have been developed to improve the quantity and quality of crops for feeding markedly increased populations and responding to the changes in user preferences. Traditional breeding technologies are primarily dependent on the natural genetic resources of each crop and typically cannot overcome the species barrier. However, with the rapid advancement of molecular biology and accompanying techniques, 'species' limitations are no longer a hindrance to the introduction of desirable traits for improving crops. For applications such as the insertion of exotic genes from a different kingdom conferring herbicide or pest resistance, genome modification technologies have made it possible to generate new organisms with reduced labour and time compared with the traditional methods (Roderick et al. 2012; Sripaoraya et al. 2006). Direct gene transfer using *Agrobacterium tumefaciens* and gene bombardment are the most widely used transformation technologies to introduce desired traits. Both technologies have facilitated the generation of transgenic plants in many crop species with comparatively low entry barriers (Azadi and Ho 2010). However, these methods have many technical problems, such as low transformation efficiency depending on the species or an uncontrollable insertion number/position leading to break(s) in the endogenous gene(s). Therefore, new technologies to modify the genome at only targeted regions without the unintended effects of uncontrollable insertion numbers and genomic position are desired. Over the last decade, genome-editing technologies that facilitate targeted gene modification have been developed, and these techniques have been applied to breed plant crops, including tomato.

J.-E. Lee • H. Ezura (✉)
Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan
e-mail: ezura@gene.tsukuba.ac.jp

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_14

14.2 General Features of Genome Editing Using Artificial Nucleases

Recently targeted genome modification using designed nucleases has been widely developed in many plants, such as *Arabidopsis*, rice, wheat, tobacco and tomato (Lioyd et al. 2005; Townsend et al. 2009; Shukla et al. 2009; Cermak et al. 2011; Mahfouz et al. 2011; Li et al. 2012, 2013; Shan et al. 2013a, b; Jiang et al. 2013; Nekrasov et al. 2013; Upadhyay et al. 2013; Miao et al. 2013; Zhang et al. 2013; Wendt et al. 2013; Gurushidze et al. 2014; Lor et al. 2014; Wang et al. 2014; Haun et al. 2014; Brooks et al. 2014; Liang et al. 2014; Feng et al. 2014; Gao et al. 2015). Artificial nucleases generally comprise controllable domains that bind to a specific target sequence and nuclease activity and generate DNA breaks near the targeted site. Basically, genome-editing technology is based on double-strand breaks (DSBs) in genomic DNA, which are mediated through designed nucleases that cut the target sequence. There are two strategies for repairing broken DNA ends: homologous recombination (HR) and nonhomologous end joining (NHEJ) (Ray and Langer 2002; Britt and May 2003). HR is based on the homologous sequence, and DNA can be restored to the original information through HR. Repair via NHEJ, in contrast, involves the ligation of broken DNA ends without any frame of reference. Thus, a random number of nucleotides are inserted and deleted to fit the ends of DNA before DNA ligation. As a result, mutation occurs at the repaired site.

Although HR presents a favourable strategy to preserve genetic information, the preferred route for DNA repair in eukaryotes, particularly in plants, is NHEJ (Ray and Langer 2002). Accordingly, in artificial nuclease systems, NHEJ can technically be used for random mutagenesis when it is designed to properly induce DSBs in the targeted loci or for large-sized deletions [as reviewed by Chen and Gao (2013)]. However, HR can be used for site-specific insertion (Li et al. 2011; Bedell et al. 2012; Zhang et al. 2013). Currently, designed artificial nucleases are widely used to induce mutations at the desired sites. In this chapter, three representative artificial nucleases, zinc -finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN) and clustered regularly interspaced short palindromic repeats/ CRISPR-associated nuclease 9 (CRISPR/Cas9) are introduced, and the current use of these technologies for genetic engineering in tomato plants is summarized.

14.2.1 Zinc-Finger Nucleases

ZFNs are progenitors of artificial chimeric nucleases in which three to four Cys_2His_2ZF proteins and the cleavage domain from *Fok* I have been combined (Kim et al. 1996, Fig. 14.1a), and over last two decades, this nuclease has received much attention as a candidate with immense potential for targeted genetic modification in many plants (Lioyd et al. 2005; Townsend et al. 2009; Shukla et al. 2009).

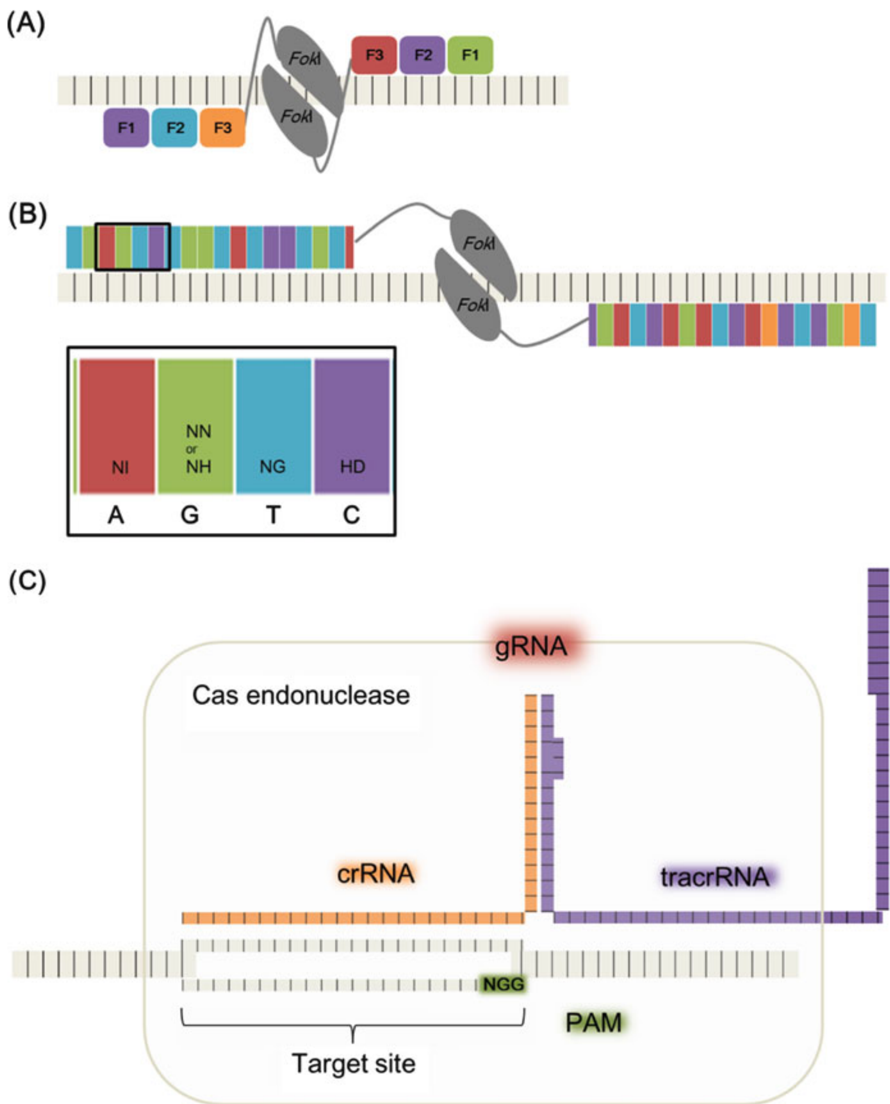


Fig. 14.1 Schematic of the three artificial nucleases and TALEs. (**a**) Zinc-finger nucleases (ZFNs). One pair of ZFNs is needed to achieve *Fok* I dimerization, which is essential to induce DSBs. One DNA-binding unit of ZFN recognizes three nucleotides, and these three units are generally assembled on one side of the ZFNs. (**b**) Transcription activator-like effector nucleases (TALENs). One pair of TALENs is also needed to induce DSBs. One DNA-binding unit of TALEN shows preference for one nucleotide, and typically over 18 units are assembled on one side of the TALENs. (**c**) CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats). crRNA and tracrRNA bind to the target site, forming a chimeric RNA molecule and signalling the endonuclease Cas. The length of the target site is only 20 nucleotides and must possess a PAM, crRNA, CRISPR RNA, tracrRNA (trans-activating crRNA), PAM (protospacer-adjacent motif) and 5-NGG-3. Technically, crRNA and tracrRNA (guide RNA) are designed and used as an RNA fusion

Each nuclease domain of ZF recognizes three nucleotides and binds to most 5-GNN-3, 5-ANN-3 and 5-TNN-3 triplets (Segal 2002; Liu et al. 2002). Because ZFN pairs function most efficiently when their binding sites are precisely 6 bp apart (Bibikova et al. 2001), a ZFN recognition sequence using only 5-GNN-3 triplets, for example, would comprise 5-NNCNNCNNC(N5-6)GNNGNNGNN-3. Theoretically, it is possible to find target sites with the published ZF domain every 0.5–1.0 kb (Segal 2002), which is sufficient to target most plant genes. ZFNs are sufficient to specify targets for plants with genome sizes of approximately 10^{10} base pairs in length, such as rice, maize, and tomato (Lioyd et al. 2005).

ZFN-induced mutations via NHEJ and HR have been reported in *Arabidopsis thaliana* (Lioyd et al. 2005; Zhang et al. 2009; de Pater et al. 2009; Osakabe et al. 2010). For example, Zhang et al. (2009) targeted two genes, *ADH1* and *TT4*, which are easy to select because phenotypic changes are observed in response to ZFN-induced mutations. Therefore, in their study, Zhang et al. measured mutations induced via NHEJ. As expected, the mutation frequencies were extremely high and showed next generation ranges from 69 to 33 % for *TT4* and *ADH1*, respectively, under the control of an oestrogen-inducible promoter. Townsend et al. (2009) also reported ZFN-based genetic modification in tobacco that targeted the *SurB* gene, which shares high similarity to *SurA*. Similarly, the mutation rate was high, particularly when the heterodimer *Fok*I was used. Mutations via NHEJ were also measured, and a relatively high range of mutations was detected, but modular assembly based on ZFNs showed higher mutation frequencies in *SurA* rather than in the target gene *SurB*. Shukla et al. (2009) reported genetic modification in *Zea mays* by using ZFNs to target the gene for *IPK1*, an enzyme that is involved in the final steps of phytate synthesis in seeds. Without no mutation at a paralogous gene *IPK2* at T_0, detected mutation inherits to next generation. Taken together, ZFNs represent artificial nucleases with potential for use in editing the plant genome. However, the occurrence of DSBs at unintended sites remains an unsolved problem; thus, target gene selection is important.

14.2.2 Transcription Activator-Like Effector Nucleases

After the first report of genomic modification using TALENs (Christian et al. 2010), TALE (transcription activator-like effector) nucleases have been rapidly developed. Reflecting the specific nucleotide preference of each unit (Boch et al. 2009; Römer et al. 2009), TALEs have received much attention as efficient instruments for genome targeting. TALEs are virulent proteins, produced from *Proteobacteria* of the genus *Xanthomonas*, which translocate to the nucleus of host cells and activate the genes involved in disease processes after binding to the promoters of these genes (Marois et al. 2002; Kay et al. 2007). TALEs typically comprise a translocation signal at the N-terminus and a nuclear localization signal (NLS) and transcription activator domain (AD) at the C-terminus (Gürlbeck et al. 2006). The DNA-binding domain in the middle of TALEs, a region that is typically referred to

as the 'repeat domain', confers the specificity for binding to target DNA sequences (Yang et al. 2000, Fig. 14.1b). The repeat domain comprises units that show nucleotide preference, and each unit comprises a highly conserved 33–34-amino acid repeat unit, except the hyper-variable 12th and 13th units, which are commonly referred to as repeat variable di-residues (RVDs) and confer nucleotide preference (Marois et al. 2002; Kay et al. 2007; Boch et al. 2009), as described in Fig. 14.1b. RVD NG shows preference for thiamine (T), HD for cytosine (C) and NI to adenine (A) (Boch et al. 2009; Moscou and Bogdanove 2009; Deng et al. 2012). The identification of exclusive RVDs for guanine (G) remains controversial, but NN, NH and NK typically recognize guanine (Christian et al. 2012; Deng et al. 2012). Streubel et al. (2012) showed the exclusive and strong binding of the RVD NN for guanine; however, this RVD also intermediately binds to adenine. Because NK showed weak binding, strong RVDs, such as HD, should be used in combination with NK. Among the identified RVDs, NH showed high guanine preference, although the binding affinity was intermediate (Streubel et al. 2012). Any sequence can be targeted through the assembly of these units on specific DNA sequences, i.e. TALENs. TALENs are also chimeric enzymes that comprise slightly modified TALEs and the cleavage domain derived from the restriction enzyme *Fok*I. After the strong affinity binding of TALEs to nucleotides was reported (Boch et al. 2009), the use of TALEs as artificial nucleases has been extensively reported in many organisms, such as humans (Cermak et al. 2011; Miller et al. 2011; Mussolino et al. 2011), zebrafish (Huang et al. 2011; Sander et al. 2011; Bedell et al. 2012; Chen et al. 2013) and fruit flies (Liu et al. 2012). Genome modification using TALENs has also been reported in plants, such as *Arabidopsis* (Cermak et al. 2011), tobacco (Mahfouz and Li 2011; Mahfouz et al. 2011; Zhang et al. 2013), rice (Li et al. 2012; Shan et al. 2013a), wheat (Shan et al. 2013a; Wang et al. 2014), soya bean (Haun et al. 2014), maize (Liang et al. 2014), barley (Wendt et al. 2013; Gurushidze et al. 2014) and tomato (Lor et al. 2014).

In preliminary studies according to species, tobacco and *Arabidopsis* were the first two plants in which TALENs were applied (Mahfouz et al. 2011; Cermak et al. 2011). Mahfouz et al. (2011) used the codon-optimized natural TALE protein Hax3 to target the natural effector-binding element (EBE), and DSBs were detected through a transient assay using tobacco leaves. Although these authors used TALEs driven from natural TAL proteins, the studies demonstrated the potential efficient application of TALEs in plants. Cermak et al. (2011) reported the first designed TALENs in plants. Each module was assembled to target a specific sequence, the *ADH*1 gene, using the golden gate cloning technique. The TALENs were introduced into protoplasts and caused random deletions at spacers, which indicated DSBs and repair via NHEJ. Li et al. (2012) reported the first *in planta* use of TALENs in rice. Interestingly, to avoid disease, these researchers induced insertion/deletion mutations in the promoter where TALE protein binding naturally occurs to yield regenerated rice without susceptibility to TALEs. Zhang et al. (2013) reported the optimization of TALENs in plants, which facilitated genetic modification via either NHEJ or HR. These authors also used tobacco protoplast with low transformation success rates using *Agrobacterium*. Using this system, the gene disruption rate

was 30 %, and the gene replacement rate was 14 %. Shan et al. (2013a) reported that TALENs induced mutations in *Brachypodium* and rice through nucleotide deletion via NHEJ and large arrangements, followed by the introduction of two pairs of TALENs in rice and wheat. The mutation occurring in one protoplast is transferred to plants with same genotype. Haun et al. (2014) reported that targeting two fatty acid desaturases in bean plants dramatically increased the production of polyunsaturated fats. Additionally, these authors showed the potential for simultaneously targeting multiple genes with a variable range of mutations and to generate plants in subsequent generations possessing mutated genes with no transgenic constructs. Moreover, Liang et al. (2014) reported TALEN-induced mutagenesis in maize. These authors targeted four genes using TALENs and showed an average targeting efficiency of 23.1 % in protoplasts and a somatic mutation rate of approximately 13–39 %. Thus, TALENs have been applied as artificial nucleases in many plants over the last 5 years and have shown many positive results. As described herein, TALENs represent a tool for the precise genetic modification of major crops, and the broad application of this technique in a range of plants demonstrates the simplicity and efficiency of TALENs.

14.2.3 Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-Associated Nuclease 9

More recently, CRISPR/Cas9, a bacterial RNA-guided DNA endonuclease system, has emerged at the centre of genome editing. The key components of CRISPR/Cas9 are a Cas9 endonuclease protein and two small RNA molecules, CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA) (Fig. 14.1c). In the type II CRISPR system, which is derived from *Streptococcus pyogenes*, short invading DNA fragments integrated into a CRISPR locus are converted into crRNA and tracrRNA. crRNA and tracrRNA bind to the target gene, thereby forming a chimeric RNA molecule and signalling the endonuclease Cas to the protospacer motif within the target DNA (Chen and Gao 2013). Technically, the chimeric RNA molecule is designed as a fusion of these two RNAs and is referred to as guide RNA (gRNA) (Cong et al. 2013; Mali et al. 2013). As described in Fig. 14.1c, only 20 nucleotides possessing the protospacer-adjacent motif (PAM), 5-NGG-3, are targeted. Because targeting is based on the intuitively understandable DNA–RNA interaction, this system was immediately adapted for genome modification. The most important feature of CRISPR/Cas9 is its simplicity of producing and targeting. After determining the optimized Cas9 for some organisms, constructs can relatively be easily produced using cloning techniques (Shan et al. 2014; Feng et al. 2014; Jiang et al. 2013). This system has an advantage over ZFN and TALEN in the construction of the desired genetic elements, which led to the rapid spread of CRISPR/Cas9-based research. Genome modification using the Cas9/CIRSPR system has also been reported in many plants, such as *Nicotiana benthamiana* (Li et al.

2013; Nekrasov et al. 2013), *Nicotiana tabacum* (Gao et al. 2015), *Arabidopsis* (Li et al. 2013; Feng et al. 2014; Jiang et al. 2013), wheat (Upadhyay et al. 2013; Shan et al. 2013b), maize (Liang et al. 2014), rice (Miao et al. 2013; Jiang et al. 2013), sorghum (Jiang et al. 2013) and tomato (Brooks et al. 2014). Li et al. (2013) reported CRISPR/Cas9-induced mutations in *Arabidopsis* and tobacco (*Nicotiana benthamiana*). After sequencing to assess the efficiency of mutagenesis through protoplast transformation (1.1–5.6 % for *Arabidopsis AtPDS* and *AtFLS*, respectively, 37.7–38.5 % for two targets within *NbPDS*), the efficiency of mutagenesis using *PDS*-targeting constructs (2.7 % for *AtPDS* and 4.8 % for *NbPDS*) was also evaluated *in planta*. Off-targeting was also estimated using *AtRACK* genes. The mutation of *AtRACKa*, which shares high similarity (only two mismatches in 12-nucleotide seed sequence) with *AtRACKb* and *AtRACKc*, was not detected. Shan et al. (2013b) also reported the use of CRISPR/Cas9 in rice using *OsPDS* and *OsBADH2* (9.6 % mutation rate for *OsPDS* and 7.1 % for *OsBADH*). However, due to the off-targeting analysis of the highly homologous target sites in *OsPDS* and *OsMPK* (one nucleotide mismatch with PDS) genes, unintended deletions were observed. Liang et al. (2014) compared CRISPR/Cas9- and TALEN-induced mutagenesis in maize. In this study, only one CRISPR/Cas9 construct was used and showed slightly better results than did TALENs targeting the same site. The mutation rates obtained using CRISPR/Cas9 were 13.1 %, whereas those obtained using TALENs were 9.1 %. Taken together, these results showed that CRISPR/Cas9 is an attractive system for genome modification in plants and that this system rapidly accumulates valuable results. Thus, among introduced the three artificial nuclease technologies described herein, CRISPR/Cas9 might represent the most preferred tool with superior simplicity.

14.2.4 Application of the Three Artificial Nucleases in Plants

The most important standard for evaluating artificial nucleases is the binding specificity to the target sequence. Off-target binding typically leads to cytotoxicity, which results from the many mutations on unwanted parts of the genome (Mussolino et al. 2011). In human cells, CCR5 was targeted using the three systems, and the off-targeting of *CCR2*, sharing high homology to *CCR5* (Mussolino et al. 2011; Cradick et al. 2013), was analysed. ZFN induced approximately 11 % off-targeting, while TALENs induced almost 1 % when the targeted site was the same (Mussolino et al. 2011). CRISPR/Cas9 showed 5 % (two nucleotides mismatch) to 20 % (no mismatch) off-target mutations according to the guide RNA sequence (Cradick et al. 2013). Thus, off-targeting remains an unsolved problem for all three systems. The occurrence of a low level of off-target effects has been reported in plants (Townsend et al. 2009; Li et al. 2013; Shan et al. 2013b; Gurushidze et al. 2014). However, at least in plants, these off-target effects might be a side issue because unwanted gene disruptions can be eliminated through backcrossing,

although it is still better to use artificial nucleases with higher binding specificity to escape cytotoxicity.

As the simplest method, CRISPR/Cas9 shows the highest accessibility because TALENs and ZFNs are primarily based on unit assembly. Notably, the design and assembly of TALEN constructs is easier compared with ZFNs. The dimerization of cleavage is an essential factor for inducing DSBs (Bitinaite et al. 1998); thus, both of the technologies should be designed in pairs. The selection of an efficient binding site for ZFNs is markedly restricted (Ramirez et al. 2008). According to a recent review (Carroll 2011), even researchers possessing the largest and well-characterized ZFs make many combinations of ZFs and extensively test for gene targeting. Additionally, many studies have suggested that the spatial effects of ZFNs counteract the affinity of these nucleases for the target sequence (Segal et al. 1999; Beumer et al. 2006). In contrast, the one-to-one correspondence between each module and nucleotide makes it relatively easy to produce TALENs compared with ZFNs. The simple modulation of the repeat domain is used to generate one pair of TALENs through the assembly of each unit to the target sequence (Weber et al. 2011; Doyle et al. 2012; Briggs et al. 2012). The selection of a target site for TALENs is also restricted, e.g. the selection of a binding site for thiamine (Streubel et al. 2012), but better than that for ZFNs. For example, Zhang et al. (2013) identified 223 available sites for TALENs targeting in a given sequence (the *SurB* gene in tobacco), with only three sites found for ZFNs using the established methods. In addition, according to Cermak et al. (2011), 90 % of predicted TALENs showed nuclease activity. Finally, the simplest rule for selecting target sites for CRISPR/Cas9 is the possession of a PAM within the target sequence. This simplicity might reflect the widespread use of CRISPR/Cas9, and the ease of identifying potential target sites is another point of accessibility. Thus, CRISPR/Cas9 is the most predominant technology compared with ZFNs or TALENs.

14.3 Genome Modification in Tomato Using TALENs and CRISPR/Cas9

Tomato is an economically important crop and one of the most produced vegetables worldwide. In addition, tomatoes are fascinating materials for research because they have a relatively small genome size that has been completely sequenced (Tomato Genome Consortium 2012). Tomato bears berry-type fruits, which are also useful for studies on fruit development or maturation (Saito et al. 2011). Accordingly, tomato plants were the first vegetable crops for which genome modifications using TALENs and the CRISRP/Cas9 system were reported (Lor et al. 2014; Brooks et al. 2014). Lor et al. (2014) applied TALENs to mutate the *PROCERA* gene, a gibberellin negative regulator in tomato, under the control of a steroid-inducible promoter to avoid cytotoxicity. Through additional tissue culture using T1 seedlings, stably heritable mutant plants were obtained that showed the

expected phenotypes. In a subsequent generation, mutants with no transgenic constructs were obtained. Brooks et al. (2014) successfully applied CRISPR/Cas9 in tomato to mutate the gene *SlAGO7*, which exhibits an immediately detectable phenotype because the first leaflets do not possess petioles and the other leaves show a rack of laminae. As expected, CRISPR/Cas9 showed efficient gene targeting. However, extensive studies to detect off-target binding showed that two sites possessing high similarity with target gene were mutated.

Unlike other widely used genetic modification methods, genetic modification using artificial nucleases has become a useful method for crop improvement without any harm to other parts of the genome. Moreover, similar to the mutations that were induced using ethyl methanesulfonate (EMS) or radiation, artificial nucleases can make sustainable genetic groups that can be used for breeding. The establishment of a stable method for the application of artificial nucleases in many plants will improve crops in the future.

References

Azadi H, Ho P (2010) Genetically modified and organic crops in developing countries: a review of options for food security. Biotechnol Adv 28(1):160–168

Bedell VM, Wang Y, Campbell JM, Poshusta TL, Starker CG, Krug RG 2nd, Tan W, Penheiter SG, Ma AC, Leung AY, Fahrenkrug SC, Carlson DF, Voytas DF, Clark KJ, Essner JJ, Ekker SC (2012) In vivo genome editing using a high-efficiency TALEN system. Nature 491(7422):114–118

Beumer k, Bhattacharyya G, Bibikova M, Trautman JK, Carroll D (2006) Efficient gene targeting in Drosophila with zinc-finger nucleases. Genetics 172:2391–2403

Bibikova M, Carroll D, Segal DJ, Trautman JK, Smith J, Kim YG, Chandrasegaran S (2001) Stimulation of homologous recombination through targeted cleavage by chimeric nucleases. Mol Cell Biol 21(1):289–297

Bitinaite J, Wah DA, Aggarwal AK, Schildkraut I (1998) FokI dimerization is required for DNA cleavage. Proc Natl Acad Sci U S A 95(18):10570–10575

Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, Lahaye T, Nickstadt A, Bonas U (2009) Breaking the code of DNA binding specificity of TAL-type III effectors. Science 326(1509):1509–1512

Briggs AW, Rios X, Chari R, Yang L, Zhang F, Mali P, Church GM (2012) Iterative capped assembly: rapid and scalable synthesis of repeat-module DNA such as TAL effectors from individual monomers. Nucleic Acids Res 40(15):e117

Britt AB, May GD (2003) Re-engineering plant gene targeting. Trends Plant Sci 8(2):90–95

Brooks C, Nekrasov V, Lippman ZB, Van Eck J (2014) Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. Plant Physiol 166(3):1292–1297

Carroll D (2011) Genome engineering with zinc-finger nucleases. Genetics 188:773–782

Cermak T, Doyle E, Christian M, Wang L, Zhang Y, Schmidt C, Baller J, Somia NV, Bogdanove AJ, Voytas D (2011) Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Res 39(17):7879

Chen K, Gao C (2013) TALENs: customizable molecular DNA scissors for genome engineering of plants. J Genet Genomics 40(6):271–279

Chen S, Oikonomou G, Chiu CN, Niles BJ, Liu J, Lee DA, Antoshechkin I, Prober DA (2013) A large-scale in vivo analysis reveals that TALENs are significantly more mutagenic than ZFNs generated using context-dependent assembly. Nucleic Acids Res 41(4):2769–2778

Christian ML, Cermak T, Doyle EL, Schmidt C, Zhang F, Hummel A, Bogdanove AJ, Voytas DF (2010) Targeting DNA double-strand breaks with TAL effector nucleases. Genetics 186(2): 757–761

Christian ML, Demorest ZL, Starker CG, Osborn MJ, Nyquist MD, Zhang Y, Carlson DF, Bradley P, Bogdanove AJ, Voytas DF (2012) Targeting G with TAL effectors: a comparison of activities of TALENs constructed with NN and NK repeat variable di-residues. PLoS One 7(9):e45383

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339(6121):819–823

Cradick TJ, Fine EJ, Antico CJ, Bao G (2013) CRISPR/Cas9 systems targeting β-globin and CCR5 genes have substantial off-target activity. Nucleic Acids Res 41(20):9584–9592

de Pater S, Neuteboom LW, Pinas JE, Hooykaas PJ, van der Zaal BJ (2009) ZFN-induced mutagenesis and gene-targeting in Arabidopsis through Agrobacterium-mediated floral dip transformation. Plant Biotechnol J 7(8):821–835

Deng D, Yan C, Pan X, Mahfouz M, Wang J, Zhu JK, Shi Y, Yan N (2012) Structural basis for sequence-specific recognition of DNA by TAL effectors. Science 335(6069):720–723

Doyle EL, Booher NJ, Standage DS, Voytas DF, Brendel VP, Vandyk JK, Bogdanove AJ (2012) TAL Effector-Nucleotide Targeter (TALE-NT) 2.0: tools for TAL effector design and target prediction. Nucleic Acids Res 40(Web Server issue):W117–W122

Feng Z, Mao Y, Xu N, Zhang B, Wei P, Yang DL, Wang Z, Zhang Z, Zheng R, Yang L (2014) Multigeneration analysis reveals the inheritance, specificity, and patterns of CRISPR/Cas-induced gene modifications in Arabidopsis. Proc Natl Acad Sci U S A 111(12):4632–4637

Gao J, Wang G, Ma S, Xie X, Wu X, Zhang X, Wu Y, Zhao P, Xia Q (2015) CRISPR/Cas9 mediated targeted mutagenesis in Nicotiana tabacum. Plant Mol Biol 87(1–2):99–110

Gürlbeck D, Thieme F, Bonas U (2006) Type III effector proteins from the plant pathogen Xanthomonas and their role in the interaction with the host plant. J Plant Physiol 163(3): 233–255

Gurushidze M, Hensel G, Hiekel S, Schedel S, Valkov V, Kumlehn J (2014) True-breeding targeted gene knock-out in Barley using designer TALE-Nuclease in haploid cells. PLoS One 9(3):e92046

Haun W, Coffman A, Clasen BM, Demorest ZL, Lowy A, Ray E, Retterath A, Stoddard T, Juillerat A, Cedrone F, Mathis L, Voytas DF, Zhang F (2014) Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. Plant Biotechnol J 12(7): 934–940

Huang P, Xiao A, Zhou M, Zhu Z, Lin S, Zhang B (2011) Heritable gene targeting in zebrafish using customized TALENs. Nat Biotechnol 29(8):699–700

Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA (2013) RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nat Biotechnol 31(3):233–239

Kay S, Hahn S, Marois E, Hause G, Bonas U (2007) A bacterial effector acts as a plant transcription factor and induces a cell size regulator. Science 318(5850):648–651

Kim YG, Cha J, Chandrasegaran S (1996) Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proc Natl Acad Sci U S A 93(3):1156–1160

Li T, Huang S, Zhao X, Wright DA, Carpenter C, Spalding MH, Weeks DP, Yang B (2011) Modularly assembled designer TAL effector nucleases for targeted gene knockout and gene replacement in eukaryotes. Nucleic Acids Res 39(14):6315–6325

Li T, Liu B, Spalding MH, Weeks DP, Yang B (2012) High-efficiency TALEN-based gene editing produces disease-resistant rice. Nat biotechnol 30(5):390–392

Li JF, Norville JE, Aach J, McCormack M, Zhang D, Bush J, Church GM, Sheen J (2013) Multiplex and homologous recombination-mediated genome editing in Arabidopsis and Nicotiana benthamiana using guide RNA and Cas9. Nat Biotechnol 31(8):688–691

Liang Z, Zhang K, Chen K, Gao C (2014) Targeted mutagenesis in Zea mays using TALENs and the CRISPR/Cas system. J Genet Genomics 41(2):63–68

Lioyd A, Plaisier CL, Carroll D, Drews GN (2005) Targeted mutagenesis using zinc-finger nucleases in Arabidopsis. Proc Natl Acad Sci U S A 102(6):2232–2237

Liu Q, Xia Z, Zhong X, Case CC (2002) Validated zinc finger protein designs for all 16 GNN DNA triplet targets. J Biol Chem 277(6):3850–3856

Liu J, Li C, Yu Z, Huang P, Wu H, Wei C, Zhu N, Shen Y, Chen Y, Zhang B, Deng WM, Jiao R (2012) Efficient and specific modifications of the Drosophila genome by means of an easy TALEN strategy. J Genet Genomics 39(5):209–215

Lor VS, Starker CG, Voytas DF, Weiss D, Olszewski NE (2014) Targeted mutagenesis of the tomato PROCERA gene using transcription activator-like effector nucleases. Plant Physiol 166(3):1288–1291

Mahfouz MM, Li L (2011) TALE nucleases and next generation GM crops. GM Crops 2(2): 99–103

Mahfouz MM, Li L, Shamimuzzaman M, Wibowo A, Fang X, Zhu JK (2011) De novo-engineered transcription activator-like effector (TALE) hybrid nuclease with novel DNA binding specificity creates double-strand breaks. Proc Natl Acad Sci U S A 108(6):2623–2628

Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM (2013) RNA-guided human genome engineering via Cas9. Science 339(6121):823–826

Marois E, Van den Ackerveken G, Bonas U (2002) The Xanthomonas type III effector protein AvrBs3 modulates plant gene expression and induces cell hypertrophy in the susceptible host. Mol Plant Microbe Interact 15(7):637–646

Miao J, Guo D, Zhang J, Huang Q, Qin G, Zhang X, Wan J, Gu H, Qu LJ (2013) Targeted mutagenesis in rice using CRISPR-Cas system. Cell Res 23(10):1233–1236

Miller JC, Tan S, Qiao G, Barlow KA, Wang J, Xia DF, Meng X, Paschon DE, Leung E, Hinkley SJ, Dulay GP, Hua KL, Ankoudinova I, Cost GJ, Urnov FD, Zhang S, Holmes MC, Zhang L, Gregory PD, Rebar EJ (2011) A TALE nuclease architecture for efficient genome editing. Nat Biotechnol 29(2):143–148

Moscou MJ, Bogdanove AJ (2009) A simple cipher governs DNA recognition by TAL effectors. Science 356(5959):1501

Mussolino C, Morbitzer R, Lütge F, Dannemann N, Lahaye T, Cathomen T (2011) A novel TALE nuclease scaffold enables high genome editing activity in combination with low toxicity. Nucleic Acids Res 39(21):9283–9293

Nekrasov V, Staskawicz B, Weigel D, Jones JD, Kamoun S (2013) Targeted mutagenesis in the model plant Nicotiana benthamiana using Cas9 RNA-guided endonuclease. Nat Biotechnol 31(8):691–693

Osakabe K, Osakabe Y, Toki S (2010) Site-directed mutagenesis in Arabidopsis using custom-designed zinc finger nucleases. Proc Natl Acad Sci U S A 107(26):12034–12039

Ramirez CL, Foley JE, Wright DA, Müller-Lerch F, Rahman SH, Cornu TI, Winfrey RJ, Sander JD, Fu F, Townsend JA, Cathomen T, Voytas DF, Joung JK (2008) Unexpected failure rates for modular assembly of engineered zinc fingers. Nat Methods 5(5):374–375

Ray A, Langer M (2002) Homologous recombination: ends as the means. Trends Plant Sci 7(10): 435–440

Roderick H, Tripatihi L, Babirye A, Wang D, Tripathi J, Urwin PE, Atkinson HJ (2012) Generation of transgenic plantain (Musa spp.) with resistance to plant pathogenic nematodes. Mol Plant Pathol 13(8):842–851

Römer P, Recht S, Lahaye T (2009) A single plant resistance gene promoter engineered to recognize multiple TAL effectors from disparate pathogens. Proc Natl Acad Sci U S A 106(48):20526–20531

Saito T, Ariizumi T, Okabe Y, Asamizu E, Hiwasa-tanase K, Fukuda N, Mizoguchi T, Yamazaki Y, Aoki K, Ezura H (2011) TOMATOMA: a novel tomato mutant database distributing Micro-Tom mutant collections. Plant Cell Physiol 52(2):283–296

Sander JD, Cade L, Khayter C, Reyon D, Peterson RT, Joung JK, Yeh JR (2011) Targeted gene disruption in somatic zebrafish cells using engineered TALENs. Nat Biotechnol 29(8):697–698

Segal DJ (2002) The use of zinc finger peptides to study the role of specific factor binding sites in the chromatin environment. Methods 26(1):76–83

Segal DJ, Dreier B, Beerli RR, Barbas CF 3rd (1999) Toward controlling gene expression at will: selection and design of zinc finger domains recognizing each of the 5′-GNN-3′ DNA target sequences. Proc Natl Acad Sci U S A 96(6):2758–2763

Shan Q, Wang Y, Chen K, Liang Z, Li J, Zhang Y, Zhang K, Liu J, Voytas DF, Zheng X, Zhang Y, Gao C (2013a) Rapid and efficient gene modification in rice and Brachypodium using TALENs. Mol Plant 6(4):1365–1368

Shan Q, Wang Y, Li J, Zhang Y, Chen K, Liang Z, Zhang K, Liu J, Xi JJ, Qiu JL, Gao C (2013b) Targeted genome modification of crop plants using a CRISPR-Cas system. Nat Biotechnol 31(8):686–688

Shan Q, Wang Y, Li J, Gao C (2014) Genome editing in rice and wheat using the CRISPR/Cas system. Nat Protoc 9(10):2395–2410

Shukla VK, Doyon Y, Miller JC, DeKelver RC, Moehle EA, Worden SE, Mitchell JC, Arnold NL, Gopalan S, Meng X, Choi VM, Rock JM, Wu YY, Katibah GE, Zhifang G, McCaskill D, Simpson MA, Blakeslee B, Greenwalt SA, Butler HJ, Hinkley SJ, Zhang L, Rebar EJ, Gregory PD, Urnov FD (2009) Precise genome modification in the crop species Zea mays using zinc-finger nucleases. Nature 459(7245):437–441

Sripaoraya S, Keawsompong S, Insupa P, Power JB, Davey MR, Srinives P (2006) Genetically manipulated pineapple: trans-gene stability, gene expression and herbicide tolerance under field conditions. Plant Breed 125(4):411–413

Streubel J, Blücher C, Landgraf A, Boch J (2012) TAL effector RVD specificities and efficiencies. Nat Biotechnol 30(7):593–595

The Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635-641

Townsend JA, Wright DA, Winfrey RJ, Fu F, Maeder ML, Joung JK, Voytas DF (2009) High-frequency modification of plant genes using engineered zinc-finger nucleases. Nature 459(7245):442–445

Upadhyay SK, Kumar J, Alok A, Tuli R (2013) RNA-guided genome editing for target gene mutations in wheat. G3 (Bethesda) 3(12):2233–2238

Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu JL (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol 32(9):947–951

Weber E, Gruetzner R, Werner S, Engler C, Marillonnet S (2011) Assembly of designer TAL effectors by golden gate cloning. PLoS One 6(5):e19722

Wendt T, Holm PB, Starker CG, Christian M, Voytas DF, Brinch-Pedersen H, Holme IB (2013) TAL effector nucleases induce mutations at a pre-selected location in the genome of primary barley transformants. Plant Mol Biol 83(3):279–285

Yang B, Zhu W, Johnson LB, White FF (2000) The virulence factor AvrXa7 of Xanthomonas oryzae pv. oryzae is a type III secretion pathway dependent nuclear-localized double-stranded DNA-binding protein. Proc Natl Acad Sci U S A 97(17):9807–9812

Zhang F, Maeder ML, Unger-Wallace E, Hoshaw JP, Reyon D, Christian M, Li X, Pierick CJ, Dobbs D, Peterson T, Joung JK, Voytas DF (2009) High frequency targeted mutagenesis in Arabidopsis thaliana using zinc finger nucleases. Proc Natl Acad Sci U S A 107(26): 12028–12033

Zhang Y, Zhang F, Li X, Baller JA, Qi Y, Starker CG, Bogdanove AJ, Voytas DF (2013) Transcription activator-like effector nucleases enable efficient plant genome engineering. Plant Physiol 161(1):20–27

Chapter 15
Toward In Silico Design and Engineering of Solanaceae and Cucurbitaceae Crops

Hiroshi Ezura

15.1 Introduction

The Solanaceae family includes the tomato (*Solanum lycopersicum*), eggplant (*S. melongena*), and pepper (*Capsicum annuum*) as fruit and vegetable crops and the potato (*S. tuberosum*) as a tuber crop. The Cucurbitaceae family includes melon (*Cucumis melo*), cucumber (*C. sativus*), watermelon (*Citrullus lanatus*), summer squash (*Cucurbita pepo*), winter squash (*C. maxima*), and bitter melon (*Momordica charantia*) as fruit and vegetable crops. These crops are produced worldwide as essential foods in every region. In addition to their role as essential foods, these crops attract consumer attention due to their health-promoting constituents. For instance, lycopene, which is present in tomato and watermelon fruits, is well known for its antioxidant function. Gamma-aminobutyric acid (GABA) has a hypotensive effect and is abundant in tomato fruits (Yoshimura et al. 2010).

Breeding programs for Solanaceae and Cucurbitaceae crops have been conducted using conventional crossbreeding technology. In the 1970s, the development of what is called biotechnology began for these two crops. In vitro embryo rescue techniques were developed, and new hybrid plants were generated. In the 1980s, a haploid breeding technique developed in several crops of these two families accelerated the breeding programs of those crops. In the 1990s, genetic engineering techniques were introduced for the improvement of these families, and the first genetically modified crop, the Flavr Savr tomato, conferred with extended shelf life, was commercialized in 1994. Since then, genetic engineering techniques have been employed to create new types of Solanaceae and Cucurbitaceae crops

H. Ezura (✉)
Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8572, Japan
e-mail: ezura.hiroshi.fa@u.tsukuba.ac.jp

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4_15

with novel traits, such as the Bt eggplant with insect resistance (Herring 2015), the purple tomato with high levels of anthocyanin (Butelli et al. 2008), and the miraculin tomato with high levels of a taste-modifying protein (Hiwasa-Tanase et al. 2012). These GM vegetables are in a commercialization pipeline. In the 2000s, the development of new plant breeding technologies such as genome editing was intensively conducted, and they have become available for use with the tomato (Brooks et al. 2014; Lor et al. 2014).

In parallel with the development of genetic engineering technologies, whole-genome sequencing projects in major crops were conducted, for example, in tomato as a model of Solanaceae crops (The Tomato Genome Consortium 2012) and melon as a model of Cucurbitaceae crops (Garcia-Mas et al. 2012). The sequence data obtained are now publicly available. To effectively utilize the whole-genome sequence data for tomato breeding and characterization, tools of functional genomics such as the collection of expressing genes (Aoki et al. 2010), large-scale mutant populations (Saito et al. 2011), and the effective isolation of mutants of target genes from the large-scale mutant population (Okabe et al. 2011) were developed and shared among research communities. The combined use of these tools makes it possible to effectively isolate a novel gene for crop breeding (Ariizumi et al. 2014). As a result of advances in DNA sequencing, we will have further large-scale genomics data, called genomic big data. By integrating such genomic big data, it will be possible to design crops in silico that are suitable for growth in a variety of environmental conditions.

In this chapter, I propose the necessary advancements in research for future in silico design and engineering of Solanaceae and Cucurbitaceae crops.

15.2 Whole-Genome Sequencing in Solanaceae and Cucurbitaceae Crops

Among the major Solanaceae crops, the whole-genome sequence was first published for the potato (*S. tuberosum*) (Potato Genome Sequencing Consortium 2011), followed by tomato (*Solanum lycopersicum*) (The Tomato Genome Consortium 2012), pepper (*Capsicum annuum*) (Kim et al. 2014), and eggplant (*S. melongena*) (Hirakawa et al. 2014). Among the major Cucurbitaceae crops, the whole-genome sequence was first published for the cucumber (*Cucumis sativus*) (Huang et al. 2009), followed by melon (*C. melo*) (Garcia-Mas et al. 2012), and watermelon (*Citrullus lanatus*) (Guo et al. 2013), and the genomes of summer squash (*Cucurbita pepo*), winter squash (*C. maxima*), and bitter melon (*Momordica charantia*) have not yet been reported, although some of them are in progress. Once whole-genome sequences are obtained, we can efficiently re-sequence diverse accessions and compare the genetic diversity and population structure of the germplasm. In watermelon, it has been revealed that genomic regions were preferentially selected, and many disease-resistant genes were found to be lost during

domestication (Guo et al. 2013). Advances in genome sequencing allow us to rapidly sequence a variety of accessions and related species with less effort, and comparative genomics analysis will provide new and useful insights and strategies for plant breeding, as shown by the case study of watermelon. Within the next decade, large-scale re-sequencing data for the two crop families will be obtained. A limiting factor will be how to extract significant data, and it will be important to collaborate in the field of information science.

15.3 Functional Genomics Tools in Solanaceae and Cucurbitaceae Crops

Large-scale artificial mutant collection is a valuable resource for plant genetics, genomics, and breeding. To artificially induce mutations, several techniques, including chemical mutagenesis, physical mutagenesis, and in vitro tissue culture, are traditionally used. If genetic transformation in the target plants is efficient, the T-DNA tagging technique is also available for establishing a mutant population. To date, chemical mutagenesis by ethyl methanesulfonate (EMS) has been widely used for the establishment of large-scale mutant populations in several species of the two crop families.

In the tomato, after a pioneering study on mutant development by chemical mutagenesis (Meissner et al. 1997), a comprehensive mutant tomato population was generated in the background of Micro-Tom, a dwarf, rapid-growth variety (Saito et al. 2011). To integrate and manage the visible phenotypes of mutants and other associated data, the in silico database TOMATOMA, a relational system interfacing modules between mutant line names and phenotypic categories, was developed. TOMATOMA is a freely accessible database, and these mutant resources are available through its website (http://tomatoma.nbrp.jp/index.jsp). To accelerate functional genomic research in the tomato, the Micro-Tom TILLING (Targeting Induced Local Lesions In Genomes) platform was developed (Okabe et al. 2011). As a tool to study pepper development, an EMS mutant collection of 1650 M2 families of the blocky *Capsicum annuum* was generated (Paran et al. 2007), but there are as yet no reports on large-scale mutant collections in the eggplant and potato.

In the melon, an EMS mutant collection of approximately 3000 M2 families of “Noy Yizre’el,” a “Galia” melon-type parental line, was generated (Tadmor et al. 2007). In the cucumber, an EMS mutant population and its TILLING collection, which can be used as an efficient reverse genetics tool, were developed (Boualem et al. 2014). Those tools will be a valuable resource for both fundamental research and the identification of agronomically important genes for crop improvement in cucurbits. There are as yet no reports of large-scale mutant collections in watermelon and squash.

Genetic transformation is another tool with which functional genomics can validate the functions of a gene of interest. Among the Solanaceae crops, standard protocols for genetic transformation have been available for tomato, potato, and eggplant (e.g., Sun et al. 2006). Although there are several reports on the genetic transformation of the pepper (Seo et al. 2014), the pepper remains a recalcitrant species for genetic transformation. Among Cucurbitaceae crops, although there are several reports on the genetic transformation of the melon (Akasaka-Kenedy et al. 2004), cucumber (Wang et al. 2015), watermelon (Ellul et al. 2003), and squash (Nanasato et al. 2011), the transformation efficiency is rather low and depends heavily on genotypes, and a protocol independent of genotypes must be developed for the practical application of this technology.

Currently, genome editing technology is gathering attention because it can create a mutation in a gene of interest that is similar to the mutations induced by conventional mutation breeding techniques. Application of genome editing technology is just beginning in the Solanaceae and Cucurbitaceae crops. Only three successful results, two in the tomato (Lor et al. 2014; Brooks et al. 2014) and one in the potato (Sawai et al. 2014), have been published so far. However, this approach will be widely used across the Solanaceae and Cucurbitaceae crops if efficient and reliable genetic transformation technology is developed for the target crops. Genetic transformation is a necessary technology for genome editing.

15.4 Hunting Agronomically Important Genes and In Silico Design Technologies for the Solanaceae and Cucurbitaceae Crops

Application of genetic engineering technologies to improve important breeding traits in crops requires genetic information accounting for the development of the important breeding traits. Biotic and abiotic stress resistance is a common breeding trait important for all types of crops. Traits related to fruit development, such as fruit setting, enlargement, and ripening, and fruit qualities such as sugar and organic acid accumulation, aroma production, and accumulation of health-promoting constituents are unique breeding traits in the Solanaceae and Cucurbitaceae crops. In the past two decades, based on molecular genetics approaches, several agronomically important genes have been isolated and characterized in Solanaceae and Cucurbitaceae crops. *IAA9,* which is responsible for fruit setting (Wang et al. 2005); *fw2.2*, which is responsible for fruit size (Cong et al. 2008); and *rin* and *nr,* which are responsible for fruit ripening (Vrebalov et al. 2002; Wilkinson et al. 1995), are well known for their agronomic importance. In addition, with the development of in silico design technology, isolation of many agronomically important genes in Solanaceae and Cucurbitaceae crops is to be expected. Those genes will be a significant target for emerging genetic engineering technology and genome editing.

15.5 Conclusion

Molecular genetics and genomics studies in the Solanaceae and Cucurbitaceae families have been conducted intensively in the past two decades. Consequently, large volumes of critical data and resources for the breeding of the two families become available and are likely to accelerate the molecular dissection of important breeding traits while allowing us to develop an advanced breeding system, an in silico crop design system, and an engineering technology. To realize this goal, it is necessary to achieve the following objectives.

The first goal is to integrate omics data, including genomics, transcriptomics, proteomics, metabolomics, and phenomics into a single platform. This integration will promote our understanding of the blueprint of plant development and responses to various environmental stimuli. The blueprint will enable in silico design of plants with the required performance. To achieve this development, it is essential to collaborate with scientists in the field of information science. The second goal is to develop a strategy for genetic engineering, for which it is essential to collaborate with scientists in a field of mechanical engineering. The final goal is to assemble an ideal plant by combining the available genetic resources and engineering resources created by conventional genetic engineering and genome editing technologies. To effectively achieve this goal, comprehensive cooperation between conventional breeders and in silico designers will be essential. In addition, it is necessary to develop genetic transformation technologies that are independent of genotypes and crop species. As mentioned previously, the genetic transformation efficiencies of major Solanaceae and Cucurbitaceae crops, except for the tomato (Lor et al. 2014; Brooks et al. 2014) and potato (Sawai et al. 2014), remain dependent on genotypes and thus remain limiting factors in the genetic engineering of the two families. Although a few successful cases of genetic engineering by genome editing technologies have been reported in the tomato and potato, it is also essential to develop genetic transformation protocols that are independent of genotypes because we must use conventional genetic transformation technology during the process of genome editing.

Upon achieving these objectives, in silico design and engineering technology will be available for the improvement of major Solanaceae and Cucurbitaceae crops within the next decade.

References

Akasaka-Kenedy Y, Tomita K, Ezura H (2004) Efficient plant regeneration and *Agrobacterium*-mediated transformation via somatic embryogenesis in melon (*Cucumis melo* L.). Plant Sci 166:763–769

Aoki K, Yano K, Suzuki A, Kawamura S, Sakurai N, Suda K, Kurabayashi A, Suzuki T, Tsugane T, Watanabe M, Ooga K, Torii M, Narita T, Shin-I T, Kohara Y, Yamamoto N,

Takahashi H, Watanabe Y, Egusa M, Kodama M, Ichinose Y, Kikuchi M, Fukushima S, Okabe A, Arie T, Sato Y, Yazawa K, Satoh S, Omura T, Ezura H, Shibata D (2010) Large-scale analysis of full-length cDNAs from the tomato (*Solanum lycopersicum*) cultivar Micro-Tom, a reference system for the Solanaceae genomics. BMC Genomics 11:210

Ariizumi T, Kishimoto S, Kakami R, Maoka T, Hirakawa H, Suzuki Y, Ozeki Y, Shirasawa K, Bernillon S, Okabe Y, Moing A, Asamizu E, Rothan C, Ohmiya A, Ezura H (2014) Identification of the tomato carotenogenic gene *PALE YELLOW PETAL 1* as an essential factor in xanthophyll esterification and yellow flower pigmentation. Plant J 79:453–465

Boualem A, Fleurier S, Troadec C, Audigier P, Kumar APK, Chatterjee M, Alsadon AA, Sadder MT, Wahb-Allah MA, Al-Doss AA, Bendahmane A (2014) Development of a *Cucumis sativus* TILLinG platform for forward and reverse genetics. PLoS One 9:e97963

Brooks C, Nekrasov V, Lippman ZB, Van Eck J (2014) Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. Plant Physiol 166:1292–1297

Butelli E, Titta L, Giorgio M, Mock HP, Matros A, Peterek S, Schijlen EGWM, Hall RD, Bovy AG, Luo J, Martin C (2008) Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. Nat Biotechnol 26:1301–1308

Cong B, Barrero LS, Tanksley SD (2008) Regulatory change in YABBY-like transcription factor led to evolution of extreme fruit size during tomato domestication. Nat Genet 40:800–804

Ellul P, Rios G, Atares A, Roig LA, Serrano R, Moreno V (2003) The expression of the Saccharomyces cerevisiae HAL1 gene increases salt tolerance in transgenic watermelon [*Citrullus lanatus* (Thunb.) Matsun. & Nakai. Theor Appl Genet 107:462–469

Garcia-Mas J, Benjak A, Sanseverino W, Bourgeois M, Mir G, Gonzalez V, Henaff E, Camara F, Cozzuto L, Lowy E, Alioto T, Capella-Gutierrez S, Blanca J, Cañizares J, Ziarsolo P, Gonzalez-Ibeas D, Rodriguez-Moreno L, Droege M, Du L, Alvarez-Tejado M, Lorente-Galdos B, Mele M, Yang L, Weng Y, Navarro A, Marques-Bonet T, Aranda M, Nuez F, Pico B, Gabaldon T, Roma G, Guigo R, Casacuberta J, Arus P, Puigdomenech P (2012) The genome of melon (*Cucumis melo* L.). Proc Natl Acad Sci U S A 109:11872–11877

Guo S, Zhang J, Sun H, Salse J, Lucas W, Zhang H, Zheng Y, Mao L, Ren Y, Wang Z, Min J, Guo X, Murat F, Ham B-K, Zhang Z, Gao S, Huang M, Xu Y, Zhong S, Bombarely A, Mueller L, Zhao H, He H, Zhang Y, Zhang Z, Huang S, Tan T, Pang E, Lin K, Hu Q, Kuang H, Ni P, Wang B, Liu J, Kou Q, Hou W, Zou X, Jiang J, Gong G, Klee K, Schoof H, Huang Y, Hu X, Dong S, Liang D, Wang J, Wu K, Xia Y, Zhao X, Zheng Z, Xing M, Liang X, Huang B, Lv T, Wang J, Yin Y, Yi H, Li R, Wu M, Levi A, Zhang X, Giovannoni J, Wang J, Li Y, Fei Z, Xu Y (2013) The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. Nat Genet 45:51–8

Herring RJ (2015) State science, risk and agricultural biotechnology: Bt cotton to Bt Brinjal in India. J Peasant Stud 42:159–186

Hirakawa H, Shirasawa K, Miyatake K, Nunome T, Negoro S, Ohyama A, Yamaguchi H, Sato S, Isobe S, Tabata S, Fukuoka H (2014) Draft genome sequence of eggplant (*Solanum melongena* L.): the representative Solanum species indigenous to the old world. DNA Res 21:649–660

Hiwasa-Tanase K, Hirai T, Kato K, Duhita N, Ezura H (2012) From miracle fruit to transgenic tomato: mass-production of the taste-modifying protein miraculin in transgenic plants. Plant Cell Rep 31:513–525

Huang S, Li R, Zhang Z, Li L, Gu X, Fan W, Lucas W, Wang X, Xie B, Ni P, Ren Y, Zhu H, Li J, Lin K, Jin W, Fei Z, Li G, Staub J, Kilian A, van der Vossen E, Wu Y, Guo J, He J, Jia Z, Ren Y, Tian G, Lu Y, Ruan J, Qian W, Wang M, Huang Q, Li B, Xuan Z, Cao J, Asan, Wu Z, Zhang J, Cai Q, Bai Y, Zhao B, Han Y, Li Y, Li X, Wang S, Shi Q, Liu S, Cho W, Kim J-Y, Xu Y, Heller-Uszynska K, Miao H, Cheng Z, Zhang S, Wu J, Yang Y, Kang H, Li M, Liang H, Ren X, Shi Z, Wen M, Jian M, Yang H, Zhang G, Yang Z, Chen R, Liu S, Li J, Ma L, Liu H, Zhou Y, Zhao J, Fang X, Li G, Fang L, Li Y, Liu D, Zheng H, Zhang Y, Qin N, Li Z, Yang G, Yang S, Bolund L, Kristiansen K, Zheng H, Li S, Zhang X, Yang H, Wang J, Sun R, Zhang B,

Jiang S, Wang J, Du Y, Li S (2009) The genome of the cucumber, *Cucumis sativus* L. Nat Genet 41:1275–1281

Kim S, Park M, Yeom SI, Kim YM, Lee JM, Lee HA, Seo E, Choi J, Cheong K, Kim KT, Jung K, Lee GW, Oh SK, Bae C, Kim SB, Lee HY, Kim SY, Kim MS, Kang BC, Jo YD, Yang HB, Jeong HJ, Kang WH, Kwon JK, Shin C, Lim JY, Park JH, Huh JH, Kim JS, Kim BD, Cohen O, Paran I, Suh MC, Lee SB, Kim YK, Shin Y, Noh SJ, Park J, Seo YS, Kwon SY, Kim HA, Park JM, Kim HJ, Choi SB, Bosland PW, Reeves G, Jo SH, Lee BW, Cho HT, Choi HS, Lee MS, Yu Y, Do Choi Y, Park BS, van Deynze A, Ashrafi H, Hill T, Kim WT, Pai HS, Ahn HK, Yeam I, Giovannoni JJ, Rose JKC, Sorensen I, Lee SJ, Kim RW, Choi IY, Choi BS, Lim JS, Lee YH, Choi D (2014) Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. Nat Genet 46:270

Lor VS, Starker CG, Voytas DF, Weiss D, Olszewski NE (2014) Targeted mutagenesis of the tomato PROCERA gene using transcription activator-like effector nucleases. Plant Physiol 166:1288–1291

Meissner R, Jacobson Y, Melmed S, Levyatuv S, Shalev G, Ashri A, Elkind Y, Levy A (1997) A new model system for tomato genetics. Plant J 12:1465–1472

Nanasato Y, Konagaya K, Okuzaki A, Tsuda M, Tabei Y (2011) Agrobacterium-mediated transformation of kabocha squash (*Cucurbita moschata* Duch) induced by wounding with aluminum borate whiskers. Plant Cell Rep 30:1455–1464

Okabe Y, Asamizu E, Saito T, Matsukura C, Ariizumi T, Bres C, Rothan C, Mizoguchi T, Ezura H (2011) Tomato TILLING technology: development of a reverse genetics tool for the efficient isolation of mutants from Micro-Tom mutant libraries. Plant Cell Physiol 52:1994–2005

Paran I, Borovsky Y, Nahon S, Cohen O (2007) The use of induced mutations to study shoot architecture in Capsicum. Israel J Plant Sci 55:125–131

Potato Genome Sequencing Consortium (2011) Genome sequence and analysis of the tuber crop potato. Nature 475:189–194

Saito T, Ariizumi T, Okabe Y, Asamizu E, Hiwasa-Tanase K, Yamazaki Y, Fukuda N, Mizoguchi T, Aoki K, Ezura H (2011) TOMATOMA: a novel tomato mutant database distributing Micro-Tom mutant collections. Plant Cell Physiol 52:283–296

Sawai S, Ohyama K, Yasumoto S, Seki H, Sakuma T, Yamamoto T, Takebayashi Y, Kojima M, Sakakibara H, Aoki T, Muranaka T, Saito K, Umemoto N (2014) Sterol side chain reductase 2 is a key enzyme in the biosynthesis of cholesterol, the common precursor of toxic steroidal glycoalkaloids in potato. Pant Cell 26:3763–3774

Seo HH, Park S, Park S, Oh BJ, Back K, Han O, Kim JI, Kim YS (2014) Overexpression of a defensin enhances resistance to a fruit-specific anthracnose fungus in pepper. PLoS One 9: e97936

Sun HJ, Uchii S, Watanabe S, Ezura H (2006) A highly efficient transformation protocol for Micro-Tom, a model cultivar of tomato functional genomics. Plant Cell Physiol 47:426–431

Tadmor Y, Katzir N, Meir A, Yaniv-Yaakov A, Sa'ar U, Baumkoler F, Lavee T, Lewinsohn E, Schaffer A, Burger J (2007) Induced mutagenesis to augment the natural genetic variability of melon (*Cucumis melo* L.). Israel J Plant Sci 55:159–169

The Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635–641

Vrebalov J, Ruezinsky D, Padmanabhan V, White R, Medrano D, Drake R, Schuch W, Giovannoni J (2002) A MADS-box gene necessary for fruit ripening at the tomato ripening-inhibitor (*Rin*) locus. Science 296:343–346

Wang H, Jones B, Li ZG, Frasse P, Delalande C, Regad F, Chaabouni S, Latche A, Pech JC, Bouzayen M (2005) The tomato Aux/IAA transcription factor IAA9 is involved in fruit development and leaf morphogenesis. Plant Cell 17:2676–2692

Wang SL, Ku SS, Ye XG, He CF, Kwon SY, Choi PS (2015) Current status of genetic transformation technology developed in cucumber (*Cucumis sativus* L.). J Integr Agric 14:469–482

Wilkinson JQ, Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ (1995) An ethylene-inducible component of signal-transduction encoded by *NEVER-RIPE*. Science 270:1807–1809

Yoshimura M, Toyoshi T, Sano A, Izumi T, Fujii T, Konishi C, Inai S, Matsukura C, Fukuda N, Ezura H, Obata A (2010) Antihypertensive effect of a gamma-aminobutyric acid-rich tomato cultivar 'DG03-9' in spontaneously hypertensive rats. J Agric Food Chem 58:615–619

Index

A
ADP-glucose pyrophosphorylase
  AGPase, 146, 147
  AgpL1, 146, 147
  AgpS1, 146, 147
  auxin response factor, 146, 149
  starch, 142, 143, 146, 147, 149
  vascular tissue, 147
  vasculature-specific expression, 147
Amplified fragment length polymorphism (AFLP), 45
Anaphase-promoting complex(APC), 115, 117, 120
Annotation, 32, 33
Anther-specific promoter, 105
Antioxidant, 106
Antisense gene silencing, 102, 104
Aphid, 199
  Aphis gossypii, 200–202, 205–206, 209
Aphis gossypii, 178
Auxin
  ARF8, 226
  AUSCIA, 227
  auxin-binding f-box protein (ABF), 226
  cell division, 226
  chalcone synthase (CHS), 227
  defH9-1aaM, 226
  embryo sac, 226
  IAA/AUX, 226
  ovule, 226
  SlARF7, 226
  SlTIR, 226
  transport inhibitor response protein 1 (TIR1), 226
Auxin/IAA (Aux/IAA), 100, 101
Auxin response factor (ARF), 100, 101, 107

B
BAC libraries, 18
Bacteria
  Acidovorax avenae subsp. *citrulli,* 180
  Erwinia tracheiphila, 180
Bacterial artificial chromosomes (BACs), 1
Balsam pear (*Momordica charantia* L.) chitinase (Mcchit1), 225
Basic Local Alignment Search Tool (BLAST), 32
Beetle, 201, 208, 212
β-carotene, 99
Bemisia tabaci, 178
Breeding trait, 254, 255
Bt toxins
  Bacillus thuringiensis, 222
  Bt crystal proteins (Cry), 222
  coleopterans, 222
  colorado potato beetle (CPB), 222
  Cry1, 222
  Cry3, 222
  Cry3A, 223
  cry1Ac, 223
  dipterans, 222

© Springer-Verlag Berlin Heidelberg 2016

H. Ezura et al. (eds.), *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops*, Biotechnology in Agriculture and Forestry 70,
DOI 10.1007/978-3-662-48535-4

Bt toxins (*cont.*)
eggplant fruit and shoot borer (EFSB), 223
lepidopterans, 222
Bulk segregant analysis, 46

C
Carbon
partitioning, 118, 128, 129
photoassimilates, 128
Carotenoid, 164–166
CC NB-LRR family, 187
CDK/CYC, 115, 117, 120, 121
CDK inhibitors, 115
Cyclin-dependent kinase (CDK), 115
Cell cycle, 114, 117, 120
G1 phase, 114, 115
G2 phase, 115
mitosis, 114
M phase, 114, 115
S phase, 114, 115, 117
4-Chlorophenoxyacetic acid (4-CPA), 99
Chromosome segment substitution lines, 51
Citrullus lanatus, 59, 65
Clustered regularly interspaced short palindromic repeats (CRISPR), 240, 244–247
CNR/*FW2.2*
fruit development, 228
fruit weight, 228
longer duration, 228
Comparative genomics, 25
Cucumber, 59, 60, 62, 65–67
Cucumber Genome Database, 63, 65
Cucumis
C. hystrix, 63
C. melo, 59, 175
C. sativus, 59, 63
C. sativus var. *hardwickii,* 63
Cucurbita
C. ecuadorensis, 65
C. maxima, 59, 65
C. moschata, 59, 65
C. pepo, 59, 65, 66
Cucurbitaceae, 31, 251, 252, 254, 255
cucumber, 33, 36, 37
Cucurbita pepo, 37
melon, 33, 37
pumpkin, 33
watermelon, 33, 36, 37
Cytological maps, 63

D
Databases, 33
Arabidopsis, 39
the Arabidopsis Information Resource (TAIR), 39
CapCyc, 37
CucCyc, 37
CucurbiGene, 37
Cucurbit Genomics Database (CuGenDB), 36
database of expressed sequence tags (dbESTs), 32
DDBJ Sequence Read Archive (DRA), 33
DNA Data Bank of Japan (DDBJ), 31
European Bioinformatics Institute-Part of the European Molecular Biology Laboratory (EMBL-EBI), 33
European Nucleotide Archive (ENA), 31
GenBank, 31
Gene Index Databases, 32–33
InParanoid, 39
International Nucleotide Sequence Databases (INSD), 31
KaFTom, 35
KaPPA-View4, 38
KEGG, 37
KNApSAcK, 38
Kyoto Encyclopedia of Genes and Genomes (KEGGs), 33
LycoCyc, 37
MelonCyc, 37
MELONOMICS, 37
MiBASE, 35, 38
MSU Rice Genome Annotation Project Database, 39
National BioResource Project (NBRP) of Japan, 36
National Center for Biotechnology Information (NCBI), 32
OrthoMCL, 39
PetCyc, 37
PhylomeDB, 39
Plant Metabolic Network (PMN), 37
Plant Omics Data Center (PODC), 40
Platform for RIKEN Metabolomics (PRIMe), 38
PotatoCyc, 37
Potato Genome Sequencing Consortium (PGSC), 36
Sequence Read Archive (SRA), 33
SGN, 36

SHared Information of GENetic resources (SHIGEN), 38
Solanum lycopersicum project of PGSB, 36
SolCyc, 37
TobaccoCyc, 37
TOMATOMA, 38
TOMATOMICS, 35, 38
UniGene database (UniGene), 32
UniProt Knowledgebase (UniProtKB), 40
Universal Protein Resource (UniProt), 31
WmnCyc, 37
DELLA, 102, 108
Designed nucleases, 240
Disease resistance, 21, 49, 50, 52, 53, 175–190
bacteria, 224
Fungi, 224
patternrecognition receptor (PRR), 224
potato virus Y (PVY), 224
siRNA, 224
viruses, 224
DNA markers, 2, 59–62, 65–68
Double-strand RNA (dsRNA)
ecdysone receptor (EcR), 223
Helicoverpa armigera, 223
metamorphosis, 223
molting, 223
RNA interference (RNAi), 223
ultraspiracle (USP) genes, 223
Durability of resistance/durability, 211–212

E
Endoreduplication, 114, 118, 120
endocycle, 117, 120, 122
endopolyploidy, 114, 117
ploidy levels, 119, 121, 122
Enzyme Commission (EC) number, 32, 33
Epigenetic mutant, 167
Epigenetic mutation, 165
Ethylene, 156
ACC oxidase (ACO), 157, 159
1-aminocyclopropane-1-carboxylase 92 synthase (ACS), 157, 158
ethylene biosynthesis pathway, 157
tomato tetratricopeptide repeat protein 1 (SlTPR1), 227
Ethylene receptor, 158
ethylene-binding sites, 160
membrane protein, 159
Ethylene signaling pathway
constitutive triple response 1 (CTR1), 162, 163
CTR1, 162
ethylene insensitive 2 (EIN2), 162, 163
Ethyl methanesulfonate (EMS), 77–80, 82, 84, 101
Euchromatin, 2
Eukaryotic translation initiation factor, 186
Experimental materials, 38
Experimental resources, 38
Expressed sequence tags (ESTs), 17, 32

F
FASCIARED (FAS)
locule number, 229
YABBY gene family, 229
F-box proteins
EIN3-binding factors, 164
EIN2 targeting proteins, 163
Flavonoid, 106
Fluorescence in situ hybridization (FISH), 3
Fruit fly, 202, 208
Fruit set
fertilization, 226
pollination, 226
Fruit size
FASCIARED (FAS), 228
fw1.1, 228
fw2.2, 228
fw2.3, 228
fw3.1, 228
fw3.2, 228
fw4.1, 228
fw9.1, 228
LOCULE NUMBER (LC), 228
OVATE, 228
SUN, 228
Fruit sweetness
Brix, 141
fruit sugar content, 143, 149
soluble sugar content, 141
total soluble solids (TSS), 141, 144–146, 149
Fungi
Alternaria cucumerina, 183
Didymella bryoniae, 183
Fusarium oxysporum f.sp. *melonis,* 181
Golovinomyces cichoracearum, 181
Monosporascus cannonballus, 181

Fungi (*cont.*)
  Podosphaera xanthii, 181
  powdery mildew, 181

G
GABA content, 166
GA20ox, 102, 107
γ-aminobutyric acid (GABA), 105
γ-ray, 101
GbTLP1, 226
Gene expression networks (GENs), 39
Gene expression profiles, 39
Gene ontology (GO), 33
Gene pyramiding, 94
Genetic linkage maps, 59, 60
  consensus map, 60, 62, 64
  integrated map, 60, 67
Genetic maps, 16
Genetic resources, 175, 255
Genetic transformation, 254, 255
Genome anchoring, 19
Genome annotation, 20
Genome duplication, 5
Genome editing, 108, 252, 254, 255
Genome-editing, 239
Genome sequence, 48
Genome sequence(s), 15, 22–25
  cucumber (*Cucumis sativus* L.), 15
  melon, 16
  watermelon, 16
Genome sequences, 44, 53
Genome sequencing, 1, 18, 32
Genome-wide association study (GWAS), 52, 53
Genomic big data, 252
Genomic selection (GS), 52, 53
Genomic tools, 16
Gibberellin
  DELLA, 227
  gib1, 227
  gib2, 227
  gib3, 227
  PROCERA/SlDELLA, 227
Glutamic acid, 105
Glycoalkaloid, 8
Greenhouse, 93

H
Heterochromatin, 2
High-throughput cDNA sequences (HTCs), 35
HMGR
  cell proliferation period, 230
  cell size, 230
  fresh weight, 230
  fruit size, 230
  3-hydroxy-3-methylglutaryl coenzyme A reductase, 230
Homologous recombination (HR), 240
Hormone, 118
  abscisic acid (ABA), 126
  auxin, 126
  cytokinin, 126
  ethylene, 126
  gibberellin (GA), 122, 126, 127

I
InDels, 35
Information science, 255
Insect, 200–202, 205, 207–208, 210, 212
Insect resistance, 199–213, 222–224
In silico crop design, 252, 255
International Cucurbit Genomics Initiative (ICuGI), 60, 61, 63, 65
Invertase, 142, 146, 148
  Brix9-2-5, 145, 147
  cell wall invertase (CWIN), 143, 144, 147
  cytoplasmic invertase (CIN), 143, 145
  invertase inhibitor, 145, 149
  LIN5, 144, 145
  vacuolar invertase (VIN), 143, 144

K
Karyoplasmic ratio theory, 117
Kip-related protein (KRP), 116
KLUH/FW3.2
  fruit weight, 228
  LA1589, 228
  SlKLUH, 228
  Yellow Stuffer (ys), 228
k-mer frequency, 4

L
LC
  locule number, 229
  WUSCHEL, 229
Leaf miner, 202, 207
Linkage disequilibrium (LD), 52, 53
Linkage maps, 43–51, 53
Lycopene, 166

M
MADS-box, 104
Magi6 peptide, 226
Map-based cloning, 43, 50
Mapping populations, 60, 67
Marker-assisted selection (MAS), 59, 60, 62, 63, 65, 67, 68, 94, 186
Melon, 59–62, 66–67, 199–213
MELONOMICS, 61
Melonworm, 202, 207
Metabolic pathways, 37–38
1-Methylcyclopropene (1-MCP), 103
microRNA (miRNA), 210, 211
Microsatellites, 46
Micro-Tom, 103
Mutant collection, 253

N
NBS-LRR gene (NBS-LRR), 210
N-(2-chloro-4-pyridyl)-*N*-phenylurea (CPPU), 94
Neoxanthin, 105
Next-generation sequencing (NGS), 9, 33, 44, 48
noncoding RNA, 21
Nonhomologous end joining (NHEJ), 240
Non-ripening mutant
  colorless non-ripening (Cnr), 165–167
  Never-ripe (Nr), 160
  non-ripening (nor), 165
  ripening-inhibitor (rin), 165, 167
Nutrition
  amino acid, 231
  anthocyanin, 231
  anthocyanins, 231
  ascorbate, 231
  beta-carotene, 231
  Brix, 231
  carotenoid, 231
  carotenoids, 231
  essential amino acids, 231
  flavonoids, 231
  fruit quality, 231
  Gamma-aminobutyric acid (GABA), 231
  GDP-D-mannose 3,5-epimerase, 231
  lycopene beta-cyclase (LYCB), 231
  MhSnRK1, 231
  miraculin, 232
  miraculin protein, 232
  taste-modifying function, 232
  vitamins, 231

O
Omics, 33, 39–40
  data, 255
  databases, 31–33
  genome, 33–37, 39
  metabolome, 39
  transcriptome, 33–37, 39
Oomycetes
  downy mildew, 181
  Pseudoperonospora cubensis, 181
  Pythium aphanidermatum, 181
  Pythium ultimum, 181
Organoleptic, 118, 127
Ortholog, 39
OVATE
  fruit shape, 229
  round-shaped fruits, 230
Ovule-specific promoter, 99

P
Parthenocarpic, 126, 127
Parthenocarpy, 51
Pattern-recognition receptors (PRRs)
  EF-Tu reception, 225
  pathogen-associated molecular patterns (PAMPs), 224
Pericarp, 118, 125
  exocarp, 120
  mesocarp, 120, 121
p35 gene, 226
Polyamines, 107
Potato *(Solanum tuberosum),* 7
Promoter(s), 108
  ovary and young-fruit-specific promoter, 100
  ovule-specific promoter, 99
Proteinase inhibitors
  Agrotis ipsilon, 224
  alpha-amylase inhibitors, 223
  CeCPI, 224
  cotton-boll weevil, 223
  digestive system, 223
  Diptera, 223
  Helicoverpa armigera, 224
  Heliothis virescens, 224
  Lepidoptera, 223
  Manduca sexta, 224
  mortality rate, 223
  phytocystatin, 224
  serine (trypsin) type, 223
  Spodoptera exigua, 224

Proteinase inhibitors (*cont.*)
Spodoptera frugiperda, 224
sporamin, 224
sugar beet serine-type proteinase inhibitor gene *(BvSTI),* 224
Protein-coding genes, 5
Pseudomolecules, 4
Pumpkin, 59, 60, 65–66

Q
Qualitative traits, 49, 51, 52
Quantitative trait loci (QTLs), 43, 49, 51, 53, 60, 62–64, 66, 106, 107, 113, 123, 168, 170, 185
fw2.2, 124, 125
fw3.2, 125
fw11.3, 125
Lin5, 128
Quantitative traits, 49, 53

R
Random amplified polymorphic DNA (RAPD), 45
Recombinant inbred lines, 51
Resequencing, 22
Resistance
inheritance of resistance/inheritance, 208, 209
molecular basis, 200, 209–211
QTL of, 203, 209, 212
sources of resistance/sources, 199, 202–208, 212
Resistance (R) genes
Bs2 gene, 224
Colletotrichum orbiculare, 224
defense signal cascade, 224
hypersensitive response (HR), 224
leucine-rich repeat (LRR), 224
nucleotide-binding leucine-rich repeat (NB-LRR), 224
Ralstonia solanacearum, 224
RPS4, 224
RRS1, 224
Xanthomonas campestris pv, 224
Restriction fragment length polymorphisms (RFLPs), 44, 45
Restriction-site-associated DNA (RAD), 48, 51
Retrotransposons, 4
Reverse genetic, 187
Risk assessment, 189–190
RNA interference (RNAi), 100, 101, 105, 106
RNA-seq, 17

S
Shelf life, 156
alpha-mannosidase (alpha-Man), 231
1-aminopropane-1-carboxylate oxidase gene 1 (ACO1), 230
1-aminopropane-1-carboxylate synthase (ACS), 230
beta-D-N-acetylhexosaminidase (beta-Hex), 231
beta-galactosidase, 230
beta-glucanase, 230
ethylene, 230
fruit softening, 230
N-glycan, 230, 231
pectin methylesterase, 230
polyamines (PAs), 230
polygalacturonase, 230
postharvest losses, 230
Short RNAs (sRNAs), 168
Simple sequence repeats (SSRs), 33, 46–48, 51
Single nucleotide polymorphisms (SNPs), 8, 33, 35, 36, 47–49, 51–53
SlAGO7, 247
Small interfering RNA (siRNA)
Cm-eIF4E, 225
4E family, 225
SOL-100, 10
Solanaceae, 1, 31, 33, 114, 124, 130, 251, 252, 254, 255
eggplant, 33
Nicotiana, 36
Nicotiana benthamiana, 33, 35
pepper, 33, 37
petunia, 33, 37
potato, 33, 35–37
Solanum tuberosum, 36
Sol Genomics Network (SGN), 35
tobacco, 33, 35–37
tomato, 33, 35–37
tomato *(Solanum lycopersicum),* 33
Solanaceae databases., 35
Solanum lycopersicum, 114
SOL Genomics Network (SGN), 10
Squash, 59, 60, 65–66
Sterility, 102, 104–106
StPUB17 (UND/PUB/ARM), 226
Sucrose synthase, 142, 143, 147
Sucrose transporter (SUT)
LeSUT1, 147
LeSUT2, 147
LeSUT4, 147, 148
phloem companion cells, 147
phloem sieve element, 147
Sugar accumulation
amino acid, 231

anthocyanin, 231
anthocyanins, 231
ascorbate, 231
beta-carotene, 231
Brix, 231
carotenoid, 231
carotenoids, 231
essential amino acids, 231
flavonoids, 231
fruit quality, 231
Gamma-aminobutyric acid (GABA), 231
GDP-D-mannose 3,5-epimerase, 231
lycopene beta-cyclase (LYCB), 231
MhSnRK1, 231
miraculin, 232
miraculin protein, 232
taste-modifying function, 232
vitamins, 231
Sulphur, 183
SUN, fruit development, 229
Synonymous nucleotide substitu-tion rates, 6
Synteny, 66–67
microsynteny, 66

T
Targeting-induced local lesions in genomes (TILLING), 17, 79–86, 108, 253
Micro-Tom, 75–76, 80
mutant, 84–86
next generation sequencing (NGS), 83, 84
TIR-NB-LRR family, 187
Tocopherols, 105
Tomato, 239, 246, 247
Transcription activator-like effector nuclease (TALENs), 240, 242–246
Transcription factors, 158
EIN3-like proteins, 163, 164
ethylene insensitive 3 (EIN3), 163, 164
ethylene response factor (ERF), 163
MADS-box, 165–167
Transgenic resistance
post-transcriptional gene silencing (PTGS), 188
ribozyme, 188
viral coat protein (CP), 188
Transposable elements (TEs), 19
Trialeurodes vaporariorum, 178
Triplet chromosomes, 6

U
Ubiquitin/26S-proteasome pathway, 158, 161, 163, 164

V
Vacuolar processing enzyme (VPE), 148
SlVPE5, 148, 149
Virus, 201, 206
algerian watermelon mosaic virus (AWMV), 178
beet pseudo-yellows virus (BPYV), 178
cucumber green mottle mosaic virus (CGGMV), 179
cucumber mosaic virus (CMV), 176
cucumber vein yellowing virus (CVYV), 179
cucurbit aphid-borne yellows virus (CABYV), 178
cucurbit leaf crumple virus (CuLCrV), 179
cucurbit yellow stunting disorder virus (CYSDV), 178
kyuri green mottle mosaic virus (KGGMV), 179
lettuce infectious yellows virus (LIYV), 178
melon necrotic spot virus (MNSV), 179
moroccan watermelon mosaic virus (MWMV), 178
papaya ringspot virus (PRSV), 178
squash mosaic virus (SqMV), 179
watermelon chlorotic stunt virus (WmCSV), 179
watermelon mosaic virus (WMV), 176
zucchini tigré mosaic virus (ZTMV), 178
zucchini yellow fleck virus (ZYFV), 178
zucchini yellow mosaic virus (ZYMV), 177

W
Wasabi defensin gene
Botrytis cinerea, 225
cysteine-rich polypeptide, 225
Watermelon, 59, 60, 62, 64–67
Whitefly/whiteflies
Bemisia tabaci, 201, 202, 206, 209, 210
Trialeurodes vaporariorum, 201, 202, 207
Whole genome sequencing, 252
Whole-genome triplication, 6
Wild tomato species, 8
S. habrochaites, 107

Z
Zinc-finger nuclease (ZFNs), 240, 242, 245, 246