Compendium of Plant Genomes
Series Editor: Chittaranjan Kole

Ryutaro Tao
Zhengrong Luo *Editors*

The Persimmon Genome

Springer

Compendium of Plant Genomes

Series Editor

Chittaranjan Kole, President, International Climate Resilient Crop Genomics Consortium (ICRCGC), President, International Phytomedomics & Nutriomics Consortium (IPNC) and President, Genome India International (GII), Kolkata, India

Whole-genome sequencing is at the cutting edge of life sciences in the new millennium. Since the first genome sequencing of the model plant *Arabidopsis thaliana* in 2000, whole genomes of about 100 plant species have been sequenced and genome sequences of several other plants are in the pipeline. Research publications on these genome initiatives are scattered on dedicated web sites and in journals with all too brief descriptions. The individual volumes elucidate the background history of the national and international genome initiatives; public and private partners involved; strategies and genomic resources and tools utilized; enumeration on the sequences and their assembly; repetitive sequences; gene annotation and genome duplication. In addition, synteny with other sequences, comparison of gene families and most importantly potential of the genome sequence information for gene pool characterization and genetic improvement of crop plants are described.

Ryutaro Tao • Zhengrong Luo
Editors

The Persimmon Genome

Springer

Editors
Ryutaro Tao
Graduate School of Agriculture
Kyoto University
Kyoto, Japan

Zhengrong Luo
Key Laboratory of Horticultural
Plant Biology
Huazhong Agricultural University
Wuhan, China

ISSN 2199-4781 ISSN 2199-479X (electronic)
Compendium of Plant Genomes
ISBN 978-3-031-05583-6 ISBN 978-3-031-05584-3 (eBook)
https://doi.org/10.1007/978-3-031-05584-3

This book series is dedicated to my wife Phullara and our children Sourav and Devleena

Chittaranjan Kole

Preface to the Series

Genome sequencing has emerged as the leading discipline in the plant sciences coinciding with the start of the new century. For much of the twentieth century, plant geneticists were only successful in delineating putative chromosomal location, function, and changes in genes indirectly through the use of a number of "markers" physically linked to them. These included visible or morphological, cytological, protein, and molecular or DNA markers. Among them, the first DNA marker, the RFLPs, introduced a revolutionary change in plant genetics and breeding in the mid-1980s, mainly because of their infinite number and thus potential to cover maximum chromosomal regions, phenotypic neutrality, absence of epistasis, and codominant nature. An array of other hybridization-based markers, PCR-based markers, and markers based on both facilitated construction of genetic linkage maps, mapping of genes controlling simply inherited traits, and even gene clusters (QTLs) controlling polygenic traits in a large number of model and crop plants. During this period, a number of new mapping populations beyond F2 were utilized and a number of computer programs were developed for map construction, mapping of genes, and mapping of polygenic clusters or QTLs. Molecular markers were also used in the studies of evolution and phylogenetic relationship, genetic diversity, DNA fingerprinting, and map-based cloning. Markers tightly linked to the genes were used in crop improvement by employing the so-called marker-assisted selection. These strategies of molecular genetic mapping and molecular breeding made a spectacular impact during the last one and a half decades of the twentieth century. But still, they remained "indirect" approaches for elucidation and utilization of plant genomes since much of the chromosomes remained unknown and the complete chemical depiction of them was yet to be unraveled.

Physical mapping of genomes was the obvious consequence that facilitated the development of the "genomic resources" including BAC and YAC libraries to develop physical maps in some plant genomes. Subsequently, integrated genetic–physical maps were also developed in many plants. This led to the concept of structural genomics. Later on, emphasis was laid on EST and transcriptome analysis to decipher the function of the active gene sequences leading to another concept defined as functional genomics. The advent of techniques of bacteriophage gene and DNA sequencing in the 1970s was extended to facilitate the sequencing of these genomic resources in the last decade of the twentieth century.

As expected, sequencing of chromosomal regions would have led to too much data to store, characterize, and utilize with the-then available computer software could handle. But the development of information technology made the life of biologists easier by leading to a swift and sweet marriage of biology and informatics, and a new subject was born—bioinformatics.

Thus, the evolution of the concepts, strategies, and tools of sequencing and bioinformatics reinforced the subject of genomics—structural and functional. Today, genome sequencing has traveled much beyond biology and involves biophysics, biochemistry, and bioinformatics!

Thanks to the efforts of both public and private agencies, genome sequencing strategies are evolving very fast, leading to cheaper, quicker, and automated techniques right from clone-by-clone and whole-genome shotgun approaches to a succession of second-generation sequencing methods. The development of software for different generations facilitated this genome sequencing. At the same time, newer concepts and strategies were emerging to handle sequencing of the complex genomes, particularly the polyploids.

It became a reality to chemically—and so directly—define plant genomes, popularly called whole-genome sequencing or simply genome sequencing.

The history of plant genome sequencing will always cite the sequencing of the genome of the model plant Arabidopsis thaliana in 2000 that was followed by sequencing the genome of the crop and model plant rice in 2002. Since then, the number of sequenced genomes of higher plants has been increasing exponentially, mainly due to the development of cheaper and quicker genomic techniques and, most importantly, the development of collaborative platforms such as national and international consortia involving partners from public and/or private agencies.

As I write this preface for the first volume of the new series "Compendium of Plant Genomes", a net search tells me that complete or nearly complete whole-genome sequencing of 45 crop plants, eight crop and model plants, eight model plants, 15 crop progenitors and relatives, and three basal plants is accomplished, the majority of which are in the public domain. This means that we nowadays know many of our model and crop plants chemically, i.e., directly, and we may depict them and utilize them precisely better than ever. Genome sequencing has covered all groups of crop plants. Hence, information on the precise depiction of plant genomes and the scope of their utilization are growing rapidly every day. However, the information is scattered in research articles and review papers in journals and dedicated web pages of the consortia and databases. There is no compilation of plant genomes and the opportunity of using the information in sequence-assisted breeding or further genomic studies. This is the underlying rationale for starting this book series, with each volume dedicated to a particular plant.

Plant genome science has emerged as an important subject in academia, and the present compendium of plant genomes will be highly useful to both students and teaching faculties. Most importantly, research scientists involved in genomics research will have access to systematic deliberations on the plant genomes of their interest. Elucidation of plant genomes is of interest not only for the geneticists and breeders but also for practitioners of an array of plant science disciplines, such as taxonomy, evolution, cytology,

physiology, pathology, entomology, nematology, crop production, biochemistry, and obviously bioinformatics. It must be mentioned that information regarding each plant genome is ever-growing. The contents of the volumes of this compendium are, therefore, focusing on the basic aspects of the genomes and their utility. They include information on the academic and/or economic importance of the plants, a description of their genomes from a molecular genetic and cytogenetic point of view, and the genomic resources developed. Detailed deliberations focus on the background history of the national and international genome initiatives, public and private partners involved, strategies and genomic resources and tools utilized, enumeration of the sequences and their assembly, repetitive sequences, gene annotation, and genome duplication. In addition, synteny with other sequences, comparison of gene families, and, most importantly, the potential of the genome sequence information for gene pool characterization through genotyping by sequencing (GBS) and genetic improvement of crop plants have been described. As expected, there is a lot of variation of these topics in the volumes based on the information available on the crop, model, or reference plants.

I must confess that as the series editor, it has been a daunting task for me to work on such a huge and broad knowledge base that spans so many diverse plant species. However, pioneering scientists with lifetime experience and expertise in the particular crops did excellent jobs editing the respective volumes. I myself have been a small science worker on plant genomes since the mid-1980s and that provided me the opportunity to personally know several stalwarts of plant genomics from all over the globe. Most, if not all, of the volume editors, are my longtime friends and colleagues. It has been highly comfortable and enriching for me to work with them on this book series. To be honest, while working on this series, I have been and will remain a student first, a science worker second, and a series editor last. And I must express my gratitude to the volume editors and the chapter authors for providing me the opportunity to work with them on this compendium.

I also wish to mention here my thanks and gratitude to the Springer staff, particularly Dr. Christina Eckey and Dr. Jutta Lindenborn for the earlier set of volumes and presently Ing. Zuzana Bernhart for all their timely help and support.

I always had to set aside additional hours to edit books beside my professional and personal commitments—hours I could and should have given to my wife, Phullara, and our kids, Sourav and Devleena. I must mention that they not only allowed me the freedom to take away those hours from them but also offered their support in the editing job itself. I am really not sure whether my dedication to this compendium to them will suffice to do justice to their sacrifices for the interest of science and the science community.

New Delhi, India Chittaranjan Kole

Preface

The genus *Diospyros* includes about 500 species, which accounts for the largest genus of the family Ebenaceae. Most of the *Diospyros* species are distributed in the tropical and subtropical regions of the world. Some of the tropical *Diospyros* species are used as ebony wood. The temperate species, such as *D. kaki*, *D. oleifera*, *D. lotus*, *D. rhombifolia*, and *D. virginiana*, have horticultural and economic importance. *D. lotus* is generally cultivated as rootstock, while *D. oleifera* is mainly used to obtain persimmon oil (kaki tannins) in China. *D. rhombifolia* is an ornamental shrub in East Asia. *D. virginiana* is native to the Eastern USA and eaten as fresh or used as rootstock. *D. kaki*, often referred to as simply kaki, or Japanese or oriental persimmon, is a major commercial species for fruit production. *D. kaki* originated in China and has been cultivated for a long time in the East Asian countries. Recently, this fruit species has been cultivated as a new and exotic fruit in Italy, Spain, Brazil, Israel, Azerbaijan, and Uzbekistan. The total amount of persimmon production has been increasing constantly every year these days. More than 2000 accessions of persimmon are preserved in China, Japan, Korea, Italy, Spain, and some other countries.

The species with precise chromosome numbers account for approximately 10% of the total *Diospyros* species. Most of the diploid species were found in the tropical or subtropical zone of the globe, while polyploid species, including *D. kaki*, are distributed in temperate regions. High ploidy (2n = 6X or 9X = 90 or 135) level of *D. kaki* is often an obstacle to efficient genetic studies for a long time. However, the recent advancement in molecular biology and DNA sequencing technologies opens the door for the utilization of genomic and genetic approaches to study and improve this species.

We here have compiled the book *The Persimmon Genome* with contributions from eminent persimmon researchers from Italy, Spain, Japan, and China to summarize the latest information on persimmon studies with a special reference to the recent status of persimmon genome studies. Each chapter of this book well describes respective aspects of conventional, molecular, and genomic studies in *Diospyros*. Although persimmons had been long regarded as an East Asian exotic fruit tree, it is now becoming a worldwide fruit tree. As the editors of this book, we hope that this book will make more scholars become interested in persimmon research, and expand the scope of persimmon production and research.

Kyoto, Japan — Ryutaro Tao
Wuhan, China — Zhengrong Luo

Contents

Abbreviations

ADH	Alcohol Dehydrogenase
AIC	Akaike Information Criterion
ALDH	Aldehyde Dehydrogenase
ANR	Anthocyanidin Reductase
ANS	Anthocyanidin Synthase
AST	Astringency
BA	Benzyl Adenine
BAC	Bacterial Artificial Chromosomes
C	Catechin
CDS	Coding Sequence
CEGMA	Core Eukaryotic Gene Mapping Approach
CG	C-3-O-gallate
CHI	Chalcone Isomerase
CHS	Chalcone Synthase
CNR	Cell Number Regulator
C-PCNA	Chinese PCNA
cpDNA	Chloroplast DNA
CPPU, KT30	N-(2-Chloro-4-pyridyl)-N′-phenylurea
CTs	Condensed Tannins
DEG	Differential Expressed Gene
DEMs	Differentially Expressed miRNA
DFR	Dihydroflavonol 4-reductase
EC	Epicatechin
ECG	EC-3-O-gallate
EGC	Epigallocatechin
EGCG	EGC-3-O-gallate
F	Inbreeding Coefficient
F3′5′H	Flavonoid 3′5′-hydroxylase
F3′H	Flavonoid 3′-hydroxylase
FAOSTAT	Statistics Division, Food and Agriculture Organization of the United Nations
FISH	Fluorescent in Situ Hybridization
FWs	Fruit Weights
GC	Gallocatechin
GCG	GC-3-O-gallate

GISH	Genomic in Situ Hybridization
GO	Gene Ontology
HKT	High-Affinity Potassium Transporter
HPLC–ESI–MS/MS	High-Performance Liquid Chromatography–Electrospray Ionization Tandem–Mass Spectrometry
HTN	Hiratanenashi
IR	Identical Inverted Repeat Regions
IRAP	Inter-Retrotransposon Amplified Polymorphism
ISSR	Inter-Simple Sequence Repeat
ISTR	Inverse Sequence-tagged Repeat
ITS	Internal Transcribed Spacer
IVIA	The Instituto Valenciano de Investigaciones Agrarias, Spain
J-PCNA	Japanese PCNA
KEGG	Kyoto Encyclopedia of Genes and Genomes
LAR	Leucoanthocyanidin Reductase
LSC	Large Single-Copy Region
LTR	Long Terminal Repeat
MAS	Marker Assisted Selection
MBW	MYB-bHLH-WD40 Complex
MDGR	Male Diospyros spp. Germplasm Resources
MeGI	Male Growth Inhibitor
ML	Maximum Likelihood
MP	Maximum Parsimony
MpV	Mid-parental Value
MSY	Male-Specific Region of the Y-chromosome
mtDNA	Mitochondrial DNA
NARO	The National Agriculture and Food Research Organization, Japan
NFGP	National Field Genebank for Persimmon
NGS	Next Generation Sequencing
NHX	Na^+/H^+ Exchanger Family
non-PCA	Non-PCA
non-PCNA	Non-PCNA
OGI	Oppressor of MeGI
PAL	Phenylalanine Ammonia-lyase
PAR	Pseudoautosomal Regions
PAs	Proanthocyanidins
PCA	Pollination Constant & Astringent
PCD	Programmed Cell Death
PCNA	Pollination Constant & Non-Astringent
PDC	Pyruvate Decarboxylase
PHYLIP	Phylogeny Inference Package
PK	Pyruvate Kinase
PVA	Pollination Variant & Astringent
PVNA	Pollination Variant & Non-Astringent
QTL	Quantitative Trait Locus
RAD seq	Restriction Site Associated DNA Sequencing

RAPD	Random Amplified Polymorphic DNA
REMAP	Retrotransposon-microsatellite Amplified Polymorphism
RFLP	Restriction Fragment Length Polymorphism
SCAR	Sequence Characterized Amplified Region
SCoT	Start Codon Targeted
SCTs	Soluble Condensed Tannins
SiMeGI	Sister of MeGI
SMRT	Single-Molecule Real-Time
SNP	Single Nucleotide Polymorphism
SOS	Salt Overly Sensitive
SRAP	Sequence-Related Amplified Polymorphism
SSAP	Sequence-Specific Amplified Polymorphism
SSC	Soluble Solids Concentration
SScR	Small Single-copy Region
SSR	Simple Sequence Repeat
TBR	Tree-Bisection-Reconnection
TDZ	Thidiazuron
TEs	Transposable Elements
TFs	Transcription Factors
TRAP	Targeted Region Amplified Polymorphism
TTN	Totsutanenashi
WAB	Weeks after Bloom
WGCNA	Weighted Gene Coexpression Network Analysis
WGD	Whole-Genome Duplication Events
WPGR	Wild Persimmon Germplasm Resources
WUE	Water Use Efficiency

History and Current Status of Worldwide Production

1

Edgardo Giordani

Abstract

Persimmon is cultivated in many regions of the globe. Worldwide yearly production is increasing in absolute values and in comparison with other fruit species since 1961. FAOSTAT Database reports for 2019, a production of 4,270,074 t, an area harvested of 992,425 ha, and an average yield of 4.3 t/ha. Asia is the continent showing the highest contribution to persimmon production (about 87%), followed by Europe (almost 10%), America (3.5%), and Oceania (less than 1%); no data are reported in the FAOSTAT Database for Africa. Americas and Europe show an increasing trend of yields since the year 2000 with the highest value in 2017 (22.1 t/ha), in Oceania, the values are lower (about 12 t/ha), while the lowest and constant yields (about 4 t/ha) were observed for Asia. The countries producing more than 10,000 t of persimmons per year from FAOSTAT Database in 2019 are China (3,092,000 t), Spain (404,131 t) Korea (298,382 t), Japan (224,900 t), Brazil (182,185 t), Azerbaijan (147,219 t), Uzbekistan (88,233 t), Italy (49,675 t), Israel (29,000 t), and Iran (24,257 t). In most of those countries, the trend of production is increasing, with the exception of Japan, Italy, and Mexico. The persimmon contribution to total fruit production at a global level is increasing (from 0.32% to 0.52% in years 1985 and 2015, respectively) and Azerbaijan, Korea, and Japan are the countries with the highest values (from 4% to almost 10%). Persimmon is placed 18th (after strawberries, dates, and avocado and before kiwifruits, apricots, and cherries, among others) in the list of 33 fruit crops itemized in the FAOSTAT Database for the year 2015.

1.1 Introduction

Persimmon is the common word adopted to indicate in the English language, the botanical species *Diospyros kaki* Thunb. Nevertheless, the origin of this term seems to be ascribable to North American native Algonquin language to indicate *Diospyros virginiana* L., known as American persimmon. *Diospyros kaki* is also termed as Kaki and Oriental persimmon. In this chapter, both persimmon and kaki will be used in reference to *D. kaki* Thunb., that represents by far the most important crop for which official production data is available, while the amount of fruits of *D. virginiana*, *D. lotus,* and other *Diospyros* species with edible fruits are negligible when compared to the first one.

E. Giordani (✉)
Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Florence, Italy
e-mail: edgardo.giordani@unifi.it

R. Tao and Z. Luo (eds.), *The Persimmon Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-05584-3_1

This chapter illustrates persimmon production data gathered from the FAOSTAT Database (FAOSTAT, 2021), with some integration obtained from published documents and personal communications. Fruit production (in metric tons), harvested surface (in hectares), and yield (metric tons/ha) will be the items of discussion. The series of data available from FAOSTAT Database from 1961 to 2019 allows to have quite a clear picture of the evolution of the cultivation and production of persimmon in quantitative terms, nevertheless, limitations of the results due to the possible absence of data and/or of data estimations at country level, must be acknowledged.

1.2 Origin and Early Diffusion

China is recognized as the center of origin and first diffusion of persimmon, where it is widely distributed in most provinces, except the cold areas (Zhang et al. 2018). The retrieval of charred seeds in the Neolithic Tianluoshan site in the lower Yangtze River valley suggests very ancient use of persimmon fruits in that Chinese geographic area (Gupta et al. 2013). Ancient written and painted records of the use of persimmon show that this crop is present since many centuries in Japan and Korea (Yamada 2005; Yamada et al. 2012). *Diospyros kaki* seems to have diffused toward western areas of the Euro-Asiatic continent through caravans along the Silk Road, reaching Turkey, and probably the Mediterranean area in ancient times. Successively, Spanish and Portuguese colonial trades and migrations from oriental countries expanded the diffusion of persimmon to the Americas, Europe, and Oceania. In the last sixty years, globalization has augmented the transport of propagation material for the planting of many fruit crops, including persimmon (Hulme 2009), hence this species spread in many areas of the world with adequate climatic conditions.

1.3 Evolution of the Worldwide Production

Worldwide, the yearly production of persimmon fruits was about 1,000,000 tons in the decades 1960, 1970, and 1980, overcoming 4,270,000 t from 2012 up to 2019 (Fig. 1.1). Within this lapse of time, the yearly amounts of produced persimmons are highly correlated ($r = 0.97$) to those of the overall fruit production ("Fruit primary" aggregated group in the FAOSTAT Database); a similar correlation was also observed for the area harvested ($r = 0.93$). The production increase fourfolds the initial amounts of 50 years ago is confirmed by the raw data and the linear trend line (Fig. 1.1). Such relevant evolution in quantitative terms can be attributed to the actual increase of production and the inclusion of the statistics of persimmon production of new countries in the FAOSTAT

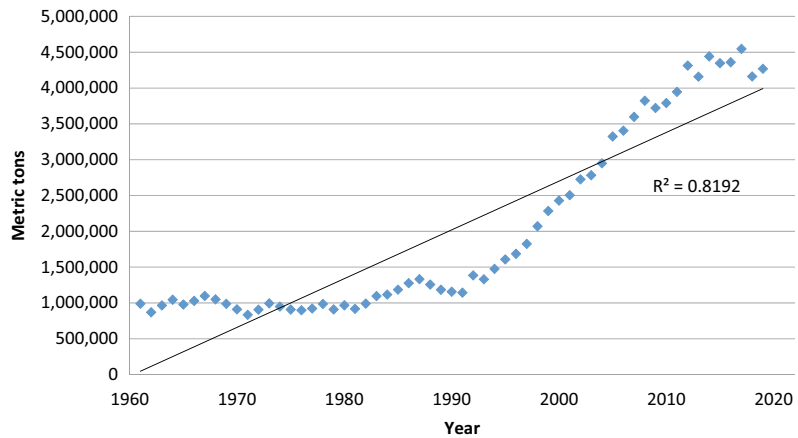

Fig. 1.1 Trend of worldwide persimmon production in the last sixty years (FAOSTA, 2021)

Database. For instance, during the period 1960–1970, the number of countries contributing to persimmon production as from FAO statistics was 6 (China mainland, China-Taiwan, Brazil, Italy, Japan, and Republic of Korea), 9 in the successive decade, and 17 after the year 2017. Taking into account that in some cases yearly data can be referred to estimations, fluctuations of production between one year and the successive resulted close to zero in many cases, while reaching percentages of plus or less than 10–12% in other consequent years. The persimmon area harvested in the 1960 decade was about 160,000 ha; it overcome 500,000 ha in year 2000 and reached almost 1,000,000 ha in 2019. The correlation between persimmon production and area harvested is linear ($r = 0.99$), thus showing a steady trend in the last sixty years of persimmon yields (~5 t/ha) as from FAO statistics. Indeed, this value does not represent the actual usually observed yields of persimmon orchards of most countries, which ranges from 15 up to 50 t/ha.

1.4 Evolution of the Production Among Continents

Persimmon is spread over the five continents, nevertheless, the amount of production widely varies among them taking into account FAOSTAT data. Figure 1.2 shows the contribution in percentage of each continent in year 2019. There is no FAOSTAT data for persimmon production in Africa, while Asia is by far the continent with the highest production all along the five decades of available data with about 1,000,000 t of yearly production in the decade 1960–1970 and over 4,000,000 t in the last few years. Europe is the second-largest producer continent, with over 300,000 t in the last five years and a very significant increase of yearly production since year 2000 substantially due to the Spanish production. The American continent is producing almost 170,000 t/year, with an increment of 0.18 t/year since the 1960 decade, reaching almost 200,000 t in 2015 but showing a minor reduction of production in the last few years. The first statistic

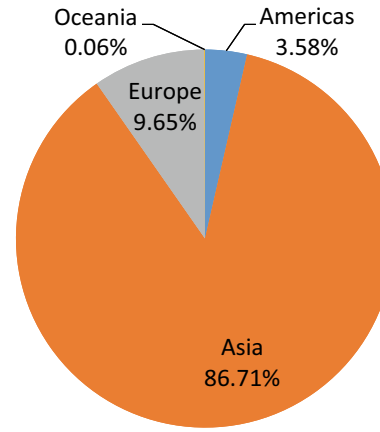

Fig. 1.2 Contribution of continents in the persimmon production in year 2019 (FAOSTA, 2021)

data on persimmon production in Oceania is dated 1983 (less than 100 t), its yearly production reached the maximum amount in the years 2000 (almost 4000 t/year) while it was below 3000 t during the last three years.

Yields (Fig. 1.3) are different among continents; Americas and Europe show an increasing trend since year 2000 with the highest values in 2017 (21.6 and 22.1 t/ha, respectively), while in Oceania the values are lower (from 7 to 12 t/ha); the lowest and stable yields (about 4 t/ha) from FAOSTAT data were found for Asia.

The amount of countries contributing to the continental production is increasing with time; during the period 1990–2015, the new entries were Chile in Americas, Uzbekistan in Asia,

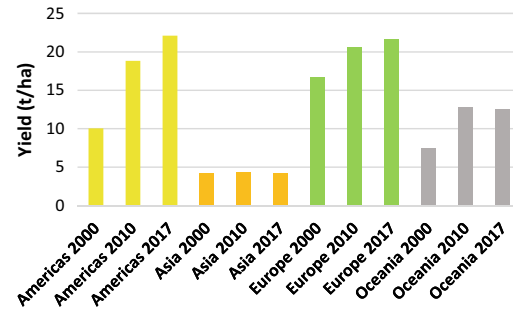

Fig. 1.3 Trend of average yields of persimmon orchards in Americas, Asia, Europe, and Oceania (FAOSTAT, 2019)

Azerbaijan, Spain, and Slovenia in Europe (FAOSTAT, 2021).

1.5 Evolution by Country

The evolution of the persimmon production by country is often hindered by the lack of available data. Indeed, FAOSTAT includes official data transmitted by countries but, as an example, Spain was not included in the FAO statistics for many years, even when its persimmon production was relevant at the national level, but also in reference to the worldwide production. In this section, production by country takes into account official data from FAOSTAT Database.

1.5.1 Persimmon Production in Asian Countries

Among the countries where persimmon is traditionally cultivated and production statistics are present in FAOSTAT Database since 1961, China (Fig. 1.4) is showing the highest yearly production in the world (3,247,068 t on a harvested area of 992,425 ha in 2019, contributing to 76% to the worldwide production) with a yearly average increase of 55,000 t/year from 1961 to 2019 and 87,000 t/year in the period 1990–2019.

The persimmon production trend in Korea (Fig. 1.5), a country with a pluricentennial tradition of cultivation of this crop, shows an increase of about 2300 t/year between 1960 and 1980 followed by a more relevant growth rate (about 10,000 t/ha) between 1980 and 2000, year in which the production resulted close to 300,000 t. In the last 20 years, the Korean persimmon production oscillated widely among years, from about 249,000 in 2003 to 430,000 t in year 2008, being the latter the highest production amount ever registered for this country. In 2019, the production reached 316,042 t with a yield of 12 t/ha (FAOSTAT, 2019).

Persimmon cultivation in Japan started very old time, nevertheless, the evolution of persimmon production in Japan in the last sixty years is characterized by a negative trend (Fig. 1.6). The average Japanese persimmon production in the decade 1960 was about 414,000 t/year, while from 2010 to 2019, it is 225,000 t/year, with a maximum value of 504,400 t in 1967 and a minimum in the year 2010 (198,400 t). The average yield of the two periods taken into account resulted constant (about 11 t/ha as from FAOSTAT Database).

Uzbekistan's statistics on persimmon production in FAOSTAT (Fig. 1.7) start from year 2000 with 16,000 t while the 2019 harvest reached 94,065 t, with a positive trend of production increment, as well as of yield (from about 6 t/ha to about 20 t/ha, in 2000 and 2019, respectively).

The trend of persimmon production in Israel is positive since 1975 (Fig. 1.8), which is the first year of data reported in the FAO Database. Starting from about 8200 t/year during the first decade of available data (1975–1985), in the last decade (2010–2019) the average yearly

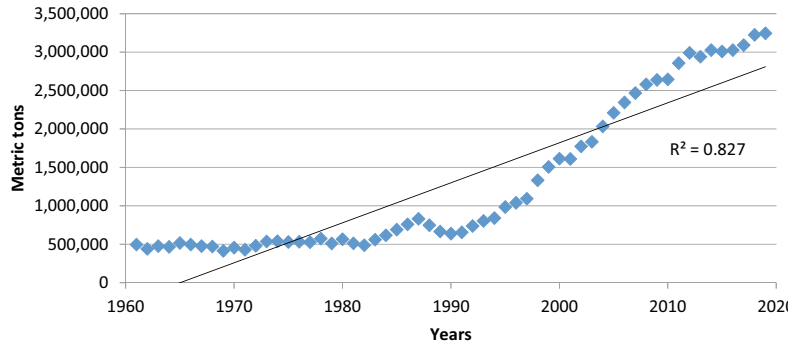

Fig. 1.4 Persimmon production trend in China (FAOSTAT, 2021)

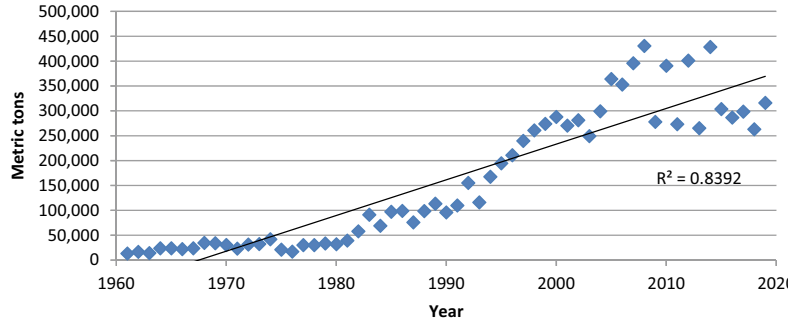

Fig. 1.5 Persimmon production trend in Korea (FAOSTAT, 2021)

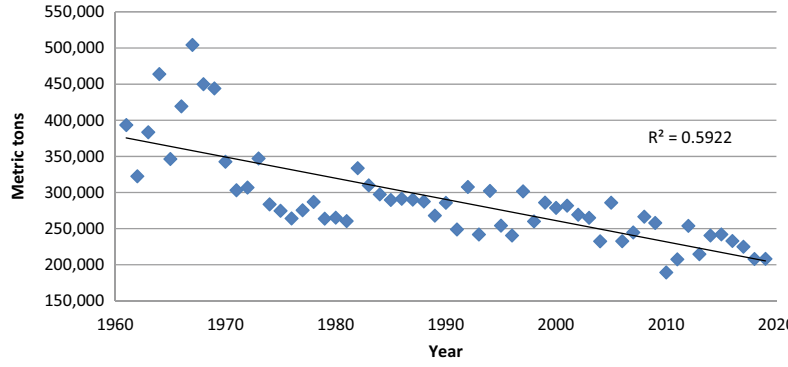

Fig. 1.6 Persimmon production trend in Japan (FAOSTAT, 2021)

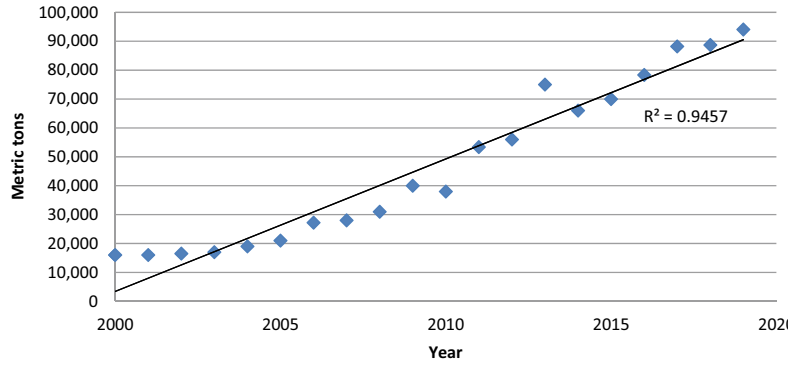

Fig. 1.7 Persimmon production trend in Uzbekistan (FAOSTAT, 2021)

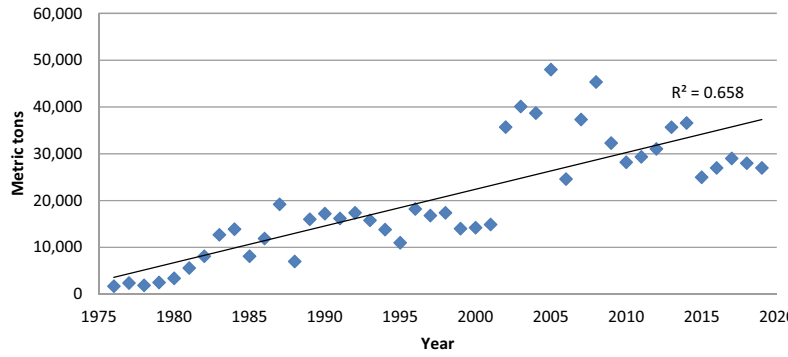

Fig. 1.8 Persimmon production trend in Israel (FAOSTAT, 2021)

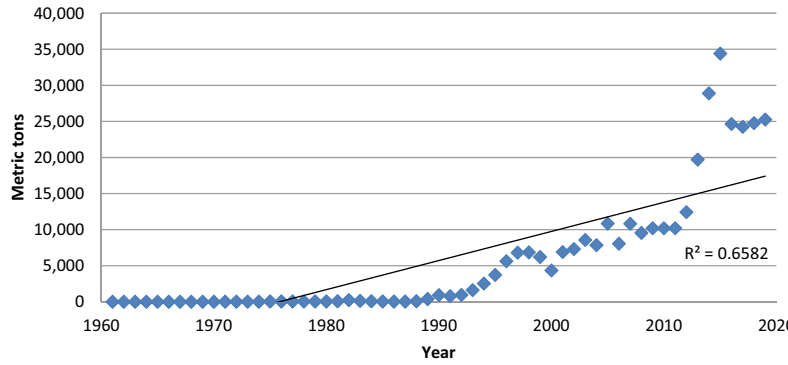

Fig. 1.9 Persimmon production trend in Iran (FAOSTAT, 2021)

production was close to 30,000 t. An increase in yield from 9 to 21 t/ha was also observed between the average values for the first and the last decades, respectively. The amount of harvested fruits in 2019 was 27,000 t.

The Iranian persimmon production reached 25,272 t in 2019 (Fig. 1.9), with an average of 21 t/year during the decade 1960 and of about 20,000 t/year in the last ten years. The yield increased from 4.3 to 14.3 t/ha in the same period.

Nepal has an increasing trend of persimmon production since 1990, when the yearly production was about 400 t; in the last decade, the amount of yearly harvested persimmons was about 2500 t.

1.5.2 Persimmon Production in European Countries

The Spanish persimmon production appeared for the first time in FAO statistics in 1991 (1222 t) reaching in 2019, a production of 404,131 t on a harvested area of 18,526 ha and a yield of 21.8 t/ha. The evolution in Spain of persimmon cultivation from the year 2000 has been exponential (Fig. 1.10). This species has been neglected for decades and fruits were only locally marketed until the birth of the industrial persimmon production due to the identification and propagation of an outstanding local variety (Rojo Brillante), and the implementation of cultivation and post-harvest techniques (Llacer and Badenes 2001).

The statistics of persimmon production from Azerbaijan appeared for the first time in the FAO database in 1992 (48,788 t) with a constant increase in yearly production till 2019 (177,130 t, with a yield of 16.2 t/ha), and with a yearly average increment of production of about 3500 t (Fig. 1.11).

In Italy, where the cultivation of persimmon started about one century ago, the production of persimmon shows a negative trend in the last sixty years. In the decade 1960–1970, the

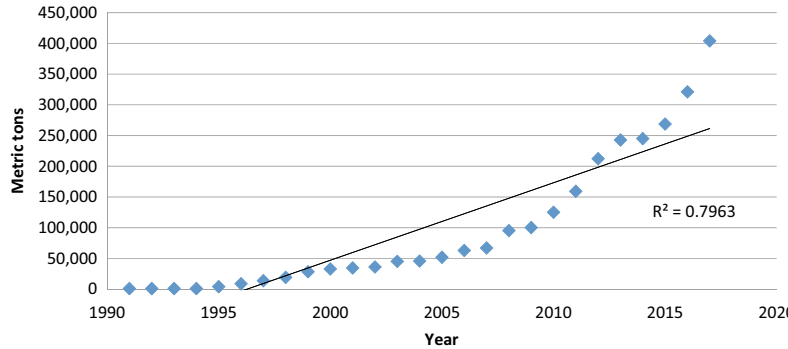

Fig. 1.10 Persimmon production trend in Spain (FAOSTAT, 2021)

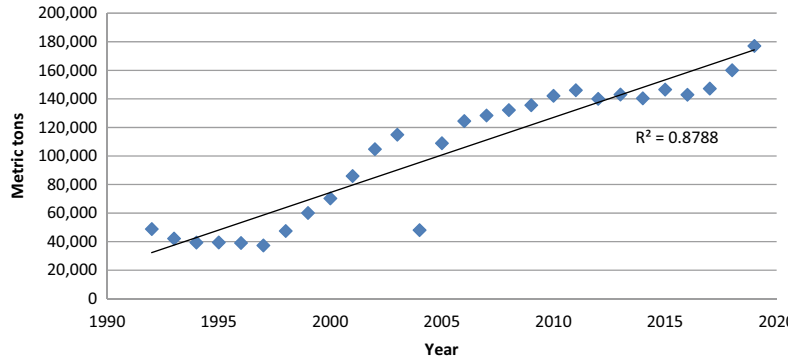

Fig. 1.11 Persimmon production trend in Azerbaijan (FAOSTAT, 2021)

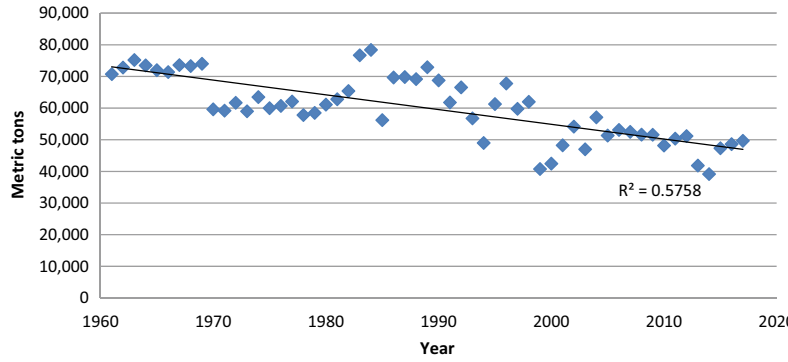

Fig. 1.12 Persimmon production trend in Italy (FAOSTAT, 2021)

average yearly production was about 73,000 t while in the period 2010–2019, it dropped to about 49,000 t. The average yield were 18 and 19 t/ha for the two periods, respectively. The highest Italian production in the last 50 years was reached in 1984 (78,400 t) while the lowest was registered in 2014 (39,149 t) (Fig. 1.12).

Slovenia shows an increasing trend in persimmon production. From the first available data (about 500 t/year), the production of the last three years overcome 2000 t/year.

1.5.3 Persimmon Production in American Countries

In Brazil, the evolution of persimmon production in the last sixty years shows a positive trend. From 18,403 t/year (average of the decade 1960), in the last ten years, Brazil has harvested about 170,000 t/year, almost tenfolds the initial value (Fig. 1.13). A relevant increase in the yield is observed, from about 6 t/ha sixty years ago to over 20 t/ha (average of the last ten years).

Mexico shows a decreasing trend in persimmon production, which is particularly relevant in the last few years. The amount of yearly harvested persimmons ranged from 200 to 500 t between years 1985 and 2010, and collapsed in the last few years to about 50 t/year (Fig. 1.14).

The Chilean persimmon production is increasing in time and almost doubled from about 300 t/year of the 1990 decade to 550 t/year in the last ten years.

1.5.4 Persimmon Production in Oceanic Countries

Persimmon production in Australia shows a positive trend, from about 300–700 t for the decades 1980 and 2010, respectively; nevertheless, the total amount of yearly harvested persimmons never reached 1000 t.

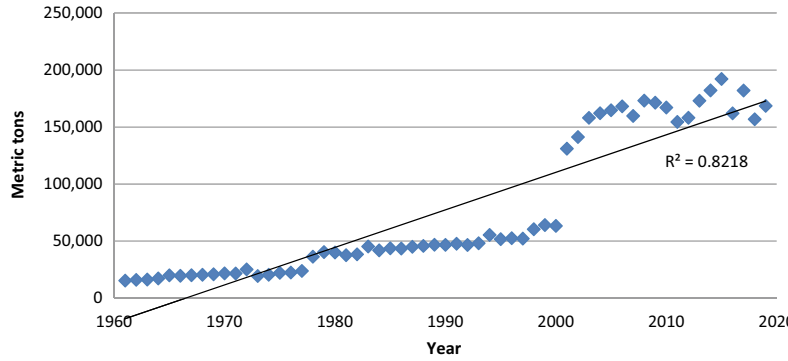

Fig. 1.13 Persimmon production trend in Brazil (FAOSTAT, 2021)

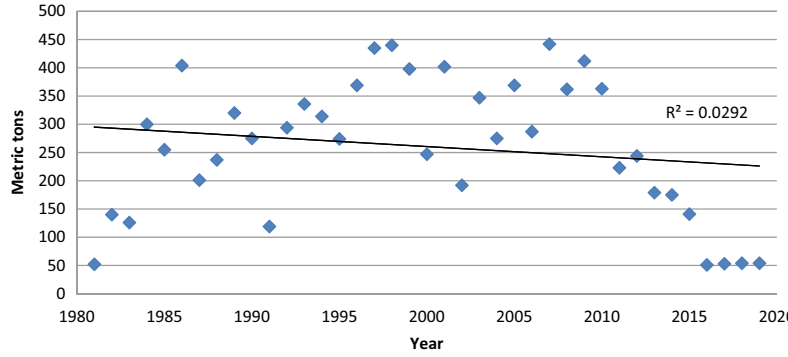

Fig. 1.14 Persimmon production trend in Mexico (FAOSTAT, 2021)

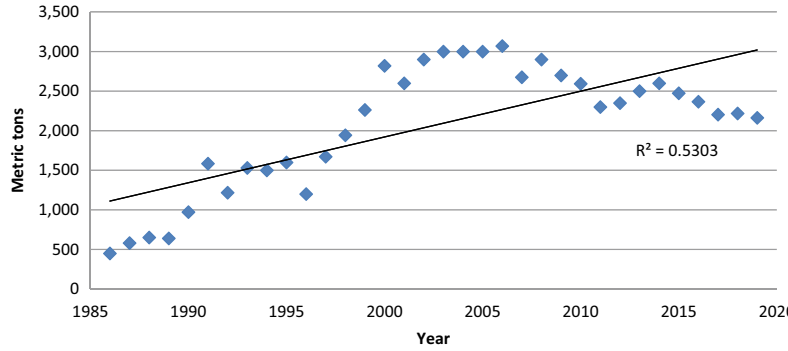

Fig. 1.15 Persimmon production trend in New Zealand (FAOSTAT, 2021)

In New Zealand, despite the positive trend over 35 years, yearly persimmon production showed a peak of about 3000 t in the period 2003–2006, and a reduction of production in the last ten years (2400 t per year as an average) with 2164 t registered in the year 2019 (Fig. 1.15). Yields increased from 2.5 (average of the decade 1986–1996) to 15.6 t/ha in the last ten years.

The trend of persimmon production at worldwide and country-level compared to the aggregate of total fruit production from FAOSTAT Database for the available data of the years 1985, 2000, and 2015 is shown in Fig. 1.16. In percentage, persimmon contribution to total fruit production at the global level is increasing (from 0.32 to 0.52% in years 1985 and 2015, respectively). Compared with other major fruit crops fruits (e.g., bananas, watermelon, apples, grapes, and oranges, contributing each one for about 10% of the total fruit production),

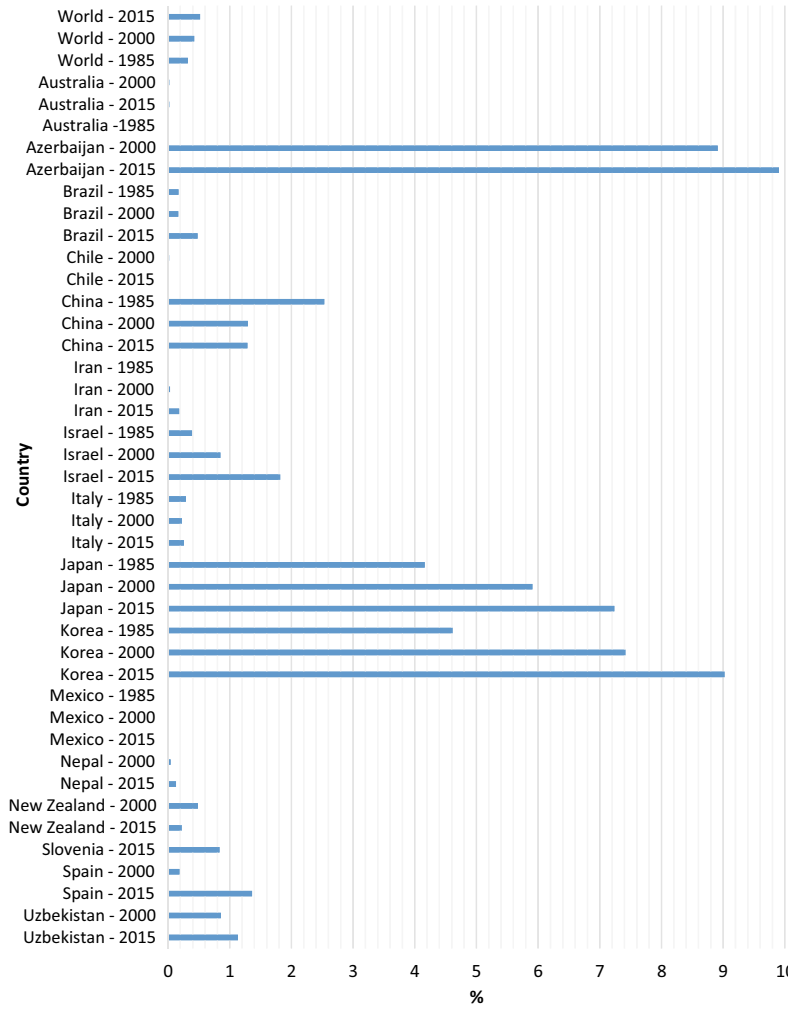

Fig. 1.16 Contribution (%) of persimmon to the total amount of fruit production in the world and per country (FAOSTAT, 2021)

persimmon is placed as 18th (after strawberries, dates, and avocado and before kiwifruits, apricots, and cherries) in the list of 33 fruit crops itemized in the FAOSTAT Database for the year 2015. The trend per country of the contribution of persimmon to the national fruit production shows different situations, being Azerbaijan, Korea, and Japan the countries with the highest (from 4% to almost 10%) and increasing values in time. More in detail, the relative weight of persimmon production in Korea is due to the increase in persimmon production, while in Japan, it is affected by the general decrease of fruit production in the observed years. In China, the percentage (about 2.5% in 1985 and 1% in 2015) is decreasing in time, essentially due to the relevant increase of fruit crops production, while in Brazil, Israel, and Spain it is increasing and in Italy it is steady.

References

FAOSTAT Database. http://www.fao.org/faostat

Gupta NS, Leng Q, Yang H, Cody GD, Fogel ML, Liu W, Sun G (2013) Molecular preservation and bulk isotopic signals of ancient rice from the Neolithic Tianluoshan site, lower Yangtze River valley, China. Org Geochem 63:85–93

Hulme PE (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization.

J App Ecology 46(1):10–18. https://doi.org/10.1111/j.1365-2664.2008.01600.x

Llacer G, Badenes ML (2001) Persimmon production and market. In: Bellini E, Giordani E. (eds.) First Mediterranean symposium on persimmon. Zaragoza. CIHEAM, 2002, 126 p (Options Méditerranéennes: Série A. Séminaires Méditerranéens, 51:9–21)

Yamada M (2005) Persimmon genetic resources and breeding in Japan. Acta Hortic 685:55–64

Yamada M, Giordani E, Yonemori K (2012) Persimmon. In: Badenes ML, Byrne D (eds) Fruit breeding. In: Handbook of plant breeding, vol 8. Springer, Boston

Zhang Q, Yang Y, Xu L, Guo D, Luo Z (2018) Current persimmon research and industry in China mainland. Acta Hortic 1195:9–16

Origin, Evolution, Taxonomy and Germplasm

2

Qinglin Zhang and Zhengrong Luo

Abstract

Persimmon (*Diospyros kaki* Thunb.) is an ancient fruit tree that originate in East Asia, especially in Southern China. Persimmon is grown in China, Japan and Korea for a long history. Persimmon can be utilized for various purposes with fruits, leaves and its derivates. The genus *Diospyros* with about 500 species accounts for the largest genus of Ebenaceae. There is a close relationship between *D. kaki*, *D. lotus*, *D. glandulosa*, *D. oleifera* and 'Yemaoshi' based on morphological and molecular evidence. The ancestor of persimmon is not clearly elucidated. Modern hexaploidy persimmon should be evolved from diploid through genome duplication, in which *2n* gametes might play an important role. Persimmon cultivars can be horticulturally classified into four types including PCNA (pollination constant and non-astringent), PVNA (pollination variant and non-astringent), PVA (pollination variant and astringent) and PCA (pollination constant and astringent). PCNA is further classified into Chinese PCNA and Japanese PCNA based on their different genetic control of astringency loss traits. Persimmon genotypes with different geographic origins can be distinguished using various molecular markers, while four persimmon cultivar types could not be well-separated by molecular detection. PCNA cultivars from China and Japan exhibit a divergent origin, interestingly, both Chinese PCNA and Japanese PCNA originate in central mountain areas of the two countries, respectively. Most of the genetic resources of Ebenaceae were distributed in tropical and subtropical regions of the world. More than 2000 accessions of persimmon were preserved in China, Japan, Korea, Italy, Spain, etc. Most of the persimmon production was derived from PCA cultivars in China, which is the largest persimmon producer globally. PCNA fruit is more attractive and new orchards prefer to plant PCNA types in Asia, Mediterranean area, Oceania and South America.

2.1 Introduction

Persimmon (*Diospyros kaki* Thunb.) is a deciduous fruit tree belonging to the genus *Diospyros* containing about 500 species distributed in tropical, subtropical and temperate zone. The genus name *Diospyros* is derived from the Greek *Dios*, for the god Zeus (Jupiter) + *pyros*, wheat or grain, alluding to the edible fruit, indicating

Q. Zhang · Z. Luo (✉)
Key Laboratory of Horticultural Plant Biology, Huazhong Agricultural University, Wuhan 430070, China
e-mail: luozhr@mail.hzau.edu.cn

Q. Zhang
e-mail: zhangqinglin@mail.hzau.edu.cn

R. Tao and Z. Luo (eds.), *The Persimmon Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-05584-3_2

that the persimmon is the 'fruit of the gods'. The English word 'persimmon' is of American Indian derivation, specifically of Algonquian origin, approximating the Cree *putchamin*, *pasimian* or *pessamin*, meaning dried fruit (seventeenth–eighteenth c.). Due to it's commercially important nature, the scientific name of persimmon—*D. kaki*, persimmon is also simply called its species name 'kaki'.

There are several species (*D. kaki*, *D. oleifera*, *D. lotus*, *D. rhombifolia*, *D. virginiana*) of *Diospyros* that are of significant horticultural or economic importance. *D. lotus* and *D. oleifera* are cultivated as fruit crops or rootstock in China. *D. oleifera* is mainly used for obtaining persimmon oil (tannins) in ancient time in China or Japan. *D. kaki* and *D. lotus* can be consumed as fresh or dried fruit in many countries. *D. rhombifolia* is an ornamental shrub in China as it bears fruits with narrow elliptic shape and orange-red skin colour. The American persimmon *D. virginiana* is native to eastern USA and eaten as fresh or processed with a small scale market, or as rootstock. *D. kaki* is the major commercial species for cultivation and marketing.

Persimmon (*D. kaki*) is believed to have originated in the mountain area forests of southern China (Wilson 1929; Grubov 1967) and has been cultivated as an important fruit crop in China, Korea and Japan for a long history, and recently cultivated as an exotic fruit in Italy, Spain, Brazil, Israel, Azerbaijan and Uzbekistan with a remarkable production increasing.

The degree of natural deastringency in PCNA (pollination constant and non-astringent) types and difficulties in artificial astringency loss of non-PCNA types are variant among cultivars. Characteristic variations also occurred among different germplasm according to persimmon shape, size, flesh texture, bottom or cover colour of pericarp, flesh brown specks, seeds number, tannin cell size and density, fruit ripening period and applicability for postharvest process. In terms of flower sexuality, persimmon exhibits bisexual (hermaphrodite) and unisexual (male and female) flowers with cymose inflorescence. Thus, gynoecious (bearing female flowers only, most cultivars), androecious (bearing male flowers only, few cultivars), monoecious (bearing both male and female flowers, few cultivars) and trimonoecious (bearing both bisexual and unisexual flowers, rare cultivars) could be found in persimmon genetic resources. Distinguishable morphological and physiological differences resulted in graft compatible and incompatible phenomenon between different persimmon cultivars and general rootstock Dateplum (*D. lotus*).

Though current commercial persimmon for consumption is only derived from the species *D. kaki*, there are abundant trait variations among persimmon cultivars for long cultivation history with artificial selection. Persimmon cultivars may produce astringent inedible fruits or non-astringent edible fruits at harvest time depending on different types. On the nature of astringency loss and genetic trait, persimmon cultivars are classified into PCNA (pollination constant and non-astringent) and non-PCNA types. PCNA is desirable for consumption for its fruit is edible and crispy with firm flesh without any extra artificial treatment to remove astringency. Novel superior PCNA cultivar is an essential goal for persimmon breeding and genetic improvement.

Most persimmon production is usually consumed as fresh fruits (soft or hard), but dried fruits are also used in oriental countries and in Europe. Persimmon fruit can also be processed into jelly, cake, chips, juices, mousses, frozen puree for ice cream preparation, vinegar, alcoholic beverages and its distillates. Persimmon leaves tea making is popular in East Asia since it helps lowering blood pressure, eliminate cholesterol accumulating, and prevent melanin accumulation (Xie et al. 2015). Extracts of persimmon leaves, calyx, crystallization (glucose, sucrose, fructose and mannitol mixture) on dried persimmon skin, have been recorded as a supplement of Chinese traditional medicines. Red persimmon leaves in autumn are attractive for tourism and garden decoration. In Korea, unripe fruit is used for dyeing garments; in Japan, persimmon tannin extracted from young fruit has long been used as an antiseptic or waterproofing of paper used for umbrellas and for fishing nets. Persimmon wood is good for furniture, ornaments and golf club heads (Sugiura 1997).

The most important trait in persimmon is astringency which is due to abundant soluble tannins (proanthocyanidin, PAs) in the vacuole of the fruit flesh. Compared to other fruits, persimmon tannin is a kind of natural polymer of flavan-3-ol units and belongs to type B proanthocyanidin group with carbon–carbon links between carbon number 4 of one component and carbon number 8 or 6 of the other unit (Matsuo and Ito 1978). Persimmon fruit contains numerous adjacent phenolic groups, which can chelate metal ions. The low-cost and environmentally friendly metal (Au, Cr, Cu, Fr, Mo, Pb, Pd, Pt, U) bioadsorbents prepared from persimmon tannin show high adsorption efficiency (Ampiaw and Lee 2020). Persimmon tannin has also been used as alleviating alcoholic hangovers, clarifying agent in the production of Japanese sake, deodorant, detergent and cosmetic additives. Persimmon tannin exhibited a very strong inhibitory effect on Chinese cobra venom (Li et al. 2013). Recently, persimmon tannin is applied for a new field such as novel biomaterials, green chemistry and fine chemicals.

2.2 Origin and Evolution of Persimmon

2.2.1 Ancestor of Persimmon

Diospyros fall into the family Ebenaceae, that consists of two subfamilies (Duangjai et al. 2006): the small neotropical Lissocarpoideae (*Lissocarpa*, 9 species) and the pantropical Ebenoideae (*Diospyros*, *Euclea*, *Royena*, 500–600 species). Molecular phylogentics of the pantropical family Ebenaceae sensu lato (s.l.) and genus *Diospyros* from New Caledonia (an island group located in the southwestern Pacific about 1300 km east of Australia) were investigated using plastid DNA or genome sequence, amplified fragment length polymorphisms (AFLP), single nucleotide polymorphism (SNP) markers (Duangjai et al. 2006, 2009; Turner et al. 2013a, b, 2016; Paun et al. 2016; Samuel et al. 2019). Geeraerts et al. (2009) applied light microscopy (LM) and scanning electron microscopy (SEM) to explore palynology variation of Ebenaceae, in which pollen is heterogeneous. The oldest *Diospyros* species are around seven million years old and the youngest ones probably much less than one million years (Turner et al. 2013a).

Genome size was estimated by flow cytometry for hexaploid and nonaploid *D. kaki* and some other species. *D. kaki* has a DNA content with 2C = 5.00–5.24 pg (hexaploid) and 2C = 7.51–8.12 pg (nonaploid). *D. lotus* (diploid, 2C = 1.85 pg), *D. olerfera* (diploid, 2C = 1.76 pg), *D. glandulosa* (diploid, 2C = 1.85 pg), *D. virginina* (hexaploidy, 2C = 5.12 pg) and *D. rhombifolia* (tetraploid, 2C = 3.76 pg) were also used for DNA contents determination and chromosome number counting (Tamura et al. 1998).

Measurements of genome size showed *D. lotus* and *D. olen* with 1C = 0.86 pg, the smallest genome of the New Caledonian *Diospyros* species examined; *D. kaki* (1C = 2.29 pg) and *D. pancheri* (1C = 2.28 pg) account for the largest genome (Turner et al. 2013a). Although chromosome counts indicate that *Diospyros* spp. are consistently diploids with $2n = 30$, extensive variation in genome size has been observed, which is due to an increase of repeat elements, including LTR/gypsy (Samuel et al. 2019). Developing firmer ideas about evolution of genome size in *Diospyros* would require many more measurements of species from throughout the phylogenetic tree.

Persimmon (*D. kaki*) was distributed worldwide with high economic benefits, the origin of persimmon has not yet been well clarified and its progenitor is not clear. Ng (1978) proposed that *D. roxburghii* (syn. *D. glandulosa*) is synonymous with *D. kaki*. Based on morphological similarities and geographical subtropical regions of Southeast Asia, Ng (1978) suggested that *D. glandulosa* might be involved in the speciation of *D. kaki*. The chromosome number for *D. glandulosa* is $2n = 2x = 30$ (Somego 1978), while for *D. kaki* $2n = 6x$, $9x = 90$, 135 (Zhuang et al. 1990a, b). If Ng's hypothesis (1978) is correct, there should be genome duplication occurrence during the modern persimmon evolution.

Polymorphism was observed among five *Diospyros* species by restriction fragment length analyses of mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA), *D. kaki*, *D. oleifera*, *D. kuroiwai*, *D. virginiana* and *D. lotus* were found to be highly related to each other, but less related to *D. rhombifolia* (Nakamura and Kobayashi 1994). Phylogenetic studies with chloroplast DNA (*rbc*L-ORF106 and *trn*T-*trn*F) indicate that *D. kaki* seems to have a common ancestor with *D. lotus* and *D. virginiana* (Yonemori et al. 1998) and is closely related to *D. ehretioides*, a diploid species from Thailand. *D. kaki* is not directly linked to *D. glandulosa*, which presented more close relationship to *D. oleifera* than *D. kaki*, *D. lotus* and *D. virginiana* in the PHYLIP (phylogeny inference package) neighbour-joining tree (Yonemori et al. 1998). But *D. kaki* was not found to be closely related to *D. ehretioides* by internal transcribed spacer (ITS) and *mat*K data sets of 15 *Diospyros* species (Yonemori et al. 2008a). Results generated from studies on the sequences of the ITS and *mat*K regions (Yonemori et al. 2008a) supported the hypothesis of Ng (1978) that a close relationship between *D. kaki* and *D. glandulosa* and *D. oleifera*.

Yamagishi et al. (2005) reported that *D. kaki* is more closely related to *D. lotus* than to *D. oleifera* and *D. rhombifolia* using RAPD markers, consistent with RFLP analyses using the cpDNA probe (Yonemori et al. 1998). Phylogenetic tree obtained using chloroplast DNA PCR–RFLP markers for 23 *D. kaki* genotypes and 6 related species suggested the close relationship between *D. kaki* and *D. lotus* with *D. glaucifolia* (Hu et al. 2008). However, chloroplast genome sequences from *D. kaki*, *D. lotus*, *D. oleifera*, *D. glaucifolia* and 'Jinzaoshi' revealed a closer relationship between *D. kaki* and *D. oleifera* (Fu et al. 2016; Li et al. 2018).

A new candidate named 'Yemaoshi' collected in Yunnan province located in Southwest of China, was suggested to be an ancestor of *D. kaki* than the other three candidates (*D. glandulosa, D. oleifera* and *D. lotus*), due to morphological similarity of fruit, calyx and leaves and the survey of specimens at the herbariums (Yonemori et al. 2012).

The genome of *D. oleifera* was assembled and may facilitate to understand genome evolution of *Diospyros* plants (Zhu et al. 2019; Suo et al. 2020). The genome sequence of *D. lotus* sheds light on the evolution path into dioecy in *Diospyros* (Akagi et al. 2020).

Up to now, the ancestor of persimmon is not clarified. More evidence such as continuous fossil or germplasm by molecular analysis is needed.

2.2.2 Evolution of Persimmon

Diospyros is widely distributed from tropical to temperate regions and contains about 500 species, most of them distributed in the humid tropics of Asia, Africa and Central-South America. The most important temperate-zone species for commercial cultivation include *D. kaki*, *D. oleifera*, *D. lotus*, *D. rhombifolia*, *D. virginiana*.

There are four kind of ploidy level for genus *Diospyros*: diploid ($2n = 2x = 30$), tetraploid ($2n = 4x = 60$), hexaploid ($2n = 6x = 90$), nonaploid ($2n = 9x = 135$) (Namikawa and Higashi 1928; Zhuang et al. 1990a, b; Yang et al. 1999; Turner et al. 2013a). Therefore, the basic chromosome number of the genus *Diospyros* is regarded as 15. Chromosome numbers for other temperate zone *Diospyros* species are $2n = 30$ for *D. lotus* and *D. oleifera* and $2n = 60$ for *D. rhombifolia* (Zhuang et al. 1990b), while *D. virginiana* is reported to have two karyotypes, $2n = 4x = 60$ and $2n = 6x = 90$ (Baldwin et al. 1941; Pomper et al. 2020). Deyangshi ($2n = 4x = 60$), a special *Diospyros* resource with a short juvenile phase distributed in Deyang city, Sichuan province, China. Species with precise chromosome number account, approximately 10% of the total *Diospyros* species (Table 2.1), in which most of the diploid species were found in tropical or subtropical zone, while polyploid species were distributed in temperate region. This indicated *Diospyros* species with high ploidy level including modern persimmon

Table 2.1 Ploidy levels of some *Diospyros* species

Ploidy level	Species
$2n = 2x = 30$	*D. chevalieri*[1], *D. confertiflora*[1], *D. decandra*[1], *D. dichrophylla*[1], *D. discolor*[1], *D. fasciculosa*[3], *D. fischeri*[1], *D. galpinii*[1], *D. glabra*[1], *D. glandulifera*[1], *D. glandulosa*[1], *D. glaucifolia*[2], *D. heudelotii*[1], *D. inconstans*[3], *D. inhacaensis*[1], *D. lotus*[1], *D. macrocarpa*[3], *D. mannii*[1], *D. mespiliformis*[1], *D. minimifolia*[3], *D. mollis*[1], *D. monbuttensis*[1], *D. montana*[1], *D. natalensis*[1], *D. oleifera*[1], *D. pentamera*[3], *D. pustulata*[3], *D. rhodocalyx*[1], *D. sanzaminika*[1], *D. scabrida*[1], *D. simii*[1], *D. soubreana*[1], *D. sumatrana*[1], *D. texana*[1], *D. tricolor*[1], *D. veillonii*[3], *D. villosa*[1], *D. wallichii*[1], *D. whyteana*[1], *D yatesiana*[3], *D. zhejiangensis*[2]
$2n = 2x$, $4x = 30$, 60	*D. lycioides*[1]
$2n = 4x = 60$	*D. ramulosa*[1], *D. rhombifolia*[1]
$2n = 2x$, $6x = 60$, 90	*D. cathayensis*[1,2]
$2n = 4x$, $6x = 60$, 90	*D. virginiana*[1]
$2n = 6x = 90$	*D. ebenum*[1]
$2n = 6x$, $9x = 90$, 135	*D. kaki*[1]

Note [1]White and Vosa (1980)
[2]Yang et al. (1999)
[3]Turner et al. (2013a, b)

(hexaploid or nonaploid) might have evolved from diploid together with genome duplication under severe environmental conditions when migrating from subtropical to temperate zone.

Unreduced gametes, which have the somatic (2n) chromosome number, are an important precursor to polyploid formation (Kreiner et al. 2017). However, even if reduced and unreduced gametophytes were fertilized successfully, the possibility of producing a zygote with subsequent normal embryo development would be very low in nature probably due to endosperm genomic imbalance (Sanford 1983). Such a zygote or an embryo, however, could be rescued successfully by means of in vitro culture.

While for the cross by using reduced pollen and unreduced egg, normal seeds could be obtained without in vitro culture (Yamada and Tao 2006, 2007).

Nonaploid persimmon was obtained by embryo rescue using unreduced pollen as a male parent (Sugiura et al. 2000). Similarly, seedless nonaploid 'Fukuoka K1 Gou' was derived from imperfect seeds of the cross between 'Fuyu' and 'Taishuu' (2*n* pollen) (Chijiwa et al. 2008). Unreduced embryo sac formation might occur in 'Fujiwaragosho' based on high frequency sexual polyploidization (Yamada and Tao 2006, 2007). Pollination on 'Fujiwara-gosho' bearing unreduced eggs using reduced pollen seems to be a promising way to produce polyploid persimmon seedlings (Fig. 2.1). Four natural nonaploid cultivars have been found thus far in the persimmon germplasm collection at Kyoto University, namely 'Hiratane-nashi', 'Tonewase', 'Miyazaki-tanenashi' and 'Watarizawa', all seedless astringent cultivars (Zhuang 1990a, b). Though the detailed information is not available about the process of modern persimmon evolution, we propose there are unreduced gametes (2*n* pollen or 2*n* egg) involved in the speciation of *D. kaki*.

There are no paleobotanical studies on the domestication and diffusion of persimmon. Recently, leaf fossil (cover page on Deciduous Fruits, vol. 3, 2021) described as '*Diospyros miokaki*' was regard as more than 18 million years old, it was now preserved in Shanwang Paleontology Fossil Museum in Linqu County, Shandong province, China. Fossil of *Diospyros* plants was also found in Japan and Korea, so persimmon was considered as native of East Asia (Kanzaki and Yonemori 2007; Yamada et al. 2013). Persimmon is also believed to have been originated in China, where wild types abound

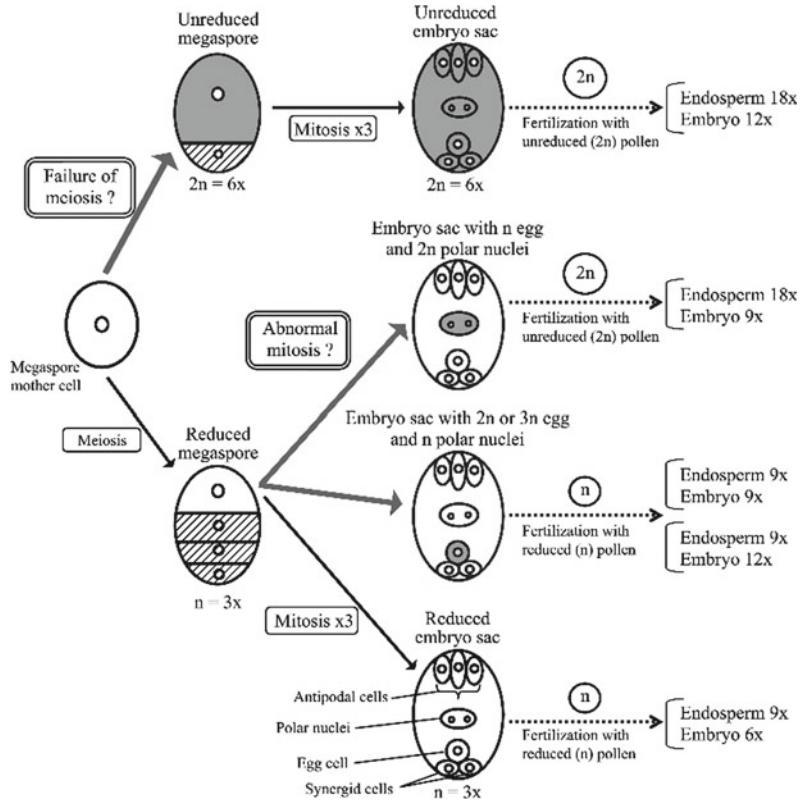

Fig. 2.1 Several patterns of embryo sacs supposedly formed in 'Fujiwara-gosho' and ploidy levels of endosperm and embryos from fertilization with reduced (*n*) or unreduced (2*n*) pollen (Yamada and Tao 2007)

and a wide range of variability in many traits, i.e. fruit colour, shape, sepal duplication, has been observed (Wilson 1929; Grubov 1967; Guan et al. 2020b).

The Chinese name of persimmon 'Shi' (*Diospyros* L.) first appeared in Li Ji·Nei Ze (around 450 BC, written by Kong Ji, grandson of Confucius), where it was considered a precious food during the Warring States Period (475–221 BC). In another famous poetry work, Shang Lin Fu (written by Sima Xiangru about 120–118 BC), the author observed that persimmon was one of the fruit crops planted in Shanglin Garden, a royal garden of the West Han Dynasty (206 BC–23 AD) (Luo and Wang 2008).

Persimmon has been cultivated for centuries in China and in the neighbourhood (Sugiura 1997); cultivars are known since the Tang dynasty (Wang et al. 1997; Luo and Wang 2008) and the most ancient descriptions of persimmon are in Chinese (Kikuchi 1948). Early in the fourteenth century, Marco Polo recorded that the Chinese traded in persimmon (Morton 1987). Hence, China is considered the primary centre of diversity (Zheven and Zhukovsky 1975). Persimmon expanded from China to Japan in the seventh century and to Korea in the fourteenth century; both are now considered secondary centres of diversification. Then persimmon was spread to many countries in Asian, European, America and Oceania continents (Zhang et al. 2020). Up to date, the origin of persimmon and its primary and secondary centre of diversity was not well defined.

D. kaki is presumed as autohexaploidy or autoallohexaploidy (Kanzaki et al. 2001) from the observation of polysomic inheritance of a molecular marker linked to natural astringency loss. *D. kaki* ($2n = 6x, 9x = 90, 135$) is suggested as an autohexaploid or autoallohexaploid by cytogenetic analysis of *Diospyros* species. Choi et al. (2002) applied in situ hybridization for genomic analysis in persimmon. GISH (genomic in situ hybridization) and FISH (fluorescent

in situ hybridization) studies show that *D. kaki* and *D. glandulosa* share many DNA sequences, which suggests that they are closely related and *D. kaki* may be either allohexaploid or autoallohexaploid (Choi et al. 2003a, b, c, d). Three repetitive DNAs were cloned from *D. kaki*, *D. oleifera* and *D. ehretioides* (Choi et al. 2003a) and used for investigating genomic similarity among *D. kaki* and nine related *Diospyros* species by FISH technique. *Eco*R V-repetitive DNA band was present in three diploid species (*D. lotus*, *D. glandulosa*, *D. oleifera*) and two hexaploidy species (*D. kaki*, *D. virginiana*). This suggested that diploid with the *Eco*RV-repetitive DNA, such as *D. lotus*, *D. glandulosa* or *D. oleifera* was involved in the speciation of *D. kaki*, due to the detection of the FISH signal of the *Eco*RV-repeat probe on the chromosomes of diploid *D. lotus* and hexaploid *D. kaki*. While for *Hind*II-digested repetitive DNA, it was also found in those five species, but missing in *D. virginiana*. Genomic evolution of *D. virginiana* after speciation was different from that of *D. kaki* and its related species, suggesting that different ancestral species might have been involved in the speciation of *D. virginiana* and *D. kaki*, as was also indicated by Nakatsuka et al. (2002) who analysed *Ty1*-copia group retrotransposons. GISH on somatic metaphase chromosomes of *D. kaki* revealed that the *D. glandulosa* probe hybridized to *D. kaki* chromosomes resulted in the strongest signal intensity among several wild species of *Diospyros* including *D. oleifera*, *D. lotus* and *D. virginiana*, indicating a close relationship between *D. kaki* and *D. glandulosa* (Choi et al. 2003b). FISH for physical mapping of 5S and 45S rDNA probes were performed in *D. kaki* and its wild relatives to visualize their signal on the somatic metaphase chromosomes (Choi et al. 2003c, d). Chromosomes of Asian diploid *D. lotus* most resembled *D. kaki* among the tested nine species by detection of 45S rDNA loci distribution on chromosomes. The four homologous chromosomes with 45S rDNA would infer that *D. kaki* may be an autoallohexaploid or at least some chromosomes are homoeologous among the different genome sets making up the *D. kaki* genome (Choi et al. 2003c). Among the Asian *Diospyros* species, the number of 5S and 45S rDNA sites seemed to increase according to ploidy level of species (Choi et al. 2003d).

Based on DNA hybridization, PCR techniques or sequencing, molecular markers have been developed and applied for *Diospyros* phylogenetic relationship analysis, genetic diversity evaluation, parental analysis, fingerprinting, marker-assisted selection. Those molecular markers include restriction fragment length polymorphism (RFLP), cpDNA or mtDNA PCR–RFLP, random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), inter-simple sequence repeat (ISSR), sequence-related amplified polymorphism (SRAP), inter-retrotransposon amplified polymorphism (IRAP), inverse sequence-tagged repeat (ISTR), retrotransposon-microsatellite amplified polymorphism (REMAP), sequence-specific amplified polymorphism (SSAP), sequence characterized amplified region (SCAR), targeted region amplified polymorphism (TRAP), start codon targeted (SCoT), restriction site-associated DNA sequencing (RADseq), DNA barcoding and single Nucleotide Polymorphism (SNP) (Zhang et al. 2020; Park et al. 2020). Phylogenetic analysis for persimmon cultivars and *Diospyros* species showed independent evolution of persimmon in China and Japan and revealed that many cultivars in Europe were introduced from Asia. Chinese, Korean and Japanese cultivars were separated by AFLP analyses, and PCNA cultivars could be separated from non-PCNA cultivars. When the effects of cultivar origin were removed, significant AFLP associations with classification type by astringency were not observed (Parfitt et al. 2015). But PCNA could not be distinctly separated from non-PCNA by using other molecular markers depending on cultivars tested (Hu and Luo 2006; Yonemori et al. 2008b; Wang et al. 2021).

2.2.3 Origin of PCNA Persimmon

PCNA-type was regarded as a recessive mutant in which the mutation has occurred on the gene

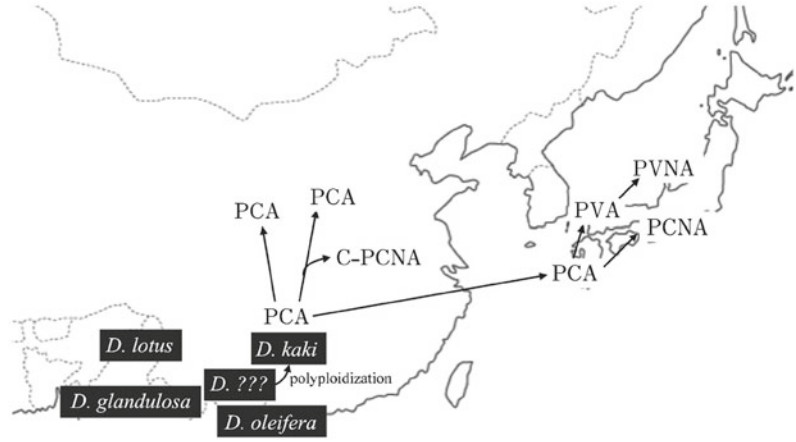

Fig. 2.2 Hypothesis of species involved in speciation of *D. kaki* and evolvement pathway of different persimmon types (Kanzaki 2016)

(s) controlling tannin accumulation (Kanzaki and Yonemori 2007; Kanzaki 2016). Chinese PCNA is distantly related to Japanese PCNA, they are of independent occurrences in China and Japan respectively (Kanzaki et al. 2000; Kanzaki 2016) (Fig. 2.2).

There are fewer PCNA cultivars than the astringent type that existed in China and Japan, (Yonemori et al. 2000, 2005). The evolution of PCNA cultivars in China seems to be earlier, since 'Luotian-tianshi' has been cultivated more than 900 years as it was recorded in 'Luotian County Annals' (1032 AD). As the PCNA in Japan, the known C-PCNA cultivars and strains (about eight varieties, e.g. 'Luotian-tianshi'), developed in a narrow geographical area in Hubei province (Wang 1983; Wang et al. 2005; Yonemori et al. 2005).

Chinese PCNA persimmons comprise various genotypes including 'Luotian-tianshi', 'Eshi 1', 'Xiaoguo-tianshi', 'Baogai-tianshi' and 'Sifang-tianshi', which are distributed in Dabieshan Mountain around the junction of three provinces (Hubei, Henan and Anhui) in central China (Yonemori et al. 2005; Yuan et al. 2011).

In Japan, the PCNA group is geographically limited to central Japan (Yamada 1993; Yamada et al. 2012) and it appears to be a recent evolution event: 'Gosho' was the first PCNA and was documented in the seventeenth century (Kikuchi 1948), while in 1214, a record of a PVNA cultivar, 'Zenjimaru', was found in Japan. The narrow genetic variability of the J-PCNA group in Japan has been confirmed based on AFLP markers (Kanzaki et al. 2000) and by progeny tests of PCNA × PCNA crosses, where lethal genes and inbreeding depression have often been observed (Yamada 2005; Sato and Yamada 2016). The PCNA cultivars, e.g. 'Daean Dangam', presumed to be native to Korea, appear to be Japanese cultivars for characteristic of 'Daean Dangam' is identical to 'Mushiroda-gosho' (PCNA of Japanese origin) (Yonemori et al. 2000; Park et al. 2005). Recently, two novel PCNA cultivars ('Jowan', 'Romang') were released in Korea, they were selected from the progenies of cross-breeding using Japanese PCNA parents or their F_1 generation (Ma et al. 2018a,b). All persimmon cultivars of presumed origin in the Mediterranean region belong to non-PCNA type (Giordani 2002) and European persimmon varieties are of close genetic relationship (Yonemori et al. 2008b).

Chinese PCNA trait is genetically controlled by dominant allele, whereas the PCNA trait of J-PCNA is recessive against non-PCNA. There are three loci that control the astringency type: (1) the locus controlling the J-PCNA trait, denoted as A-a which controls tannin accumulation; (2) the locus controlling the C-PCNA trait, denoted by B-b, which probably results in suppression of tannin accumulations; (3) the locus controlling the PV trait, denoted by C–c conferring exudation of acetaldehyde. The PV

trait has not been demonstrated to be controlled by a single locus, so its designation as a single locus here is hypothetical (Yamada 2013; Sato and Yamada 2016).

Inbreeding depression influences fruit weight, vigour and productivity and cause a serious problem in the 1980s breeding program aimed at developing early ripening J-PCNA cultivars (Yamada 1993; Sato and Yamada 2016). J-PCNA trait is recessive to the non-PCNA trait and is qualitatively inherited. Crosses among PCNA cultivars/selections of Japanese origin yielded only PCNA F_1 offspring because the parents are homozygous for the recessive allele, whereas almost no F_1 PCNA offspring resulted from crosses between PCNA and non-PCNAs (Ikeda et al. 1985; Yamada and Sato 2002). Backcrosses of non-PCNA F_1 offspring derived from non-PCNA × J-PCNA to J-PCNA yielded only about 15% PCNA progenies (Ikeda et al. 1985). The J-PCNA/non-PCNA genotype is controlled by a single locus (designated *AST/ast* or A/a), but the hexaploid nature of persimmon complicates the segregation ratios (Sato and Yamada 2016).

Natural astringency loss in J-PCNA fruits is due to the dilution of soluble tannins through fruit growth because of cessation of the development of tannin cells in the early stages of fruit development, also called 'dilution effects' (Yonemori and Matsushima 1985; Yonemori et al. 2003). Natural deastringency of C-PCNA persimmon can be accomplished only before full ripening. Besides 'dilution effects', soluble tannins conversion into insoluble tannins with acetaldehyde released in fruit flesh (called 'coagulation effects'), also mediates in natural astringency loss in C-PCNA (Mo et al. 2016; Xu et al. 2016). Chinese PCNA cultivar has the potential as an important cross-parent for PCNA breeding because it is expected to produce 50% PCNA offspring in the F_1 generation in crosses with either J-PCNA or non-PCNA. Through this approach, superior novel PCNA genotypes are expected to be created by using J-PCNA or non-PCNA as a female parent together with C-PCNA pollen donor (Pei et al. 2015; Xu et al. 2017). A reliable protocol conferring traditional cross-breeding, embryo rescue, astringency and sexuality identification by marker assist selection (MAS), in vitro shoot top-grafting, multiply nursery land and producing areas simultaneous evaluation for C-PCNA rapid genetic improvement was established (Xu et al. 2017). Additionally, it is important to breed new PCNA persimmon compatible with rootstock *D. lotus* for the sustainable industry.

2.3 Taxonomy of Persimmon

Ebenaceae sensu lato (s.l.) contains Ebenaceae sensu stricto (s.s.) and Lissocarpaceae, which are a medium-sized pantropical family with the greatest number of species in Asia and the Indo-Pacific region (White 1983). Infrafamilial classifications of Ebenaceae s.s. have been proposed by de Candolle (1844), Hiern (1872), Bakhuizen (1936–1955), White (1980, 1983, 1993) and Singh (2005). The earliest infrafamilial classification for Ebenaceae s.s. on a worldwide scale was that of de Candolle (1844), who recognized eight genera: *Cargillia*, *Diospyros*, *Euclea*, *Gunisanthus*, *Maba*, *Macreightia*, *Rospidios* and *Royena*. Hiern (1872) placed the 249 then-recognized species in five genera, *Diospyros*, *Euclea*, *Maba*, *Royena* and *Tetraclis*. In contrast to de Candolle's system, he proposed a new Madagascan endemic, *Tetraclis*, and lumped *Cargillia*, *Gunisanthus*, and *Rospidios* with *Diospyros* and *Macreightia* with *Maba*. Bakhuizen (1936–1955) in his regional revision united *Maba* with *Diospyros*, thus recognizing only four genera, *Diospyros*, *Euclea*, *Royena* and *Tetraclis* (the last three genera only according to the literature; they were not in the area he covered). Bakhuizen also divided *Diospyros* into five subgenera (*Cargillia*, *Cargillia*, *Hierniodendron*, *Maba*, *Mabacea*). White (1980, 1983) had a much broader generic concept, reducing *Royena* and *Tetraclis* to synonymy with *Diospyros*; he thus recognized only two genera, *Diospyros* and *Euclea*, in Ebenaceae s.s. Singh (2005), while dealing with Indian Ebenaceae, followed the generic concepts of White (1980, 1983). He divided Indian *Diospyros* species into 27 sections.

The botanical scientific name of persimmon is *Diospyros kaki*, but the paternity of the name is assigned to Carl Linnaeus (Linneo, Linn. or L.), to his son, known as Carl Linnaeus the Younger (L. f.), and to Carl Peter Thunberg (Thunb.); hence in referring to persimmons, the two names commonly used are *D. kaki* L.f. and *D. kaki* Thunb. (Yonemori et al. 2000; Giordani 2002). The authority for persimmons should be Thunberg due to his reference to *D. kaki* in the Nova Acta Regiae Societatis Scientiarum Upsaliensis, Vol. 3, p. 208, issued in 1780 and '*D. kaki* Thunb'. should be correct binomial nomenclature.

It is clear that modern persimmon subjects to *D. kaki* in accordance with botanical taxonomy. However, there are different pomological classifications for persimmon. In early period, European classification on fruit trees did not contain some fruit species of Asian origin like persimmon, jujube, Chinese bayberry. At the end of nineteenth century, Japanese pomologist Toru Fujii categorized persimmon into pome fruit (Qu and Sun, 1990). Later, Prof. Akio Kikuchi proposed to group persimmon into pseudo-pome fruit (Yu 1979). On the basis of fruit anatomy and consumption character, Yu (1979) classified persimmon into berry fruit.

Although there were some literatures (Wang et al. 1998) on persimmon classification by using morphology (Martinez-Calvo et al. 2013; Yilmaz et al. 2017), isozyme (Tao and Sugiura 1987; Sugiura et al. 1988), molecular markers (Luo et al. 1995; Maki et al. 2001), it is still difficult to distinguish cultivars accurately. Astringency is caused by water-soluble tannins, which are found in large special tannin cells in the fruit flesh. Fruit astringency at harvest is the main trait for practical pomological classification on persimmon.

Hume (1914) divides astringent and non-astringent types into two subgroups: variant and constant type, depending on the effect of the presence of seeds on flesh colour. In variant types, flesh colour changes to dark when seeds are present in the fruit, while in constant types flesh colour is not affected by the presence of seeds. Gradually, other scholars advanced the persimmon classification based on Hume (1914).

Kajiura (1946) classified persimmon into two major groups with four subgroups: (i) non-astringent or astringent at maturity whether seeded or not, flesh colour is not influenced by seed. Subgroup PCNA (pollination constant & non-astringent) fruits keep natural deastringency and flesh presented some brown specks, subgroup PCA (pollination constant and astringent) fruits keep astringent flesh without brown specks; (ii) astringency loss depending on seeds number. Subgroup PVNA (pollination variant and non-astringent) fruits can remove astringency partially or totally with only one or few seeds and resulted brown flesh colour (soluble tannin coagulation occurs with acetaldehyde and ethanol, insoluble tannin oxidized in the flesh, and the flesh colour darkens), subgroup PVA (pollination variant and astringent) fruits are astringent when they have several seeds and brown flesh colour only restricted around the seed at maturity.

Sugiura (1984) proposed a new classification system on persimmon taking into account the quality of tannin in the flesh and the capability of seeds of releasing volatile compounds during fruit development. Persimmon cultivars are divided into a volatile-independent group (VIG) (corresponding to the PCNA type) and a volatile-dependent group (VDG), which consist of PCA, PVA and PVNA types. In pollination variant cultivars, seeds exude acetaldehyde, which causes the soluble tannins to be condensed or coagulated and to become insoluble and oxidized. The seeds of PVNA types exude so much that the fruit becomes non-astringent, but those of PVA exude so little that the fruit is astringent; whereas, those of PCA do not exude at all. These fruits can only be eaten firm after the astringency has been removed by carbon dioxide gas or ethanol treatment.

The genetic traits of the PCNA type are controlled by two independent loci: the *AST* locus and the *CPCNA* locus. The Japanese PCNA (J-PCNA) type is nulliplex at the *AST* locus, while the Chinese PCNA (C-PCNA) type holds at least one dominant allele (*CPCNA*) at the *CPCNA* locus (Akagi et al. 2011a). Hence, combining these two classification methods (Kajiura 1946;

Sugiura 1984), persimmon cultivars are divided into PCNA type and non-PCNA type. PCNA fruits can lose astringency on the tree during fruit development and become edible at maturity under qualitative genetic control. Fruit astringency in non-PCNA is quantitative trait. PCNA comprise Chinese PCNA (C-PCNA) and Japanese PCNA (J-PCNA) type. Regarding C-PCNA, the genetic trait concerning astringency is controlled by dominant locus against non-PCNA trait; while for J-PCNA, when all six alleles are recessive for *ast* locus, it could exhibit PCNA trait that was qualitatively inherited because of hexaploid nature of persimmon. Non-PCNA include pollination variant & non-astringent (PVNA), pollination variant and astringent (PVA) and pollination constant & astringent (PCA) types (Yonemori et al. 2000; Akagi et al. 2011). This classification system was carried out according to the mode of astringency loss and heritage distinctness between J-PCNA and C-PCNA (Fig. 2.3).

Chinese persimmon scientist Wang, Renzi suggested another classification method for persimmon cultivars (2021, private communication) (Fig. 2.4). Professor Wang supposed that PCA should be the originally existed persimmon type, other persimmon types were generated from PCA during evolution.

2.4 Germplasm of *Diospyros* and Persimmon

2.4.1 Genetic Resources of *Diospyros*

The number of species recognized in Ebenaceae s.s. has remarkable variation. de Candolle (1844) described 158 species in Ebenaceae, while Hiern (1872) recognized 262 species and fossil species. Lee proposed there were 500 species in Ebenaceae with three tropical genera and one genus in China (Lee et al. 1996). In Wallnöffer's (2001) opinion, Ebenaceae is a family that comprises more than 500 species. Yonemori et al. (2000) suggested 400 species in the genus *Diospyros*. IPNI (Internatioal Plant Index) collected more than 1,700 names of *Diospyros* plants. Website

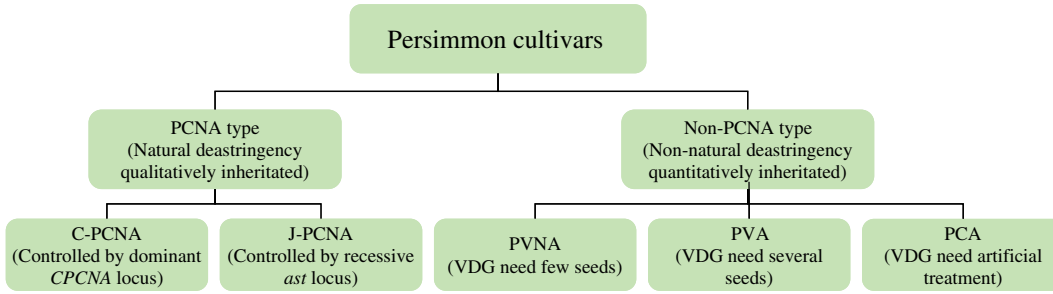

Fig. 2.3 Pomological classification of persimmon (Modified from Akagi et al. 2011)

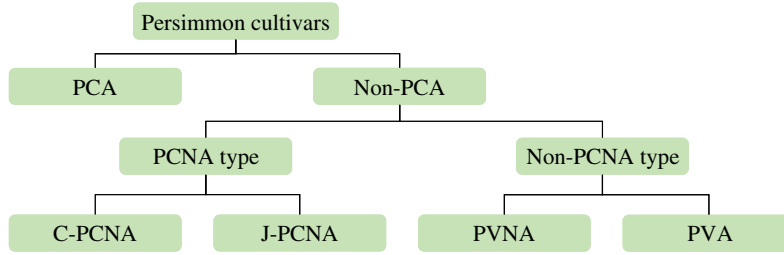

Fig. 2.4 Classification of persimmon (Modified from Renzi Wang, private communication)

of the Plant List (http://www.theplantlist.org) and species2000 (http://www.sp2000.org) recorded 780 and 300 names of *Diospyros* plants, respectively (Tang et al. 2019). It's difficult to know the precise number of *Diospyros* plants.

A total of 60 species with detailed morphological character was distributed in China. Most of them existed in Southeast and Southwest China (Lee et al. 1996; Tang et al. 2019). 'Jinzaoshi', a persimmon-like tree mainly distributed in the southern part of Zhejiang Province (China), has a long cultivation history and had been regarded as a cultivar of *D. kaki*. Through ITS and *mat*K sequence together with morphology observation, Tang et al. (2014) proposed 'Jinzaoshi' did not belong to *D. kaki* and other *Diospyros* species, it was recommended to be a new species of *Diospyros*. Another two new species *D. brandisiana* Kurz from southeastern Yunnan province of China, *D. penghuae* from Danxiashan in Guangdong province of China were identified by diagnostic characters, habitat and distribution record.

In Thailand, 60 *Diospyros* species have been described by Phengklai (1978). Many of them distributed in the forest throughout Thailand, some of them may contribute to the evolution of modern persimmon (Utsunomiya et al. 1998). There are 31 species distributed in New Caledonia island in the southwestern Pacific (Duangjai et al. 2009).

2.4.2 Genetic Resources of Persimmon

There are abundant persimmon cultivar diversity in East Asia counties including China, Japan and Korea. In China, more than 900 cultivars are known, however, only 40 cultivars are planted. Currently, more than 1000 persimmon accessions derived from different counties or regions are preserved in National Field GenBank for Persimmon located in Yangling, Shaanxi province of China (Yang et al. 2013). All the known cultivars are astringent types, with the exception of several PCNA types including 'Luotiantianshi' newly found in Central China (Wang et al. 1997; Yuan et al. 2011). Most of persimmon in commercial orchard belong to non-PCNA with a percentage of approximately 90%. Five androecious persimmon genetic resources collected in Luotian county, Hubei province of China contain RO2 marker, which linked to the dominant *CPCNA* locus controlling natural deastringency of C-PCNA (Pei et al. 2013). A newly found millennial androecious persimmon germplasm collected in Jiangxi province of China, also holds RO2 marker. Those precious androecious persimmon genotypes may serve as a parent for PCNA breeding (Guan et al. 2020a).

The national institute in Japan conserves around 600 cultivars/selections in Akitsu, Hiroshima. However, only 18 PCNA cultivars of Japanese origin, excluding bud sports, synonyms and newly released cultivars, are conserved in Akitsu (Yamada 2005). The first recorded PVNA cultivar was 'Zenjimaru', which was mentioned in a thirteenth-century document, whereas the word 'kaki' was mentioned as over-ripened or dried fruits in a tenth-century document (Kikuchi 1948), suggesting that the fruits in the tenth century were astringent type. PVNA local cultivars in Japan show wide variation, comparable to that of the PCA cultivars, and are distributed throughout the country, suggesting rapid development and dissemination of PVNA cultivars (Yamada et al. 1994). PVNA offspring are commonly found among the open-pollinated seeds of PVNA genotypes (Ikeda et al. 1985). These open-pollinated chance seedlings may have contributed to the rapid development of PVNA cultivars (Yamada et al. 1994). J-PCNA cultivars are distributed mostly within narrow geographic areas, including the Kinki and Tokai districts (Yamada 2005; Yamada et al. 2012). Only one J-PCNA cultivar, 'Gosho', was widely grown 200 years ago and assumed to be the oldest J-PCNA (Kikuchi 1948). Currently, 31% of all the production area is cultivated with PCNA types with 71% based on 'Fuyu' and its bud mutant 'Matsumo-wase Fuyu'. Novel nonaploid 'Akiou' formally named as Fukuoka K1 Gou was created and released from imperfect seeds of the cross using female parent 'Fuyu' and 'Taishuu' as unreduced pollen donor (Chijiwa et al. 2008).

In Korea, 233 local cultivars were collected at the branch of Experimental Station at Kim-hae during 1959–1969, and 74 superior cultivars were selected for persimmon cultivation after identifying the name of 188 cultivars among these local cultivars. In Korea, interest in persimmon cultivation is increasing and two experimental stations for persimmon have been established, the one for non-astringent persimmon was established in 1994 and the other for astringent persimmon established in 1995 (Cho and Cho 1965; Sato and Yamada 2016).

In Europe and outside Asian countries, persimmon is considered a secondary fruit tree species; only few countries, located in the Mediterranean area, are interested in a large-scale production (Giordani 2002). Spain, Italy, Israel and Brazil are now producing important amounts and these countries have developed their own cultivars such as 'Rojo Brillante' (PVA) in Spain, 'Kaki Tipo' (PVNA) in Italy, 'Triumph' (PVA, commercial name 'Sharon') in Israel and 'Rama Forte' (PVNA) in Brazil, 'Coroa de Rei' (non-PCNA) in Portugal. Recently, Australia and New Zealand have started to produce PCNA persimmon of 'Fuyu' and 'Jiro' mainly for export, and the USA is also producing persimmon on a small scale (Giordani 2002; Yamada 2012; Nissen and Roberts 2015). In Italy and Spain, under GENRES29 Project, 160 accessions were preserved; the collection in Florence has been increased by entering the most important Spanish accessions, for a total of 96 accessions (Bellini and Giordani 2005). In Nikita Botanical Gardens of Russia, there is a germplasm collection of 87 cultivars and genotypes of *D. kaki*, *D. virginiana* and *D. lotus* (Khokhlov and Plugatar 2018). A germplasm collection with 93 accessions was established at the Instituto Valenciano de Investigaciones Agrarias (I.V.I.A.) (Martinez-Calvo et al. 2018).

References

Akagi T, Katayama-Ikegami A, Yonemori K (2011) Proanthocyanidin biosynthesis of persimmon (*Diospyros kaki* Thunb.) fruit. Sci Hort 130:373–380

Akagi T, Shirasawa K, Nagasaki H, Hirakawa H, Tao R, Comai L, Henry IM (2020) The persimmon genome reveals clues to the evolution of a lineage-specific sex determination system in plants. PLoS Genet 16: e1008566

Ampiaw RE, Lee W (2020) Persimmon tannins as biosorbents for precious and heavy metal adsorption in wastewater: a review. Int J Environ Sci Technol 17:3835–3846

Bakhuizen van den BRC (1936–1955) Revisio ebenacearum Malayensium. Bull Jard Bot Buitenzorg, III 15:1–515

Baldwin JT Jr, Culp R (1941) Polyploidy in *Diospyros virginiana* L. Amer J Bot 28(10):942–944

Bellini E, Giordani E (2005) Germplasm and breeding of persimmon in Europe. Acta Hort 685:65–76

Chijiwa H, Kuwahara M, Hirakawa N, Tetsumura T (2008) Generation of nonaploid persimmons (*Diospyros kaki* Thunb.) by embryo culture of imperfect seeds derived from a cross between 'Fuyu' and 'Taishuu'. J Jpn Soc Hort Sci 77:358-363

Cho SK, Cho TH (1965) Studies on the local varieties of persimmon in Korea. Res Rep RDA 8:147–190 (in Korean with English summary)

Choi YA, Tao R, Yonemori K, Sugiura A (2002) Multicolor genomic *in situ* hybridization identifies parental chromosomes in somatic hybrids of *Diospyros kaki* and *D. glandulosa*. HortScience 37:184–186

Choi YA, Tao R, Yonemori K, Sugiura A (2003a) Genomic distribution of three repetitive DNAs in cultivated hexaploid *Diospyros* spp. (*D. kaki* and *D. virginiana*) and their wild relatives. Gene Genet Syst 78:301–308

Choi YA, Tao R, Yonemori K, Sugiura A (2003b) Genomic *in situ* hybridization between persimmon (*Diospyros kaki*) and several wild species of *Diospyros*. J Jpn Soc Hort Sci 72:385–388

Choi YA, Tao R, Yonemori K, Sugiura A (2003c) Physical mapping of 45s rDNA by fluorescent *in situ* hybridisation in persimmon (*Diospyros kaki*) and its wild relatives. J Hort Sci Biotech 78:265–271

Choi YA, Tao R, Yonemori K, Sugiura A (2003d) Simultaneous visualization of 5S and 45S rDNAs in persimmon (*Diospyros kaki*) and several wild relatives (*Diospyros* spp.) by fluorescent *in situ* hybridization (FISH) and multicolor FISH (MCFISH). J Amer Soc Hort Sci 128:736–740

de Candolle A (1844) Ordo CXXV. Ebenaceae. In: de Candolle AP, de Candolle A (eds) Prodromus systematis naturalis regnivegetabilis, vol 8. Treuttel & Würtz, Paris, pp 209–243

Duangjai S, Samuel R, Munzinger J, Forest F, Wallnöfer B, Barfuss MHJ, Fischer G, Chase MW (2009) A multi-locus plastid phylogenetic analysis of the pantropical genus *Diospyros* (Ebenaceae), with an emphasis on the radiation and biogeographic origins of the New Caledonian endemic species. Mol Phylogenet Evol 52:602–620

Duangjai S, Wallnoefer B, Samuel R, Munzinger J, Chase MW (2006) Generic delimitation and

relationships in Ebenaceae sensu lato: evidence from six plastid DNA regions. Amer J Bot 93:1808–1827
Fu JM, Liu HM, Hu JJ, Liang YQ, Liang JJ, Wuyun TN, Tan XF (2016) Five complete chloroplast genome sequences from *Diospyros*: genome organization and comparative analysis. PLoS ONE 11:e0159566
Geeraerts A, Raeymaekers JAM, Vinckier S, Pletsers A, Smets E, Huysmans S (2009) Systematic palynology in Ebenaceae with focus on Ebenoideae: morphological diversity and character evolution. Rev of Palaeobot Palynol 153:336–353
Giordani E (2002) Varietal assortment of persimmon in the countries of the Mediterranean area and genetic improvement. In: Bellini E, Giordani E (eds) Options Mediterraneennes. First Mediterranean Symposium on Persimmon. Zaragoza: CIHEAM-IAMZ, pp 23–37
Grubov VI (1967) Family Ebenaceae. In: Shishkin BK, Bobrov EG (eds) Flora of the USSR. Israel Program for Scientific Translations, Jerusalem, pp 349–355
Guan CF, Zhang PX, Wu MH, Zeng M, Chachar S, Ruan XF, Wang RZ, Yang Y (2020a) Discovery of a millennial androecious germplasm and its potential in persimmon (*Diospyros kaki* Thunb.) breeding. Sci Hort 269:109392
Guan CF, Zhang YF, Zhang PX, Chachar S, Wang RZ, Du XY, Yang Y (2020b) Germplasm conservation, molecular identity and morphological characterization of persimmon (*Diospyros kaki* Thunb.) in the NFGP of China. Sci Hort 272:109490
Hiern WP (1872) A monograph of Ebenaceae. Trans Cambridge Philos Soc 12:27–300
Hu DC, Luo ZR (2006) Polymorphisms of amplified mitochondrial DNA non-coding regions in *Diospyros* spp. Sci Hort 109:275–281
Huang Y, Luo Z, Zhang Q (2013) Phylogenetic analysis of some androecious genotypes native to China and related *Diospyros* spp. using chloroplast fragments. Acta Hort 996:103–109
Hume HH (1914) A kaki classification. J Hered 5:400–406
Ikeda I, Yamada M, Kurihara A (1985) Inheritance of astringency in Japanese persimmon (*Diospyros kaki* Thunb.). J Jpn Soc Hort Sci 54:39–45 (in Japanese with English summary)
Kajiura M (1946) Persimmon cultivars and their improvement 2. Breed Hort 1:175–182 (in Japanese)
Kanzaki S, Yonemori K (2007) Persimmon. In: Cole C (ed) Genome mapping and molecular breeding in plants. Fruits and nuts, vol 4. Springer, Berlin, pp 353–358
Kanzaki S, Yonemori K, Sato A, Yamada M, Sugiura A (2000) Analysis of the genetic relationships among pollination-constant and non-astringent (PCNA) cultivars of persimmon (*Diospyros kaki* Thunb.) from Japan and China using amplified fragment length polymorphism (AFLP). J Jpn Soc Hort Sci 69:702–704
Kanzaki S (2016) The origin and cultivar development of Japanese persimmon (*Diospyros kaki* Thunb.). Nippon Shokuhin Kagaku Kogaku Kaishi 63:328–330 (in Japanese with English summary)
Khokhlov S, Plugatar Y (2018) Persimmon collection in Nikita Botanical Gardens: its history and pratical use. Acta Hort 1195:23–27
Kikuchi A (1948) Pomology: Part I. Yokendo, Tokyo, pp 347–400 (in Japanese)
Kreiner JM, Kron P, Husband BC (2017) Evolutionary dynamics of unreduced gametes. Trends Genet 33:583–593
Lee SK, Gilbert MG, White F (1996) Ebenaceae. Flora of China 15:215–234
Li CM, Zhang Y, Yang J, Zou B, Dong XQ, Hagerman AE (2013) The interaction of a polymeric persimmon proanthocyanidin fraction with Chinese cobra PLA_2 and BSA. Toxicon 67:71–79
Li WQ, Liu YL, Yang Y, Xie XM, Lu YZ, Yang ZR, Jin XB, Dong WP, Suo ZL (2018) Interspecific chloroplast genome sequence diversity and genomic resources in *Diospyros*. BMC Plant Biol 8:210
Luo ZR, Wang RZ (2008) Persimmon in China: domestication and traditional utilizations of genetic resources. Adv Hort Sci 22:239–243
Luo ZR, Yonemori K, Sugiura A (1995) Evaluation of RAPD analysis for cultivar identification of persimmon. J Jpn Soc Hort Sci 64:535–541 (in Japanese with English summary)
Ma KB, Cho KS, Kang SS, Han JH, Kim YK, Hwana HS, Shin IS (2018a) A New Sweet Persimmon: 'Romang.' J Amer Pomolog Soc 72:74–79
Ma KB, Lee IB, Kim YK, Won KH, Cho KS, Choi JJ, Lee BHN, Kim MS (2018b) 'Jowan', an early maturing PCNA (pollination constant non-astringent) persimmon (*Diospyros kaki* Thunb.). Acta Hort 1195:61–64
Maki S, Oyama K, Kurahashi T, Nakahira T, Kawabata T, Yamada T (2001) RFLP analysis for cultivar identification of persimmons. Sci Hort 91:407–412
Martinez-Calvo J, Climent J, Naval MM, Badenes ML (2018) The I.V.I.A. germplasm collection of persimmon (*Diospyros kaki* Thunb.). Acta Hort 1195:55–59
Martinez-Calvo J, Naval M, Zuriaga E, Llacer G, Badenes ML (2013) Morphological characterization of the IVIA persimmon (*Diospyros kaki* Thunb.) germplasm collection by multivariate analysis. Genet Resour Crop Evol 60:233–241
Matsuo T, Ito S (1978) The chemical structure of kakitannin from immature fruit of the persimmon (*Diospyros kaki* L.). Agric Biol Chem 42:1637–1643
Mo RL, Yang SC, Huang YM, Chen WX, Zhang QL, Luo ZR (2016) *ADH* and *PDC* genes involved in tannins coagulation leading to natural de-astringency in Chinese pollination constant and non-astringency persimmon (*Diospyros kaki* Thunb.). Tree Genet Genomes 12:17
Morton J (1987) Japanese persimmon. In: Morton JF (ed) Fruits of Warm Climates. Creative Resource Systems Inc., Miami, Florida, pp 411–416
Nakamura Y, Kobayashi S (1994) DNA restriction fragment length variability in *Diospyros kaki* and related *Diospyros* species. HortScience 29:809–811
Nakatsuka A, Iwami N, Matsumoto S, Itamura H, Yamagashi M (2002) *Ty1*-copia group retrotransposons in

persimmon (*Diospyros kaki* Thunb.). Genes Genet Syst 77:131–136

Namikawa I, Higashi M (1928) On the number of chromosomes in *Diospyros kaki* L.f. and *Diospyros lotus* L. Bot Mag 42:436–438

Ng FSP (1978) *Diospyros roxburghii* and the origin of *Diospyros kaki*. Malays Forester 41:43–50

Nissen R, Roberts R (2015) History, origin and classification of persimmon cultivars. J Amer Pomolog Soc 69:31–44

Parfitt DE, Yonemori K, Honsho C, Nozaka M, Kanzaki S, Sato A, Yamada M (2015) Relationships among Asian persimmon cultivars, astringent and non-astringent types. Tree Genet Genomes 11:24

Park JH, Kim CH, Suh HD, Hwang KS (2005) Fruit characteristics of local persimmon native to Jeju, Korea. Acta Hort 685:93–98

Park YO, Choi ST, Son JY, Kim EG, Ahn GH, Park JH, Joung WK, Jang YH, Kim DW (2020) The application of single nucleotide polymorphism markers for discrimination of sweet persimmon cultivars. J Life Sci 30:614–624 (in Korean with English summary)

Paun O, Turner B, Trucchi E, Munzinger J, Chase MW, Samuel R (2016) Processes driving the adaptive radiation of a tropical tree (*Diospyros*, Ebenaceae) in New Caledonia, a biodiversity hotspot. Syst Biol 65:212–227

Pei X, Zhang QL, Guo DY, Liu JH, Luo ZR (2015) Development of genetic improvement in Chinese PCNA persimmon. J Fruit Sci 32:313–321 (in Chinese with English summary)

Pei X, Zhang QL, Guo DY, Luo ZR (2013) Effectiveness of the RO_2 marker for the identification of non-astringency trait in Chinese PCNA persimmon and its possible segregation ratio in hybrid F_1 population. Sci Hort 150:227–231

Phengklai C (1978) Ebenaceae of Thailand. Thai for Bull 11:1–103

Pomper KW, Lowe JD, Crabtree SB, Vincent J (2020) Ploidy level in American persimmon (*Diospyros virginiana*) cultivars. HortScience 55:4–7

Qu ZZ, Sun YW (1990) Survey of Fruit Species. China Agriculture Press, Beijing, China, pp 1–8 (in Chinese)

Samuel R, Turner B, Duangjai S, Munzinger J, Paun O, Barfuss MHJ, Chase MW (2019) Systematics and evolution of the Old World Ebenaceae, a review with emphasis on the large genus *Diospyros* and its radiation in New Caledonia. Bot J Linn Soc 189:99–114

Sanford JC (1983) Ploidy manupilations. In: Moore JN, Janick J (eds) Methods in Fruit Breeding. Purdue University Press, West Lafayette, pp 100–123

Sato A, Yamada M (2016) Persimmon breeding in Japan for pollination-constant non-astringent (PCNA) type with marker-assisted selection. Breed Sci 66:60–68

Singh V (2005) Monograph on Indian *Diospyros* L. (persimmon, ebony) Ebenaceae. Botanical Survey of India, Kolkata, India

Somego M (1978) Chromosome number of *Diospyros roxburghii*. Malays Forester 41:51–52

Sugiura A (1997) Keynote Address. Acta Hort 436:15–19

Sugiura A, Ohkuma T, Choi YA, Tao R, Tamura M (2000) Production of nonaploid (2n = 9x) persimmons (*Diospyros kaki*) by pollination with unreduced (2*n* = 6x) pollen and embryo rescue culture. J Amer Soc Hort Sci 125:609–614

Sugiura A, Tao R, Tomana T (1988) Distinguishing between Japanese persimmon cultivars (*Diospyros kaki* L.) by means of pollen isozymes. Sci Hort 36:67–77

Sugiura A (1984) The origin of persimmon and its cultivar differentiation. In: Ohmura T, Yomogihara Y, Ito H, Kinoshita T, Kadota J (eds) Recent Advances in Plant Breeding, vol 25. Keigaku Publishers, Tokyo, pp 30–37 (in Japanese)

Suo YJ, Sun P, Cheng HH, Hann WJ, Diao SF, Li HW, Mai YN, Zhao X, Li FD, Fu JM (2020) A high-quality chromosomal genome assembly of *Diospyros oleifera* Cheng. GigaScience 9:giz164

Tamura M, Tao R, Yonemori K, Utsunomiya N, Sugiura A (1998) Ploidy level and genome size of several *Diospyros* species. J Jpn Soc Hort Sci 67:306–312

Tang DL, Hu Y, Zhang QL, Yang Y, Luo ZR (2014) Discriminant analysis of "Jinzaoshi" from persimmon (*Diospyros kaki* Thunb.; Ebenaceae): a comparative study conducted based on morphological as well as ITS and *mat*K sequence analyses. Sci Hort 168:168–174

Tang DL, Zhang QL, Xu LQ Guo DY, Luo ZR (2019) Number of species and geographical distribution of *Diospyros* L. (Ebenaceae) in China. Hortic Plant J 5:59-69

Tao R, Sugiura A (1987) Cultivar identification of Japanese persimmon by leaf isozyme. HortScience 22:932–935

Tian XY, Chen WH, Shui YM, Yu ZY, Zhu XT (2014) *Diospyros brandisiana* Kurz (Ebenaceae), A newly recorded species from China. J Trop Subtrop Bot 22:31–33

Turner B, Munzinger J, Duangjai S, Temsch EM, Stockenhuber R, Barfuss MHJ, Chase MW, Samuel R (2013a) Molecular phylogenetics of New Caledonian *Diospyros* (Ebenaceae) using plastid and nuclear markers. Mol Phylogenet Evol 69:740-763

Turner B, Paun O, Munzinger J, Chase MW, Samuel R (2016) Sequencing of whole plastid genomes and nuclear ribosomal DNA of *Diospyros* species (Ebenaceae) endemic to New Caledonia: many species, little divergence. Ann Bot 117:1175–1185

Turner B, Paun O, Munzinger J, Duangjai S, Chase MW, Samuel R (2013b) Analyses of amplified fragment length polymorphisms (AFLP) indicate rapid radiation of *Diospyros* species (Ebenaceae) endemic to New Caledonia. BMC Evol Biol 13:269

Utsunomiya N, Subhadrabandhu B, Yonemori K, Oshida M, Kanzaki S, Nakatsubo F, Sugiura A (1998) *Diospyros* species in Thailand: their distribution, fruit morphology and uses. Econ Bot 52:343–351

Wallnöfer B (2001) The biology and systematics of Ebenaceae: a review. Ann Naturhist Mus Wien 103B:485–512

Wang LY, Li HW, Suo YJ, Han WJ, Diao SF, Mai YN, Sun P, Fu JM (2021) Development of EST-SSR markers and their application in the genetic diversity of persimmon (*Diospyros kaki* Thunb.). Trees 35:121–133

Wang R (1983) The origin of 'Luotiantianshi.' China Fruits 2:16–19 (in Chinese)

Wang R, Yang Y, Li G (1997) Chinese Persimmon Germplasm Resources. Acta Hort 436:43–50

Wang WS, Luo ZR, Cai LH (1998) Advances in the identification and taxonomy of persimmon (*Diospyros kaki* L. f.) cultivars. Acta Hort Sin 25:44–50 (in Chinese with English summary)

White F (1980) Notes on the Ebenaceae, VIII: the African section of *Diospyros*. Bull Jard Bot Nat Belg 50:445–460

White F (1983) Ebenaceae. In: Launert E (ed) Flora Zambesiaca, vol 7. Flora Zambesiaca Managing Committee, London, UK, vol 1, pp 248–300

White F (1993) Twenty-two new and little-known species of *Diospyros* (Ebenaceae) from New Caledonia with comments on section *Maba*. Adansonia 14:179–222

White F, Vosa CG (1980) The chromosome cytology of African Ebenaceae with special reference to polyploidy. Bol Soc Brot II 53:275–298

Wilson EH (1929) China—Mother of Gardens. Stradford, Boston

Xie CY, Xie ZS, Xu XJ, Yang DP (2015) Persimmon (*Diospyros kaki* L.) leaves: a review on traditional uses, phytochemistry and pharmacological properties. J Ethnopharmacol 63:229–240

Xu JC, Gan JP, Xiang J, Zhang QL, Xu LQ, Guo DY, Luo ZR (2017) Advance of genetic improvement technology for Chinese PCNA persimmon. Acta Hort Sin 44:987–998 (in Chinese with English summary)

Xu JC, Zhang QL, Xu LQ, Guo DY, Luo ZR (2016) Recent developments in deastringency mechanism of persimmon fruit. Acta Hort Sin 43:1653–1664 (in Chinese with English summary)

Yamada A, Tao R (2006) High frequency sexual polyploidisation observed in hexaploid Japanese persimmon (*Diospyros kaki*) 'Fujiwaragosho.' J Hortic Sci Biotech 81:402–408

Yamada A, Tao R (2007) Controlled pollination with sorted reduced and unreduced pollen grains reveals unreduced embryo sac formation in *Diospyros kaki* Thunb. 'Fujiwara-gosho.' J Jpn Soc Hort Sci 76:133–138

Yamada M (1993) Persimmon breeding in Japan. Jpn Agr Res Quart 27:33–37

Yamada M (2005) Persimmon genetic resources and breeding in Japan. Acta Hort 685:51–64

Yamada M (2013) Breeding goals, trait heredity and genetic improvement of persimmon in Japan. Acta Hort 996:77–88

Yamada M, Giordani E, Yonemori K (2012) Persimmon. In: Badenes ML, Byrne DH (eds) Fruit breeding, Handbook of plant breeding, vol 8. Springer Science +Business Media, LLC, New York, pp 663–693

Yamada M, Sato A (2002) Segregation for fruit astringency type in progenies derived from crosses of 'Nishimurawase' × pollination constant non-astringent genotypes in Oriental persimmon (*Diospyros kaki* Thunb.). Sci Hort 92:107–111

Yamada M, Yamane H, Sato A, Hirakawa N, Wang R (1994) Variations in fruit ripening time, fruit weight and soluble solids content of Oriental persimmon cultivars native to Japan. J Jpn Soc Hort Sci 63:485–491

Yang Y, Ruan X, Wang R (2013) Indigenous persimmon germplasm resources in China. Acta Hort 996:89–96

Yang Y, Wang RZ, Li GC, Wang W (1999) Study on chromosome numbers of *Diospyros* and their varieties. Acta Agric Boreali-Occidentalis Sin 8:64–67 (in Chinese with English summary)

Yilmaz B, Genc A, Cimen B, Incesu M, Yesiloglu T (2017) Characterization of morphological traits of local and global persimmon varieties and genotypes collected from Turkey. Turk J Agric for 41:93–102

Yonemori K, Honsho C, Kanzaki S, Ino H, Ikegami A, Kitajima A, Sugiura A, Parfitt DE (2008a) Sequence analyses of the ITS regions and the *mat*K gene for determining phylogenetic relationships of *Diospyros kaki* (persimmon) with other wild *Diospyros* (Ebenaceae) species. Tree Genet Genomes 4:149–158

Yonemori K, Honsho C, Kitajima A, Aradhya M, Giordani E, Bellini E, Parfitt DE (2008b) Relationship of European persimmon (*Diospyros kaki* Thunb.) cultivars to Asian cultivars, characterized using AFLPs. Genet Resour Crop Evol 55:81–89

Yonemori K, Ikegami A, Kanzaki S, Sugiura, (2003) Unique features of tannin cells in fruit of pollination constant non-astringent persimmons. Acta Hort 601:31–35

Yonemori K, Ikegami A, Kitajima A, Luo ZR, Kanzaki S, Sato A, Yamada M, Yang Y, Wang RZ (2005) Existence of several pollination constant non-astringent type persimmons in China. Acta Hort 685:77–84

Yonemori K, Kanzaki S, Parfitt DE, Utsunomiya N, Subhadrabandhu S, Sugiura A (1998) Phylogenetic relationship of *Diospyros kaki* (persimmon) to *Diospyros* spp. (Ebenaceae) of Thailand and four temperate zone *Diospyros* spp. from an analysis of RFLP variation in amplified cpDNA. Genome 41:173–182

Yonemori K, Matsushima J (1985) Property of development of the tannin cell in non-astringent type fruits of Japanese persimmon (*Diospyros kaki*) and its relationship to natural astringency. J Jpn Soc Hort Sci 54:201–208 (in Japanese with English summary)

Yonemori K, Yamada M, Luo ZR, Kanzaki S, Yang Y, Wang RZ (2012) New candidate for the ancestor of Japanese persimmon (*Diospyros kaki* Thunb.) found in Yunnan province of China. Acta Hort 948:121–128

Yonemori K, Yamada M, Sugiura A (2000) Persimmon genetics and breeding. Plant Breeding Reviews 19:191–225

Yu DJ (1979) Taxonomy of Chinese Fruit Trees. China Agriculture Press, Beijing, China. pp 243–249 (in Chinese)

Yuan LX, Zhang QL, Guo DY, Luo ZR (2011) Characteristics of Chinese PCNA types and their roles in

science and industry of oriental persimmon. Acta Hort Sin 38:361–370 (in Chinese with English summary)
Zhang YF, Yang Y, Guo J, Hu CQ, Zhu RS (2016) Taxonomic status of Deyangshi based on chromosome number and SRAP markers. Sci Hort 207:57–64
Zhang QL, Tetsumura T, Tao R, Luo ZR (2020) 8.1 *Diospyros kaki* Persimmo. In: Litz RE, Pliego-Alfaro F, Hormaza JI (eds) Biotechnology of fruit and nut crops (2nd edition). Oxfordshire, Boston, pp 164–190
Zhao WY, Fan Q, Chen F, Huang CY, Chen ZX, Hou RF, Liao WB (2018) *Diospyros penghuae*, a new species of Ebenaceae from Danxiashan mountain in Guangdong, China. Acta Sci Nat Univ Sunyatseni 57:98–103 (in Chinese with English summary)
Zheven AC, Zhukovsky PM (1975) Dictionary of cultivated plants and their centres of diversity. Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands
Zhu QG, Xu Y, Yang Y, Guan CF, Zhang QY, Huang JW, Grierson D, Chen KS, Gong BC, Yin XR (2019) The persimmon (*Diospyros oleifera* Cheng) genome provides new insights into the inheritance of astringency and ancestral evolution. Hort Res 6:138
Zhuang DH, Kitajima A, Ishida M (1990a) Chromosome number of the original tree and open-pollinated progenies of Japanese persimmon cv Hiratanenashi. J Jpn Soc Hort Sci 59:479–485 (in Japanese with English summary)
Zhuang DH, Kitajima A, Ishida M, Sobajima Y (1990b) Chromosome numbers of *Diospyros kaki* cultivars. J Jpn Soc Hort Sci 59:289–297 (in Japanese with English summary)

Scion Cultivar Breeding

3

Akihiko Sato

Abstract

Oriental persimmon (*Diospyros kaki*) originated in Eastern Asia and many local landrace cultivars have been developed in China, Japan, and Korea. In these countries, persimmon cultivars had been developed initially from chance seedlings and subsequently by bud mutants and crossbreeding. At present, crossbreeding, particularly pollination-constant non-astringent (PCNA) breeding, is being conducted mainly in Japan, Korea, China, Italy, Uzbekistan, and Spain. In Japan, the crossbreeding program started at the national institute in 1938 with the aim of releasing PCNA cultivars with early ripening, high quality, and less-cracking characteristics. So far, 12 PCNA and two PVA cultivars for table use have been released by the institute. Because the PCNA genotype is recessive to the other three non-PCNA genotypes and recessive PCNA alleles are not accumulated in most cultivars, crosses between PCNA and local non-PCNA local cultivars produce only non-PCNA F_1 offspring. Therefore, PCNA-type F_1 offspring are obtained exclusively by crosses among PCNA genotypes, Thus, crosses among PCNA are the most efficient method for obtaining PCNA offspring. However, the number of superior PCNA cross-parents is limited. Consequently, inbreeding depression became obvious in the program in the late 1980s, especially in fruit size, tree vigor, and productivity. To overcome inbreeding, a backcross program [(PCNA × non-PCNA) × PCNA] was started in the 1990s. This process, however, was time-consuming and inefficient because only 15% of PCNA offspring were segregated from the crosses. Therefore, molecular markers linked to PCNA/non-PCNA locus were developed for discriminating PCNA and have been applied to practical breeding in Japan. Chinese PCNA, which has a different origin and mechanism of removal of astringency from the Japanese PCNA, is dominant to the non-PCNA trait. The molecular marker linked to the Chinese PCNA has also been developed. The marker for discriminating Chinese PCNA has also been applied to practical breeding in Japan and China. Persimmon breeding has been conducted at several institutes around the world, including the Huazhong Agricultural University in China, Sweet Persimmon Research Institute, Gyeongsangnam-do Agricultural Research and Extension Service, and Pear Research Station, National Institute of Horticultural & Herbal Science in Korea, University of Florence in Italy, Instituto Valenciano de

A. Sato (✉)
Experimental Farm, Kindai University, Wakayama, Japan
e-mail: satoaki@nara.kindai.ac.jp

R. Tao and Z. Luo (eds.), *The Persimmon Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-05584-3_3

Investigacions Agrarias in Spain, Nikita Botanical Garden, Nova Kachocka Experimental Research Station, and Grishko Botanical Garden in Ukraine, and Instituto Agronomico, Campinas, State of Sao Paulo in Brazil, with goals for releasing highly adaptable cultivars in each country.

3.1 Introduction

Persimmon originated in east Asia and has been a major fruit crop, especially in Japan, China, and Korea for a long time (Agricultural Research Station 1912; Cho and Cho 1965; Wang et al. 1997). Today, persimmon is produced in many countries, including China, Spain, Korea, Japan, Azerbaijan, Brazil, Uzbekistan, Italia, Israel, and Iran (Food and Agriculture Organization of the United Nations 2020). Despite its large production in the world, persimmon crossbreeding program has been only conducted in a few countries. Several studies have reviewed the breeding and genetics of persimmon, including those on the programs in Japan by Yamada (1993, 2005, 2013), in China by Xu et al. (2017), and those in worldwide efforts by Yonemori et al. (2000), Yamada et al. (2012a), and Yesiloglu et al. (2018). This chapter focuses on the recent activities in practical persimmon breeding, particularly PCNA breeding, the research associated with this breeding, and its achievements.

3.2 Seedling and Bud Mutant Selection

3.2.1 Seedling Selection

An early stage of cultivar development in persimmon is assumed to be based on selections from seedlings of natural hybridization. According to Kikuchi (1948), 'cultivar names' were mentioned in Chinese literature in the 1100s and in Japanese literature in the 1600s. In Japan, there were many local cultivars all over the country, except in the Hokkaido and Okinawa islands, most of which were selected from the seedlings and clonally propagated. In 1912, the Department of Horticulture of the National Agricultural Research Station at Okitsu, Shizuoka (presently, Citrus Research Station, Institute of Fruit Tree and Tea Science, National Agriculture Research Organization, NARO), collected persimmon cultivars, classified them based on astringency type and fruit shape, and has reported that there were more than 1000 local cultivars, including synonyms (Agriculture Research Station 1912). The report showed that several superior cultivars, including 'Fuyu', 'Jiro', and 'Hiratanenashi', were selected from the local cultivars and were recommended for cultivation. These have been commercially grown as leading cultivars today.

3.2.2 Natural Bud Mutant

Natural bud mutants have been found based on the changes in visible traits such as ripening time, fruit size and shape, fruit color, or tree growth habits in grower's orchards. In persimmon, particularly, bud mutants with early ripeness and large fruits have contributed to the development of persimmon production, especially after the twentieth century (Table 3.1).

For early ripeness, several mutants with a difference of two weeks in ripening from original cultivars have been found in the orchards in Japan. This phenomenon suggests that the genetic effect of a single mutation change in an allele of a QTL-controlling fruit ripening time may be two weeks, or that humans can detect two weeks difference in ripeness by visual observation. These bud mutant cultivars contributed to an expansion of sale period of persimmon fruits.

For fruit size, 'Sunami' and 'Ohtanenashi' with large fruits were selected from 'Fuyu' and 'Hiratanenashi', respectively. 'Totsutanenashi' is a small-sized early ripening and dwarf type bud mutant of 'Hiratanenashi' (Yamane et al. 2008).

Bud mutant cultivars sometimes manifested some associated small changes in other traits. For example, 'Matsumoto Wase Fuyu' shows less productivity and tree vigor, and more sensitivity to cracking at the calyx end than 'Fuyu' (Yamada

Table 3.1 Representative bud mutant cultivars and their production in Japan

Bud mutant cultivar	Original cultivar	Major visible changes	Accompanied small changes	Production area in Japan (ha)[a]
Matsumoto Wase Fuyu	Fuyu	Early ripeness	Increase of cracking at calyx end, less vigorous and productivity	703.3 (Fuyu:3353.1)[b]
Uenishiwase	Matsumoto Wase Fuyu	Early ripeness		64.7 (Matsumoto Wase Fuyu:703.3)
Sunami	Fuyu	Large fruit		1.9 (Fuyu:3472.3)
Maekawa Jiro	Jiro	Early ripeness	Decrease of cracking at fruit apex	484.3[c] (Jiro:303.5)
Aishuho	Maekawa Jiro	Large fruit	Increase of cracking at fruit apex	4.5 (Maekawa Jiro:484.3)
Tonewase	Hiratanenashi	Early ripeness		2170.5 (Hiratanenashi:2268.9)
Ishibashiwase	Hiratanenashi	Early ripeness	Less vigorous	10.3 (Hiratanenashi:2268.9)
Nakataniwase	Tonewase	Early ripeness		185.2 (Tonewase:2170.5)
Hasshu[d]	Hiratanenashi	Seed formation, decrease of fruit size, dwarfing		–

[a]Acarage was in 2018, based on the statistical data by the Ministry of Agriculture, Forestry, and Fisheries, Japan (2021)
[b]Within parenthesis shows the original cultivars and their acreage
[c]Data includes other early ripening cultivar such as 'Ichikikei Jiro', but the production area of those cultivars are likely to be very small
[d]Yakushiji et al. (2016, 2017)

1995). 'Jiro' also produced several early ripening bud mutant cultivars. 'Maekawa Jiro', an early ripening bud mutant, shows less cracking at fruit apex than 'Jiro' (Yamada 1995) and has been substituted for 'Jiro' due to both its early ripeness and reduced cracking. Therefore, 'Maekawa Jiro' is advantageous for commercial growing compared to other early ripening bud mutants. Although 'Jiro' produced other early ripening bud mutants such as 'Ichikikei Jiro', 'Yaizu Wase Jiro', these cultivars have similar cracking characteristics as 'Jiro' (Yamada 1995).

Bud mutants sometimes exhibit changes in ploidy level. An octoploid bud mutant, 'Hasshu' ($2n = 8x = 120$) was found in the orchard of 'Hiratanenashi' ($2n = 9x = 135$) (Yakushiji et al. 2016, 2017). The mechanism of transformation from nonaploid to octoploid is unknown. 'Hasshu' has small size and seeded fruits and the short internode in shoots compared to original 'Hiratanenashi'. Seeds of 'Hasshu' are well-germinated rendering it potential for being used as a cross-parent for breeding seedless heptaploid seedlings by hybridization of their pollen with hexaploid persimmon.

An astringent cultivar, 'Saijo', which grows in Chugoku district in Japan and has a long cultivation history (Kikuchi 1948), has wide genetic variations in ripening time, fruit shape and size, shelf life after removal of astringency. Isoda (1983) has shown the characteristics of over 40 'Saijo' selections. Mochida and Itamura (2007) selected a 'Saijo' selection with tolerance against preharvest fruit softening among six 'Saijo' mutants.

Among the bud mutant cultivars in Japan, 'Matsumoto Wase Fuyu', 'Maekawa Jiro', and 'Tonewase' have remained as leading cultivars. 'Nakataniwase', an early ripening bud mutant of 'Tonewase' is now gradually increasing in

popularity (Table 3.1). Thus, finding bud mutants is still an important breeding procedure, and these cultivars contribute to the persimmon industry.

3.2.3 Artificial Bud Mutant

In fruit breeding, gamma irradiation has been used as a genetic modification method. Nishida (1970) found that the lethal gamma irradiation dose for persimmon plants was lower than that for grape, apple, peach, and Japanese pear. Moreover, the lethal dose to 'Jiro' and 'Yotsumizo' was lower than to 'Fuyu', suggesting that there was a varietal difference in lethal irradiation doses. Ray (2002) also summarized that a wide range of viability was obtained when persimmon cuttings, seeds, and pollen were irradiated with gamma-ray doses at 5–10 kR. In Spain, gamma irradiation technique has been applied to 'Rojo Brillante' to extend genetic variation (Naval et al. 2013). The optimal gamma irradiation dose combining survival rate and mutation induction was determined as 20 Gy on an astringent cultivar, 'Rojo Brillante'.

3.3 Genetics of Fruit Traits

3.3.1 Astringency Type

Persimmon cultivars are classified into pollination constant and variant, which are subdivided into astringent and non-astringent, resulting in four types denoted as PCA, PCNA, PVNA, and PVA (Yonemori et al. 2000). Mature fruits of astringent types have no palatability due to their severe astringent taste. Mature fruits of non-astringent types can be eaten with no or very little astringency. Genetically, the trait is composed of two subtraits: PCNA and non-PCNA, and non-PCNA are classified into PVNA, PVA, and PCA, depending on the quantitative capability of acetaldehyde and ethanol production in seeds (Ikeda et al. 1985). In addition, there are two types of PCNA: Japanese and Chinese types, which are characterized by small tannin cells in contrast with non-PCNA with bigger size tannin cells (Ikegami et al. 2006). Yamada (2013) summarized the inheritance of the astringent types.

The Japanese PCNA trait is recessive to the non-PCNA trait (Ikeda et al. 1985). Crosses among Japanese PCNA cultivars/selections produce PCNA F_1 offspring solely, and almost no F_1 PCNA offspring were obtained from crosses between PCNA and local non-PCNA cultivars (Ikeda et al. 1985; Yamada and Sato 2002). Only around 15% PCNA offspring were obtained from backcrosses [PCNA × (PCNA × non-PCNA)] (Ikeda et al. 1985). The Japanese PCNA/non-PCNA genotype is controlled by a single locus (*AST/ast*, or *A/a*), but the hexaploid nature of persimmon complicates the segregation ratios. The presence of one dominant *AST* allele is sufficient to express the non-PCNA phenotypes, whereas the PCNA phenotype is expressed only when all alleles are homozygous recessive for *ast* (denoted as *aaaaaa*) (Akagi et al. 2010).

The Chinese PCNA is quite different from the Japanese PCNA in genetic behavior (Ikegami et al. 2004, 2006). In terms of tannin cells, all offspring from crosses among Japanese PCNA cultivars/selections contained only small tannin cells, resulting in PCNA phenotypes, while those from crosses between non-PCNA and Japanese PCNA cultivars had mostly large tannin cells with non-PCNA phenotypes due to the recessive nature of the Japanese PCNA trait. Meanwhile, offspring between Chinese PCNA ('Luotian Tianshi') and Japanese PCNA cultivars segregated into two offspring groups with small and large tannin cells, respectively (Ikegami et al. 2004). Crosses between Chinese PCNA and non-PCNA cultivars produced about 50% of the PCNA phenotype (Ikegami et al. 2006). These results indicate that the Chinese PCNA trait of 'Luotian Tianshi' is genetically dominant to the non-PCNA trait and 'Luotian Tianshi' is likely heterozygous (Ikegami et al. 2006).

Complete loss of astringency in the fruits of the Japanese PCNA cultivar requires a high temperature during the fruit development stage (Chujo 1982). There are genetic differences in the natural loss of astringency among

Japanese PCNA cultivars, which results from the temperature required for complete loss of astringency in each cultivar. Because 'Fuyu' showed ease in the natural loss of astringency, and with the added advantage of its high quality, this cultivar has been grown for commercial production in a large scale in Japan. In contrast, 'Luotian Tianshi', a Chinese PCNA, has constantly displayed slight astringency at the orchard of NIFTS at Akitsu, Hiroshima (Yamada et al. 1993a). Sato et al. (2018a) showed that crosses with Chinese PCNA segregated higher percentage of PCNA individuals with little or more astringent taste than crosses among Japanese PCNA segregated. At present, it is not obvious whether incomplete loss of astringency in Chinese PCNA is influenced by temperature during the fruit development stage like in Japanese PCNA. To clarify if environmental factors affected the loss of astringency in Chinese PCNA, further studies on adaptability to wide ranges of temperature in fruit growing season will be required. Therefore, to select a new Chinse cultivar with broad local adaptability, breeders need to conduct severe selection for sensory astringency in Chinese PCNA, particularly in cool regions.

PCNA traits in both Japanese and Chinese types influence fruit weight or fruit size. Sato et al. (2013) showed that fruit weight in Japanese PCNA is significantly lower than that in non-PCNA offspring in two backcross families. Katayama-Ikegami et al. (2013) also showed that the fruit shape of Chinese PCNA offspring was flatter than those of non-PCNA offspring, although the differences in fruit weight between Chinese PCNA offspring and non-PCNA were not significant. Further studies on PCNA gene expression and its genetic association with other traits are needed for breeding superior PCNA that combine desirable characteristics, including large fruit weight.

3.3.2 Fruit Ripening Time

Fruit ripening time of original local Japanese PCNA cultivars was later than other astringent types (Yamada et al. 1994a). Therefore, PCNA with early ripening has been an important breeding target in Japan (Yamada 1993). Inheritance of fruit ripening time is under solely additive and quantitative control (Yamada et al. 1995). Fruit ripening time had high broad-sense heritability in a population of cultivars/selections used as cross-parents in the 1970s and 1980s at the national institute (Yamada et al. 1993b). Yamada et al. (1995) showed that the proportions of offspring with early ripening were very low in crosses using late-ripening PCNA cultivars/selections as parents due to the narrow segregation in the cross. Fruit ripening time in PCNA breeding populations gradually shifted toward early ripening through several cycles of selection over 50 years (Fig. 3.1).

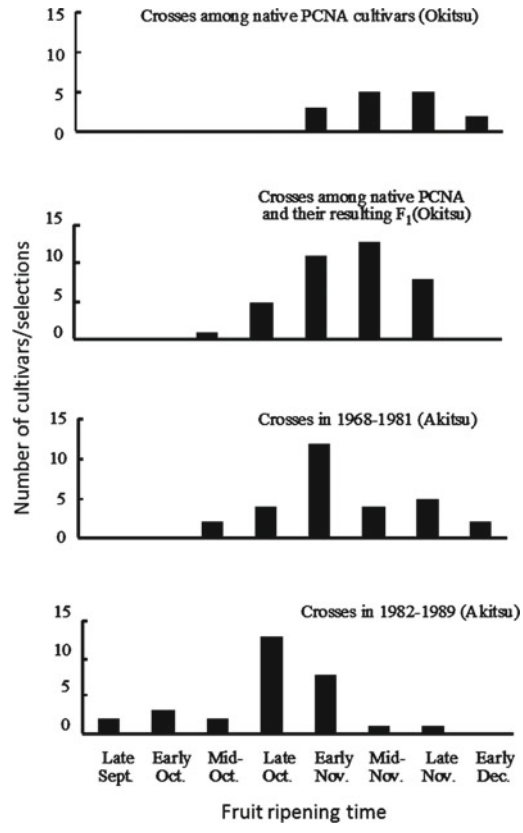

Fig. 3.1 Fruit ripening time of cultivars and selections of PCNA or derived from PCNA, used as parents at Okitsu and Akitsu. Citation from Yamada (1993)

3.3.3 Fruit Weight

Large fruit size is an important character for the market because large-sized persimmons are sold at a higher price. Fruit weight is a quantitative character and shows higher broad-sense heritability than soluble solids concentration (Yamada et al. 1993b). The genetic differences among family means could be explained mostly by the multiple regression on the inbreeding coefficient (F) and mid-parental value (MP), indicating that breeders can estimate the proportion of offspring with desirable fruit weight accuracy based on F and MP (Yamada et al. 1994b; Yamada and Yamane 1997). It should be emphasized that fruit weight is influenced more by F than by MP and that fruit weight of family mean in offspring is greatly reduced by inbreeding. Unfortunately, the family mean in offspring was smaller than the MP even when F = zero in crosses among Japanese PCNA cultivars/selections. As described later, local PCNA cultivars have a narrow genetic background. Repeated crossing within a small number of those local Japanese PCNA cultivars/selections leads to inbreeding, which explains the reason why family means in offspring were smaller than MP values, even in crossings with F = zero (Yamada et al. 1994b).

3.3.4 Cracking Habits

Local Japanese PCNA cultivars have relatively high cracking habits against the three other astringent types of local cultivars in Japan (Yamada et al. 1988). High frequency of offspring with high cracking habits from crosses among local Japanese PCNA cultivars had severely hindered Japanese PCNA breeding. There are two types of cracking in persimmon: cracking at calyx end and at fruit apex. Both types are inherited independently and quantitatively and are influenced by the environment and fluctuate greatly from year to year (Yamada et al. 1986, 1987). For cracking at calyx end, genotype × year interaction was the largest component among environmental factors (Yamada et al. 2002). The non-cracking cultivars are likely to be homozygous whereas cultivars with cracking are heterozygous (Yamada et al. 1988). Consequently, crosses among crack-resistant parents are desirable, but it was difficult to carry out crosses with most Japanese PCNA parents as they are all crack-susceptible. In the early generations of the breeding, therefore, breeders should make a special emphasis on the selection of cracking habit at the calyx and/or fruit apex (Yamada et al. 1988). However, careful selections over the two decades have resulted in increases in crack-resistant offspring (Yamada and Sato 2003). As shown by Onoue et al (2018b), long-term selections against crack susceptibility over generations has been successfully conducted in Japanese PCNA breeding.

3.3.5 Soluble Solids Concentration

Sweetness is an important quality factor for increase in palatability. Soluble solids concentration (SSC) is a quantitative trait, which fluctuates markedly depending on the year, trees, and fruits (Yamada et al. 1993b). SSC has a very large within-family variance in offspring in a cross, indicating that SSC variation in segregating population is much larger than the fruit weight and fruit ripening time variations (Yamada et al. 1997). Even when MP = 16.5%, the proportion of offspring that have an SSC of more than 18% is estimated at 32%, while when MP = 17.5% it is estimated at 50%. Thus, it is not difficult to obtain fruits with high SSC in a cross of parents with low SSC.

3.3.6 Flesh Firmness and Juiciness

Soft and juicy flesh is a desirable textural property in persimmon (Yamada et al. 2012a). Flesh firmness and juiciness are quantitative traits. In the breeding program at NARO, Japan, flesh firmness and juiciness have been evaluated as sensory scores since modern breeding started. Ban et al. (2010) estimated the proportion of offspring with soft and juicy flesh in various

crosses using logistic regression based on sensory scores. While the proportion of offspring with soft flesh was largely estimated only by MP of firmness, the proportion of offspring with juicy flesh could be estimated both by MP of juiciness and firmness. It showed that sensory juiciness was affected by firmness and that the proportion of offspring with juicy flesh increased as firmness in MP decreased even in the same MP value in juiciness.

3.3.7 Seedlessness Caused by Changes in Ploidy Level

Seedlessness is an important fruit character in persimmon because seedless fruits have higher palatability than seeded ones. Fruits of some cultivars such as 'Jiro' and its bud mutant cultivars in Japan, 'Kaki Tipo' in Italy, 'Rojo Brillante' in Spain, and 'Triumph' in Israel are sold commercially as seedless fruits. Seedless fruits in these cultivars are produced in orchards without pollinizers. However, these cultivars produce seeded fruits when pollinizers are planted around them. Thus, the production of seedless fruits in these cultivars is influenced by pollinizers.

Seedlessness in persimmon is also induced by polyploidy. Cytogenetic studies revealed that seedless cultivars, 'Hiratanenashi', 'Tonewase', 'Miyazakitanenashi', and 'Watarizawa' were nonaploid ($2n = 9x = 135$) whereas others are hexaploid ($2n = 6x = 90$) (Zhuang et al. 1990a, 1992). In these nonaploid seedless cultivars, seed abortion occurs at an early stage of fruit developmental stage so that fruits become seedless regardless of the presence or absence of pollinizers. Zhuang et al. (1990b) also confirmed that the chromosome number of roots in the original tree of 'Hiratanenashi' was $2n = 9x = 135$, suggesting that 'Hiratananashi' was derived from a natural hybridization between reduced ($n = 45$) and unreduced gametes ($2n = 90$).

Unreduced pollen is frequently produced under natural conditions in Japanese persimmon cultivars and could be sorted from reduced pollen based on the grain size (Sugiura et al. 2000). Using unreduced pollen and an in vitro embryo rescue technique, nonaploid plantlets have been obtained by artificial hybridization. In a practical breeding, nonaploid individuals were obtained by embryo culture of imperfect seeds from a cross between hexaploid persimmon. Chijiwa et al (2008) reported that two nonaplaiod plants raised by embryo culture of 68 imperfect seeds derived from a cross between 'Fuyu' and 'Taishuu', were both hexaploid. SSR analysis suggested that the two plants were derived from fertilization of a reduced female gamete with an unreduced male gamete. Thus, seedless nonaploid individuals can be produced using unreduced pollen in hybridization or embryo culture of imperfect seeds from a cross among normal hexaploid cultivars.

3.4 Marker-Assisted Selection (MAS) for Discriminating PCNA

3.4.1 Japanese PCNA

The processes for developing DNA markers for discriminating Japanese PCNA have been reviewed by Sato and Yamada (2016) and Yamada et al. (2012a). This section focuses mainly on the background of the development of the DNA marker, current status in marker development, and the practical uses of the marker.

As has been described in several reviews (Yonemori et al. 2000; Yamada et al. 2012a; Sato and Yamada 2016), the local Japanese PCNA were found in a narrow geographical area. The first documented Japanese PCNA cultivar in the literature, 'Gosho', was assumed to be originated only 200 years ago (Kikuchi 1948), indicating a short cultivation history. Yamada (2005) also showed that there were only 18 PCNA cultivars, except for bud mutant, in the germplasm collection of the national institute. Moreover, Japanese PCNA cultivars are characterized by a late-ripening time, flat fruit shape with wrinkles, and sensitivities to fruit cracking both at calyx end and at fruit apex (Yamada 1993; Yamada et al. 2012a; Sato and Yamada 2016). AFLP

(Kanzaki et al, 2000a; Yonemori et al. 2008) and SSR analysis (Naval et al. 2010) also suggested that Japanese PCNA cultivars show high genetic similarities. Thus, the original PCNA cultivars seem to have had a narrow genetic background.

The persimmon breeding program in the national institute began at Okitsu (currently Citrus Research Station, Institute of Fruit Tree and Tea Science, NARO) in 1938 and moved to Akitsu (currently Grape and Persimmon Research Station, Institute of Fruit Tree and Tea Science, NARO) in 1968 (Yamada et al. 2012a). The program has been focusing on the release of superior PCNA cultivars. Since the breeding started, crossings have mostly been conducted among PCNA cultivars/selections. In particular, the breeding program has been aiming at developing superior early ripening PCNA cultivars because ripening of local PCNA was late. Through several cycles of selection over 50 years, fruit ripening time in breeding populations gradually shifted toward early ripening as shown in Fig. 3.1.

A small number of superior PCNA cultivars have been used as parents for selections over many years for early ripeness, which has resulted in inbreeding. Inbreeding depressions such as reduction of fruit size and vigor became a serious problem in the late 1980s (Fig. 3.2; Yamada 1993; Sato and Yamada 2016). Approaches to avoid inbreeding was initiated in the1990s. An approach included obtaining Japanese PCNA from backcrosses (Japanese PCNA × (Japanese PCNA × non PCNA)). This type of crossing, however, produces usually only 15% of PCNA offspring because of the recessive nature of PCNA (Ikeda et al. 1985).

To solve this inefficiency, the development of molecular markers linked to the *AST /ast* locus began in 1997 as a cooperative study between the national institute and Kyoto University, and subsequently with Kindai University. After a series of studies (Kanzaki et al. 2000b, 2001, 2009, 2010), PCR-based SCAR markers that can be applied to the offspring from several types of parents with different *Ast* allele (Fig. 3.3) were developed. These SCAR markers have been applied to offspring from two backcross families using 'Taigetsu' (Japanese PCNA × 'Kurokuma') (Yamada et al. 2012b) and 'Taiten' (Japanese PCNA × 'Kurokuma') (Yamada et al. 2012c). The effectiveness of these markers was confirmed in practical persimmon breeding (Mitani et al. 2014a, b). Moreover, Kono et al (2016) estimated the genotypes of astringency in 'Taiten' and 'Taigetsu' as *AAaaaa*.

Kono et al (2016) also found that the region linked to the *AST* locus showed high polymorphism, including microsatellites. The analysis of the size of PCR-amplified fragments revealed 12 different *Ast* alleles from 14 non-PCNA cultivars and the Chinese PCNA, 'Luotian Tianshi'. In addition, Onoue et al (2018a) demonstrated 21 *Ast* alleles and five *ast* alleles from 237 germplasm accessions. Among the five *ast* alleles, four were found not only in Japanese non-PCNA cultivars but also in Chinese, Korean, Turkish, Italian, and New Zealand non-PCNA cultivars. Although the fact that some Chinese and Korean non-PCNA cultivars have *ast* alleles was shown by q-PCR (Akagi et al. 2010), this study showed that the *ast* alleles are widely distributed in non-PCNA cultivars of non-Japanese origin. The polymorphism can be applied to cultivar identification and a choice of non-PCNA parents in breeding.

3.4.2 Chinese PCNA

As described in Sect. 3.3.1, the Chinese PCNA trait of 'Luotian Tianshi' is dominant and is controlled by a different locus (the *B/b* locus) compared to the Japanese PCNA (Ikegami et al. 2006), showing that this trait has a different origin from Japanese PCNA. Moreover, AFLP analysis revealed that 'Luotian Tianshi' was genetically far from the group of Japanese PCNA (Kanzaki et al. 2000a). Therefore, the use of Chinese PCNA as a cross-parent is suggested to be advantageous for avoiding inbreeding depression. For discriminating Chinese PCNA, a PCR-based SCAR marker that is tightly linked to the Chinese PCNA dominant allele was developed by Ikegami et al. (2011).

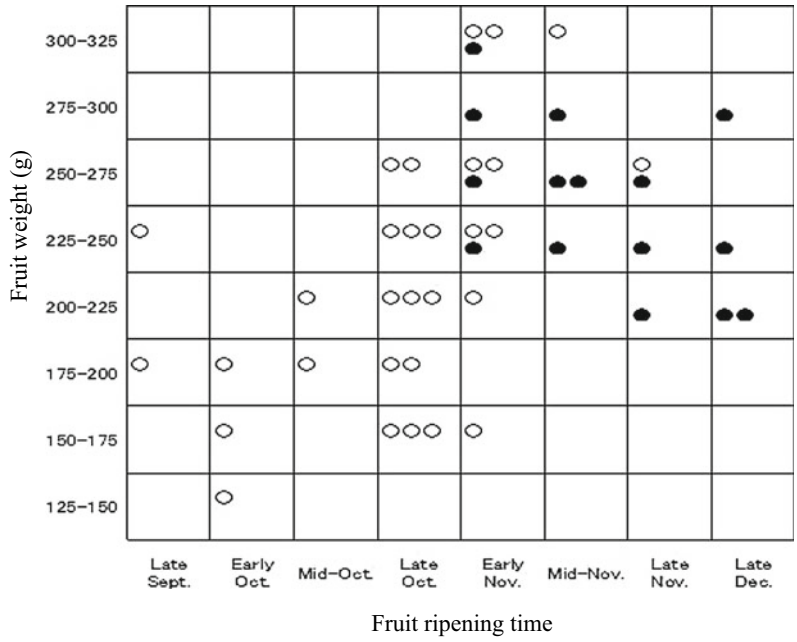

Fig. 3.2 Relationship between fruit ripening time and fruit weight in cross-parents at Okitsu and Akitsu. Filed circle: PCNA native cultivars used as parents at Okitsu. Open circle: PCNA used as parents at Akitsu. Citation from Yamada (1993)

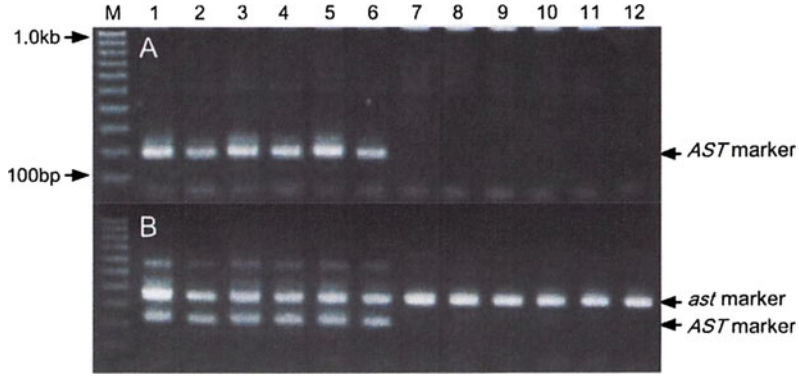

Fig. 3.3 Segregation of the SCAR markers in offsprig in a backcross [('Kurokuma' × 'Taishuu') × 109–27] family). Lane 1–6: non-PCNA type offspring and lane 7–12: PCNA type offspring. M: 100 bp ladder size marker. A: polymorphisms detected by the SCAR primer pair AST-F/AST-R. The 190-bp fragment was presented in all non-PCNA type offspring, but not in PCNA type offspring. B: Amplified fragments of multiplex PCR by the primer pair AST-F/PCNA-F/5R3R. All offspring showed the *ast*-linked 350-bp fragment while only non-PCNA type offspring showed the *AST*-linked 220 bp fragment. Citation from Kanzaki et al. (2010)

3.5 Breeding Activities and Achievements

3.5.1 Japan

The Japanese persimmon breeding program has been focused on releasing superior PCNA cultivars. Because there were only two leading Japanese PCNA cultivars ('Fuyu' and 'Jiro'), both of which were late ripening, one of the most important breeding objectives was to develop superior early ripening PCNA cultivars. Other considerations included no cracking habits, large fruit size, high eating quality (soft, juicy, and non-mealy flesh), high productivity, long shelf life, and good fruit appearance. Since 1950s, crosses had been mainly made among Japanese PCNA cultivars and selections. At Okitsu, 'Suruga' (Iikubo et al. 1961) with late ripening and 'Izu' with early ripening (Hirose et al. 1971) were selected from the first and second generation, respectively. After the program moved to Akitsu, 'Shinsyuu' (Yamane et al. 1991a), 'Soshu' (Yamada et al. 2004), 'Kanshu' (Yamada et al. 2006), 'Kishu' (Yamada et al. 2009). 'Taiga' (Sato et al. 2019a), and 'Reigyoku' (Sato et al. 2019b) were released as PCNA cultivars with early ripening. In addition, 'Youhou' (Yamane et al. 1991b), 'Taishuu' (Yamane et al. 2001), 'Yubeni' (Yamada et al. 2003), and 'Taiho' (Sato et al. 2018b) were released as medium to late-ripening PCNA cultivars (Table 3.2). All of them were produced from crosses among PCNAs.

As mentioned above, back cross strategies have been adopted to relieve inbreeding depression in PCNA breeding since the 1990s at Akitsu. In the process, superior non PCNA cultivars, 'Taigetsu' (Yamada et al. 2012b) and 'Taiten' (Yamada et al. 2012c) were selected from the first generation between PCNA ('Taishuu') × non-PCNA ('Kurokuma'). Because 'Taiten' and 'Taigetsu' have large fruits and high productivities, they have been used as parents in backcrosses and their offspring have been applied to MAS. So far, MAS has been applied to over 12,000 and 1300 seedlings for discriminating Japanese and Chinese PCNA, respectively. Several superior seedlings in both PCNA types have been selected and are continuing to be evaluated.

At present, PCNA breeding at NARO has three strategies in crosses for obtaining new PCNA: (1) crosses among PCNA (PCNA cross), (2) backcrosses of PCNA × non-PCNA to PCNA (BC1), and (3) crosses of Chinese PCNA × non-PCNA or Japanese PCNA (Chinese PCNA cross). Table 3.3 shows a comparison of the proportion of individuals with desirable characteristics in the original 18 PCNA cultivars and in PCNA seedlings fruited in the breeding population in 2014. In PCNA crosses, except for fruit weight, selections toward desirable characteristics were effective probably due to the additive effect in these traits. However, genetic improvement in fruit weight was not observed despite long-time selections over generations. This suggests that fruit weight in persimmon was controlled considerably by a dominant effect that leads to inbreeding depression. In BC1 crosses, the proportion of PCNA individuals with fruit weight more than 250 g was 78% and higher than that of the original PCNA cultivars. This was probably due to mitigation of inbreeding depression in BC1 crosses. In Chinse PCNA cross, the proportion of PCNA individuals that showed no cracking was the highest, but those with high SSC was the lowest among the type of crosses. These characteristics were suggested to be derived from the Chinese PCNA parents, 'Luotian Tianshi' and 'Tianbaogai'.

Recently, PCNA breeding has been conducted at several prefectural research stations (Table 3.4). The Fukuoka Prefectural Research Center released the nonaploid seedless PCNA cultivar, 'Fukuoka K1 Gou' (Chijiwa et al. 2013). The Gifu Prefectural Agricultural Technology Center released a new PCNA cultivar, 'Neo sweet' with sweet and juicy flesh, derived from a cross between 'Shinsyuu' and 'Taishuu' (Niikawa et al. 2018). The Wakayama Prefectural Experiment Station of Agriculture, Forestry and Fisheries also released a new PCNA cultivar, 'Kishu temari' with early ripening (Furuta et al.

Table 3.2 PCNA cultivars released by NARO, Japan

Cutivar	Cross	Astringency type	Characteristics	Reference
Suruga	Okugosho × Hanagosho	PCNA	Late ripening, large fruit, sensitive to cracking at calyx end, high parthenocarpy	Iikubo et al. (1961)
Izu	Fuyu × Okitsu 1	PCNA	Early ripening, medium-sized fruit, less vigorous	Hirose et al. (1971)
Shinsyuu	Okitsu 20 × Okitsu 1	PCNA	Early ripening, high sugar content, skin stain	Yamane et al. (1991a)
Youhou	Fuyu × Jiro	PCNA	Medium ripening, high parthenocarpy, high productivity	Yamane et al. (1991b)
Taishuu	Fuyu × IIiG-16	PCNA	Medium ripening, large fruit, crisp and very juicy flesh	Yamane et al. (2001)
Yubeni	Matsumoto Wase Fuyu × F-2	PCNA	Late ripening, attractive red skin, few seeds, high parthenocarpy	Yamada et al. (2003)
Soshu	Izu × 109–27	PCNA	Very early ripening, less cracking, less vigorous	Yamada et al. (2004)
Kanshu	Shinsyuu × 18–4	PCNA	Early ripening, high sugar content, resistant to cracking, high parthenocarpy	Yamada et al. (2006)
Kishu	Izu × Akitsu 5	PCNA	Early ripening, large fruit, resistant to cracking at calyx end	Yamada et al. (2009)
Reigyoku	Kanshu × Akitsu 19	PCNA	Early ripening, good appearance, high sugar content, soft and juicy flesh, less cracking, high parthenocarpy	Sato et al. (2019b)
Taiga	Kanshu × Akitsu 19	PCNA	Early ripening, large fruits, soft and juicy flesh, less cracking, high parthenocarpy	Sato et al. (2019a)
Taiho	Okitsu 20 × Taishuu	PCNA	Late ripening, large fruit, crisp and juicy flesh, less cracking, high parthenocarpy	Sato et al. (2018b)
Taiten	Kurokuma × Taishuu	PVA	Extremely large fruit, less cracking, crisp and juicy flesh, high productivity	Yamada et al. (2012c)
Taigetsu	Kurokuma × Taishu	PVA	Medium ripening, very large fruit, soft and juicy flesh, less cracking, high parthenocarpy, high productivity	Yamada et al. (2012b)

Okitsu 1: selfing of Okugosho, Okitus 20: Fukurogosho × Hanagosho, IIiG-16: Jiro × (Okugosho × Hanagosho), F-2: Jiro × Okugosho, 109–27: (Fuyu × Okugosho) × (Okugosho × Fukurogosho), 18–4: Fuyu × (Okugosho × Hanagosho), Akitsu 5: Fuyu × (Okugosho × Hanagosho), Akitsu19: Ogosho × Taishuu

2019), which was derived from a cross between 'Soshu' and 'Taishuu'. In addition, the Tottori Horticultural Experiment Station bred the PCNA cultivar 'Kitaro', in cooperation with NARO. It was derived from a cross between 'Taromaru' and 'Kanshu'. In general, new PCNA cultivars released by these prefectural institutes have cultivars bred by NARO as the parents. Use of non-PCNA cultivars as cross-parents, therefore, is still important for getting superior offspring with high productivity in PCNA breeding. It will expand the genetic background in PCNA breeding population.

3.5.2 China

The Chinese PCNA cultivar 'Luotian Tianshi' has a long cultivation history of over 900 years (Wang 1983). Wang et al. (2005) reported

Table 3.3 Comparison of proportion of individuals with desirable characters between the original local PCNA cultivars and PCNA seedling population in NARO breeding program

Type of crosses	The proportion of individuals with desirable character in each trait (%)					
	Cracking at fruit apex:	Cracking at calyx end:	Fruit weight	SSC	Flesh firmness	Juiciness
	None	None	> 250 g	> 18%	Soft	Juicy
	Original local PCNA cultivars					
	28	28	50	33	0	44
	Seedling population at NARO breeding program (2014)					
PCNA[a]	83	76	48	68	39	54
BC1[b]	87	78	74	48	52	81
CPCNA[b]	90	97	58	23	48	81

[a]PCNA: PCNA individuals derived from PCNA × PCNA
[b]BC1: PCNA individuals derived from PCNA × (PCNA × non-PCNA)
[c]CPCNA: PCNA individuals derived from Chinese PCNA parents

Table 3.4 New PCNA cultivars recently released by prefectural institute in Japan

Breeder	Cutivar	Cross	Astringency type	Characteristics	Reference
Fukuoka Pref	Fukuoka K.1 Gou (Akiou)	Fuyu × Taishuu	PCNA	2n = 9x, seedless, large fruit, crisp and juicy flesh, high sugar content	Chijiwa et al. (2008)
Gifu Pref	Neo sweet	Shinsyuu × Taishuu	PCNA	Early ripening, crisp and juicy flesh, high sugar content	Niikawa et al. (2018)
Wakayama Pref	Kishu temari	Soshu × Taishuu	PCNA	Early ripening, good appearance, large fruits, soft and juicy flesh, less cracking	Furuta et al. (2019)
Tottori Pref. and NARO	Kitaro	Sodawase × Kanshu	PCNA	Early ripening, large fruits, less cracking	

several Chinese PCNA cultivars, 'Tianbaogai', 'Qiuyan', 'Sifangtianshi', and 'Xiaoguotianshi' around the south Dabie Mountains in Hubei province, where 'Luotian Tianshi' was found. These Chinese PCNA cultivars varied in fruit size and shape (Yonemori et al. 2005), probably due to the dominant nature of Chinese PCNA.

A persimmon research group of Huazhong Agricultural University focused on the origin, mechanism of natural removal of astringency, and genetic diversity of Chinese PCNA. Luo et al. (2005) surveyed and collected persimmon cultivars and individuals, including trees that bear only male flowers, and studied their origin and economic value. Consequently, a Chinese PCNA cultivar, 'Eshi 1' was selected as a promising cultivar from the germplasm in Luotian County, Hubei Province. This cultivar ripens early to mid-October in Luotian County, and has fruits weighing 180 g, larger than 'Luotian Tianshi', and with few seeds (Yi et al. 2004). As for trees with only male flowers, 'Male 8' was confirmed to be a Chinese PCNA from the progeny tests (Zhang et al. 2016). It is suggested to be a useful pollen donor for genetic improvement of Chinese PCNA. Attempts are being made at breeding new Chinese PCNA cultivars combining crossbreeding, embryo rescue, astringency type, and sexuality identification by marker assist selection, young-tip grafting,

expansion of nursery land, and producing areas (Xu et al. 2017).

3.5.3 Korea

In Korea, big holidays affect the breeding objectives of persimmon. There is a big Korean holiday season named the Chuseok from early September to early October. During this season, people offer many kinds of fruits, including persimmon, pear, apple, jujube, and chestnuts, to their ancestors and consume them, making it a season with high consumption of fruits (Ma et al. 2013, 2018). Therefore, breeding of early ripening cultivars, particularly of non-astringent type, is a main objective in Korea. In addition to early ripening, large fruit size, less physiological disorders, high quality including soft and juicy flesh and sweetness, are also important objectives (Ma et al. 2013).

Korean persimmon breeding programs has been conducted at the Sweet Persimmon Research Institute, Gyeongsangnam-do Agricultural Research and Extension Service, and at the Pear Research Station, National Institute of Horticultural & Herbal Science. Recently, 'Chuyeaon', an early ripening PVNA (Ma et al. 2013), 'Jowan', an early ripening PCNA (Ma et al. 2018), 'Gamnuri', a PCA with large fruit (Kim et al. 2018), 'Dannuri', a PCNA with high sugar content (Kim et al. 2019), 'Chosi', an early ripening PVNA (Ma et al. 2019), 'Olnuri', an early ripening PCNA (Kim et al. 2020), were released by these two institutes (Table 3.5).

3.5.4 Italy, Spain, Ukraine, and Brazil

In Italy, persimmon cultivars were introduced in the nineteenth century (Bellini and Giordani 2005). The persimmon breeding program started in 1971 at the University of Florence (Giordani 2002; Bellini and Giordani 2005). Some of the breeding objectives were: release of PCNA types, low tree vigor to adapt to severe European pruning, trees with only female flowers, early ripening, high productivity, resistance to chilling injury, compatibility on both *D. kaki* and *D. lotus,* and tree hardness. As for fruit traits, medium to large size, round or slightly flat shaped fruits, orange-red skin color, hard orange flesh without black spots, suitability to conservation and industrial transformation (drying), low susceptibility to cracking at fruit apex and calyx end, have also been important objectives in the program (Giordani 2002; Bellini and Giordani 2005).

In Spain, documents that place persimmon from the sixteenth century are available (Badenes et al. 2013). A PVA cultivar, 'Rojo Brillante' has been dominant and accounts for 88% of the total Spanish production (Badenes 2013), enjoying an almost monoculture status in persimmon. For this reason, the breeding program at the Instituto Valenciano de Investigacions Agrarias (IVIA) started in 2002 with the aim of increasing the range of cultivars by producing new cultivars as an alternative to 'Rojo Brillante'. The objective was also to extend the harvest season and to obtain new PCNA cultivars (Badenes et al. 2013).

In Ukraine, persimmon seeds were introduced in the nineteenth century. An intensive breeding program was started at the Nikita Botanical Garden at Yalta (Grygorieva et al. 2009) and the first new cultivar, 'Sputnik', was bred in 1945 at the Garden. Thereafter, breeding programs, including hybridization between kaki and *Diospyros virginiana*, have been conducted at the Nikita Botanical Garden, Nova Kachocka Experimental Research Station, and Grishko Botanical Garden. A breeding objective in Ukraine was to obtain new cultivars with adaptation to steppe climate to extremely low winter temperature and relatively high summer temperature.

In Brazil, 'Pomelo', an early ripening PCA cultivar, was developed from a breeding program at the Instituto Agronomico, Campinas, State of Sao Paulo (Rigitano et al. 1984; Ojima et al. 1985). From this program, 'Fuyuhana', a PCNA cultivar made between 'Fuyu' and 'Hanagosho', 'Rubi' (PCA), and 'Kaoru' (PVA) were released as early ripening cultivars with high productivity (Rigitano et al. 1984).

Table 3.5 New cultivars recently released in Korea

Breeder	Cultivar	Cross	Astringency type	Characteristics	Reference
Pear Research Station[a]	Chuyeon	Nishimurawase × Johongsi	PVNA	Early ripening, high quality	Ma et al. (2013)
Pear Research Station	Jowan	Shinsyuu × Taishuu	PCNA	Early ripening, very high quality	Ma et al. (2018)
Sweet Persimmon Research Institute [b]	Gamnuri	Sunami × Johongsi	PCA	Large fruit size, long keeping quality on tree	Kim et al. (2018)
Sweet Persimmon Research Institute	Dannuri	Danyeon 104 × Taishuu	PCNA	Large fruit size, high sugar content	Kim et al. (2019)
Pear Research Station	Chosi	Johongsi × Nishimurawase	PVNA	Early ripening	Ma et al. (2019)
Sweet Persimmon Research Institute	Olnuri	Shinsyuu × Taishuu	PCNA	Early ripening, high sugar content	Kim et al. (2020)

[a]Pear Research Station, National Institute of Horticultural & Herbal Science
[b]Sweet Persimmon Research Institute, Gyeongsangnam-do Agricultural Research and Extension Service

References

Agricultural Research Station (1912) Investigation on persimmon cultivars. Bull Agr Res Sta (extra) 28:1–46 (in Japanese)

Akagi T, Takeda Y, Yonemori K, Ikegami Kono A, Yamada M, Kanzaki S (2010) Quantitative genotyping for the astringency locus in hexaploid persimmon cultivars using quantitative real-time PCR. J Amer Soc Hortic Sci 135:59–66

Badenes ML, Martines-Calvo J, Naval MM (2013) The persimmon breeding program at IVIA: alternatives to conventional breeding of persimmon. Acta Hortic 996:71–75

Ban Y, Kono A, Mitani N, Sato A (2010) Estimation of the proportion of offspring having soft and juicy fruits as evaluated by sensory tests in Japanese persimmon. Scientia Hortic 126:145–151

Bellini E, Giordani E (2005) Germplasm and breeding of persimmon in Europe. Acta Hortic 685:65–75

Chijiwa H, Kuwahara M, Hirakawa N, Tetsumura T (2008) Generation of nonaploid persimmons (*Diospyros kaki* Thunb.) by embryo culture of imperfect seeds derived from a cross between 'Fuyu' and 'Taishuu'. J Jpn Soc Hortic Sci 77:358–363

Chijiwa H, Asakuma H, Ishizaka A (2013) Development of seedless PCNA persimmon (*Diospyros kaki* Thunb.) cv. 'Fukuoka K1 Gou' and the effect of gibberellin spray and/or disbudding on fruit set. Hortic Res (Japan) 12:263–267 (In Japanese with English abstract)

Cho SK, Cho TH (1965) Studies on the local varieties of persimmon in Korea (in Korean with English summary). Res Rep RDA 8:147–190

Chujo T (1982) Studies on the effects of thermal conditions on the growth and quality of fruits of Fuyu kaki. Memories of Faculty of Agric Kagawa Univ 37:1–63

Food and Agriculture Organization of the United Nations (2020) FAOSTAT. http://www.fao.org/faostat/en/#home

Furuta T, Kumamoto S, Nonaka A, Komatsu H (2019) New persimmon cultivar 'Kishu temari'. Bull Wakayama Agric Forest Fisheries Exp Stn 7:83–91

Giordani E (2002) Varietal assortment of persimmon in the countries of the Mediterranean area and genetic improvement. Options Mediterraneennes; Serie A seminaires Mediterraneens. n. 51:23–37.

Grygorieva O, Klymenko S, Brindza J, kochanova Z, Toth D, Derevjanko V, Grabovecka O (2009) Introduction, breeding and use of persimmon species (*Diospyros* spp.). Acta Hortic 833:57–62

Hirose K, Yamamoto M, Sato T, Ohata T, Nishida T, Ikeda I, Shimura I, Siba S, Yagi M, Tominaga N (1971) New Japanese persimmon variety 'Izu'. Bull Hortic Res Sta B 11:1–17 (in Japanese with English summary)

Iikubo S, Sato T, Nishida T (1961) New Japanese persimmon variety 'Suruga'. Bull Hortic Sta Natl Tokai-Kinki Agr Exp Sta. 6:33–37 (in Japanese with English summary)

Ikeda I, Yamada M, Kurihara A, Nishida T (1985) Inheritance of astringency in Japanese persimmon (*Diospyros kaki* Thunb.). J Jpn Soc Hortic Sci 54:39–45 (in Japanese with English summary)

Ikegami A, Yonemori K, Sugiura Sato A, A, Yamada M, (2004) Segregation of astringency in F_1 progenies derived from crosses between pollination-constant non-astringent persimmon cultivars. HortScience 39:371–374

Ikegami A, Eguchi S, Yonemori K, Yamada M, Sato A, Mitani N, Kitajima A (2006) Segregation of astringent progenies in the F_1 population derived from crosses

between a Chinese pollination-constant nonasteringent (PCNA) 'Luo Tian Tian Shi', and Japanese PCNA and pollination-constant astringent (PCA) cultivars of Japanese origin. HortScience 41:561–563

Ikegami A, Eguchi S, Akagi T, Sato A, Yamada M, Kanzaki S, Kitajima A, Yonemori K (2011) Development of molecular markers linked to the allele associated with the non-astringent trait of the Chinese persimmon (*Diospyros kaki* thumb.). J Jpn Soc Hortic Sci 80:150–155

Isoda R (1983) Difference of fruit shapes and quality in Saijo Japanese persimmon. Bul. Hiroshima Agric College 7:165–180 (In Japanese with English summary)

Kanzaki S, Yonemori K, Sato A, Yamada M, Sugiura A (2000a) Analysis of the genetic relationships among pollination-constant and non-astringent (PCNA) cultivars of persimmon (*Diospyros kaki* Thunb.) from Japan and China using amplified fragment length polymorphism (AFLP). J Jpn Soc Hortic Sci 69:665–670

Kanzaki S, Yonemori K, Sato A, Yamada M, Sugiura A (2000b) Evaluation of RFLP analysis for discriminating PCNA genotype in some persimmon cultivars. J Jpn Soc Hortic Sci 69:702–704

Kanzaki S, Yonemori K, Sato A, Yamada M, Sugiura A (2001) Identification of molecular markers linked to the trait of natural astringency loss of Japanese persimmon (*Diospyros kaki* Thunb.) fruit. J Am Soc Hortic Sci 126:51–55

Kanzaki S, Yamada M, Sato A, Mitani N, Utsunomiya N, Yonemori K (2009) Conversion of RFLP markers fir the selection of pollination-constant and non-astringent type persimmon (*Diospyros kaki* Thunb.) into PCR-based markers. J Jpn Soc Hortic Sci 78:68–73

Kanzaki S, Akagi T, Masuko T, Kimura Yamada M, Sato A, Mitani N, Utsunomiya N, Yonemori K (2010) SCAR markers fir practical application of marker-assisted selection in persimmon (*Diospyros kaki* Thunb.) breeding. J Jpn Soc Hortic Sci 79:150–155

Katayama-Ikegami A, Yonemori K, Sato A, Yamada M, Habu T, Kitajima A (2013) Relationship between astringency type and fruit shape in progenies of Chinese PCNA persimmon. Hortic Res 12:29–34

Kikuchi A (1948) Pomology-part I. Yokendo, Tokyo, pp. 347–400 (in Japanese)

Kim EG, Choi ST, Son JY, Ahn GH, Kim SC, Hong KP (2018) Breeding of the Gamnuri (*Diospyros kaki* Thunb.) cultivar for astringent persimmon with large-sized quality fruits. Korean J Breed Sci 50:340–343 (In Korean with English abstract)

Kim, EG, Son JY, Ahn GH, Joung WK, Hong KP (2019) A large sized sweet persimmon (*Diospyros kaki* Thunb.) cultivar, 'Dannuri' with high sugar content. Abstracts of 2019 Annual Meeting of the Korean Breeding Society, 283

Kim EG, Son JY, Choi ST, Ahn GH (2020) An early ripening large sized sweet persimmon (*Diospyros kaki* Thunb.) cultivar, 'Olnuri'. Korean J Breeding Sci Conf Issue (2020.8):324

Kono A, Kobayashi S, Onoue N, Sato A (2016) Characterization of a highly polymorphic region closely linked to the *AST* locus and its potential use in breeding of hexaploidy persimmon (*Diospyros kaki* Thunb.). Mol Breeding 36:56

Luo Z, Zhang Q, Guo D (2005) General situation on science and industry pf persimmon in China mainland. Acta Hortic 685:29–36

Ma KB, Cho KS, Kang SS, Kim YK, Choi JJ, Cho KH, Lee HC (2013) Breeding of Sweet persimmon cultivar 'Chuyeon' with high quality for early Korean Thanksgiving Day. Acta Hortic 996:327–331

Ma KB, Lee IB, Kim YK, Won KH, Cho KS, Choi JJ, Lee BHN, Kim MS (2018) 'Jowan', an early maturing PCNA (pollination constant non-astringent) persimmon (*Diospyros kaki* Thinb.). Ancta Hortic 1195:61–64

Ma KB, Cho KS, Kang SS, Kim YK, Hwang HS, Choi JJ, Han JH (2019) Chosi', early ripening sweet persimmon (*Diospyros kak*i thumb.) for 'Chuseok. Korean J Breed Sci 51:424–427 (In Korean with English abstract)

Mitani N, Kono A, Yamada M, Sato A, Kobayashi S, Ban Y, Ueno T, Shiraishi M, Kanzaki S, Tsujimoto T, Yonemori K (2014a) Application of marker-assisted selection in persimmon breeding of PCNA offspring using Scar markers among the population from the cross between non-PCNA 'Taigetsu' and PCNA 'Kanshu.' HortScience 49:1132–1135

Mitani N, Kono A, Yamada M, Sato A, Kobayashi S, Ban Y, Ueno T, Shiraishi M, Kanzaki S Tsujimoto T, Yonemori K (2014b) Practical marker-assisted selection using two SCAR markers for fruit astringency type in crosses of 'Taiten' x PCNA cultivars in persimmon. Scientia Hortic 170: 219–223

Mochida K, Itamura H (2007) Cause of pre-harvest fruit softening and comparison among early−ripening strains of Japanese Persimmon 'Saijo'. Hortic Res Japan 6:97–103 (In Japanese with English summary)

Navel MM, Zuriaga E, Pecchioli S, Llacer G, Giordani E, Badness ML (2010) Analysis of genetic diversity among persimmon cultivars using microsatellite markers. Tree Genet Genomes 6:677–687

Naval MM, Zuriaga E, Badenes ML (2013) AFLP analysis of mutations induced by gamma irradiation in 'Rojo Brillante' persimmon. Acta Hortic 996:117–121

Niikawa T, Sugiura M, Ozeki T, Suzuki T (2018) New persimmon cultivar 'Neo sweet.' Bull Gifu Agric Tech Center 18:7–13

Nishida T (1970) Radiation breeding for perennial crops. J Agricul Sci 25:564–569 In Japanese

Ojima M, Dall'orto FAC, Barbosa W, Tombolato AFC, Rigitano O (1985) Alternate baring in persimmon—CV. Pomelo (in Portuguese with English summary). Bragantia, Campinas 44:481–486

Onoue N, Kobayashi S, Kono A, and Sato A (2018a) SSR-based molecular profiling of 237 persimmon (*Diospyros kaki* Thunb.) germplasm using an

ASTRINGENCY-linked marker. Tree Genet Genomes 14:28

Onoue N, Kono A, Sato A, Yamada M (2018b) Reduction of fruit cracking in persimmon breeding at the national institute of Japan. Acta Hortic 1195:75–80

Ray PK (2002) Persimmon. In: Breeding tropical subtropical fruits. Narosa Publishing House. New Delhi, pp 316–329

Rigitano O, Ojima M, Dall'Orto FAC, Tombolate AFC, Barbora, Scarani HJ, Martins FP (1984) 'Fuyuhana': A new non-astringent persimmon for Sao Paulo (in Spanish). VIIth Brazilian Fruitculture Congr 288–294

Sato A, Kono A, Mitani N, Ban Y, Yamada M (2013) Comparison of fruit traits between pollination constant non-astringent (PCNA) and non-PCNA offspring derived from two Backcrosses (non-PCNA x PCNA) x PCNA. Acta Hortic 996:123–126

Sato A, Yamada M (2016) Persimmon breeding in Japan for pollination-constant non-astringent (PCNA) type with marker-assisted selection. Breed Sci 66:60–68

Sato A, Kono A, Onoue N, Yamada M (2018a) Incomplete loss of astringency in pollination-constant non-astringent (PCNA) offspring derived from Japanese and Chinese PCNA persimmon. Acta Hortic 1195:14

Sato A, Yamada M, Kono A, Mitani N, Yamane H, Iwanami H, Ueno Y, Shiraishi M, Ban Y. Hirakawa N, Onoue N, Yoshioka M, Nakajima I (2018b) New persimmon cultivar 'Taiho'. Bull NARO Fruit Tree and Tea Sci 2:19–31

Sato A, Yamada M, Kono A, Mitani N, Ban Y, Ueno T, Shiraishi M, Onoue N, Iwanami H, Yoshioka M (2019a) New persimmon cultivar 'Taiga'. Bull NARO Fruit Tree and Tea Sci 3:61–73

Sato A, Yamada M, Kono A, Mitani N, Ban Y, Ueno T, Shiraishi M, Onoue N, Iwanami H, Yoshioka M (2019b) New persimmon cultivar 'Reigyoku'. Bull NARO Fruit Tree and Tea Sci 3:75–89

Sugiura A, Ohkuma T, Choi YA, Tao R, Tamura M (2000) Production of nonaploid (2n=9x) Japanese persimmon (*Diospyros kaki*) by pollination with unreduced (2n=6x) pollen and embryo rescue culture. J Amer Soc Hortic Sci 125:609–614

Wang R (1983) The origin of 'Luo Tian Tian Shi'. Chinese Fruit Tree 2:16–19 (In Chinese)

Wang R, Yang Y, Li G (1997) Chinese persimmon germplasm resources. Acta Hortic 436:43–50

Wang R, Yang Y, Ruan X, Li G (2005) Native non-astringent persimmons in China. Acta Hortic 685:99–102

Xu J, Gan J, Xiang J, Zhang Q, Xu L, Guo D, Luo Z (2017) Advance of genetic improvement technology for Chinese PCNA persimmon. Acta Hortic Sinica 44:987–998 (In Chinese with English abstract.)

Yakushiji H, Kobayashi YA, S, Kaneyoshi J Azuma A, Sugiura H, Sato A, (2016) Characteristics of a dwarf octoploid mutation arising from a nonaploid persimmon cultivar. Hortic J 85:209–216

Yakushiji H, Yamasaki A, Kobayashi S Azuma A, Sugiura H, Sato A (2017) 'Hasshu', a new pollination -variant astringent persimmon cultivar. Bull NARO Fruit Tree and Tea Sci 1:27–35 (In Japanese with English summary)

Yamada M, Yamane H, Hirabayashi T (1986) Studies on crossbreeding of Japanese persimmon (*Diospyros kaki* Thunb.), V. Variation of "Hetasuki" (fruit cracking under the calyx) Bull Fruit Tree Res Stn E6:21–30 (in Japanese with English summary)

Yamada M, Yamane H, Hirabayashi T (1987) Yearly fluctuations of two types of fruit cracking in seedling populations of Japanese persimmon (*Diospyros kaki* Thunb). J Jpn Soc Hortic Sci 56:287–292

Yamada M (1993) Persimmon breeding in Japan. Jpn Agr Res Quart 27:33–37

Yamada M, Ikeda I, Yamane H, Hirabayashi T (1988) Inheritance of fruit cracking at the calyx and stylar end in Japanese persimmon (*Diospyros kaki* Thunb.). J Jpn Soc Hortic Sci 57:8–16 (in Japanese with English summary)

Yamada M, Sato A, Yakushiji H, Yoshinaga K, Yamane H, Endo M (1993a) Characteristics of 'Luo Tian Tian Shi', a non-astringent cultivar of oriental persimmon (*Diospyros kaki* Thunb.) of Chinese origin in relation to non-astringent cultivars of Japanese origin. Bull Fruit Tree Res Stn 25:19–32 (in Japanese with English summary)

Yamada M, Yamane H, Yoshinaga K, Ukai Y (1993b) Optimal spatial and temporal measurement repetition for selection in Japanese persimmon breeding. HortScience 28:838–841

Yamada M, Yamane H, Sato A, Hirakawa N, Wang R (1994a) Variations in fruit ripening time, fruit weight and soluble solids content of oriental persimmon cultivars native to Japan. J Jpn Soc Hortic Sci 63:485–492

Yamada M, Yamane H, Ukai Y (1994b) Genetic analysis of Japanese persimmon fruit weight. J Amer Soc Hortic Sci 119:1298–1302

Yamada M (1995) Persimmon. In: Kozaki I, Ueno I, Tsuchiya S, Kajiura I (eds) The fruit in Japan. Yokendo. Tokyo, pp 174–209

Yamada M, Yamane H, Ukai Y (1995) Genetic analysis of fruit ripening time in Japanese persimmon. J Amer Soc Hortic Sci 120:886–890

Yamada M, Yamane H (1997) Relationship between the observed and predicted distribution of offspring for fruit ripening time and fruit weight in Japanese persimmon. Scientia Hortic 69:157–167

Yamada M, Yamane H, Takano Y, Ukai Y (1997) Estimation of the proportion of offspring having soluble solids content in fruit exceeding a given critical value in Japanese persimmon. Euphytica 93:119–126

Yamada M, Sato A (2002) Segregation for fruit astringency type in progenies derived from crosses of 'Nishimurawase'×pollination constant non-astringent genotypes in Oriental persimmon (*Diospyros kaki* Thunb.). Scientia Hortic 92:107–111

Yamada M, Sato A, Ukai Y (2002) Genetic differences and environmental variations in calyx-end fruit cracking among Japanese persimmon cultivars and selections. HortScience 37:164–167

Yamada M, Sato A (2003) Status of persimmon breeding at the National Institute of Fruit Tree Science, Japan. Options Mediterraneennes Series A 51:75–79

Yamada M, Yamane H, Kurihara K, Nagata K, Sato A, Kishi T, Matsumoto R, Yoshinaga K, Hirakawa N, Iwanami H, Kakutani M, Ozawa T, Sumi T, Hirabayashi T, Kanato K, Nakajima I (2003) New Japanese persimmon cultivar 'Yubeni'. Bull Natl Inst Fruit Tree Sci 2:65–75 (in Japanese with English summary)

Yamada M, Yamane H, Sato A, Iwanami H, Hirakawa N, Yoshinaga K, Ozawa T, Nakajima I (2004) New Japanese persimmon cultivar 'Soshu'. Bull Natl Inst Fruit Tree Sci 3:53–66 (in Japanese with English summary)

Yamada M (2005) Persimmon genetic resources and breeding in Japan. Acta Hortic 685:51–64

Yamada M, Sato A, Yamane H, Yoshinaga K, Hirakawa N, Iwanami H, Ozawa T, Kakutani M, Mitani N, Yoshioka M, Nakajima I (2006) New Japanese persimmon cultivar 'Kanshu'. Bull Natl Inst Fruit Tree Sci 5:95–106 (in Japanese with English Summary)

Yamada M, Yamane H, Sato A, Yoshinaga K, Hirakawa N, Iwanami H, Kakutani M, Ozawa T, Hirabayashi T, Mitani N, Shiraishi M, Sumi T, Yoshioka M, Nakajima I (2009) New Japanese persimmon cultivar, 'Kishu'. Bull Natl Inst Fruit Tree Sci 8:25–38 (in Japanese with English Summary)

Yamada M, Giordani E, Yonemori K (2012a) Persimmon. In: Badness ML, Byrne DH (eds) Fruit breeding. Springer, New York, pp 663–693

Yamada M, Sato A, Yamane H, Mitani N, Iwanami H, Shiraishi M, Hirakawa N, Ueno T, Kono A, Yoshioka M, Nakajima I (2012b) New persimmon cultivar 'Taigetsu'. Bull NARO Inst Fruit Tree Sci 14–25–38.

Yamada M, Sato A, Yamane H, Mitani N, Iwanami H, Shiraishi M, Hirakawa N, Ueno T, Kono A, Yoshioka M, Nakajima I (2012c) New persimmon cultivar 'Taiten'. Bull NARO Inst Fruit Tree Sci 14-39-52

Yamada M (2013) Breeding goals, trait heredity and genetic improvement of persimmon in Japan. Acta Hortic 996:77–88

Yamane H, Kurihara K, Nagata K, Yamada M, Kishi T, Yoshinaga K, Matsumoto R, Ozawa T, Sumi T Hirabayashi T, Kakutani M (1991a) New Japanese persimmon cultivar 'Shinsyuu'. Bull Fruit Tree Res Stn 19:13–27 (in Japanese with English summary)

Yamane H, Kurihara K, Nagata K, Yamada M, Kishi T, Yoshinaga K, Matsumoto R, Kanato K, Sumi T, Hirabayashi T, Ozawa T, Hirose K, Yamamoto M, Kakutani M (1991b) New Japanese persimmon cultivar 'Youhou'. Bull Fruit Tree Res Stn 20:49–61

Yamane H, Yamada M, Kurihara K, Sato A, Yoshinaga K, Nagata K, Matsumoto R, Hirakawa N, Kakutani M, Sumi OT, T, Hirabayashi T, Iwanami H, (2001) New Japanese persimmon cultivar 'Taishuu'. Bull Fruit Tree Res Stn 35:57–73

Yamane H, Ichiki M, Tao R, Esumi T, Yonemori K, Niikawa T, Motosugi H (2008) Growth characteristics of a small-fruit dwarf mutant arising from bud sport mutation in Japanese persimmon *(Diospyros kaki* Thunb.). HortScience 43:1726–1730

Yesiloglu, T, Cimen B, Incesu M, Yilmaz B (2018) Genetic Diversity and Breeding of persimmon. https://doi.org/10.5772/intechopen.74977. IntecOpen

Yi Z, Luo Z, Pan D, Wu H, Zhou J (2004) 'Eshi 1' – a new pollination xonstant and non-astringent cultivar of Chinese persimmon. Acta Hortic Sinica 31:699

Yonemori K, Sugiura A, Yamada M (2000) Persimmon genetics and breeding. Plant Breeding Rev 19:191–225

Yonemori K, Ikegami A, Kitajima A, Luo Z, Kanzaki S, Sato A, Yamada M, Yang Y, Wang R (2005) Existence of several pollination constant non-astringent type persimmons in China. Acta Hortic 685:77–83

Yonemori K, Honsho C, Kitajima A, Aradhya M, Giordani E, Bellin E, Parfitt DE (2008) Relationship of Europian persimmon (*Diospyros kaki* Thunb.) to Asian cultivars, characterized using AFLPs. Genet Resour Crop Evol 55:91–89

Zhang N, Xu J, Mo R, Zhang Q, Luo Z (2016) Androecious genotype 'Male 8' carries the CPCNA gene locus controlling natural deastringency of Chinese PCNA persimmons. Hortic Plant J 2:309–314

Zhuang DH, Kitajima A, Ishida M, Sobajima Y (1990a) Chromosome numbers of Diospyros kaki Cultivars. Jpn Soc Hortic Sci 59:289–297 (In Japanese with English summary)

Zhuang DH, Kitajima A, Ishida M (1990b) Chromosome number of the original tree and open-pollinated progenies of Japanese persimmon cv. Hiratananashi. J Jpn Soc Horict Sci 59:479–485 (In Japanese with English summary)

Zhuang DH, Kitajima A, Ishida M (1992) Seed development and chromosome number in seedless cultivar of Japanese Persimmon. J Jpn Soc Hortic Sci 60:747–754 (In Japanese with English summary)

Rootstock Breeding and Propagation

4

Maria L. Badenes, Francisco Gil-Muñoz, and Takuya Tetsumura

Abstract

Persimmon grafting into rootstocks is a common practice for improving management and yield. Several *Diospyros* species are used worldwide as rootstocks, each of them with their own advantages and drawbacks. Characteristics required for persimmon rootstock include dwarfing, salt tolerance, and graft compatibility. As a result of years of research and investigation, several varieties of persimmon rootstock have recently been released. Dwarfing clones of *D. kaki* have been selected either by use as rootstocks or interstock graft with *D. lotus* for improved orchard management and increased yield. Research on genes related to dwarfness is necessary as well as the promotion of these varieties. Salinity tolerance has been also a trait explored among different persimmon species, showing a variability in tolerance mechanisms that can be inherited among interspecific crosses. However, the yield and compatibility of these new salt-stress tolerant rootstocks has not been tested yet. Since clonal propagation of persimmon was thought to be impossible by cuttings, micropropagation techniques were intensively developed at the end of the 20th century. However, further improvement is needed for the practical use of micropropagation of rootstocks because transplanting to ex vitro conditions remains difficult.

4.1 Introduction

Persimmon as a fruit tree species is grown grafted on rootstocks; they provide the adaptability to the soil conditions and allow selection of the scion according to the fruit characteristics chosen by the growers. Persimmon scion is being grafted mainly in three *Diospyros* species: *D. kaki, D. lotus*, and *D. virginiana*. Graft compatibility should be taken into account when *D. lotus*, and *D. virginiana* are used. Several graft incompatibilities have been described between persimmon varieties and *D. lotus* (Tanaka 1930) and *D. virginiana* (Cohen et al. 1991).

The most common rootstocks used for persimmon are seedlings from *D. kaki*. This species as being the same that the scion, it does not produce any graft incompatibility. However, *D. kaki* is highly sensitive to lime-filled soils, where it produces tap roots with few lateral roots, which are rather fine and broke easily, all together makes

M. L. Badenes (✉) · F. Gil-Muñoz
Instituto Valenciano de Investigaciones Agrarias, CV 315 Km 10,5, 46113 Moncada, Valencia, Spain
e-mail: badenes_mlu@gva.es

F. Gil-Muñoz
e-mail: Francisco.gil.munoz@slu.se

T. Tetsumura
Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen Kinbanadai-nishi, Miyazaki, Japan
e-mail: tetsumur@cc.miyazaki-u.ac.jp

R. Tao and Z. Luo (eds.), *The Persimmon Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-05584-3_4

difficult the plant management in the nurseries (Bellini 2002). *D. kaki* is highly sensitive to salinity (de Paz et al. 2016; Visconti et al. 2017). Consequently, seeds from *D. kaki* are not used in the Mediterranean Basin countries, but it is the most used in Japan, California, Brazil, Australia, and New Zealand. Seedlings from *D. virginiana* species are used in USA, Israel, and Spain. This species is tolerant to salinity (Gil-Muñoz et al. 2018) and performs well on lime-filled soils, but confers too much vigor to the plant, and produces many root-suckers, thus hindering crop management (Incesu et al. 2014). *D. virginiana* resulted graft-incompatible with a wide range of varieties, hence the graft-compatibility needs to be checked for each variety (Bellini 2002). Moreover, persimmon trees grafted on *D. virginiana* seedlings were reported to show several decline symptoms (Cohen et al. 1991). The most commonly used rootstocks for persimmon production in the Mediterranean basin are seedlings from *D. lotus* species, because of its tolerance to lime-filled soils and its adaptability to the Mediterranean soil conditions. *D. lotus* is cold hardier than *D. kaki* and trees grafted on *D. lotus* seedlings grow faster after field establishment. *D. lotus* seedling is commonly used persimmon rootstock in China and is used as well in the cold regions of Japan because of its cold hardiness. However, trees on *D. lotus* rootstocks usually are larger than those on *D. kaki* and sometimes excessive fruit shedding is a problem (Choi et al. 2008; Tao and Sugiura 1992).

Recently, *D. lotus* resulted highly sensitive to salinity which is becoming an increasing problem in some Mediterranean orchards. Additionally, the species *D. rhombifolia* is used as dwarfing rootstocks in some areas, but it showed graft incompatibility with some astringent varieties and all the PCNA types. *D. rhombifolia* is a bushy plant and used as garden shrub and bonsai in Japan. Some researchers studied the possibility of persimmon dwarfing rootstock and interstock and found the trees on *D. rhombifolia* seedlings showed dwarfing growth (Koshita et al. 2006, 2007; Yakushiji et al. 2008; Yamada et al. 1997). However, *D. rhombifolia* is not commercially used as persimmon either rootstock or interstock, because many trees died soon after planting and the productivity was very low (Koshita et al. 2007; Yakushiji et al. 2008).

All the rootstocks used to come from seedlings produced by cross-pollination. This origin implies a high genetic heterozygosity among rootstocks, which confers high variability among the trees in the orchards. This problem can be solved by the use of clonal rootstocks from breeding. Currently, there are two breeding rootstocks programs with different objectives: a breeding program for dwarfing rootstocks in Japan, mainly at NARO, National Agriculture and Food Research Organization, and a breeding program for adaptability to climate change (Gil-Muñoz et al. 2018). In order to keep the production in these areas, the availability of rootstocks tolerant to salinity is required (Forner-Giner and Ancillo 2013).

4.2 Breeding

4.2.1 Breeding for Dwarfing Rootstocks

Persimmon tends to grow to a large tree, and growers often encounter difficulties in orchard management, especially when it is grown on hilly slopes (Tao and Sugiura 1992). To make orchard management easier, researches on cultivation techniques aiming at reduction of tree height such as cutting back scaffold branches (Suzuki and Suganuma 2002), restricting root zone (Fumuro and Utsunomiya 1999; Matsumura and Ozeki 1998), and training to horizontal trellis (Hayashi et al. 2004) were conducted in Japan.

Persimmon trees grafted on *D. kaki* seedlings usually grow into large trees, as mentioned above. Moreover, micropropagated and own-rooted 'Nishimurawase' persimmon trees grew more vigorously than the trees grafted on *D. kaki* seedlings (Tetsumura et al. 1999, 2004). Meanwhile, each seedling of *D. kaki* has a different genomic composition. Very occasionally, we can find a persimmon tree with extremely lower height in the orchard, despite the same scion cultivar, the same conditions such as soil, temperature, and water, and the same cultivation

management. The rootstocks of such low trees are considered to have the genetic ability to dwarf scions. Therefore, in Japan, the search for dwarfed trees in the persimmon orchards has been conducted since the 1980s (Kimura et al. 1985). As a result, some candidates for dwarfing rootstocks were found, whereas in the early days of search for dwarfing rootstocks, methods for clonal propagation of persimmon rootstock had not been developed, except for root cutting which was a low-efficiency propagation (Yamada et al. 1988). Therefore, the selected elite dwarfing rootstocks could be propagated only in experimental scale, not in large quantities.

4.2.2 Selection of Dwarfing Interstocks

During the late twentieth century, when the clonal mass propagation of rootstocks was difficult, the selections were made from the scion cultivars known as less vigorous, the scions as interstocks were grafted on *D. kaki* seedlings before double grafting, and the nursery stocks consisting of the different interstocks, the same scion, and *D. kaki* seedlings, the rootstock, were planted in orchards for comparing the orchard performance. Although the height of 13 years old 'Maekawajiro' tree grafted on *D. kaki* seedling was 3.5 m and the canopy volume was 34.6 m^3, 'Shidare-kaki' interstocks made the tree height 2.7 m and the canopy volume 17.7 m^3 (Manago et al. 2000). The yield efficiency per canopy area of trees with 'Shidare-kaki' interstocks was 118% of that without the interstocks, although the yield per tree with 'Shidare-kaki' interstocks was 78% of that without the interstocks. 'Nishimurawase' interstocks made the 'Maekawajiro' tree height 2.8 m and the canopy volume 23.9 m^3, although 'Fudegaki' interstocks did not dwarf 'Maekawajiro' tree (Manago et al. 2000). These results were obtained in the public research institution in Aichi prefecture, and the other public research institutions in Japan also studied the effects of interstocks and found that the interstocks of 'Izu', 'Shakokushi', and 'Nishimurawase' respectively made the trees of 'Hiratanenashi', 'Aizumishirazu', and 'Fuyu' smaller (Gotou et al. 1997). Even after tissue culture has enabled clonal propagation of the candidates for dwarfing rootstock, the interstock tests were continued. In NARO, some candidates for dwarfing interstocks with 'Aogaki' *(D. kaki)* seedling as rootstock and 'Fuyu' scion were tested, and Ac-1 and Y were found to reduce the tree size without the reductions of yield efficiency and photosynthetic rate (Koshita et al. 2006, 2007). Recently, 'Horakudai' and 'MKR1', which were developed as dwarfing rootstocks, were shown to be effective as dwarfing rootstocks for 'Saijo' and 'Fuyu', respectively (Ohata et al. 2018). In China, 'Nantong-xiaofangshi' persimmon is a dwarf cultivar found in Nantong during 'the Fruit Tree Resources Survey of Jiangsu Province' in 1982 (Jiang et al. 1992), and after test and research, some excellent traits were found, such as small stature, early bearing, high and stable yield (Tu et al. 2013). Recently, the field evaluation of 'Nantong-xiaofangshi' interstock grafted on *D. lotus* seedling rootstock started by using Japanese PCNA scions as well as Chinese cultivars (Fig. 4.1).

4.2.3 Selection of Dwarfing Rootstocks

Studies on micropropagation of persimmon have progressed since the 1980s, and studies on cutting propagation since 2000s. Both studies have effectively developed the clonal propagation of candidates for dwarfing rootstocks which were already selected at that time. After grafted scion cultivars, they were planted in experimental orchards and then have been evaluated as dwarfing rootstocks (Yakushiji et al. 2008). To date, four cultivars, 'Shizukadai1go', 'Shizukadai2go', 'Hourakudai' and 'MKR1' are registered under Japan's Plant Variety Protection and Seed Act and expected to be used as dwarfing rootstocks.

Fig. 4.1 A 'Jiro' tree with 'Nantong-xiaofangshi' interstock grafted on *D. lotus* seedling in Xixi botanical garden, Dongtai city, Jiangsu province

'Shizukadai1go' and 'Shizukadai2go'

In 2014, Shizuoka prefecture released two dwarfing rootstock cultivars, 'Shizukadai1go' and 'Shizukadai2go'. Fifty-nine 'Maekawajiro' nursery stocks grafted on Yamagaki (*D. kaki*) seedling rootstocks were planted in orchards of the Shizuoka Research Institute of Agriculturure and Forestry between 1977 and 1979, and the field evaluation had been conducted for 12 years (Hattori et al. 2015). After the primary selection of dwarfing rootstocks, the seven rootstock strains were micropropagated by using shoot tips of their root suckers, and three of them were successfully established in vitro. The second selection was made by comparing 'Maekawajiro' trees grafted on the micropropagated rootstocks with those grafted on Yamagaki seedling rootstocks. Finally, a semi-dwarfing rootstock with higher yield efficiency was selected and named 'Shizukadai1go', and a dwarfing rootstock 'Shizukadai2go'.

Fig. 4.2 A eleven-year-old 'Fuyu' tree on 'Hourakudai' in the Grape and Persimmon Research Station, NARO Institute of Fruit Tree and Tea Science

'Hourakudai'

In 2016, National Agriculture and Food Research Organization, NARO, and Shimane prefecture jointly registered a dwarfing persimmon rootstock as 'Hourakudai', which originated in Shimane prefecture as a dwarfing rootstock for 'Saijo' persimmon and was selected from a seedling of unknown parents. 'Fuyu' nursery stocks grafted on 'Horakudai' were planted in the orchard of Grape and Persimmon Research Station, NARO Institute of Fruit Tree and Tea Science in 2007, and for more than ten years the field performance had been evaluated comparing with those grafted on the other two dwarfing rootstock candidates and 'Aogaki' seedling, a conventional seedling rootstock in Japan (Yakushiji et al. 2021). The dwarfing effect of 'Hourakudai' produced trees with a tree canopy volume of approximately 40% of those grown on conventional rootstocks (Fig. 4.2). Considering dwarfing ability, yield efficiency, and ease of propagation by cuttings, 'Hourakudai' was the most promising of the dwarf rootstocks tested. In Shimane Agricultural Technology Center, it was also confirmed that 'Saijo' trees on 'Hourakudai' rootstocks were dwarfed. 'Horakudai' is easily

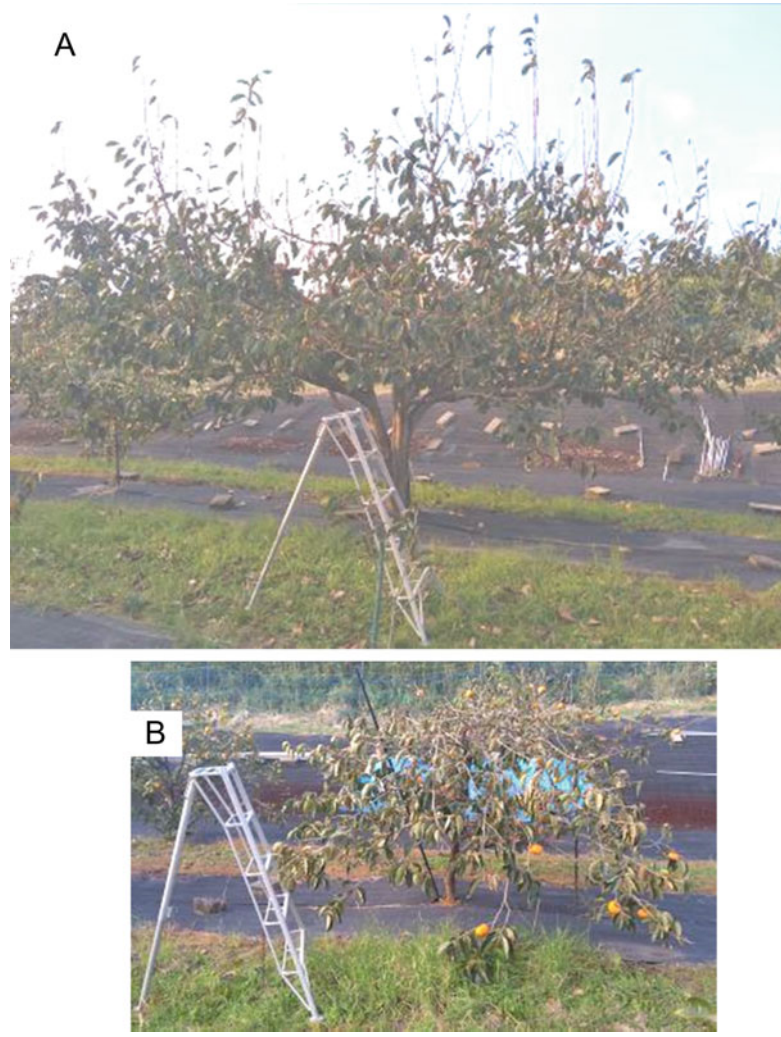

Fig. 4.3 Eighteen-year-old 'Hiratanenashi' trees in the orchard of University of Miyazaki (**A**: a seedling rootstock, **B**: 'MKR1' rootstock)

propagated by softwood cutting (Yakushiji et al. 2021) and tissue culture (Azuma et al. 2011)..

'MKR1'

In 2015, University of Miyazaki released a dwarfing rootstock cultivar, 'MKR1', formerly named Rootstock b and OD-1. The original tree was a 'Saijo' tree 1 m in height in the orchard of Okayama Prefectural Agriculture Center, in which the height of normal-sized 'Saijo' tree was 4.5 m (Tetsumura et al. 2003). Root suckers spontaneously sprouting from the roots of original tree were collected for softwood cutting propagation, and then the rooted cuttings were grafted with 'Fuyu', 'Hiratanenashi', 'Soshu', and 'Taishuu'. The characteristics of 'Fuyu' and 'Hiratanenashi' trees on 'MKR1' rootstocks were as follows: (1) less vigorous shoot growth and almost no secondary elongation, (2) precocity and high proportion of flowering branches, (3) high yield efficiency, and (4) strong graft union (Tetsumura et al. 2010). 'Taishuu' tree on 'MKR1' showed almost the same characteristics as the 'Fuyu' and 'Hiratanenashi' trees (Tetsumura et al. 2019). The early fruit drop of trees of the four cultivars on 'MKR1' drastically decreased (Tetsumura et al. 2013). These

characteristics had not changed for more than 20 years after planting (Fig. 4.3). 'MKR1' is easily propagated by softwood cutting and tissue culture (Hejazi et al. 2018; Tetsumura et al. 2009).

An adaptability test of persimmon dwarfing rootstocks for persimmons using these four cultivars was initiated in 17 public research organizations in Japan in 2016. The scion cultivars used varied with the organizations, and fruit quality as well as tree growth has been evaluated. Apart from those, FDR-1 became a promising persimmon dwarfing rootstock. A 'Fuyu' persimmon tree grafted on FDR-1 in the orchard of Fukuoka Agriculture and Forestry Research Center showed a semi-dwarfing growth habit. The dwarfing effect of FDR-1, which was propagated in vitro from the roots, on 'Taishuu' scion was similar to that of 'MKR1' (Tetsumura et al. 2019). An effective propagation by softwood cutting of FDR-1 was also developed (Tetsumura et al. 2017).

4.2.4 Future Perspectives

In addition to dwarfing effect on scion, desired traits for persimmon dwarfing rootstock are graft compatibility, high yield efficiency, reduction in biennial bearing, precocity, easy clonal propagation, maintaining or improving fruit quality, adaptability of various soil and environmental conditions, disease and pest resistance, life-span to a certain degree, and so on.

In general, persimmons are not as high yielding as some other fruit crops, especially PCNA cultivars (Kitagawa and Glucina 1984). Hence, yield efficiency is an important factor in developing dwarfing rootstocks, and in dwarfing rootstock selection some of the candidates were excluded from the perspective of productivity (Kimura et al. 1985; Yakushiji et al. 2021).

Biennial bearing is a major problem with persimmons (Kitagawa and Glucina 1984). 'Hiratanenashi' and 'Fuyu' grafted on 'MKR1' continued to produce efficiently with no observed biennial bearing more than 10 years after planting (Tetsumura et al. 2015). Although there was no report on the persimmon dwarfing rootstock making biennial bearing worse, it should be needed for more tests of the combination of rootstock and scion. Persimmon is particularly prone to transplantation shock (Izaki et al. 1960; Kitagawa and Glucina 1984). Moreover, persimmon has a long time to become mature trees which produce enough fruit, and thus, it is considered that persimmon orchard soil management is important for young trees to grow fast and become mature smoothly (Iwamoto et al. 1995). Similar to other fruit trees, *Diospyros* is susceptible to certain diseases and insects. The candidate dwarfing persimmon rootstock was excluded from the selection because the trunks were often damaged by the larvae of persimmon bark borer *(Euzophera batangensis*) during the evaluation (Yakushiji et al. 2016a).

Persimmon has been considered difficult to root (Tao and Sugiura 1992). Hence, a high rooting ability is the vital trait for dwarfing rootstock. Although the clonal propagation of persimmon dwarfing rootstocks, to be discussed later, has long been improved, commercial large-scale production has not been done yet. Hence, it is desirable that QTL associated with rooting ability as well as dwarfing one will be found. Recently, 'Hasshu' was discovered as a dwarf budsport originating from a seedless and vigorous cultivar, 'Hiratanenashi' (Yakushiji et al. 2016b). 'Hasshu' is octoploid and not a seedless cultivar probably because meiosis is not disturbed, while nonaploid 'Hiratanenashi' is a seedless cultivar. Moreover, half of the seedlings showed dwarf growth, and thus the dwarfing trait of 'Hasshu' is most likely to be dominantly inherited (Yakushiji et al. 2017). Hence, the octoploid dwarf 'Hasshu' may open the way for genetic analysis of its dwarfism.

4.2.5 Breeding for Salinity Tolerance

Persimmon (*Diospyros kaki*) production in the Mediterranean Basin countries has been very much affected by climate change modifying rainfall distribution and increased salt content in the irrigation water (Visconti et al. 2015). Saline

Fig. 4.4 Persimmons affected by salt stress

stress symptoms in the orchards are endangering persimmon production (de Paz et al. 2016; Visconti et al. 2017) (Fig. 4.4). Availability of rootstocks tolerant to salinity can help to keep the production in these areas.

Rootstocks for persimmon production in the Mediterranean basin are based on seedlings from *Diospyros lotus* species, because of its tolerance to lime-filled soils. However, this species is highly sensitive to salinity (de Paz et al. 2016; Visconti et al. 2017). Other species used as rootstocks in this region is *D. virginiana,* which is tolerant to salinity and performs well on lime-filled soils. However, its use is limited because it confers too much vigor to the plant and produces suckers making crop management difficult (Incesu et al. 2014; de Paz et al. 2016).

Despite the most used rootstock around the world are seedlings from *D. kaki*, this species is highly sensitive to lime-filled soils, thus seeds from *D. kaki* are not used in the Mediterranean Basin countries. Neither clonal rootstocks from *D. lotus*, *D. virginiana*, nor *D. kaki* adapted to the Mediterranean conditions are available. To solve this problem, a breeding program focused in tolerance to salinity is in progress at the IVIA (Gil-Muñoz et al. 2018).

4.2.5.1 Selection of Salt-Tolerant Genotypes

Selection of salt tolerance in plants is made by hydroponic culture and irrigation with different saline solutions (Fig. 4.5). For identifying the saline-stress performance in persimmon species, several salt-tolerance trials have been performed at the Valencian Institute of Agricultural Research (IVIA) in populations of *D. kaki*, *D. lotus*, *D. virginiana,* and a backcross between *D. kaki* and *D. virginiana.*

In all the trials, differences were found within species regarding salt stress tolerance. It was confirmed that *D. virginiana* presents the highest salt stress tolerance among the tested populations, in agreement with previous field observations (Visconti et al. 2017). Salt stress in *D. virginiana* only reduced plant weight, probably because of the limitation in water availability due to the increase in water osmotic pressure. However, in *D. lotus* and *D. kaki* species, besides the reduction of plant weight, leaf tip and margin burns and defoliation were observed and some plants died eventually. Interestingly, the *D. kaki* x *D. virginiana* backcross population showed surprisingly high levels of salt tolerance in some phenotypes (Fig. 4.6), this result suggests a

Fig. 4.5 Greenhouse salt-stress trial in persimmon seedlings for salt-tolerance selection

Fig. 4.6 Effects of saline stress on *D. virginiana*, *D. kaki* and *D. kaki* x *D. virginiana* backcross after 60 days of 40 mM NaCl irrigation

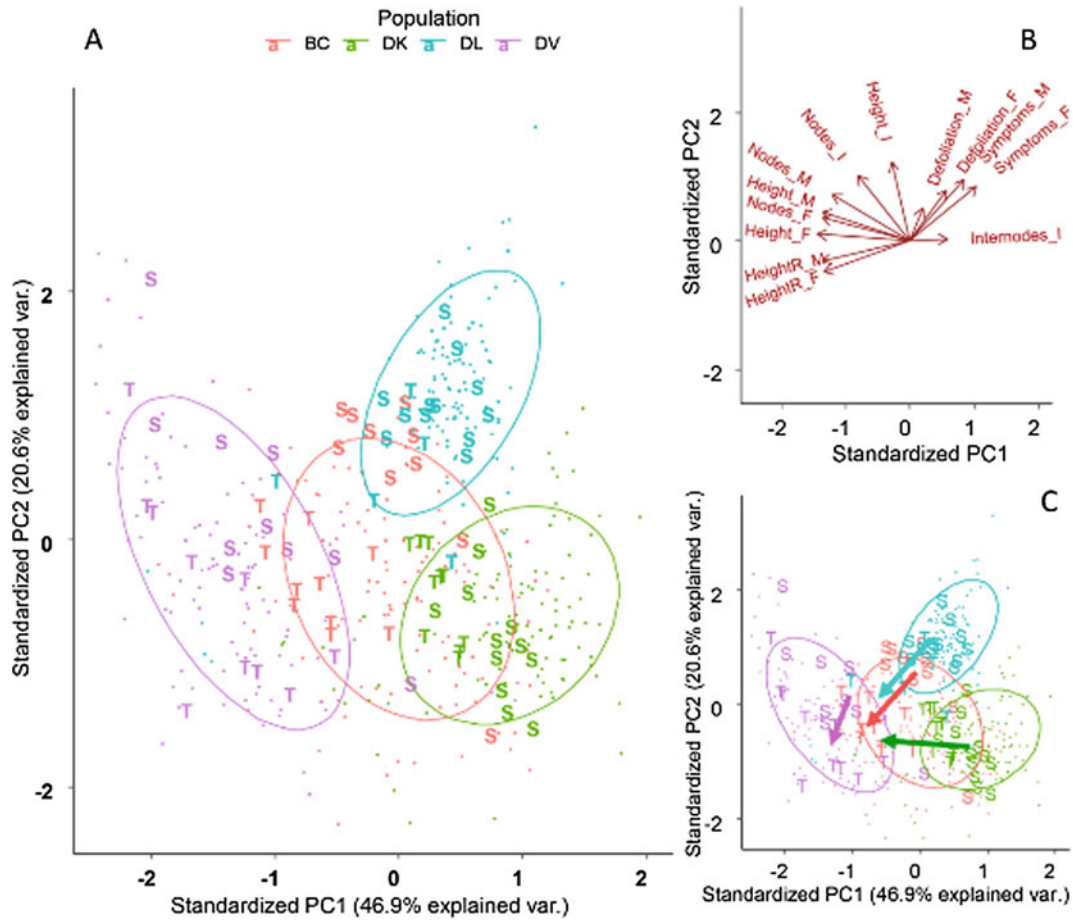

Fig. 4.7 Plot of the first 2 components from Principal Component Analysis (PCA) of the morphological variables. Populations of seedlings from *D. kaki* x *D. virginiana* backcross (BC), *D. kaki* (DK), *D. lotus* (DL) and *D. virginiana* (DV) are included. Plants selected as Tolerant (T) and Sensitive (S) according its phenotype have been represented. Projection of the variables on the first 2 components (B). Dimensional division between tolerant and sensitive subsets for each population (C). From: Gil-Muñoz et al., (2020a)

strong genetic control of salt stress tolerance trait based on a few genes. Selection of the tolerant individuals based on phenotype parameters followed by a multivariate analysis resulted in clustering of the genotypes according to its salinity tolerance (Fig. 4.7).

Besides the effect of salinity on morphological parameters, ion content accumulation varies greatly depending on the *Diospyros* species used as rootstock (de Paz et al. 2016; Visconti et al. 2017; Gil-Muñoz et al. 2018, 2020a). Salt stress experiments in several *Diospyros* populations have demonstrated that both Cl^- and Na^+ leaf accumulation greatly varies among species. Furthermore, differences in Na^+ accumulation have been found within *D. kaki* x *D. virginiana* backcross population and *D. virginiana* (Fig. 4.8). The only species that showed differences within Cl- accumulation was *D. lotus*. However, its levels were still the highest compared to the other species studied.

Plants with different levels of tolerance to salinity were selected from all the populations studied and are currently micropropagated for further field trials in which grafted plants will be tested for fruit quality, production, and graft

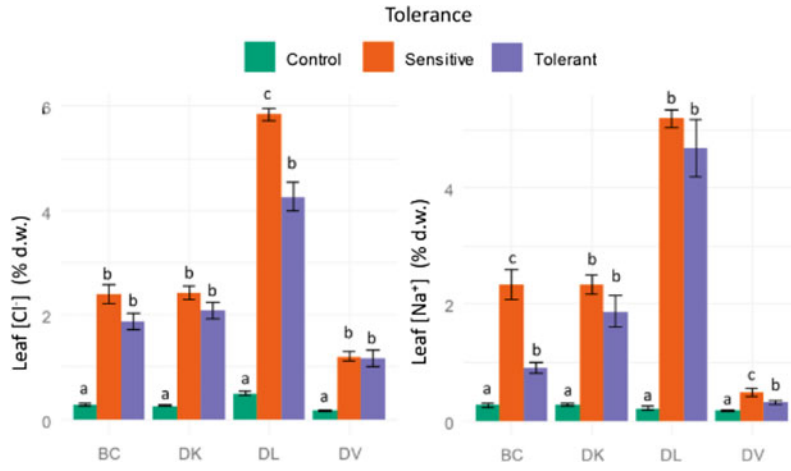

Fig. 4.8 Leaf Na^+ and Cl^- content after 60 days of salinity treatment. *D. kaki* x *D. virginiana* backcross (BC), *D. kaki* (DK), *D. lotus* (DL) and *D. virginiana* (DV). Adapted from: Gil-Muñoz et al. (2020a)

compatibility. The trials included astringent and non-astringent varieties under different irrigation conditions.

4.2.5.2 Mechanisms of Salinity Tolerance

Salt stress tolerance is a polygenic and quantitative trait highly influenced by the environment (Tiwari et al. 2016). Therefore, discovering the salinity tolerance mechanism or even the genes underlying the tolerant phenotypes might help to avoid the environment effect in breeding programs. Furthermore, the studies at gene level of these mechanisms can lead to the identification of allelic variability and its use in Marker Assisted Selection (MAS).

Regulation of water transport, small solutes, and hydric balance through the plant is a physiological mechanism to overcome salinity stress. In *Diospyros* genus, this physiological mechanism varies depending on the species. On *D. kaki* and *D. lotus*, both salt-sensitive species, present variability on the salt response through stomatal conductance, the sensitive plants present stomatal closure after 60 days of salt stress (Gil-Muñoz et al. 2020a). However, this fact is a consequence of the salt stress level of sensitive plants and not a tolerance mechanism. On the other hand, *D. kaki* x *D. virginiana* backcross population shows variability for the Water Use Efficiency (WUE) values between tolerant and sensitive genotypes (Fig. 4.9). This result opens the possibility of using these genotypes in water

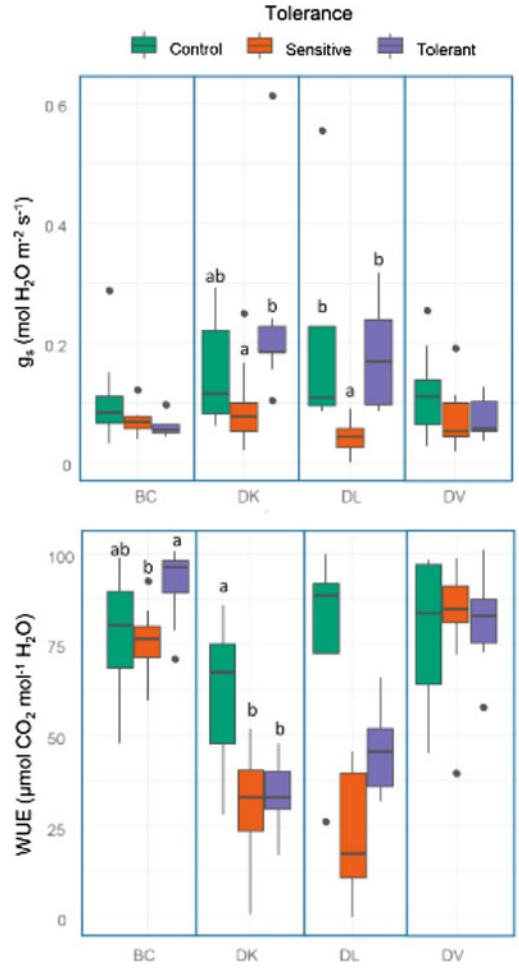

Fig. 4.9 Leaf stomatal conductance (g_s) and intrinsic water use efficiency (WUE) after 60 days of salt treatment on *D. kaki* x *D. virginiana* backcross (BC), *D. kaki* (DK), *D. lotus* (DL) and *D. virginiana* (DV) populations. Adapted from: Gil-Muñoz et al. (2020a)

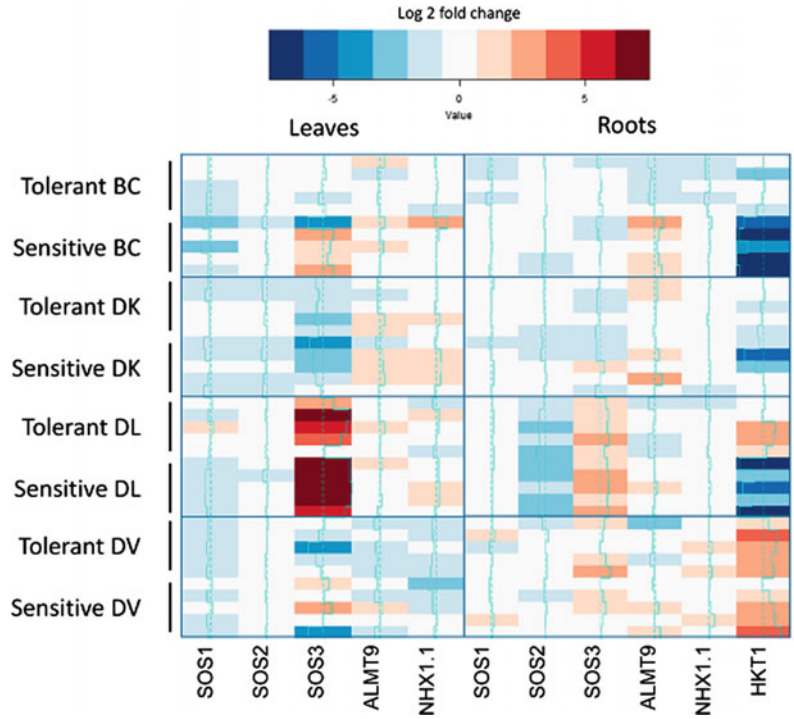

Fig. 4.10 Expression analysis of salt tolerance related-like genes from control and treated subsets of tolerant and sensitive plants from each population: *D. kaki* x *D. virginiana* backcross (BC), *D. kaki* (DK), *D. lotus* (DL) and *D. virginiana* (DV). Only significant differences between control and treated subsets are colored. From: Gil-Muñoz et al. 2020a

scarcity conditions or for its use in sustainable orchards.

Several genes have been identified in other species related to salt tolerance; mainly with ionic stress overcame by avoiding toxic ion inflow, transport, or by balancing ion homeostasis. As example, the Salt Overly Sensitive (SOS) signaling pathway is responsible of maintaining ion homeostasis during salt stress (Zhu et al. 1998). Other genes previously related with salt stress tolerance participate in different tolerance mechanisms. Genes from HKT (High-affinity potassium transporter) family have been related to Na^+ exclusion (Almeida et al. 2013; Byrt et al. 2007; Isayenkov and Maathuis 2019; Munns and Tester 2008). Also, a member from the Na^+/H^+ exchanger family (NHX) has been liked to salinity tolerance (Apse et al. 1999). In the case of *Diospyros* genus, salt tolerance has been linked to differences in root expression of an HKT-1 like gene (Gil-Muñoz et al. 2020a, 2020b). The levels of root expression of this gene have been linked to salt tolerance. Accordingly, *D. virginiana* presents higher expression levels during salt stress, whereas in *D. kaki* x *D. virginiana* and *D. lotus*, differences in the expression of this gene are present between salt-tolerant and salt-sensitive plants (Fig. 4.10). These differences in gene expression among tolerant and sensitive plants are not present in *D. kaki*. In the case of SOS3-like gene, it is believed that its differences in expression are related to the level of stress present in the plant. In agreement with this result, *D. lotus* presented the higher levels of expression in leaves and roots.

New generation transcriptomic techniques have been applied in order to elucidate the reasons behind intra-specific variability of Cl^- leaf accumulation variability in *D. lotus*. Results of an RNA-seq performed on tolerant and sensitive plants after 60 days of salt stress suggest that chloride channel expression levels and/or H^+-ATPases expression might play a role in explaining these differences in chloride accumulation (Gil-Muñoz et al. 2020c). Both genes can explain the tolerance variability present in *D. lotus*. Chloride channels have been proposed as key transporters of Cl^- into the higher parts of

the plant (Isayenkov and Maathuis 2019). The H^+- ATPases allow to generate the adequate electrochemical proton gradient that allows these antiporters to perform the Na^+ extrusion (Palmgren 2001) into the vacuole. Furthermore, the upregulation of this protein under salt conditions has been previously reported (Niu et al. 1993; Quan et al. 2007; Sahu and Shaw 2009; Vera-Estrella et al. 1994) and its critical role on the Na^+ extrusion has been confirmed using transgenic plants (Gévaudant et al. 2007; Shen et al. 2011).

4.3 Micropropagation of Persimmon Rootstocks

At the beginning of research on micropropagation using shoot tips as explants, scion cultivars were mainly used (Cooper and Cohen 1984; Fukui et al. 1989; Murayama et al. 1989; Sugiura et al. 1986). However, in the last decade of the twentieth century, the methods of micropropagation of dwarfing rootstocks were developed (Ito-Ogawa et al. 2001; Kagami 1995; Kagami et al. 1995).

4.3.1 Culture Establishment

Two types of explants have been used for shoot tip culture establishment. One is actively growing shoots (Cooper and Cohen 1984; Fukui et al. 1989; Sarathchandra and Burch 1991; Wardani et al. 2019); the other is dormant winter buds that are fully satisfied with the chilling requirements (Bellini and Giordani 1997; Fumuro et al. 1988; Kagami et al. 1995; Murayama et al. 1989; Matsumoto and Yamada 1993; Sugiura et al. 1986; Tao et al. 1994; Tetsumura et al. 1991). The advantage of using the actively growing shoots is immediate use after collecting from the field during the growing season. However, the growth speed soon after the culture establishment varied with collecting time, and there is appropriate timing of the year to start shoot tip culture (Fukui et al. 1990). On the other hand, the dormant buds cannot be used soon after collection from the field if their chilling requirements are not satisfied, and in such a case, they should be stored in a refrigerator for a while. However, micropropagation using them can start at any time of the year because they are stored in a refrigerator for up to one year without losing their viability.

Main factors in the medium affecting the culture establishment of shoot tip are basal salt mixture (basal medium) and cytokinin. Modified Murashige and Skoog (MS) medium (Murashige and Skoog 1962) with NH_4NO_3 and KNO_3 reduced to half the original strength, MS (1/2 N), have been usually used for culture establishment (Bellini and Giordani 1997; Fukui 1990; Hu et al. 2009; Kagami et al. 1995; Matsumoto and Yamada 1993; Sugiura et al. 1986; Tetsumura et al. 1991), although there are some reports, in which WPM or MS medium for culture establishment was successfully used (Cooper and Cohen 1984; Sarathchandra and Burch 1991; Wardani et al. 2019). A natural cytokinin, zeatin, is especially effective for culture establishment as well as shoot proliferation of micropropagation, including rootstocks (Kagami et al. 1995). Although some reports showed that a synthetic cytokinin, BA (6-Benzyladenine, 6-Benzylaminopurine, BAP), could establish in the culture of some cultivars (Murayama et al. 1989; Sugiura et al. 1986; Wardani et al. 2019), more cultivars could be established in culture and proliferated on the media supplemented with zeatin than on those with BA (Tetsumura et al. 1991).

4.3.2 Shoot Multiplication

Once the cultures have been established, shoots are proliferated on MS (1/2 N) medium supplemented with the same kind of cytokinin used in the culture establishment stage. The media with BA produce many rosette shoots, whereas the media with zeatin produce one elongated shoot when one bud is planted on the medium (Fig. 4.11). The longer shoots are better suited for rooting treatment, while the shoot multiplication rate was about the same between the two cytokinins. However, zeatin is more than 100

Fig. 4.11 Shoots of 'MKR1' dwarfing rootstock growing on MS(1/2 N) medium supplemented with BA (left) and zeatin (right) 20 days after planted in the fresh media

times higher price than BA, and hence, considering the cost performance, the shoots should be proliferated on the media with BA and be transferred to the media with zeatin to ensure shoot elongation just before rooting treatment if the shoots can be proliferated on the media with BA (Fumuro et al. 1988).

4.3.3 Rooting

Persimmon is one of the difficult-to-root fruit species and propagation by cuttings was very difficult (Tao and Sugiura 1992). As for microcuttings, short time immersion of cutting base in high-concentration auxin, quick-dip, was conducted in most of the reports (Fumuro et al. 1988; Ito-Ogawa et al. 2001; Kagami 1999; Kagami et al. 1995; Murayama et al. 1989; Sugiura et al. 1986; Tao et al. 1994; Tetsumura et al. 1991). Murayama et al. (1989) showed that quick dip of 250 mg/L 3-indolebutyric acid (IBA) and initial 9 days dark treatment were the best for rooting of 'Hiratanenashi' microcuttings and resulted favorable rooting of some cultivars. Usually, the multiplication medium is solidified with agar, while the rooting medium solidified with gellan gum (Fig. 4.12) induced better rooting of microcuttings than that solidified with agar (Kagami et al. 1995).

4.3.4 Transplanting to Ex Vitro Conditions

Persimmon plantlets regenerated in vitro are not easy to adapt to field conditions (Tao and Sugiura 1992). Tetsumura et al. (1993) studied on acclimatization of 'Nishimurawase' shoots and found that most shoots treated with high-

Fig. 4.12 Microcuttings of 'MKR1' dwarfing rootstock rooting in half-strength MS (1/2 N) medium solidified with gellan gum 20 days after IBA 1.25 mM quick-dip treatment

Fig. 4.13 Microcuttings of 'MKR1' dwarfing rootstock directly rooting in peat pellets 40 days after IBA 1.25 mM quick-dip treatment

concentration auxin and cultured in rooting medium for a certain period survived, while all shoots transplanted to vermiculite immediately after the quick-dip rooting treatment, direct rooting method, died. The difficulties of acclimatization to ex vitro conditions may result from brittle roots, which are probably injured when transplanted from agar-solidified rooting medium to potting soil. Improvements in direct rooting method for avoiding transplantation shock from agar-solidified medium to pot could bring about not only a high success rate of acclimatization but also a quick growth of plantlet ex vitro (Fig. 4.13).

4.3.5 Future Perspectives

The micropropagation techniques of persimmon cultivars have been applied to those of rootstocks. As with the scion cultivars, responses to treatments differ depending on rootstock genotypes and strains, so treatments are necessary to vary with those if optimal micropropagation efficiency is needed. Meanwhile, cheap clonal propagation technique, cutting propagation, has rapidly developed, and starts to put to practical use. Nevertheless, multiplication rates of persimmon rootstock by micropropagation are very high. Azuma et al. (2011) theoretically produced 1837 plantlets of the lowest efficiency dwarfing rootstock and 1,222,941 plantlets of the highest efficiency dwarfing rootstock in one year from one bud. Hence, when there are not enough materials, for example, a newly developed rootstock, micropropagation may be applied to mass production of the rootstock.

References

Almeida P, Katschnig D, de Boer AH (2013) HKT transporters-state of the art. Int J Mol Sci 14:20359–20385

Apse MP, Aharon GS, Snedden WA, Blumwald E (1999) Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in *Arabidopsis*. Science 285:1256–1258

Azuma A, Yakushiji Y, Koshita Y, Yamasaki A (2011) Mass propagation of dwarfing rootstocks in Japanese persimmon by tissue culture. Hortic Res (japan) 10 (Suppl 2):349 (In Japanese)

Bellini E, Giordani E (1997) *In vitro* culture establishment and shoot elongation of "Kaki Tipo" (*D. kaki* L.) dormant buds. Acta Hortic 436:129–134

Bellini E (2002) Cultural practices for persimmon production. CIHEAM Options Mediterr 39–52

Byrt CS, Platten JD, Spielmeyer W, James RA, Lagudah ES, Dennis ES, Tester M, Munns R (2007) HKT1;5-like cation transporters linked to Na^+ exclusion loci in wheat, *Nax2* and *Kna1*. Plant Physiol 143:1918–1928

Choi ST, Kim SC, Park DS, Kang SM (2008) Tree growth and physiological fruit drop of 'Hachiya' persimmon on *D. kaki* and *D. lotus* rootstocks. Acta Hortic 772:345–349

Cohen Y, Gur A, Barkai Z, Blumenfield A (1991) Decline of persimmon (*Diospyros kaki* L.) trees on *Diospyros virginiana* rootstocks. Sci Hortic 48:61–70

Cooper PA, Cohen D (1984) Micropropagation of Japanese persimmon (*Diospyros kaki*). Comb Proc Intl Plant Prop Soc 34:118–124

de Paz JM, Visconti F, Chiaravalle M, Quiñones A (2016) Determination of persimmon leaf chloride contents using near-infrared spectroscopy (NIRS). Anal Bioanal Chem 408:3537–3545

Forner-Giner MA, Ancillo G (2013) Breeding salinity tolerance in citrus using rootstocks. In: Parvaiz A, Azooz MM, Prasad MNV (eds) Salt stress in plants: Signalling, omics and adaptations. Springer, New York, pp 355–376

Fukui H, Sugiyama M, Nakamura M (1989) Shoot tip culture of Japanese persimmon (*Diospyros kaki* Thunb.). J Jpn Soc Hortic Sci 58:43–47

Fukui H, Nishimoto K, Murase I, Nakamura M (1990) Annual changes in responsiveness of shot tip cultures to cytokinin in Japanese persimmon. J Jpn Soc Hortic Sci 59:271–274

Fumuro M, Utsunomiya N (1999) Shoot growth and fruit development as affected by warming the soil in forcing culture of 'Tonewase' persimmon under restricted root volume. Hortic Res (jpn) 2:179–182 (In Japanese with English summary)

Fumuro M, Murayama H, Tao R, Murata R, Sugiura A (1988) Studies on rearing of dwarfing rootstocks for Japanese persimmon. 1. In vitro propagation of a dwarf strain of cv. Nishimurawase. Bull Shiga Agric Expt Stn 29:20–30 (In Japanese with English summary)

Gévaudant F, Duby G, Von Stedingk E, Zhao R, Morsomme P, Boutry M (2007) Expression of a constitutively activated plasma membrane H^+-ATPase alters plant development and increases salt tolerance. Plant Physiol 144:1763–1776

Gil-Muñoz F, Peche PM, Climent J, Forner MA, Naval MM, Badenes ML (2018) Breeding and screening persimmon rootstocks for saline stress tolerance. Acta Hortic 1195:105–110

Gil-Muñoz F, Pérez-Pérez JG, Quiñones A, Naval MM, Badenes ML (2020a) Intra and inter-specific variability of salt tolerance mechanisms in *Diospyros* genus. Front Plant Sci 11:1132

Gil-Muñoz F, Pérez-Pérez JG, Quiñones A, Primo-Capella A, Cebolla J, Forner-Giner MA, Badenes ML, Naval MM (2020b) A cross population between *D. Kaki* and *D. Virginiana* shows high variability for saline tolerance and improved salt stress tolerance. PLoS One 15:e0229023

Gil-Muñoz F, Delhomme N, Quiñones A, Naval M, Badenes ML, García-Gil MR (2020c) Transcriptomic analysis reveals salt tolerance mechanisms present in date-plum persimmon rootstock (*Diospyros lotus* L.). Agronomy 10:1703

Gotou M, Matumura H, Ozeki T (1997) Studies on the dwarfed tree training of Japanese persimmon 'Fuyu'. 2. Effects or interstock. J Jpn Soc Hortic Sci 66 (Suppl 2):4–5 (In Japanese)

Hattori N, Kamada N, Isobe T, Anma S, Kagami H, Araki Y, Taneishi M (2015) Development of new dwarfing persimmon (*Diospyros kaki* Thunb.) rootstock cultivars 'Shizukadai1go' and 'Shizukadai2go.' Bull Shizuoka Res Inst Agric Forestry 8:61–67 (In Japanese with English summary)

Hayashi K, Ushijima K, Chijiwa H, Himeno S (2004) Comparison of yields and fruit quality between 'Matsumotowase-Fuyu' Japanese persimmon trees trained to a horizontal trellis and open center free-standing ones. J Jpn Soc Hortic Sci 73:346–353 (In Japanese with English summary)

Hejazi Z, Ishimura S, Honsho C, Tetsumura T (2018) Effects of planting time, irrigation system, rooting medium, and IBA concentration on cutting propagation of the persimmon dwarfing rootstock 'MKR1.' Hort J 87:184–192

Hu H, Zhou R, Miao W, Li G, Zhou J (2009) The filtration and evaluation of subculture media for the nonastringent persimmon. Acta Hortic 833:193–198

Incesu M, Cimen B, Yesiloglu T, Yilmaz B (2014) Growth and photosynthetic response of two persimmon rootstocks (*Diospyros kaki* and *D. virginiana*) under different salinity levels. Not Bot Horti Agrobot Cluj-Napoca 42:386–391

Isayenkov SV, Maathuis FJM (2019) Plant salinity stress: Many unanswered questions remain. Front Plant Sci 10:80

Ito-Ogawa R, Manago N, Sakanishi K (2001) Rooting of dwarf rootstocks propagated *in vitro* in Japanese persimmon. Res Bull Aichi Res Agric Ctr 33:169–174 (In Japanese with English summary)

Iwamoto K, Ohnishi Y, Koyama K, Fukui T, Kosugi S, Mae E (1995) Investigation of fast maturing method persimmon tree field. Bull Nara Agric Expt Stn 26:7–16 (In Japanese with English summary)

Izaki M, Itakura A, Kkashimura K (1960) Studies on the reduction of the transplanting injury in Japanese persimmon trees: II. Effect of potassium α-naphthaleneacetate on rooting and new shoot growth of transplanted persimmon trees. J Jpn Soc Hortic Sci 29:219–222 (In Japanese with English summary)

Jiang D, Xue X, Sheng B, Dai W, Xu C (1992) The rare dwarf persimmon cultivar—'Nantong-xiaofangshi.' Jiangsu Agric Sci 8:49–50 (In Chinese)

Kagami H (1995) Growth enhancement of micropropagated young plants of Japanese persimmon. Bull Shizuoka Citrus Expt Stn 26:17–22 (In Japanese with English summary)

Kagami H (1999) Effect of sugars on rooting of shoots of Japanese persimmon propagated *in vitro*. Plant Biotechnol 16:371–374

Kagami H, Shikano E, Araki Y, Anma S (1995) Proliferation of Japanese persimmon rootstocks by shoot tip culture. Bull Shizuoka Citrus Expt Stn 26:7–16 (In Japanese with English summary)

Kimura N, Kawabuchi A, Aoki M, Okada N, Manago N, Suzuki S (1985) On the search for the dwarfing stocks for kaki and their utilization (I). Res Bull Aichi Res Agric Ctr 17:273–281 (In Japanese with English summary)

Kitagawa H, Glucina PG (1984) Persimmon culture in New Zealand. Sci Info Publ Ctr, Wellington

Koshita Y, Morinaga K, Tsuchida Y (2006) The early growth and photosynthetic rate of Japanese persimmons (*Diospyros kaki* L.) grafted onto different interstocks. Sci Hortic 109:138–141

Koshita Y, Morinaga K, Tsuchida Y, Asakura T, Yakushiji H, Azuma A (2007) Selection of interstock for dwarfing Japanese persimmon (*Diospyros kaki* Thunb.) trees. J Jpn Soc Hortic Sci 76:288–293

Manago N, Yoshida Y, Honmi Y, Banno M, Kimura N, Sakakibara M (2000) Influence of various interstem on growth of Japanese persimmon cv. Maekawajiro. Res Bull Aichi Res Agric Ctr 32:129–133 (In Japanese with English summary)

Matsumoto T, Yamada K (1993) Micropropagation of a Japanese persimmon rootstock (*Diospyros kaki* Thunb.) by stem tip culture. Bull Shimane Agric Expt Stn 27:41–46 (In Japanese with English summary)

Matsumura H, Ozeki K (1998) Container planting cultivation of Japanese persimmon (*Diospyros kaki* Thunb.) (Part 1) The recommendable cultivar and the suitable kind and proper quantity of soil. J Soc High Tech Agric 10:21–26 (In Japanese with English summary)

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Murayama H, Tao R, Tanaka T, Sugiura A (1989) In vitro shoot proliferation and rooting of several Japanese persimmon cultivars. J Jpn Soc Hortic Sci 58:55–61 (In Japanese with English summary)

Niu X, Narasimhan ML, Salzman RA, Bressan RA, Hasegawa PM (1993) NaCl regulation of plasma membrane H^+-ATPase gene expression in a glycophyte and a halophyte. Plant Physiol 103:713–718

Ohata K, Takahashi H, Kanda M, Takahashi T, Yakushiji T, Kurahashi T, Mochida K (2018) Growth characteristics of 'Saijo' persimmon trees grafted on 'Horakudai' persimmon interstock. Hortic Res (jpn) 17(Suppl 1):96 (In Japanese)

Palmgren MG (2001) Plant plasma membrane H^+-ATPases: Powerhouses for nutrient uptake. Annu Rev Plant Physiol Plant Mol Biol 52:817–845

Quan R, Lin H, Mendoza I, Zhang Y, Cao W, Yang Y, Shang M, Chen S, Pardo JM, Guo Y (2007) SCABP8/CBL10, a putative calcium sensor, interacts with the protein kinase SOS_2 to protect *Arabidopsis* shoots from salt stress. Plant Cell 19:1415–1431

Sahu BB, Shaw BP (2009) Salt-inducible isoform of plasma membrane H^+ATPase gene in rice remains constitutively expressed in natural halophyte, *Suaeda maritima*. J Plant Physiol 166:1077–1089

Sarathchandra SU, Burch G (1991) Micropropagation of Japanese persimmon (*Diospyros kaki* Thun.) cv. 'Hiratanenashi.' New Zealand J Crop Hortic Sci 19:113–120

Shen P, Wang R, Jing W, Zhang W (2011) Rice phospholipase Dα is involved in salt tolerance by the mediation of H^+-ATPase activity and transcription. J Integr Plant Biol 53:289–299

Sugiura A, Tao R, Murayama H, Tomana T (1986) In vitro propagation of Japanese persimmon. HortScience 21:1205–1207

Suzuki H, Suganuma K (2002) Effect of cutting back scaffold branches and ringing shoots upon growth, yield, and hours required for harvest of Japanese persimmon cv Maekawajiro. Res Bull Aichi Agric Res Ctr 34:139–146 (In Japanese with English summary)

Tanaka C (1930) Experiments on the rootstocks for the Kaki or Japanese persimmon (*Diospyros kaki* L.). J Okitsu Hortic Soc 25:1–30 (In Japanese)

Tao R, Sugiura A (1992) Micropropagation of Japanese persimmon (*Diospyros kaki* L.). In: Bajaj YPS (ed) Biotechnology in agriculture and forestry 18: High-tech and micropropagation II. Springer, Berlin, pp p424-440

Tao R, Ito J, Sugiura A (1994) Comparison of growth and rooting characteristics of micropropagated adult plants and juvenile seedlings of persimmon (*Diospyros kaki* L.). J Jpn Soc Hortic Sci 63:537–541

Tetsumura T, Tao R, Sugiura A (1991) Effect of cytokinin types on the *in vitro* propagation of Japanese persimmon (*Diospyros kaki* Thunb.). Plant Tissue Cult Lett 8:209–211 (In Japanese with English summary)

Tetsumura T, Tao R, Yukinaga H (1993) Factors influencing acclimatization of 'Nishimurawase' Japanese persimmon micropropagules and their field performance. J Jpn Soc Hortic Sci 62:533–538 (In Japanese with English summary)

Tetsumura T, Tao R, Yukinaga H (1999) Orchard growth, flowering and fruiting of micropropagated Japanese persimmon trees. J Hortic Sci Biotechnol 74:251–253

Tetsumura T, Tao R, Sugiura A, Fujii Y, Yoda S (2003) Cutting propagation of some dwarfing rootstocks for persimmons. Acta Hortic 601:145–149

Tetsumura T, Koyanagi Y, Ito S, Habu T, Kawase K (2004) Comparative field performance of mature Japanese persimmon trees grafted on seedling rootstocks vs. micropropagated ones. J Jpn Soc Hortic Sci 73:134–136

Tetsumura T, Haranoushiro S, Honsho C (2009) Improvement of rooting of cuttings of a dwarfing rootstock for kaki and its micropropagation. Acta Hortic 833:177–182

Tetsumura T, Haranoushiro S, Marume T, Torigoe C, Omori T, Kurogi Y, Uchida Y, Honsho C (2010) Orchard growth, flowering and fruiting of 'Fuyu' and 'Hiratanenashi' Japanese persimmon trees grafted on potentially dwarfing rootstocks propagated by cutting. J Japan Soc Hortic Sci 79:327–334

Tetsumura T, Ishimura S, Hidaka T, Hirano E, Kuroki S, Uchida Y, Honsho C (2013) MKR1, a dwarfing rootstock for kaki, decreases early fruit drop. Acta Hortic 996:257–263

Tetsumura T, Ishimura S, Hidaka T, Hirano E, Uchida H, Kai Y, Kuroki S, Uchida Y, Honsho C (2015) Growth and production of adult Japanese persimmon (*Diospyros kaki*) trees grafted onto dwarfing rootstocks. Sci Hortic 187:87–92

Tetsumura T, Ishimura S, Honsho C, Chijiwa H (2017) Improved rooting of softwood cuttings of dwarfing rootstock for persimmon under fog irrigation. Sci Hortic 224:150–155

Tetsumura T, Ishimura S, Takita T, Funaki S, Uchida H, Hidaka T, Haranoushiro S, Udatsu Y, Matsuo M, Honsho C, Asakuma H (2019) Tree growth, flowering, and fruiting of 'Taishuu' Japanese persimmon grafted onto dwarfing rootstocks. Hort J 88:57–66

Tiwari S, Sl K, Kumar V, Singh B, Rao A, Mithra SV A, Rai V, Singh, AK, Singh NK (2016) Mapping QTLs for salt tolerance in rice (*Oryza sativa* L.) by bulked segregant analysis of recombinant inbred lines using 50K SNP chip. PLoS One 11:e0153610

Tu X, Zhang Z, Zhang S, Luo C, Chen X, Qua S (2013) A dwarf cultivar, 'Nantong-xiaofangshi.' Acta Hortic 996:139–114

Vera-Estrella R, Barkla BJ, Higgins VJ, Blumwald E (1994) Plant defense response to fungal pathogens (Activation of host-plasma membrane H^+-ATPase by elicitor-induced enzyme dephosphorylation). Plant Physiol 104:209–215

Visconti F, de Paz JM, Bonet L, Jordà M, Quiñones A, Intrigliolo DS (2015) Effects of a commercial calcium protein hydrolysate on the salt tolerance of *Diospyros kaki* L. cv. "Rojo Brillante" grafted on *Diospyros lotus* L. Sci Hortic 185:129–138

Visconti F, Intrigliolo DS, Quiñones A, Tudela L, Bonet L, de Paz JM (2017) Differences in specific chloride toxicity to *Diospyros kaki* cv. "Rojo Brillante" grafted on *D. lotus* and *D. virginiana*. Sci Hortic 214:83–90

Wardani NC, Yuniastuti E, Sulandjari (2019) Micropropagation protocol of juvenile buds derived from mature persimmon (*Diospyros kaki* L.) tree. AIP Conf Proc 2120:1.5115609

Yakushiji H, Koshita Y, Azuma A, Tsuchida Y, Asakura T, Morinaga K (2008) Growth performance of Japanese persimmon "Fuyu" grafted on different dwarfing rootstocks and relationship between canopy volume and shoot length. Acta Hortic 772: 385–387

Yakushiji H, Azuma A, Sugiura H, Yamasaki A, Koshita Y (2016a) Comparison of promising dwarfing rootstocks for 'Fuyu' Japanese persimmon trees. Acta Hortic 1130:469–472

Yakushiji H, Yamasaki A, Kobayashi S, Kaneyoshi J, Azuma A, Sugiura H, Sato A (2016b) Characteristics of a dwarf octoploid mutation arising from a non-aploid cultivar of persimmon. Hort J 85:209–216

Yakushiji H, Yamasaki A, Sugiura H, Azuma A, Nishimura R (2017) Dwarf heptaploids obtained from cross between octoploid 'Hasshu' and hexaploid persimmons. Hortic Res (jpn) 16(Suppl 2):158 (In Japanese)

Yakushiji H, Sugiura H, Yamasaki A, Azuma A, Koshita Y (2021) Tree growth, productivity, and fruit quality of 'Fuyu' persimmon trees onto different dwarfing rootstocks. Sci Hortic 278:109869

Yamada M, Sumi T, Kurihara A, Yamane H (1988) Effects of various factors on the regeneration of Japanese persimmon (*Diospyros kaki* Thunb.) from root cuttings. Bull Fruit Tree Res Stn E 7:9–30 (In Japanese with English summary)

Yamada M, Yoshinaga K, Yamane H, Sumi T, Kurihara A (1997) Dwarfing effect of the rootstock of *Diospyros rhombifolia* Hemsl. on the tree growth of "Fuyu" Japanese persimmon. Acta Hortic 436:295–304

Zhu JK, Liu J, Xiong L (1998) Genetic analysis of salt tolerance in arabidopsis: Evidence for a critical role of potassium nutrition. Plant Cell 10:1181–1191

Diospyros Lotus Genome

5

Takashi Akagi

Abstract

Crop genomics and genetics have been radically progressed in this past decade, as a whole genome-wide sequencing era. Notwithstanding, it is still hard to exploit the genome sequence information in most of the highly polyploid crops, including hexaploid persimmon (*Diospyros kaki*). In this chapter, we focus on the genome sequences of a diploid persimmon, *D. lotus*, which is a close wild relative of *D. kaki*, and discuss their applications and contribution to evolutionary aspects. The whole-genome sequences of a male *D. lotus* were drafted recently with one of the next generation sequencing (NGS) technologies, PacBio sequencing, to be defined with 15 pseudomolecules consistent with the basic chromosome numbers of the genus *Diospyros*. As one of the representative characteristics in plant genomes, the *Diospyros* genome also underwent at least two genome-wide duplication events. One of the two, named *Dd*-α, is a lineage-specific duplication that occurred at approx. 60–70 million years ago. This duplication is thought to drive some lineage-specific neofunctionalization in both protein (*trans*-acting) and expression (*cis*-regulatory) functions, including an important transition into separated sexualities. Furthermore, the *D. lotus* genome sequences provided critical insights into sex chromosome evolution. The draft genome sequences have been already widely applied as an alternative reference of the *D. kaki* genome, in transcriptomic, genomic, and epigenomic analyses. Further exploration in the frontiers of various *Diospyros* genomes for comparative approaches would expand the possibilities of genomic analysis in persimmon, and shed light on more for the evolution of the genus *Diospyros*.

5.1 Potential of Genome Information in the Wild Relatives of Persimmon

Recent progress in biotechnology, especially molecular biological techniques, has allowed various genetic and genomic approaches in non-model plant species, including tree crops. In tree crops, long juvenile phases generally prevent rapid selection of the bred lines, to which the marker-assisted selection (MAS) based on their genomic information has been proposed as a good solution since the 1990s (Ribaut and Hoisington 1998; Collard and Mackill 2008). In contrast to mostly diploid lineages, such as tree

T. Akagi (✉)
Graduate School of Environmental and Life Science, Okayama University, Okayama 700-8530, Japan
e-mail: takashia@okayama-u.ac.jp

T. Akagi
Japan Science and Technology Agency (JST), PRESTO, Kawaguchi-shi, Saitama 332-0012, Japan

R. Tao and Z. Luo (eds.), *The Persimmon Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-05584-3_5

crops in the genus *Citrus* or the family Rosaceae, polyploid species have been thought to be hard to be studied with genetic or genomic approaches, due to its nature of genetic complexity and difficulty in genome sequencing. Persimmon, as a highly polyploid species ($2n$ = 6X or 9X = 90 or 135), would also be an annoying target of genetic/genomic analysis. Notwithstanding, previous studies have managed to develop genetic markers mainly for the understanding of cultivar/variety differentiations (Luo et al. 1995; Badenes et al. 2003; Yonemori et al. 2008; Parfitt et al. 2015) or selection of specific traits, such as fruit astringency derived from proanthocyanidin accumulation (Kanzaki et al. 2001, 2010). While, in hexaploid species, it would be still quite hard to construct a genome-wide sequence database, at least with the current sequencing technology (although further next-generation techniques may easily enable that, considering the progress in these 10 years). For genome-wide analysis in hexaploid persimmon, one feasible approach would be the utilization of genomes in diploid close relatives. This concept would not be limited to persimmon, but has been acceptable to other polyploid crop species, such as strawberry (Shulaev et al. 2011) or sweet potato (Wu et al. 2018). Previous phylogenetic analyzes suggested that diploid *Diospyros lotus* and *Diospyros oleifera* would be candidates for the alternative whole-genome sequences resource (Yonemori et al. 2008). The use of *D. lotus* genome information indeed successfully resulted in the identification of genetic markers linked to the sex determination locus (Akagi et al. 2014) or of the region including the *ASTRINGENCY* locus (Nishiyama et al. 2018). Although we should be careful about the phenotypic differences in these wild relatives and persimmon (or *D. kaki*), alternative use of their genomes would often allow various objectives involving genetic research or actual molecular breeding in hexaploid persimmon.

Another importance of the genome information of diploid *Diospyros* species would be understanding the evolutionary scenario for persimmon. Recent draft genome sequencing in diversified crops has unveiled an "unexpected" (and "undesirable," as well) possibility for the conservation of gene functions amongst the lineages. In contrast to animal taxa, plants have undergone frequent whole-genome duplication events (WGD) in lineage-specific manner (Van de Peer et al. 2017), which are thought to have provided opportunities for the appearance of new traits representing each species. For instance, in horticultural crops, functional differentiation between paralogs, which had been derived from WGD, resulted in the establishment of specific ripening characteristics in tomato fruits (The Tomato Genome Consortium 2012), specific oil composition in olive (Unver et al. 2017), and specific sex-determination system in kiwifruit (Huang et al. 2013; Akagi et al. 2018, 2019). These are also consistent with an evolutionary theoretical framework that the gene redundancy provided by the presence of duplicate copies allows one copy to be neofunctionalized without loss of the original function (Flagel and Wendel, 2009). In other words, proper (or reliable) draft genome sequences of each crop or its relatives would be indispensable for understanding their lineage-specific traits, which would often involve their commercial importance.

5.2 Draft Genome Sequences in *D. Lotus*

So far, a few of the genome sequences were drafted in diploid *Diospyros*, such as *D. oleifera* (introduced in Chap. 6; Zhu et al. 2019; Suo et al. 2020) or *D. lotus*. Here, I introduce the characteristics and evolution of *D. lotus* genome, often associating the sex determination systems (as related to Chaps. 9 and 10). The current version (on 31st Aug 2021) of the *D. lotus* cv. Kunsenshi-male (male, 2A + XY) draft genome (http://persimmon.kazusa.or.jp/), of which the expected haploid genome size is 907 Mb from flow cytometry (Tamura et al. 1998) or 877.7 Mb from kmer analysis in PacBio long-read data, are constituted of 3073 primary contigs totaling 746.1 Mb, and 5901 "secondary" contigs, which are putative allelic contigs to the primary contigs. With the segregating F1 populations (N = 314

and 119, Akagi et al. 2013), a total of 5,959 markers derived from GBS/ddRAD sequencing allowed anchoring of the ca 61.8% scaffolds into 15 pseudomolecules, which is consistent with the basic chromosome numbers in the *Diospyros* (Akagi et al. 2020). The draft genome (or primary contigs) includes 40,532 predicted gene locations, of which the numbers would be comparable to those in other asterid plant species, such as tomato (N = 34,879) (The Tomato Genome Consortium 2012) or kiwifruit (N = 39,040) (Huang et al. 2013).

Potential whole genome-wide duplications would be one of the important characteristics which would be detectable from mainly two indexes; (i) long syntenic relationships within the genomic regions, and (ii) distribution of silent divergence [or four-fold synonymous (degenerative) third-codon transversion (4DTv), to be more strict] in the duplicated paralogous gene pairs. Previous characterization of the genetic diversities and the distribution of silent divergence (*Ks* or *dS*) values in limited numbers of the genes in the genera *Actinidia*, *Diospyros*, and *Camellia* suggested lineage-specific genome-wide duplications in the genus *Actinidia* and *Diospyros* (Shi et al. 2010). Consistent with this assumption, in the *D. lotus* draft genome, the described two indexes suggested at least two genome-wide duplication events, which would correspond to the hexaploidization γ (*Hex*-γ) (Jaillon et al. 2007) common in the eudicot taxa, and a novel one, named *Dd*-α (Akagi et al. 2020) (Fig. 5.1). This result was also supported by comparative genomics in the genome drafting of tea (*Camellia sinensis*), which is nested into the order Ericale, as well as the genus *Diospyros* (Wang et al. 2021). While, the timing of the later lineage-specific genome-wide duplication (or *Dd*-α) might remain to be examined more because the species divergences in the ancestral state of the order Ericale is still ambiguously defined (Akagi et al. 2020; Wang et al. 2021). Importantly, the *Dd*-α occurred concurrently with previously reported whole-genome duplication events in the asterids (Huang et al. 2013; Iorizzo et al. 2016; Reyes-Chin-Wo et al. 2017), as well as across the angiosperms (Vanneste et al. 2014; Van de Peer et al. 2017), concentrated around the K-Pg (Cretaceous-Paleogene) boundary, which is hypothetically 60–70 million years ago (mya) (Fig. 5.2).

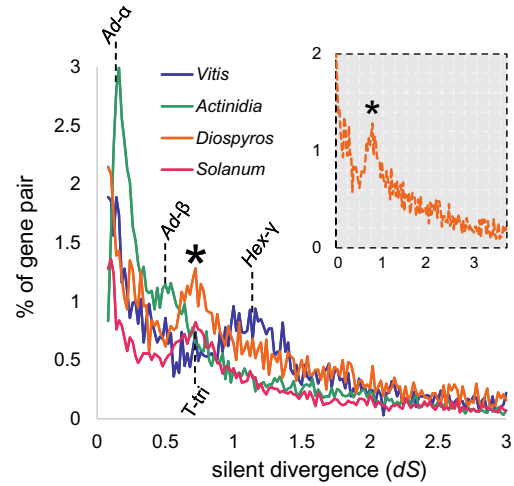

Fig. 5.1 Distribution of silent divergences in the genome-wide homologous gene pairs *(this panel is cited from* Akagi et al. 2020 *PLoS Genet 16(2): e1008566).* The peaks correspond putative genome-wide (or very large) duplication events, in *Vitis* (purple), *Actinidia* (green), *Diospyros* (orange), and *Solanum* (red) genomes. The silent divergence would be a proxy age. For the *Diospyros* genome, the most representative peak, given as the asterisk, was detected almost concurrently with the *Solanum*-specific (tomato-specific) hexaploidization, named *T-tri*, which was supposed to occur ca 60–70 mya. The Hex-γ is thought to be a common event to the (eu)dicot species (Jaillon et al. 2007) including the described four species, while recent paleo-ploidizations would tend to mask the genomic traces of Hex-γ

5.3 New Functions Established via the Lineage-Specific Genome-Wide Duplications

Paleo-genome duplication events, as described, would provide good chances to establish new gene functions, as functional redundancy of the duplicates can facilitate the plasticity, often resulting in subfunctionalization, neofunctionalization, or pseudogenization (Flagel and Wendel, 2009; Van de Peer et al. 2017). Neofunctionalization in expression pattern (or *cis*-evolution) would be quite simple to define if either of the

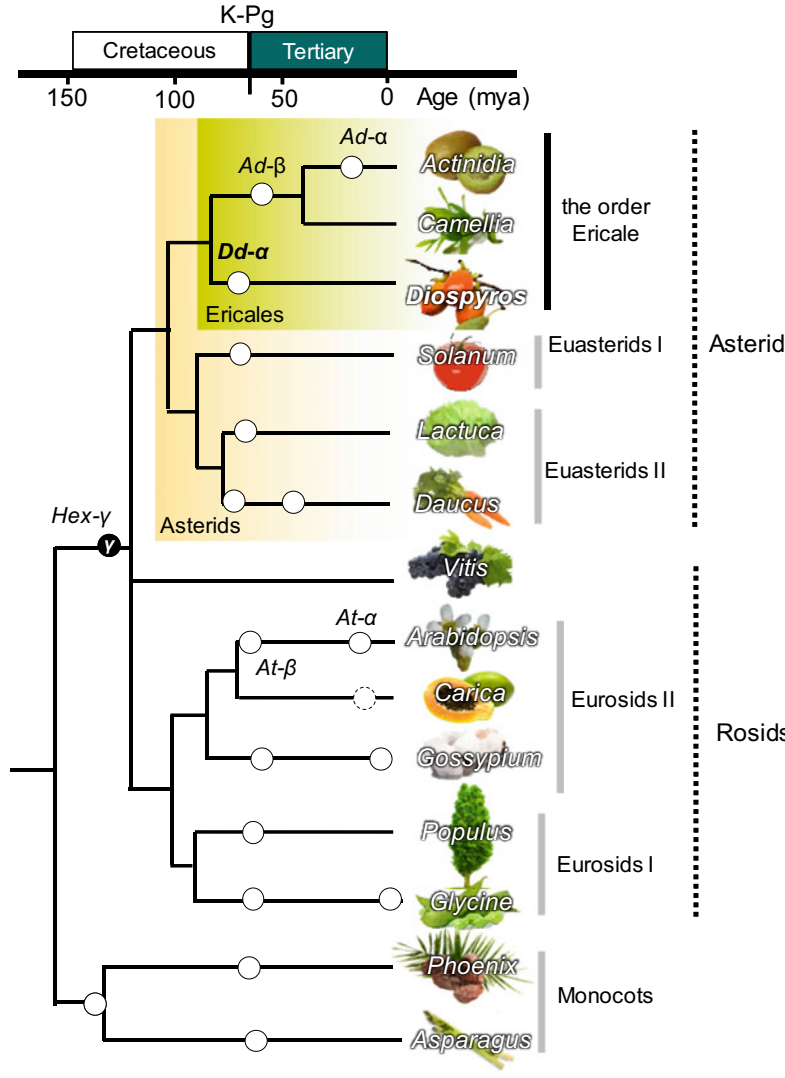

Fig. 5.2 Lineage-specific whole-genome duplication events in representative angiosperm. Putative whole genome-wide duplication events, given in open circles, and their time scales are referred from previous reports (Van de Peer 2009; Huang et al. 2013; Vanneste et al. 2014; Iorizzo et al. 2016; Reyes-Chin-Wo et al. 2017; Harkess et al. 2017; Van de Peer et al. 2017; Akagi et al. 2020). K-Pg, Cretaceous-Paleogene boundary

duplicated pair exhibits novel expression behaviors. The evolution in *cis*-regulatory elements is thought to be a more rapid and fundamental reaction after duplication events, than that in *trans*-acting elements (Lynch and Conery 2000; Roulin et al 2013). Consistent with this theoretical framework, in *D. lotus* genome, the expression patterns of the duplicated pairs derived from the *Dd*-α showed substantially differentiated, even in focusing only on the expressions between male and female flower buds (Akagi et al. 2020). On the other hand, it might be hard to directly define neofunctionalization in *trans*-acting elements (or protein function) via duplication events. A potential index to call a novel beneficial function (for surviving) that was triggered by duplication, would be the transition of evolutionary rate (*dN/dS* values). New beneficial amino-acid mutations are often under positive selections ($dN/dS \ll 1.0$), then followed by strong purifying selections ($dN/dS \ll 1.0$) to be genetically fixed. Application of the model considering this situation, called "episodic positive selection," to the *D. lotus* genome found some candidate genes that acquired a novel function immediately after the *Dd*-α whole genome-wide duplication event (Fig. 5.3). It is worthy to note that a sex-

determining gene, *MeGI* (see Chap. 8 for sex-determination system in persimmon) derived from its paralogous gene, named *Sister of MeGI* (*SiMeGI*), was included in the list of the putatively neofunctionalized genes that underwent episodic positive selection after the *Dd*-α (Akagi et al. 2020). Importantly, this assumption has been experimentally validated with the transformation of *Nicotiana tabacum*, where constitutive induction of *MeGI* resulted in repressed androecium development, or feminization. This feminizing function is not conserved in the paralogous genes, *SiMeGI*, and its orthologous gene in the other plant species, such as barley (Komatsuda et al. 2007; Sakuma et al. 2013), Arabidopsis (González-Grandío et al. 2017), and maize (Whipple et al. 2011), and also tomato (Lin et al. 2008). Hence, detection of the adaptive evolution of a sex-determining gene, *MeGI*, by using evolutionary indexes would be a good example for exploitation of the whole-genome information to figure out lineage-specific traits or gene functions. Still, there would be massive untouched gene resources that possibly have evolved *Diospyros*-specific new functions via lineage-specific duplication events. Considering the potential of this frontier, rapid gene evaluation systems, such as with precocious flowering lines as in the case of kiwifruit nested into the same order Ericale (Varkonyi-Gasic et al. 2019), would be the next breakthrough.

5.4 Evolution of the Sex Chromosome

The first finding of the sex chromosome in flowering plants was made independently in white champion (*Silene latifolia,* and *Silene* spp.), sorrel (*Rumex acetosa*), hop (*Humulus lupulus*), and so on, in 1923 (Kihara and Ono 1923; Winge 1923; Blackburn 1923). After that, sex chromosomes have been researched over a century, while yet their nucleotide sequence contexts and evolution of the male(or female in ZW system)-specific regions have been little assessed (Renner and Müller 2021). The genus *Diospyros* is mostly dioecious, except potentially polygamous polyploids or minorities, which are determined by an XY (or heterogametic male) system (see Chaps. 8 and 9). So far, no reports suggested heteromorphic sex (or XY) chromosomes in *Diospyros*, and thus, their male-specific region of the Y-chromosome (MSY) is thought to be very small (Akagi et al. 2014). The draft genome of *D. lotus* successfully anchored some pseudo-autosomal sex-linked scaffolds to chromosome 15 (Akagi et al. 2020). On the other hand, the MSY, including *OGI,* has not been anchored using genetic markers, presumably due to the large structural variation between the X and Y chromosome and the resultant Y-specific hemizygosity surrounding the *OGI* gene. Instead, with bacterial artificial chromosomes (BAC) walking started from the seed of *OGI* (Akagi et al. 2014), Y-chromosomal supercontigs covering most of the MSY were physically anchored (Akagi et al. 2020). The regions surrounding the *OGI* are male-specific (or hemizygous) and hyper-repetitive, often including palindrome-like structures. These sequence contexts are consistent with those of sex chromosomes in animal taxa (Bachtrog et al. 2014). In the outer regions of the MSY, putative pseudo-autosomal regions (PAR), which include both X- and Y-allelic genes, are partially scattered into the MSY, and dominantly appear only 200–300 kb apart from the single-sex determinant, *OGI*. Considering the relatively long history of the Y chromosome (or the sex determinant; ca 20–30 million years estimated from the putative silent divergence between *MeGI* and *OGI*) in *Diospyros*, such small MSY (up to 600–1000 kb) might be inconsistent with the conventional hypothesis for sex chromosome evolution in plants, where distinct MSY can form in only a few million years (Ming et al. 2011). On the other hand, recent characterization of various plant sex chromosomes is uncovering their evolutionary diversities, providing some examples for small MSY, such as in *Populus*, *Vitis*, and *Actinidia*, which would be consistent with the sex chromosome evolution in *Diospyros* (Renner and Müller 2021).

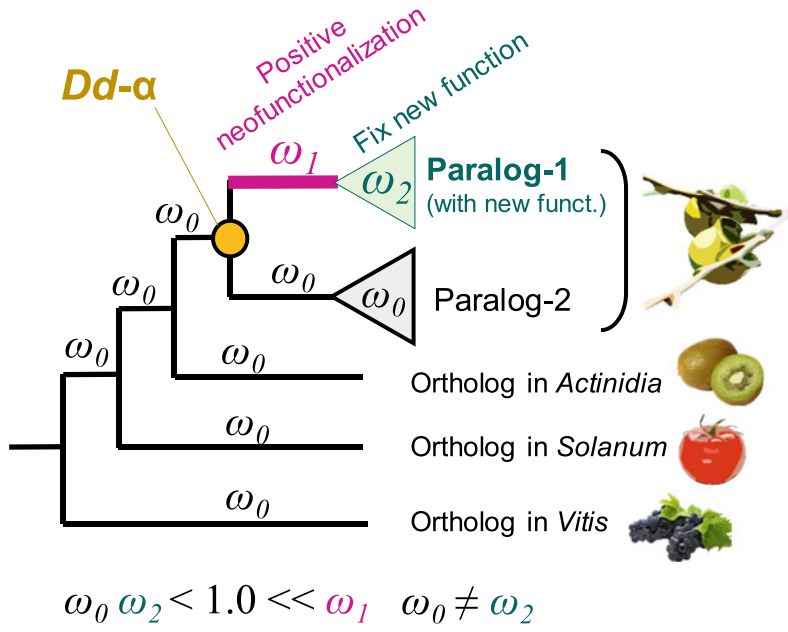

Fig. 5.3 Evolutionary model to detect episodic positive selections driven imeediately after the *Dd*-α. We targeted strong positive selection on either of the duplicates derived from the *Dd*-α ($\omega_1 \gg 1$, on the thick pink branch), which was not undergone in the background branches and the counter part paralog ($\omega_0 < 1$). After establishment of an adaptive new function, the evolutionary rate should be reduced to be under purifying selection, with distinct selective patterns from the original orthologs ($\omega_2 < 1$, $\omega_2 \neq \omega_0$, green triangle)

In the MSY of *D. lotus*, although the boundary to the PAR would be quite ambiguous, some genes were predicted in the hemizygous Y-allelic regions, while they are thought to be mostly the components of transposable elements, which is consistent with the MSY evolution in animal taxa. In the PAR-like sequences, which still include male-specific hemizygous islands, the silent divergence rate between the putative X and Y alleles of the genes is decreased, in inverse proportion to the distance from *OGI*. This situation is expectable, which reflects historical recombination between the perfectly conserved Y factor, *OGI*, and the surrounding regions, often forming evolutionary strata (Bergero et al. 2007). The actual *dS* rate in 1-Mb from *OGI*, ranges from approx. 0.2 (at *OGI*) to 0.07, which is comparable to the interspecific *dS* rate between *D. lotus* and distant *Diospyros* species (such as in *D. mespiliformis*, *dS* = ca 0.07 against *D. lotus*), implying suppressed recombination in the regions flanking *OGI* before the divergence of some *Diospyros* species. Although *OGI* is thought to arise via duplications from the ancestral *MeGI* (Akagi et al. 2014), long syntenic collinearity among the regions surrounding them is not conserved. This means that a local segmental duplication (or simple gene duplication) event followed by an inversion, triggered the establishment of the dioecious sex-determination system of this genus.

5.5 D. Lotus Genome Shed Light on the Insights into the Transition into Dioecy Associated with Duplication Events

Summarizing so far, the *D. lotus* genome information would raise the following hypothesis for the transition into dioecy in *Diospyros*. A lineage-specific WGD event, *Dd*-α contributed to derive the first twin, proto-*MeGI*, and *SiMeGI*,

and the proto-*MeGI* specifically underwent positive selection to be neofunctionalized to act as a feminizing factor to dominantly suppress androecium development. This was followed by a segmental duplication to derive *MeGI* and a Y-encoded *OGI*, which dominantly suppresses the expression of *MeGI* (Akagi et al. 2014, 2020). It would be worthy to note that this type of evolutionary pattern would be reminiscent of potential generality or commonality for the establishment of dioecy in other plant species. In the establishment of dioecy in garden asparagus, the Y-encoded sex-determining gene, *SOFF*, is thought to have originated from an *Asparagus*-specific segmental or whole genome-wide duplication, which was followed by the acquisition of its function as a dominant suppressor of feminization (SuF) (Harkess et al. 2017). One of the two Y-encoded sex determinants in kiwifruit (*Actinidia* spp.), *Shy Girl*, which is also a dominant suppressor of feminization (Akagi et al. 2018, Varkonyi-Gasic et al. 2021), arose via an *Actinidia*-specific duplication event (Akagi et al. 2018, 2019), potentially involving one of the two *Actinidia*-specific WGD events, *Ad*-α (Huang et al. 2013). Furthermore, in the family *Salicaceae*, multiple Y-encoded sex determinants, which are mostly small-RNAs acting as dominant suppressors of the "master sex regulator" of *ARR17*, are independently derived from lineage-specific duplication events (Müller et al. 2020). These parallel paths towards the independent evolution of these sex determinants are probably not coincidental, but consistent with the theoretical framework described above. In flowering plants, transition into separated sexuality would require the appearance and selection of a gain-of-function event in order to acquire a dominant suppressor(s) (Charlesworth and Charlesworth 1978a, 1978b). Although a dominant suppressor would be hard to generate from a non-redundant (or single) gene due to the importance of its original function, while whole-genome duplication events provide good opportunities to relax purifying selective pressure for abundant genes to be neofunctionalized into dominant suppressors.

5.6 Application of the *D. Lotus* Genome Information and Future Prospects

One of the simplest usages of the *D. lotus* genome is as a reference for transcripts or genome mapping from *D. kaki*. As in many cases of the transcriptomic or whole-genomic assessments in polyploid species, alternative use of a diploid close relative for mapping would be an effective way to reduce the issues derived from their genomic/genetic complexities. Although the *D. lotus* genome information has not been opened until recently, some genome-wide studies already utilized that for the database in *D. kaki*. Transcriptomic analyses in *D. kaki* focusing on unlocked male flower production (Masuda et al. 2020a), fruit shape diversity among the cultivars (Maeda et al. 2019), or dwarfism involving gibberellin metabolism (Dong et al. 2021), applied the *D. lotus* whole-gene sets, and successfully found the candidate genes or molecular mechanisms. Considering the conservation of the genes and their homologies between *D. lotus* and *D. kaki*, transcriptomic approaches should have no serious issues. As a genomic reference map, Masuda et al. (2020b) mapped captured gDNA Illumina reads (ddRAD-Seq data, more specifically) from *D. kaki* segregated population to the *D. lotus* pseudomolecule and scaffold contigs, to identify genetic regions contributing to the bias in the female/male flower ratio. Importantly, the Y-chromosome (or the Y-encoded sex determinant, *OGI*) itself and/or its allelic dosage had a substantial association with the male flower ratio. The *D. lotus* genome sequences were derived from a male (2A + XY), and the Y-specific regions have been well examined as described, so that it would be especially suitable for the studies involving the persimmon sexualities. Epigenetic studies in *D. kaki* are also acceptable with the *D. lotus* genome as a reference. Fluctuation of the genome-wide DNA methylation levels, which is potentially associated with male flower production in a genetically female cultivar, were successfully characterized with *D. lotus* genome as the reference (Masuda et al. 2020c).

On the other hand, if we target more "specific" sequences, such as short *cis*-regulatory elements, in *D. kaki*, the *D. lotus* genome might not be applicable, considering the evolutionary distance between them. The genome sequences of *D. oleifera*, which showed a little bit closer relationship to *D. kaki* than *D. lotus* (Yonemori et al. 2008), would be an alternative option, as described in Chap. 6. Still, we should be careful about the hexaploidy in *D. kaki*, which exhibited substantial sequence variations among the hexaplex alleles. Also, the evolutionary distances (or sequences homologies) between two species would be very flexible among the genomic regions, especially in tree crops, where it would be hard to define uniform distance per genome, mainly due to frequent introgressions (Cornille et al. 2012; Numaguchi et al. 2020). Case-by-case selection of a reference genome, depending on the research purposes, would be key. With the current technologies, construction of a draft genome with nearly perfect quality, such as chromosome-scale assembly, would be becoming quite easier for very cheaper costs. We suppose that it would be reasonable not to persist with the limited genomes information, but to explore the frontiers of other *Diospyros* genomes for various objectives. Comparative genomic or population genetic approaches with diversified *Diospyros* species would shed light on more evolutionary aspects, such as the domestication path of *D. kaki*.

Acknowledgements We thank Dr. Deborah Charlesworth for the extensive discussions on the interpretation of *D. lotus* genome evolution, especially on the parts involving sex determination, and the many thoughtful pieces of advice provided through this work.

References

Akagi T, Henry IM, Tao R, Comai L (2014) A Y-chromosome–encoded small RNA acts as a sex determinant in persimmons. Science 346(6209):646–650

Akagi T, Pilkington SM, Varkonyi-Gasic E, Henry IM et al (2019) Two Y-chromosome-encoded genes determine sex in kiwifruit. Nat Plants 5(8):801–809

Akagi T, Shirasawa K, Nagasaki H, Hirakawa H, Tao R, Comai L, Henry IM (2020) The persimmon genome reveals clues to the evolution of a lineage-specific sex determination system in plants. PLoS Genet 16(2): e1008566

Akagi T, Kajita K, Kibe T, Morimura H, Tsujimoto T, Nishiyama S, Kawai T, Tao, R (2013) Development of molecular markers associated with sexuality in *Diospyros lotus* L. and their Application in *D. kaki* Thunb J Jpn Soc Hort Sci, CH-109

Akagi T, Henry IM, Ohtani H, Morimoto T, Beppu K, Kataoka I, Tao R (2018) A Y-encoded suppressor of feminization arose via lineage-specific duplication of a cytokinin response regulator in kiwifruit. Plant Cell 30 (4):780–795

Bachtrog D, Mank JE, Peichel CL, Kirkpatrick M, Otto SP, Ashman TL, Hahn MW, Kitano J, Mayrose I, Ming R, Perrin N, Ross L, Vamosi, Tree of Sex Consortium (2014) Sex determination: why so many ways of doing it?. PLoS Biol 12(7):e1001899

Badenes M, Garcés A, Romero C, Romero M, Clavé J, Rovira M, Llácer G (2003) Genetic diversity of introduced and local Spanish persimmon cultivars revealed by RAPD markers. Genet Resour Crop Evol 50(6):579–585

Bergero R, Forrest A, Kamau E, Charlesworth D (2007) Evolutionary strata on the X chromosomes of the dioecious plant Silene latifolia: evidence from new sex-linked genes. Genetics 175(4):1945–1954

Blackburn KB (1923) Sex chromosomes in plants. Nature 112(2819):687–688

Charlesworth B, Charlesworth D (1978a) A model for the evolution of dioecy and gynodioecy. Am Nat 112 (988):975–997

Charlesworth D, Charlesworth B (1978b) Population genetics of partial male-sterility and the evolution of monoecy and dioecy. Heredity 41(2):137–153

Collard BC, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philos Trans R Soc Lond B Biol Sci 363 (1491):557–572

Cornille A, Gladieux P, Smulders MJ, Roldan-Ruiz I, Laurens F, Le Cam B, Nersesyan A, Clavel J, Olonova M, Feugey L, Gabrielyan I, Zhang XG, Tenalillon M, Giraud T (2012) New insight into the history of domesticated apple: secondary contribution of the European wild apple to the genome of cultivated varieties. PLoS Genet 8(5):e1002703

Dong Y, Ye X, Xiong A, Zhu N, Jiang L, Qu S (2021) The regulatory role of gibberellin related genes DKGA2ox1 and MIR171f_3 in persimmon dwarfism. Plant Sci 310:110958

Flagel LE, Wendel JF (2009) Gene duplication and evolutionary novelty in plants. New Phytol 183(3):557–564

González-Grandío E, Pajoro A, Franco-Zorrilla JM, Tarancón C, Immink RG, Cubas P (2017) Abscisic acid signaling is controlled by a BRANCHED1/HD-ZIP I cascade in Arabidopsis axillary buds. Proc Natl Acad Sci USA 114(2):E245–E254

Harkess A, Zhou J, Xu C, Bowers JE et al (2017) The asparagus genome sheds light on the origin and evolution of a young Y chromosome. Nat Commun 8 (1):1–10

Huang S, Ding J, Deng D, Tang W et al (2013) Draft genome of the kiwifruit Actinidia chinensis. Nat Commun 4(1):1–9

Iorizzo M, Ellison S, Senalik D, Zeng P et al (2016) A high-quality carrot genome assembly provides new insights into carotenoid accumulation and asterid genome evolution. Nature Genet 48(6):657–666

Jaillon O, Aury JM, Noel B, Policriti A, Clepet C, Cassagrande A, Wincker P (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449(7161):463–467

Kanzaki S, Yonemori K, Sugiura A, Sato A, Yamada M (2001) Identification of molecular markers linked to the trait of natural astringency loss of Japanese persimmon (*Diospyros kaki*) fruit. J Am Soc Hortic Sci 126(1):51–55

Kanzaki S, Akagi T, Masuko T, Kimura M, Yamada M, Sato A, Mitani N, Ustunomiya N, Yonemori K (2010) SCAR markers for practical application of marker-assisted selection in persimmon (*Diospyros kaki* Thunb.) breeding. J Jpn Soc Hort Sci 79(2):150–155

Kihara H, Ono T (1923) Cytological studies on rumex L. II on the relation of chromosome number and sexes in rumex acetosa L. Bot Mag Tokyo 37:147–149

Komatsuda T, Pourkheirandish M, He C, Azhaguvel P et al (2007) Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. Proc Natl Acad Sci USA 104 (4):1424–1429

Lin Z, Hong Y, Yin M, Li C, Zhang K, Grierson D (2008) A tomato HD-Zip homeobox protein, LeHB-1, plays an important role in floral organogenesis and ripening. Plant J 55(2):301–310

Luo ZR, Yonemori K, Sugiura A (1995) Evaluation of RAPD analysis for cultivar identification of persimmons. J Jpn Soc Hort Sci 64(3):535–541

Lynch M, Conery JS (2000) The evolutionary fate and consequences of duplicate genes. Science 290 (5494):1151–1155

Maeda H, Akagi T, Onoue N, Kono A, Tao R (2019) Evolution of lineage-specific gene netorks underlying the considerable fruit shape diversity in persimmon. Plant Cell Physiol 60(11):2464–2477

Masuda K, Akagi T, Esumi T, Tao R (2020a) Epigenetic flexibility underlies somaclonal sex conversions in hexaploid persimmon. Plant Cell Physiol 61(2):393–402

Masuda K, Fujita N, Yang HW, Ushijima K, Kubo Y, Tao R, Akagi T (2020b) Molecular mechanism underlying derepressed male production in hexaploid persimmon. Front Plant Sci 11:567249

Masuda K, Yamamoto E, Shirasawa K, Onoue N, Kono A, Ushijima K, Kubo Y, Tao R, Henry IM, Akagi T (2020c) Genome-wide study on the polysomic genetic factors conferring plasticity of flower sexuality in hexaploid persimmon. DNA Res 27(3): dsaa012

Ming R, Bendahmane A, Renner SS (2011) Sex chromosomes in land plants. Annu Rev Plant Biol 62:485–514

Müller NA, Kersten B, Leite Montalvão AP, Mähler N, Bernhardsson C, Bräutigam K, Lorenzo ZC, Hoenicka H, Kumar V, Mader M, Pakull B, Robinson KM, Sabatti M, Vettori C, Ingvarsson PK, Cronk Q, Street NR, Fladung M (2020) A single gene underlies the dynamic evolution of poplar sex determination. Nature Plants 6(6):630–637

Nishiyama S, Onoue N, Kono A, Sato A, Yonemori K, Tao R (2018) Characterization of a gene regulatory network underlying astringency loss in persimmon fruit. Planta 247(3):733–743

Numaguchi K, Akagi T, Kitamura Y, Ishikawa R, Ishii T (2020) Interspecific introgression and natural selection in the evolution of Japanese apricot (*Prunus mume*). Plant J 104(6):1551–1567

Parfitt DE, Yonemori K, Honsho C, Nozaka M, Kanzaki S, Sato A, Yamada M (2015) Relationships among Asian persimmon cultivars, astringent and non-astringent types. Tree Genet Genomes 11(2):24

Renner SS, Müller NA (2021) Plant sex chromosomes defy evolutionary models of expanding recombination suppression and genetic degeneration. Nat Plants 7 (4):392–402

Reyes-Chin-Wo S, Wang Z, Yang X, Kozik A et al (2017) Genome assembly with in vitro proximity ligation data and whole-genome triplication in lettuce. Nat Commun 8(1):1–11

Ribaut JM, Hoisington D (1998) Marker-assisted selection: new tools and strategies. Trends Plant Sci 3 (6):236–239

Roulin A, Auer PL, Libault M, Schlueter J, Farmer A, May G, Stacey G, Doerge RW, Jackson SA (2013) The fate of duplicated genes in a polyploid plant genome. Plant J 73(1):143–153

Sakuma S, Pourkheirandish M, Hensel G, Kumlehn J, Stein N, Tagiri A, Yamaji N, Ma JF, Sassa H, Koda T, Komatsuda T (2013) Divergence of expression pattern contributed to neofunctionalization of duplicated HD-Z ip I transcription factor in barley. New Phytol 197 (3):939–948

Shi T, Huang H, Barker MS (2010) Ancient genome duplications during the evolution of kiwifruit (*Actinidia*) and related Ericales. Ann Bot 106(3):497–504

Shulaev V, Sargent DJ, Crowhurst RN et al (2011) The genome of woodland strawberry (*Fragaria vesc*a). Nat Genet 43(2):109–116

Suo Y, Sun P, Cheng H, Han W, Diao S, Li H, Mai Y, Zhao X, Fu J (2020) A high-quality chromosomal genome assembly of *Diospyros oleifera* Cheng. GigaScience 9(1):giz164

Tamura M, Tao R, Yonemori K, Utsunomiya N, Sugiura A (1998) Ploidy level and genome size of several *Diospyros* species. J Jpn Soc Hort Sci 67 (3):306–312

Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485(7400):635

Unver T, Wu Z, Sterck L, Turktas M et al (2017) Genome of wild olive and the evolution of oil biosynthesis. Proc Natl Acad Sci USA 114(44):E9413–E9422

Van de Peer Y, Mizrachi E, Marchal K (2017) The evolutionary significance of polyploidy. Nat Rev Genet 18(7):411–424

Vanneste K, Baele G, Maere S, Van de Peer Y (2014) Analysis of 41 plant genomes supports a wave of successful genome duplications in association with the Cretaceous-Paleogene boundary. Genome Res 24 (8):1334–1347

Varkonyi-Gasic E, Wang T, Voogd C, Jeon S, Drummond RS, Gleave AP, Allan AC (2019) Mutagenesis of kiwifruit CENTRORADIALIS-like genes transforms a climbing woody perennial with long juvenility and axillary flowering into a compact plant with rapid terminal flowering. Plant Biotechnol J 17(5):869–880

Varkonyi-Gasic E, Wang T, Cooney J, Jeon S, Voogd C, Douglas MJ, Pilkington SM, Akagi T, Allan AC (2021) Shy Girl, a kiwifruit suppressor of feminization, restricts gynoecium development via regulation of cytokinin metabolism and signalling. New Phytol 230(4):1461–1475

Wang Y, Chen F, Ma Y, Zhang T, Sun P, Lan M, Li F, Fang W (2021) An ancient whole-genome duplication event and its contribution to flavor compounds in the tea plant (*Camellia sinensis*). Hortic Res 8(1):1–12

Whipple CJ, Kebrom TH, Weber AL, Yang F, Hall D, Meeley R, Schmidt R, Doebley J, Brutnell TP, Jackson DP (2011) *grassy tillers1* promotes apical dominance in maize and responds to shade signals in the grasses. Proc Natl Acad Sci USA 108(33):E506–E512

Winge O (1923) On sex chromosomes, sex determination and preponderance of female in some dioecious plants. Comp Rend Trav Lab Carlsberg 15(5):1–25

Wu S, Lau KH, Cao Q et al (2018) Genome sequences of two diploid wild relatives of cultivated sweetpotato reveal targets for genetic improvement. Nat Commun 9(1):1–12

Yonemori K, Honsho C, Kanzaki S, Ino H, Ikegami A, Kitajima A, Sugiura A, Parfitt DE (2008) Sequence analyses of the ITS regions and the mat K gene for determining phylogenetic relationships of *Diospyros kaki* (persimmon) with other wild *Diospyros* (Ebenaceae) species. Tree Genet Genomes 4(2):149–158

Zhu QG, Xu Y, Yang Y, Guan CF, Zhang QY, Huang JW, Grierson D, Chen KS, Gong BC, Yin XR (2019) The persimmon (*Diospyros oleifera* Cheng) genome provides new insights into the inheritance of astringency and ancestral evolution. Hortic Res 6(1):1–15

D. Oleifera Genome

6

Yujing Suo and Jianmin Fu

Abstract

Diospyros oleifera is closely related to the hexaploid cultivated persimmon, making it a promising model system for research *D. kaki*. As such, efforts to develop genomic resources for *D. oleifera* have the potential to enable hexaploidy persimmon genome assembly and to clarify the molecular basis for key developmental or agronomic traits of interest. The integration of Illumina sequencing, single-molecule real-time sequencing, and high-throughput chromosome capture has enabled the assembly of two *D. oleifera* genome versions (DOL_v1.0 and DOL_v2.0). The DOL_v1.0 and DOL_v2.0 genomes were 849.53 Mb and 812.32 Mb in length, respectively with an N50 scaffold length of 1.42 Mb and 3.36 Mb, respectively, of which 94.1% (799.71 Mb) and 88.81% (721.45 Mb) were anchored to 15 pseudochromosomes, respectively. In total, 64.96% and 54.8% of the DOL_v1.0 and DOL_v2.0 genomes, respectively, were composed of identified repeat sequences. Through de novo sequencing and comparisons with other species of plants, 32,516 gene candidates (average length: 6773.92 bp) were identified within the DOL_v1.0, of which 95.95% were functionally annotated. Similarly, 30,530 candidate protein-coding genes (average length: 7105.40 bp) were identified within the DOL_v2.0 genome, of which (93.61%) harbored conserved functional motifs or annotated terms. Through a 4DTv analysis, the DOL_v1.0 genome was found to have undergone only an evolutionarily ancient γ whole-genome duplication (WGD) event, while both the DOL_v2.0 and *D. lotus* (4dtv = 0.36 $\sim$ 0.27–0.42) genomes have undergone a second WGD event. Based on the DOL_v2.0 genome, phylogenetic analyses suggested that the split between *D. oleifera* and *D. lotus* likely arose $\sim$9.0 million years ago. Furthermore, these two genome versions were predicted to encode 57 and 171 respective genes associated with proanthocyanidin biosynthesis and insolubilization, with chalcone synthase (*CHS*) genes having undergone expansion in the DOL_v2.0 genome in comparison to the *D. lotus* genome, and with chalcone isomerase (*CHI*) gene having undergone positive selection. Overall, the assembly of a high-quality *D. oleifera* draft genome offers new insight regarding the evolution of persimmon species, and provides a promising foundation for future efforts to develop germplasm resources and to decrease the astringency of cultivated fruits.

Y. Suo · J. Fu (✉)
Non-Timber Forest Research Institude, Chinese Academy of Forestry, No. 3 Weiwu Road, Jinshui District, Zhengzhou 450003, China
e-mail: fjm371@163.com

Y. Suo
e-mail: suoyujing1988@126.com

R. Tao and Z. Luo (eds.), *The Persimmon Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-05584-3_6

6.1 Introduction

Diospyros oleifera Cheng is a diploid species ($2n = 2x = 30$), economically important member of the Ebenaceae family that produces fruits containing high levels of tannins. *D. oleifera* is more closely related to *D. kaki* than other relevant diploid species, and this is reflected by greater similarity in the phenotypic characteristics of the fruits. The flower sexual diversity and instability of *D. oleifera* is also more similar to that of *D. kaki* than that of *D. lotus* with respect to rates of androecy, gynoecy, monoecy, androgynomonoecy, and andromonoecy (Fig. 6.1). Efforts to more fully the complex molecular determinants of these varied sexual phenotypes in *D. oleifera* may thus provide fundamental insights into the regulation of these processes in cultivated *D. kaki.* Phylogenetic assessments conducted on the basis of the chloroplast genome and on protein-coding, intergenic, and intronic sequence data have also confirmed the close relationship between *D. kaki* and *D. oleifera,* underscoring the potential value of the latter species as a model for research focused on cultivated persimmon plants (Fu et al. 2016). Given that most cultivars of *D. kaki* are either hexaploid ($2n = 6 \times = 90$) or nonaploid ($2n = 9 \times = 135$), details regarding the related progenitors, origins, and strategies of polyploidization are poorly understood, limiting associated molecular breeding efforts. Therefore, further efforts are needed to assemble and analyze the diploid *D. oleifera* genome in order to provide guidance for the assembly of the hexaploidy *D. kaki* genome and to guide research focused on the evolution and origins of persimmon species, as well as efforts to influence the genetics that regulate key agronomic traits of interest.

Here, we describe and compare two published versions of the *D. oleifera* genome and associated annotations. The first of these two genomes (DOL_v1.0, PRJNA562043) was published in

Fig. 6.1 *D. oleifera*. Mature tree (**a**), gynoecious type (**b**), androecious type (**c**), andromonoecious type (**d**), monoecious type (**e**), fruit (**f**). (From Suo et al. 2020)

Horticultural Research (Zhu et al. 2019), while the second (DOL_v2.0, PRJNA532832) was published in *GIGA Science* (Suo et al. 2020).

6.2 *D. Oleifera* Genome de Novo Assembly

Prior to sequencing, the size of the *D. oleifera* genome was estimated based upon measurements of nuclear weight conducted via flow cytometry and K-mer analysis. The DOL_v1.0 and DOL_v2.0 projects predicted respective genome sizes of 853.3 Mb and 868.41 Mb, with the latter project having predicted heterozygosity of 1.08%. The platforms of Illumina HiSeq (Illumina, USA) and PacBio Sequel were used to sequence the *D. oleifera* for both of these projects, with the Hi-C technique being used for subsequent scaffold assembly. In total, the DOL_v1.0 and DOL_v2.0 projects produced ~86 Gb of high-quality sequences and 443.59 Gb of raw sequence data (510.81-fold genome coverage), respectively, with final assembled genome sizes of 849.53 Mb (DOL_v1.0) and 812.32 Mb (DOL_v2.0), respectively corresponding to 99.56% and 93.54% of genome as estimated through initial flow cytometry and k-mer analyzes. Overall, the DOL_v1.0 genome was composed of 4728 scaffolds with an N50 of 42.43 Mb and 5919 contigs with an N50 of 890.84 kb, while the DOL_v2.0 genome consisted of 2812 scaffolds with an N50 of 3.36 Mb and 2986 contigs with an N50 of 2.94 Mb. Hi-C sequencing information was then associated with assembled scaffolds, with LACHESIS being used to cluster these scaffolds into chromosomes such that 799.71 Mb (94.14% of 849.53 Mb) and 721.45 Mb (88.81% of 812.32 Mb) were anchored to 15 pseudochromosomes for the DOL_v1.0 and DOL_v2.0 genomes, respectively (Fig. 6.2). These results indicated that the overall integrity and continuity of both versions of the *D. oleifera* genome were better than those for the reported *D. lotus* genome, which exhibited a final size of 945.63 Mb with contigs N50 of 0.65 Mb, of which 746.09 Mb (78.9%) was anchored to 15 pseudochromosomes (Akagi et al. 2019).

The quality of these assembled genomes was estimated by using Burrows-Wheeler Alignment (BWA) to map short reads to the consensus genome, with overall mapping rates of 98.58% (DOL_v1.0) and 98.19% (DOL_v2.0), indicating that the genomic information of assembled genomes contains were comprehensive. The Core Eukaryotic Gene Mapping Approach (CEGMA) and BUSCO approaches were employed to appraise the completeness of the assembled genomes, with the former approach indicating that the DOL_v1.0 and DOL_v2.0 genomes, respectively, covered 191 (77.02%) and 232 (93.55%) of 248 core eukaryotic genes while 89.9% and 89.4% of complete BUSCOs were detectable and 6.9% and 6.6% were absent in these two versions of the genome, consistent with both exhibiting high levels of completeness.

6.3 Genomic Annotation

Repetitive sequences comprising transposable elements (TEs) and tandem repeats within the *D. oleifera* genome were annotated, with different programs having been used for this annotation process for the two different versions of the genome specifically, TEs in the DOL_v2.0 genome were identified using RepeatMasker based on a known repeat library as well as a de novo library of repeats derived from RepeatModeler, RepeatScout, Piler, and LTR FINDER, while Tandem Repeats Finder was used for tandem repeat identification. Overall, this approach indicated that 54.8% of the DOL_v2.0 genome was composed of repetitive sequences, of which 53.03% were TEs. The most common TEs included long terminal repeat (LTR) retro-transposons (46.73%) and DNA TEs (4.17%), with LTRs being further subdivided into Ty3/Gypsy (26.63%) and Ty1/Copia (14.40%). In contrast, repetitive sequences were found to account for 64.96% of the DOL_v1.0 genome, of which LTRs were the most common (45.31%), with 24.98% and 19.51% of these LTRs being, respectively, identified as Ty3/Gypsy and Ty1/Copia.

Annotations of protein-coding genes within the *D. oleifera* genome were made through

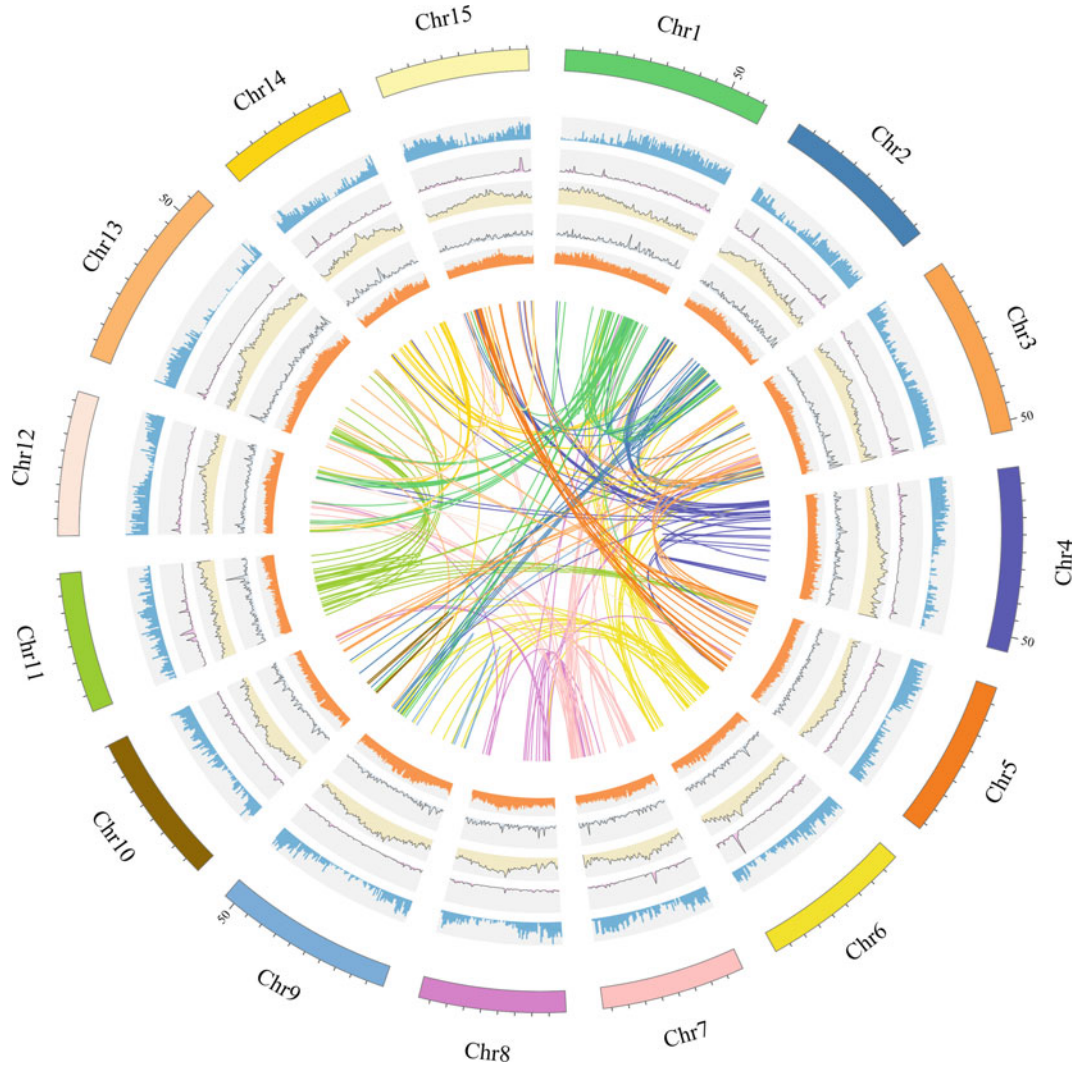

Fig. 6.2 Features of the *D. oleifera* genome. The outermost tracks correspond to gene density distributions, while the next outermost tracks correspond to LINE retrotransposon density, the next track corresponds to DNA transposon density, the next to GC density, and the innermost track corresponds to syntenic blocks. (citation from Suo et al. 2020)

homolog-based, de novo, and RNA-seq predictive analyses using the Augustus, Genescan, Geneid, GlimmerHMM, and SNAP software packages. Briefly, protein-coding sequences corresponding to 9 species (Table 6.1) were downloaded from the NCBI or Ensembl databases, with TBLASTN being utilized to align homologous sequences within these datasets to the *D. oleifera* genome. Gene models were predicted based upon aligned sequences with Genewise. For RNA-seq data, unique transcript sequences were plotted to the *D. oleifera* genome with Cufflinks and TopHat, or RNA-seq data were assembled with Trinity, and PASA was implemented to ameliorate gene structures. The models of all genes estimated employing the three pathways listed above were merged with EVM to generate a non-redundant, weighted gene set that was further adjusted with PASA. In total, 32,516 predicted genes were identified in

Table 6.1 Annotation of two versions of the *D. oleifera* genome

Method	Gene set	Gene number	
		DOL_v1.0	DOL_v2.0
De novo	Augustus	47,353	40,622
	GlimmerHMM	69,582	79,952
	SNAP	45,643	83,029
	Geneid	72,918	82,410
	Genscan	38,463	54,764
Homolog	*Actinidia chinensis*	/	53,571
	Arabidopsis thaliana	24,266	41,064
	Camellia sinensis	32,561	17,857
	Daucus carota	/	19,497
	Primula veris	/	22,892
	Rhododendron delavayi	/	32,588
	Solanum lycopersicum	/	34,666
	Oryza sativa	23,371	/
	Ziziphus jujuba	23,660	/
RNAseq	PASA	40,638	99,230
	Cufflinks	/	76,536
	TransDecoder	86,842	/
	GeneMarkS-T	48,654	/
EVM		32,516	44,212
Pasa-update		/	43,917
Final set		32,516	30,530

the DOL_v1.0 genome with an average gene length of 6773 bp, of which 80.53% exhibited homology with known genes (Zhu et al. 2019). Similarly, the DOL_v2.0 genome comprised 30,530 predicted protein-coding genes with an average transcript size 7105.4 bp, an average coding sequence length of 1080.74 bp, and an average of 4.62 exons per gene (Suo et al. 2020). Both of these genomes coded for fewer predicted annotated genes than those identified within the *D. lotus* genome (40,532 genes) (Akagi et al. 2019).

The SwissProt, NCBI non-redundant (NR), and TrEMBL protein databanks were subsequently utilized for the annotation of the putative protein-coding genes within the assembled *D. oleifera* genomes via a BLASTP search, with the best hit being used for annotation. In addition, InterProScan was used to search the PRINTS, Pfam, ProDom, PROSITE, and SMART InterPro databanks for motifs and domains that were subsequently annotated, while Gene Ontology terms corresponding to individual genes were identified and annotated with Blast2GO, and gene classification was further facilitated by mapping them to KEGG pathways. Genes included within the DOL_v1.0 genome were further mapped to the COG, KOU, TrEMBL, and NT databases. Overall, functional annotation was achieved for 31,198 genes (95.95% of 32,516 genes) in the DOL_v1.0 genome, although just 25,379 (78.05%) of these genes were successfully anchored to the 15 pseudochromosomes. The self-alignment of these anchored genes further led to the identification of 3612 paralogous gene groups and 137 gene syntenic blocks, suggesting the instance of frequent interchromosomal fusion and segmental duplication events over

Table 6.2 *D. oleifera* genome annotation statistics derived from various databanks

Database	DOL_v1.0		DOL_v2.0	
	Number	Percent (%)	Number	Percent (%)
Swissprot	21,317	65.56	22,135	72.5
NR	30,292	93.16	28,098	92.03
KEGG	10,271	31.59	21,739	71.21
GO	16,976	52.21	20,826	68.21
Pfam	25,804	79.36	22,172	72.62
COG	13,716	42.18	/	/
KOG	17,212	52.93	/	/
TrEMBL	29,941	92.08	/	/
NT	29,103	89.5	/	/
Annotated	31,198	95.95	28,580	93.61

the course of the evolution of the persimmon genome. For the DOL_v2.0 genome, functional motifs or annotations were attributed to 28,580 protein-coding genes (93.61% of 30,530 genes) (Table 6.2).

The tRNAscan-SE software was utilized to predict tRNAs using default parameters, while rRNAs annotation was achieved using BLASTN based on homology with rRNAs from several other species. INFERNAL was used to identify snRNA and miRNA fragments through a search of the Rfam database. Overall, this analysis led to the identification of 107 miRNAs, 600 tRNAs, and 833 rRNAs in the DOL_v1.0 genome, as well as 564 miRNAs, 507 tRNAs, 2207 rRNAs, and 803 snRNAs in the DOL_v2.0 genome, with average respective lengths of 114.69, 74.82, 161.40, and 111.54 bp in the latter genome (Table 6.3).

Tannins (PAs) are important compounds characteristic of persimmon species, and the biosynthesis of these tannins has previously been linked to the phenylpropane metabolic, chorismic acid, flavonoid-anthocyanin, and proanthocyanidin-specific pathways. Through the alignment of reference genes from NCBI or *Arabidopsis* resources, synthase genes associated with these and closely related pathways were identified with BLASTP, as were associated transcription factors (TFs) such as WD40 and WIP-ZF. Overall, 57 PA biosynthesis-related genes were identified in the DOL_v1.0 genome, with 33.96% of these genes being encoded on chromosome 1 in a gene cluster enriched for these genes. Moreover, 171 genes and 380 TFs of interest were identified in the DOL_v2.0 genome, with 13, 59, and 21 of these genes being, respectively, associated with the phenylpropane metabolic, flavonoid-anthocyanin, and proanthocyanidin-specific pathways. Additionally, 18 transporters closely associated with tannin transmembrane transport, including glutathione S-transferase (GST) and multidrug toxic compound extrusion transporter (MATE), were identified. Certain key acetaldehyde metabolism genes related to persimmon deastringency were also identified, including

Table 6.3 Noncoding RNA statistics in assembles *D. oleifera* genomes

RNA classification	DOL_v1.0	DOL_v2.0	
	Number	Number	Average length (bp)
miRNA	107	564	114.69
tRNA	600	507	74.83
rRNA	833	2207	161.4
snRNA	/	803	111.54

ADH (10), ALDH (19), and PDC (5). These efforts to identify genes and TFs related to tannin biosynthesis will present a foundation for subsequent molecular breeding efforts aimed at modifying persimmon tannin content.

6.4 Comparative Genomic and Evolutionary Analyses

Gene family cluster analyses of the overall DOL_v1.0 (*D. oleifera*), Arabidopsis (*A. thaliana*), apple (*M. domestica*), and grape (*V. vinifera*) gene sets were conducted, revealing the presence of 25,199 gene families within the *D. oleifera* genome that were subdivided into 13,406 gene clusters, with 7567 of these clusters being divided through all four analyzed entities. For the DOL_v2.0 genome, an evolutionary analysis was conducted based on the sequences of *D. oleifera* and 11 plant species comprising 8 asterids (*D. lotus*, *Coffea canephora*, *Primula veris*, *Daucus carota*, *Camellia sinensis*, *Rhododendron delavayi*, *Solanum lycopersicum,* and *Actinidia chinensis*) and 3 rosids (*A. thaliana*, *Cucumis melo,* and *V. vinifera*). These clustering analyzes led to the identification of 19,722 gene families in *D. oleifera* and these other 11 species, with all 12 species sharing 221 single-copy orthologs and 5599 gene families. Among the 5 analyzed Ericales species (*D. oleifera*, *D. lotus*, *R. delavayi*, *A. chinensis*, and *C. sinensis*), 177 gene families composed of 312 genes were unique to *D. oleifera*. GO enrichment analyses of these genes suggested that 98 were enriched in GO terms including proteolysis, zinc ion binding, and nutrient reservoir activity. Moreover, 4 and 1 of these genes were respectively associated with carbohydrate and aldehyde metabolic processes, indicating that they can perform tasks in deastringency and carbohydrate accumulation within *D. oleifera* fruits.

Phylogenetic trees were further generated for the DOL_v1.0 and DOL_v2.0 genomes that incorporated 13 and 11 other sequenced plant species, respectively, with these trees being based upon single-copy genes. The DOL_v1.0 tree indicated a close relationship between *D. oleifera*, *A. chinensis* (kiwifruit), and *S. lycopersicum* (tomato), with *D. oleifera* having diverged from *A. chinensis* ~77.80 million years ago (Mya), following the divergence from *S. lycopersicum* at 104.47 Mya. In contrast, the DOL_v2.0 phylogenetic tree suggested *D. oleifera* and *D. lotus* to be most closely related to *C. sinensis* (tea tree), followed by *A. chinensis* (kiwifruit), with all of these species belonging to Ericales. while *S. lycopersicum* was divided into the lamiids branch that is more distantly related to *D. oleifera*. The split between *D. oleifera* and *D. lotus* was evaluated to have occurred ~9.0 Mya (Fig. 6.3).

Gene family expansion and contraction were assessed through comparisons of differences in cluster sizes between ancestors and individual species as conducted with the CAF´E program. In total, 328 and 493 gene families within the DOL_v1.0 genome respectively exhibited evidence of expansion and contraction after divergence from kiwifruit. KEGG analyzes revealed a majority of the expanded genes to be associated with processes including starch/sucrose metabolism, phenylpropanoid biosynthesis, and plant-pathogen interactions. In contrast, contracted gene families were associated with KEGG pathways such as phytohormone signal transduction, triterpenoid and sesquiterpenoid biosynthesis, and beta-alanine metabolism. Relative to the last common ancestor shared by *D. lotus* and *D. oleifera,* 175 gene families (1896 genes) and 333 gene families (1021 genes) were found to have undergone expansion and contraction, respectively, in the DOL_v2.0 genome. The majority of the expanded genes were associated with the pathways of KEGG comprising ABC transporters, ubiquitin-mediated proteolysis, and carbon fixation in photosynthetic organisms, while contracted genes were enriched in KEGG pathways comprising plant-pathogen interactions, cyanoamino acid metabolism, and phenylpropanoid biosynthesis. Overall, the functional annotation of these expanded and contracted gene families suggests that these evolutionary shifts are related to specific persimmon traits including adaptability, disease resistance, and high levels of sugar and flavonoid content.

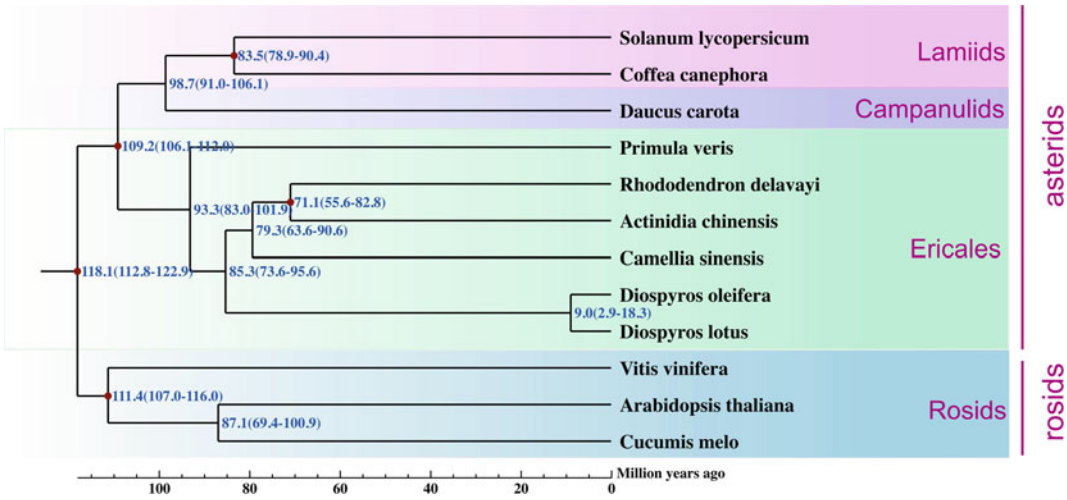

Fig. 6.3 The phylogenetic associations and the estimated timing of divergence between *D. oleifera* (DOL_v2.0) and other plant entities. Numbers outside of brackets correspond to the estimated time of divergence, with confidence ranges being noted in parentheses. (from Suo et al. 2020)

Tannin synthase gene sequences in the DOL_v2.0 genome were additionally used to conduct BLAST analyses in an effort to detect homologous genes encoded by *D. lotus*, *C. sinensis*, and *V. vinifera*. Relative to these three other species, chalcone synthase genes (*CHS*) had undergone expansion within the *D. oleifera* genome (11 genes in *D. oleifera*, 7 in *D. lotus*, 3 in *C. sinensis*, and 1 in *V. vinifera*). Given the key role of CHS during the initial stages of the flavonoid-anthocyanin pathway, such CHS gene expansion may partially explain the high levels of tanning production observed in *D. oleifera*. Conversely, laccase genes (*LAC*), which mediate persimmon tannin monomer polymerization (Zhao et al. 2010; Hu et al. 2013) were found to have undergone contraction in *D. oleifera* relative to *V. vinifera* (21 vs. 53).

In an effort to explore the evolutionary history of *D. oleifera,* adaptive gene evolution was examined through a positive selection analysis. To that end, MUSCLE was used to conduct the coding sequence (CDS) alignment of 789 single-copy gene families in *D. oleifera* (DOL_v2.0), *D. lotus*, *A. chinensis*, *P. veris*, *S. lycopersicum,* and *R. delavayi*, revealing 186 genes to have undergone positive selection in *D. oleifera*. Included among these positively selected genes was the key flavonoid-anthocyanin pathway enzyme chalcone isomerase (*CHI*) gene (ID:evm.model.original scaffold 909.101), suggesting that the positive selection of this gene can partially account for the differences in tannin production between *D. oleifera* and closely related species.

Instances of potential whole-genome duplication (WGD) or species split events were next identified through an analysis of transversion substitutions at four-fold degenerate sites (4dtv) rates for all syntenic genes. This analysis of the DOL_v2.0 genome suggested that, besides the ancient γ event (divided by all core eudicots, 4dtv = 0.66), a second WGD event took place in *D. lotus* and *D. oleifera* (4dtv = 0.33/0.36), potentially contributing to the divergence of Ebenaceae from *C. sinensis* and *A. chinensis* (Fig. 6.4). These findings align with prior studies (Akagi et al. 2019). In contrast, a corresponding analysis of the DOL_v1.0 genome only revealed a peak 4DTv value at ∼ 0.65 for *D. oleifera*, suggesting that this lineage had only been subject to the ancient γ event.

In total, 431 syntenic blocks were identified through a microsynteny analysis of the *D. oleifera* (DOL_v2.0) and *D. lotus* genomes. In general, a high degree of conservation was observed between these two genomes with the exception of

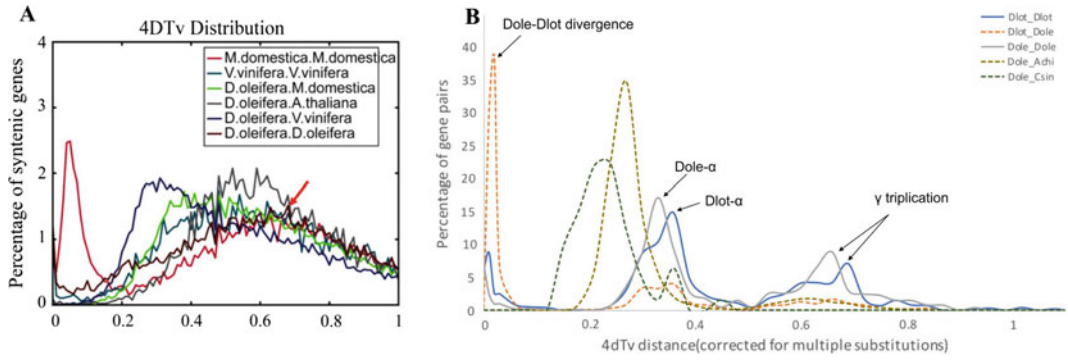

Fig. 6.4 Whole-genome duplication analysis of *D. oleifera* genome. A, DOL_v1.0. B, DOL_v2.0. (from Zhu et al. 2019; Suo et al. 2020)

certain translocations, and the *D. lotus* genome was also found to lack certain regions associated with each chromosome, likely owing to the loss of these sequences during contig anchoring to the 15 different pseudochromosomes. Overall, these findings further support the accuracy and integrity of the assembled *D. oleifera* genome assembly.

6.5 Conclusions

Here, the assembly and annotation of two high-quality *D. oleifera* draft genomes (DOL_v1.0 and DOL_v2.0) were discussed at the chromosome level. These two genomes were 849.53 Mb and 812.3 Mb in size with respective N50 scaffolds lengths of 1.42 Mb and 3.36 Mb. After combining Hi-C outcomes with the assembled draft genomes for the generation of the chromosome-length scaffolds, 94.14% (799.71 Mb) and 88.81% (721.5 Mb) of the DOL_v1.0 and DOL_v2.0 genomes, respectively, were successfully anchored to 15 pseudochromosomes. These two genomes were respectively predicted to encode 32,516 and 30,530 protein-coding genes, with average respective gene lengths of 6773.92 bp and 7105.40 bp, and functional annotations being available for 95.95% and 93.61% of these genes, respectively. The DOL_v1.0 and DOL_v2.0 genomes were found to respectively be composed of 64.96% and 54.8% repeat sequences. The DOL_v1.0 genome was predicted to encode 57 genes related to PA biosynthesis, with 33.96% of these being encoded on chromosome 1, whereas DOL_v2.0 was found to encode 171 candidate genes associated with the synthesis of tannin and deastringency, among which the CHS genes were found to have undergone expansion within the *D. oleifera* genome in comparison to the *C. sinensis, D. lotus, and V. vinifera* genomes. In total, 186 positively chosen genes were detected within the DOL_v2.0 genome, with the key flavonoid-anthocyanin pathway gene *CHI* being one such gene. While 4DTv analysis indicated that only a single γ WGD event was evident in the DOL_V1.0 genome, the DOL_v2.0 and *D. lotus* genomes exhibited evidence of two WGD events (γ event and a second WGD event [4dtv = 0.36 ~0.27–0.42]). Moreover, phylogenetic analyses suggested the split between *D. lotus* and *D. oleifera* to have possibly took place ~9.0 million years ago. While similar, the results of these two different genomic assembly and annotation efforts are not identical, enabling researchers to select the assembly best suited to their needs. Overall, the assembly of a premium quality chromosome-level *D. oleifera* genome will help guide further research exploring key agronomic traits of *Diospyros* species and genome assembly efforts for hexaploidy persimmon species.

References

Akagi T, Shirasawa K, Nagasaki H et al (2019) The persimmon genome reveals clues to the evolution of a lineage-specific sex determination system in plants. bioRxiv 2019:628537

Fu JM, Liu HM, Hu JJ et al (2016) Five complete chloroplast genome sequences from Diospyros: genome organization and comparative analysis. PLoS ONE 11(7):e0159566

Hu QN, Luo C, Zhang QL et al (2013) Isolation and characterization of a laccase gene potentially involved in proanthocyanidin polymerization in oriental persimmon (Diospyros kaki Thunb.) fruit. Mol Biol Rep 40(4):2809–2820

Suo Y, Sun P, Cheng H et al (2020) A high-quality chromosomal genome assembly of Diospyros oleiferaCheng. GigaScience 9(1)

Zhao J, Pang Y, Dixon RA (2010) The mysteries of proanthocyanidin transport and polymerization. Plant Physiol 153(2):437–443

Zhu QG, Xu Y, Yang Y et al (2019) The persimmon (Diospyros oleifera Cheng) genome provides new insights into the inheritance of astringency and ancestral evolution. Hortic Res 6:138

Chloroplast Genome of Diospyros Species

7

Jianmin Fu

Abstract

At present, the genetic relationships among individual species within the *Diospyros* genus remain poorly defined, as do the progenitor, origins, and mechanisms of polyploidization that gave rise to hexaploidy *D. kaki* persimmons. Chloroplast (cp) genomes offer significant value in evolutionary and taxonomic studies. Here, cp genome assembly and corresponding annotation for five *Diospyros* species (*D. kaki*, *D. glaucifolia*, *D. oleifera*, *D. lotus*, and *D.* 'Jinzaoshi') were conducted. The range of cp genomes is from 157,300 to 157,784 bp in length and exhibited a standard quadripartite structure composed of one big and one tiny single-copy region. In total, 134 genes were annotated for each of these cp genomes, comprising 80 protein-coding genes, 31 tRNAs, and 4 rRNAs. Moreover, four hypervariable regions were detected within these cp genomes, including the intergenic *trnQ_rps16*, *trnV_ndhC*, and *psbD_trnT* regions as well as an intronic region within the *ndhA* gene. Through phylogenetic analyses of the full-length cp genome sequences, intergenic and intronic sequences, and protein-coding sequences, *D. oleifera* was found to be closely related to *D. kaki,* demonstrating that it can offer value as a model for studies of cultivated persimmon. Moreover, two large (301 bp and 140 bp) deletions were detected within the *D.* 'Jinzaoshi' cp genome, consistent with its status as a new species within the *Diospyros* genus. Phylogenetic analyses of 19 taxa reaffirmed the basal position of Ericales within the Asterids clade, in addition to supporting Ebenaceae as being monophyletic in Ericales.

7.1 Introduction

Diospyros is the biggest genus in the Ebenaceae family, consisting of over 500 species, many of which are highly economically valued. Owing to the presence of both natural and artificial interspecific hybrid species, complex chromosome numbers ($2n = 2\times, 4\times, 6\times, 9\times = 30, 60, 90, 135$), and a high degree of morphological similarity among species, classifying individual *Diospyros* species remains challenging. The major part of cultivated persimmon (*D. kaki*) cultivars are hexaploid, with a limited number being nonaploid. However, the progenitors, origins, and mechanisms of polyploidization that have given rise to these *D. kaki* cultivars remain poorly understood, hampering persimmon molecular breeding efforts. Related species with a clearly defined genetic background generally

J. Fu (✉)
Non-Timber Forest Research Institude, Chinese Academy of Forestry, No. 3 Weiwu Road, Jinshui District, Zhengzhou 450003, China
e-mail: fjm371@163.com

R. Tao and Z. Luo (eds.), *The Persimmon Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-05584-3_7

serve as both invaluable breeding parent sources and as tools for studies focused on gaining insight into the origins of persimmon cultivation. Prior studies have attempted to establish phylogenetic relationships among *Diospyros* species based upon morphological characteristics (Venkatasamy et al. 2006) or molecular markers (Hu et al. 2008; Yonemori et al. 2008). However, the results of these analyses can vary depending on the markers used, likely owing to differences in sequence diverse ratios and the methods of tree propagation employed. The general findings of these prior analyses suggest that the diploid *D. oleifera*, *D. glaucifolia*, *D. lotus*, and *D.* 'Jinzaoshi' species are all relevant to *D. kaki* (Du et al. 2009; Guo and Luo 2011). While *D.* 'Jinzaoshi' has generally been regarded as a *D. kaki* cultivar, recent morphological analyses and the assessment of its matK and internal transcripted sequence (ITS) regions have suggested that it may instead represent a new species (Tang et al. 2014). Genetic relationships among *Diospyros* species remain unclear, necessitating the use of more precise tools to clarify these associations and to identify a diploid species strongly relevant to *D. kaki* that can be employed as a model in further studies of cultivated persimmons.

Chloroplasts (cp) are vital photosynthetic organs and organelles within plant cells that exhibit a long evolutionary history. Moreover, cp genome sequences and structural characteristics can offer insight into the origins, evolution, and genetic relationships among plant species. At a high level, the cp genome consists of a conserved quadripartite configuration with two identical inverted repeat (IR) areas that separate a single small single-copy region (SSC) and a single large single-copy areas (LSC) (Jansen et al. 2005). In angiosperms, cp genomes generally exhibit a great degree of conservation with respect to the genes encoded therein and the order of these genes, with 110–130 protein-coding, rRNA, and tRNA genes typically being present within these genomic sequences (Chumley et al. 2006). However, several large genomic rearrangements including both gene loss and gene gain have occurred among monocot and dicot species, introducing further interspecific diversity (Guisinger et al.2011).

Owing to their conserved structural and genetic composition, small size, and maternal patterns of inheritance, cp genomes are valuable tools for use in taxonomic and evolutionary studies (Parks et al. 2009). More detailed cp genome analyzes are essential to permit accurate phylogenetic analyses of angiosperm species. Moreover, these cp genomes could be used for DNA barcoding, genetic transformation, and the improvement of agricultural traits (Fazekas et al. 2008). Indeed, as it is associated with better gene containment and higher transgene expression levels, cp genome transformation is greater than nuclear transformation (Daniell et al. 2004).

In this chapter, the genetic and phylogenetic relationships among persimmon species within the Ericales branch were analyzed through the sequencing and comparative analysis of cp sequences from five *Diospyros* species (*D. kaki*, *D. lotus*, *D. glaucifolia*, *D.* 'Jinzaoshi', and *D. oleifera*).

7.2 Genome Sequencing, Assembly, and Validation

To facilitate cp genome sequencing, young healthy leaves were procured from mature trees of the five target persimmon species cultivated in the Persimmon Germplasm Bank (Yuanyang County, Henan Province, China; 34°55.30′–34°56.45′ N, 113°46.24′–113°47.59 E). In total, a DNeasy Plant Mini Kit (Qiagen, CA, USA) was utilized for the extraction of DNA from 50 g of fresh leaf tissue. Purified DNA was then subjected to random fragmentation to facilitate paired-end (PE) library construction, with Illumina Hiseq and Miseq platforms subsequently being used for library sequencing. Raw sequencing data with quality scores <20 were then filtered out, with clean PE reads subsequently being overlapped with FLASH and aligned to the database of cp with Burrows–Wheeler Aligner (BWA) computer program. The assembling of reads into contigs was executed employing Celera Assembler, and these contigs were in turn assembled with SSPACE into scaffolds. LASTZ was then used for mapping

assembly generation, with *Camellia yunnanensis* (NC_013707) serving as a reference sequence. Fill in the gaps with GapFiller for acquiring the complete genome. Whole cp genome sequence validation was conducted using 101 primer pairs that had been designed based upon these assembly results, with four of these pairs covering the junctions between SC and IR regions. Sanger sequencing of the resultant PCR products was then conducted, and the resultant sequences were aligned to *Diospyros* cp genomes.

The Illumina Hiseq and Miseq platforms were employed for sequencing these five cp genomes, yielding 477–1150 Mb high-quality shor

t reads, respectively. Reference genome alignment was successfully achieved for 18.2–58.9 Mb of the generated data, with an overall sequencing depth of 116–376 × (Table 7.1). Of these five species, the *D. kaki* cp genome sequence was the largest (157,784), followed by that of *D. oleifera* (157,760), *D. glaucifolia* (157,610), and *D. lotus* (157,597), with *D.* 'Jinzaoshi' exhibiting the smallest cp genome (157,300). These five *Diospyros* cp genomes were similarly sized to those of other angiosperm species (Yang et al. 2010). The AT content within *Diospyros* cp genomes was greater than the GC content (63% vs. 37%), consistent with prior sequencing data from asterid plastid genomes (Yi and Kim 2012).

7.3 Gene Annotation and Repeat Identification

The Dual Organellar GenoMe Annotator (DOGMA) tool was used to annotate the cp gene, followed by manual correction based upon corresponding annotations published in online databases. OGDraw was used to generate a circular distribution map. REPuter was implemented to analyze the forward, reverse, palindromic, and complement repeats. MISA was used to detect microsatellites, which were defined as 1/10, 2/6, 3/5, 4/4, 5/3, and 6/3 (unit size/minimum number of repeats). A high degree of conservation was observed when comparing *Diospyros* cp genomes to those of other angiosperms in relation to the structure of genomes, gene content, and gene order. As expected, these *Diospyros* cp genomes exhibited a traditional quadripartite structure comprised of two IRs (26,079–26,119 bp) separated through the regions of SSC (18,076–18,532 bp) and LSC (86,948–87,059 bp) (Fig. 7.1; Table 7.1). All five sequences *Diospyros* cp genomes were comparable regarding the gene content and order. In total, predictive analyzes identified 134 genes encoded within these genomes, of which 115 were unique (comprising 80 protein-coding, 31 rRNA, and 4 rRNA genes), and 19 were duplicated genes within the regions of IR. Trans-splicing of the *rps*12 gene was observed such that its 3' and 5' ends were placed in the IR and LSC regions, respectively, in line with reported cp genome sequences from *Actinidia chinensis* (Yao et al. 2015). Several non-canonical start codons were also detected, including GTG and ACG in the *rps*19 and *ndhD* genes, accordingly. Moreover, *ycf*1 was discovered to form a pseudogene at the SSC-IRb boundary, resulting in its expansion in the corresponding position within IRb as has been reported for the plastid genomes of other angiosperms (Yang et al. 2010). 58% and 42% of the sequences *Diospyros* cp genomes

Table 7.1 Statistics corresponding to the sequencing and annotation of cp genomes from five *Diospyros* species

Sample	Clean data (M)	Matched data (M)	Genome size (bp)	Coverage (×)	LSC length (bp)	SSC length (bp)	IR length (bp)	Accession no
D. kaki	968	58.9	157,784	376	87,059	18,505	26,110	KT223565
D. lotus	477	31.2	157,597	199	86,948	18,411	26,119	KM522849
D. 'Jinzaoshi'	577	18.2	157,300	116	87,010	18,076	26,107	KM522848
D. oleifera	1000	49.7	157,760	317	87,034	18,532	26,097	KM522850
D. glaucifolia	1150	49.3	157,610	314	86,965	18,407	26,119	KM504956

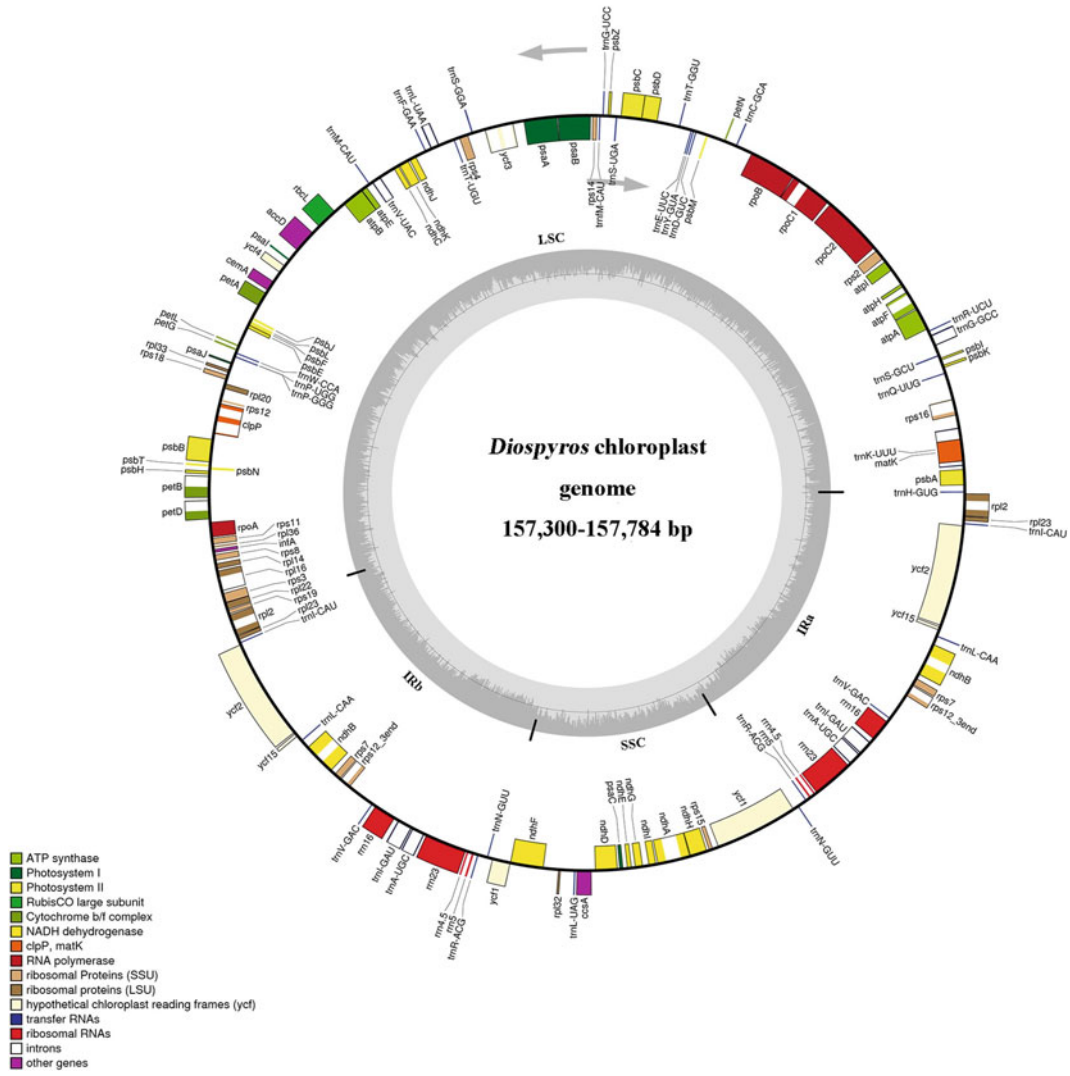

Fig. 7.1 *Diospyros* chloroplast genome gene maps

corresponded to coding and non-coding regions. Conclusively, although there is frequent large-scale genome rearrangement, gene loss or acquisition events in some lineages of land plants, the chloroplast genome of Persimmon is highly conserved, and its gene composition, gene sequence, and genome structure characteristics are highly consistent among different species.

Genes on the outside and inside of the larger circle are, respectively, transcripted in a clockwise and counterclockwise manner. All genes are color-coded based upon the related function. Dashed regions correspond to GC content within the chloroplast genome. (Citation from Fu et al. 2016).

7.4 Analysis of Codon Usage in the Chloroplast Protein-Coding Genes of *Diospyros* Spp.

Codons play an important role in the transmission of genetic information, and accurate identification of codons encoding different amino acids

is the key to ensure the correct expression of genetic information. Multiple codons encoding the same amino acid are called synonymous codons. Codon usage varies between different species or between different genes of the same species, that is, some codons are used more frequently than other synonymous codons, which are called codon usage bias. Using chloroplast genome as the receptor for transgene can avoid the safety problems caused by pollen escape in nuclear transgene systems. Codon usage bias can affect the expression level of foreign genes in the host. Analyzing the sequence characteristics of chloroplast genome and codon usage bias to screen out suitable codons is of great significance to improve the efficiency of chloroplast genome genetic transformation.

Based on the five *Diospyros* cp genome information, CDS sequences less than 300 bp in length were removed. Finally, 55 CDS sequences of each species were used for analysis, and the stop codons that did not encode any amino acids in each CDS sequence were removed. CodonW and CUSP were used to analyze the codon usage patterns of chloroplast coding genes of each species. The results showed that the GC content of the third codon base in five *Diospyros* cp genome ranged from 26.9% to 27.8%, and the effective codon number (NEC value) ranged from 49.58 to 50.18, showing weak codon preference (Table 7.2). The NEC values of codon in different positions of cp genome were different, ranging from 32.36 to 59.64. The results of neutral plot analysis and ENC-plot plot analysis showed that the codon usage bias of *Diospyros* cp genome was mainly affected by selection. By combining high-frequency codon analysis with high-expression superior codon analysis, 23 optimal codons were screened out, and these codons mainly ended with A and U bases.

REPuter was employed for the detection of forward, reverse, complementary, and palindromic repeats (>20 bp, >90% similarity), revealing 179 such repeats across the five sequenced cp genomes of which 100 were divided across all genomes, while 2, 7, 5, and 4 were detected only within the *D. glaucifolia, D.* 'Jinzaoshi', *D. oleifera*, and *D. kaki* cp genomes, accordingly. The most common repeats were palindromic (49%), followed by forward (40%) and reverse (10%) repeats. Just a single 20 bp complementary repeat was identified within the region of LSC in the *D.* 'Jinzaoshi' cp genome. A majority of these repeats were present within non-coding regions, with only a subset being present in the coding regions of the *ndhH, ycf2*, *trnS-GCU, ndhC, trnfM-CAU, trnS-UGA, trnS-GGA, trnV-UAC, trnA-UGC,* and *trnP-GGG* genes. Moreover, 62, 55, 61, 52, and 53 single sequence repeat (SSR) loci were, respectively, discovered in the *D. glaucifolia, D. oleifera, D. lotus, D.*'Jinzaoshi' and *D. kaki* cp genomes. With respect to mononucleotide repeats, 278 were stretches of A/T repeats whereas just a single G stretch and a single C stretch were respectively identified in the *D. glaucifolia* and *D. locus* cp genomes. Three tetranucleotide repeats (AAAT) were detected within the *D. oleifera, D.* 'Jinzaoshi', and *D. kaki* cp genomes, while no hexa-, penta-, tri-, or dinucleotide repeats were observed. Most of the mononucleotide repeats were A/T stretches, which account for the abundant A/T content in the Diospyros cp genome, indicating that most cp

Table 7.2 The third base of codons in chloroplastic genes of *Diospyros* spp

Species	T_{3s} /%	C_{3s} /%	A_{3s} /%	G_{3s} /%	GC /%	GC_{3s} /%	N_{EC}
D. kaki	46.79	17.15	43.32	18.17	37.8	26.9	49.65
D. oleifera	45.23	17.88	43.34	18.51	37.9	27.8	50.18
D. lotus	46.77	17.22	43.33	18.15	37.8	26.9	49.58
D. jinzaoshi	45.23	17.88	43.34	18.51	37.9	27.8	50.18
D. glaucifolia	46.70	17.29	43.37	18.19	37.8	27.0	49.65

SSRs were short polyadenine (polyA) or polythymine (polyT) repeat. Of the identified SSRs, 25, 49, and 209 were located in the IR, SSC, and LSC regions, with 67% being located within intergenic regions, suggesting a high degree of variation within these regions. *Diospyros* cp microsatellites may offer value in further evolutionary and ecological research.

7.5 Comparison of Whole Chloroplast Genomes Sequences Among Ericales

mVISTA was used for the global alignment of *D. kaki* and other published Ericales family members (*C. yunnanensis, A. deliciosa, A. polysticta, Vaccinium macrocarpon*) (Fig. 7.2). The *V. macrocarpon* cp genome in Ericaceae differed significantly from that of *D. kak*i, likely owing to the presence of several structural rearrangements. A higher degree of conservation was observed for IR regions as compared to SCs, probably because of copy correction by gene conversion when mutations are introduced into IRs. A higher degree of variability among species was observed in non-coding sequences relative to coding sequences. High levels of variation were detected in the *atpI_atpH, trnQ_rps16, ndhF_rpl32, psbJ_petA, trnV_ndhC, rpl32_trnL, and psbD_trnT* intergenic regions, which were located within 13 hotspot regions that have been reported in several plastid genomes (Shaw et al. 2007). These regions may offer value for further development as interspecific DNA markers for the phylogenetic analysis in Ericales.

7.6 Indel Identification and Relationships Among *Diospyros* cp Genomes

Clustalw was used to perform multiple sequence alignment, with a maximum parsimony (MP) tree being fabricated with PAUP* employing the heuristics investigation and tree-bisection-reconnection (TBR) settings for branch-swapping. RAxML was used for ML reconstructions utilizing the best corresponding model (Stamatakis 2014). Bootstrap analyses with 1000 replicates were used for both ML and MP trees. The construction of these ML and MP trees for the five *Diospyros* species was based upon the full sequences, intergenic/intronic sequences, and protein-coding sequences of the analyzed cp genomes. The results indicated that *D. kaki* was more strongly relevant to *D. oleifera*, while *D. lotus* was more strongly relevant to *D. glaucifolia* (Fig. 7.3). This finding aligns well with prior reports based upon SSR and ITS regions (Liang et al. 2015; Fu et al. 2015), and with morphology-based taxonomic reports given that the flowers and fruits of *D. kaki* are morphologically similar to those of *D. oleifera*.

Insertions and deletions (Indels) offer value when studying evolutionary history, offering insight regarding the origins of domesticated species (Wills and Burke 2006), patterns of biogeographic movement (Ickert-Bond and Wen 2006), and the complex relationships that exist among different species (Shaw and Small 2005). Multiple sequence alignments for these five highly conserved *Diospyros* cp genomes were thus used to identify indels (>5 bp) as a means of analyzing interspecific variation. Generally, 66 indel loci were discovered through this analysis (Fig. 7.4), of which five were located within the *trnQ_rps16* intergenic region, consistent with it being the most changing region, followed by the *ndhAintron* (4), *trnV_ndhC* (4), and *psbD_trnT* (3) regions. The biggest indels were 301 bp and 140 bp deletions located in *rpl32_trnL* and *trnQ_rps16*, respectively, in the *D.* 'Jinzaoshi' cp genome, potentially having arisen through slipped-strand mispairing or illegitimate recombination events. A major part of the identified indels was placed within intergenic LSC and SSC regions, in line with prior reports suggesting IR regions to be more conserved compared with the regions of SC (Kim et al. 2009). The identified hotspots may offer value as tools when analyzing the molecular phylogeny of different species *of Diospyros*. The two great deletions in the D. 'Jinzaoshi' cp genome, together with the generated phylogenetic tree, provide its identity as a novel species, consistent with prior reports

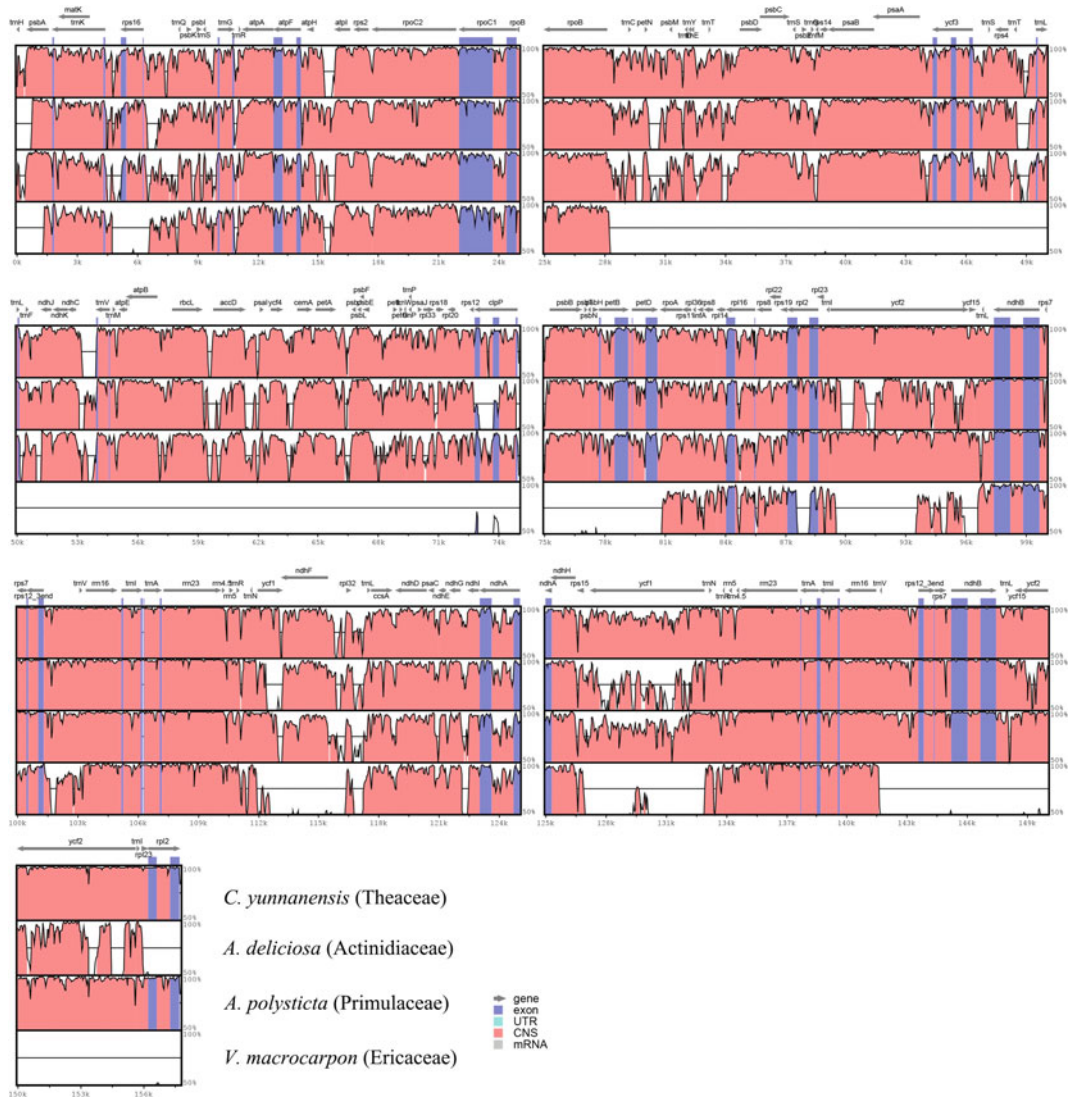

Fig. 7.2 Global alignment of Ebenaceae and other released Ericales cp genomes. The Y-axis corresponds to the variety of identity (50–100%), and *D. kaki* served as a reference. (Citation from Fu et al. 2016)

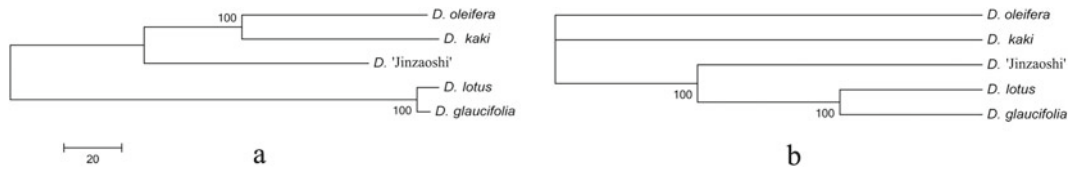

Fig. 7.3 Phylogenetic trees on the basis of *Diospyros* whole-genome sequences. **a** Maximum likelihood tree, **b** The tree of maximum parsimony. (Citation from Fu et al. 2016)

based upon morphological findings and ITS/*matK* sequence analyzes (Tang et al. 2014).

7.7 Exploitation of cpDNA Molecular Markers and Genetic Variation of *Diospyros* spp.

Diospyros species have differentiated many valuable resources during long-term cultivation. At present, there are few molecular markers applied to study the genetic diversity of *Diospyros* plants, especially the markers applied to study the intraspecific genetic variation are not specific enough to distinguish the cultivated persimmon species with various varieties. Therefore, it is urgent to develop specific molecular markers to study and evaluate its germplasm resources and genetic diversity. Based on the cp genome sequences of five *Diospyros* plants, the polymorphic Indel loci with differences of more than 20 bp were selected to develop molecular markers. A total of three suitable Indel loci were screened, which are located in the *ycf4_cemA* and *trnT_trnL* intergenic regions in the LSC region, and in the *rps15* gene in the SSC region. In addition, a sequence of *trnH_psbA* intergenic region in the LSC region was selected to develop cp marker as control. The above four sequences were extracted together with the flanking sequences. For the upstream and downstream of the target fragment, Primer 5.0 was used to design specific primers, and Oligo7.0 was used to evaluate and screen the primers, and the optimal-specific primers were screened (Table 7.3). Then, 16 persimmon materials were used to verify its feasibility in interspecific and intraspecific persimmon species, including 5 closely related species and 11 persimmon varieties from different regions.

Using MEGA software, the sequence length and variation site of PCR products obtained in 16 materials for 4 markers were analyzed. The length of the amplified fragment of CP1 was 409–427 bp, CP3 was the shortest (234–285 bp), and CP4 was the longest (518–571 bp). Since the

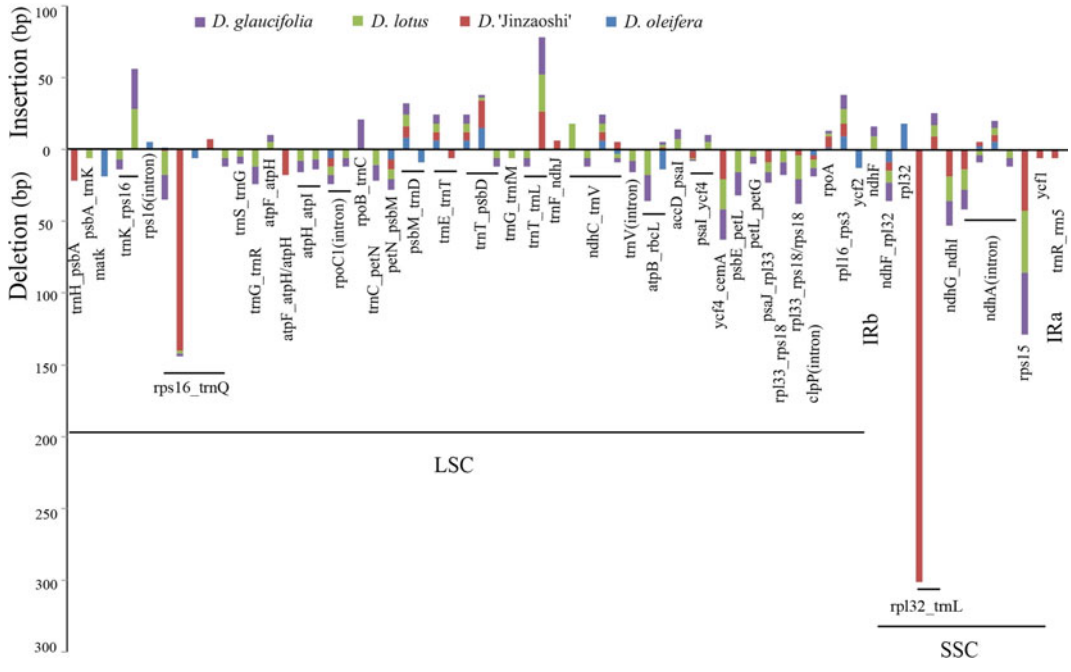

Fig. 7.4 Indel identification based upon multiple sequence alignment of five *Diospyros* cp genomes. deletions and insertions are, respectively, below and above the horizontal axis, and *D. kaki* served as a reference. (Citation from Fu et al. 2016)

CP2, CP3, and CP4 markers were developed based on the Indel locus, after sequencing the amplified fragments, it was found that the a 21 bp sequence was missing in the CP2 amplified fragments of *D.* 'Jinzaoshi', *D. glaucifolia, D. lotus,* and *D. artrotricha,* and a 43 bp sequence was missing in the CP3 amplified fragments. While, in the fragments amplified by CP4 marker, both *D. kaki* and *D. oleifera* lacked a 28 bp sequence. The three missing sequences were compared with the three Indel sequences, and the results were completely consistent, that is, the authenticity of the three Indel loci was verified. In addition, there was no significant difference in the length of CP1 amplified fragments between *D. kaki* and 4 related species. There was also no significant difference in the sequence lengths of the amplified fragments of the 4 molecular markers among the 11 *D. kaki* varieties. For the four cpDNA markers, 31, 27, 7, 25 variable sites were detected, respectively, and the parsimony-informative sites were 14, 5, 2, 13 (Table 7.3).

Cluster analysis of four markers respectively showed that: CP1 marker can completely separate 11 *D. kaki* varieties and five related species, and the distance between *D. kaki* and *D. oleifera* is the closest, indicating that this marker is suitable for the identification of interspecific resources of the *Diospyros* genus, but not within *D. kaki* species. However, CP2, CP3, and CP4 markers could not completely distinguish *D. kaki* varieties from their related species, but multiple mutation, insertion, and deletion sites were detected among 11 *D. kaki* varieties, and most of the *D. kaki* varieties had different mutation sites, which could be considered for studying genetic variation within *D. kaki* species.

7.8 IR Analyzes

The regions of IR perform a fundamental task in plastid genome structural stabilization (Maréchal and Brisson 2010). While these regions of IR are considerably conserved, their contraction and expansion are common evolutionary procedures that account for the majority of length variability among plastid genomes (Wang et al. 2008). Comparative analyses of IR/SSC and IR/LSC boundary positions in Ebenaceae cp genomes and those of four other families (Actinidiaceae, Theaceae, Primulaceae, and Ericaceae) were conducted. In Ebenecaceae, the IRa/SSC border was located in the 3' portion of *ycf1* gene, resulting in *ycf1* pseudogene formation at the IRb/SSC border, in line with the results from Primulaceae, Theaceae, and Actinidiaceae although differing markedly from those observed for Ericaceae. IRb/SSC boundaries were located upstream of *ndhF* in all families other than Primulaceae, in which this boundary was located within the *ndhF* 5' region. The IRa/LSC junctions in Theaceae and Ebenaceae were placed in an upstream area of *trnH-GUG*, whereas in Ericaceae, Actinidiceae, and most monocot cp

Table 7.3 Details of four polymorphic Indel loci developed from *Diospyros* cpDNA

Marker name	region	Marker sequences (5'-3')	Length/bp	Variable sites	Parsim-info sites
CP1	*trnH_psbA*	F-AACCCTGTAGACCATCCC	409–427	31	14
		R-GAGTTGTTATGCCTTTGT			
CP2	*ycf4_cemA*	F-TCGACTTTTCTTTTGTGCTCCT	459–489	27	5
		R-CGGATTGTCTAGTATTCCACCA			
CP3	*rps15*	F-ATTGATGGGTGGTGAGGA	234–285	7	2
		R-GAGAGGTCTGCGGAAAATTCT			
CP4	*trnT_trnL*	F-TTAGATAGTTTTTTTCGTTT	518–571	25	13
		R-GGCCCATATTTGAGGAGA			

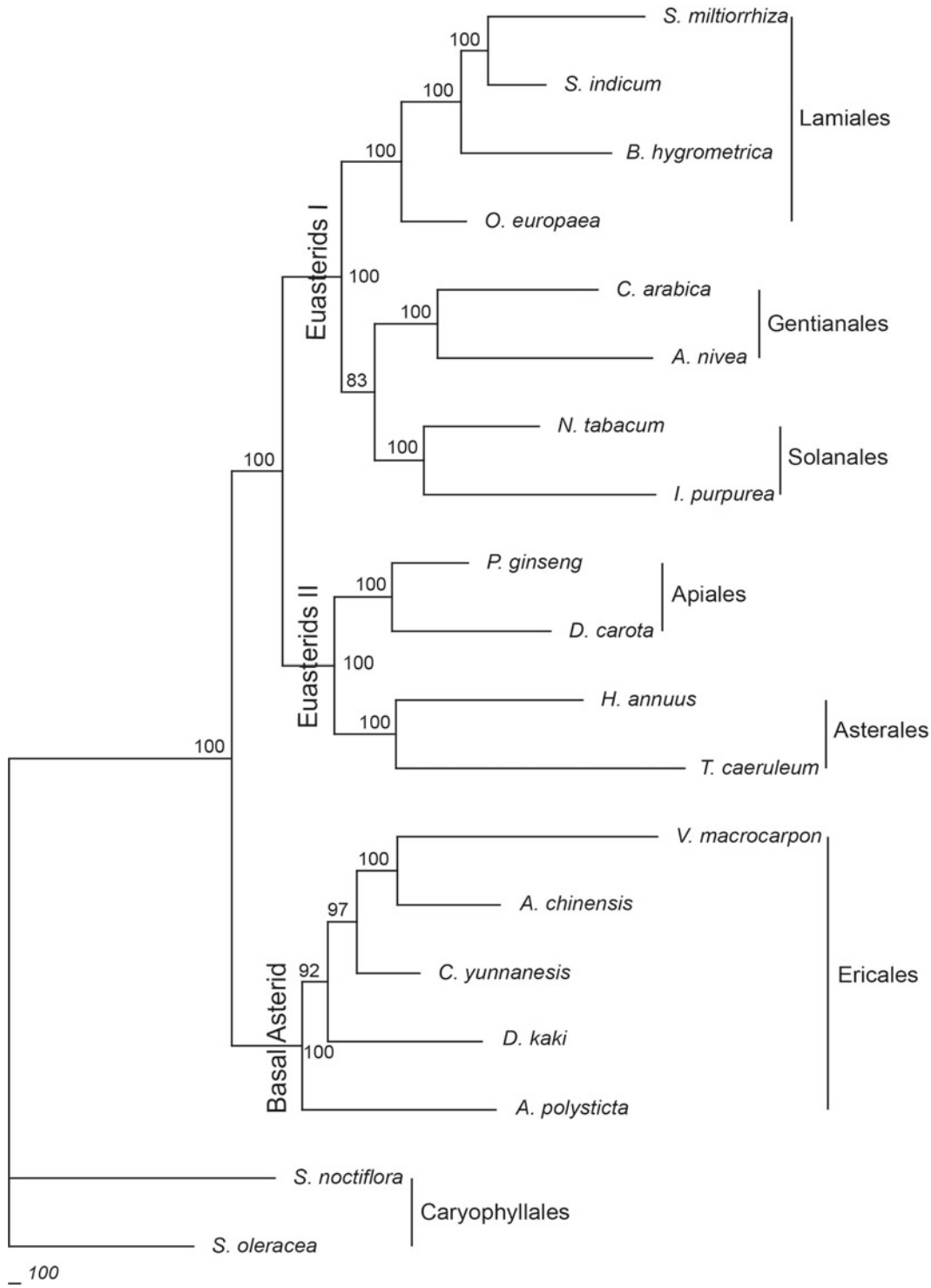

Fig. 7.5 Asterid clade phylogenetic tree. This tree was constructed using 61 protein-coding sequences shared among 19 angiosperm species. (Citation from Fu et al. 2016)

genomes this gene was instead located within IR regions (Huotari and Korpelainen 2012). IRb/LSC junctions in Primulaceae and Ebenaceae were located within *rps19* without any corresponding regional copy generation. In summary, these data suggest a high degree of cp genome conservation among closely related species, whereas greater diversity is evidence for species belonging to different families, as in the case of the large inversions present within the *Eucommia ulmoides* cp genome (Wang et al. 2016) or the inverted repeat loss reported in the *Astragalus membranaceus* cp genome (Lei et al. 2016).

7.9 Phylogenetic Analysis

Phylogenetic relationships among *Diospyros* and other asterid species were explored by querying the NCBI database and downloading 18 released cp genome sequences, with the cp genomes of *Spinacia* and *Silene* of the order Caryophyllales being incorporated as outgroup taxa. The 61 protein-coding gene sequences that were common across these cp genomes were used to construct an MP phylogenetic tree. The MP tree strongly indicated that Ericales was a basal sister order to the subdivision of euasterids (euasterids I and II; Fig. 7.5), consistent with the monophyletic placement of Ebenaceae in Ericales. Gentianales, Solanales, and Lamiales were clustered within the euasterids I subsection, while Asterales and Apiales were members of the euasterids II subsection. When tree topology was constructed via the ML technique, it was in agreement with the topology of the tree of MP. These results are in line with a prior published phylogenetic assessment on the basis of the complete cp genomes from 15 asterid species and a single outgroup (Yao et al. 2015). Overall, 100% bootstrap support was achieved for 13/16 nodes in the MP tree, consistent with the use of appropriate settings during tree construction. The monophyletic nature of Ebenaceae in this analysis was in agreement with the outcomes of a prior assessment of five genes from mitochondrial and plastid genomes (Anderberg et al. 2002).

7.10 Conclusion

The complete *Diospyros* cp genomes offer value as a reference source for the sequencing of other Ebenaceae or Ericales species. The plastid genome contains sufficient phylogenetic information to resolve interspecific relationships and elucidate the origin of persimmons. As the majority of *D. kaki* cultivars exhibit hexaploidy, whole-genome sequencing is poorly suited to studying its associated genetic background. The *Diospyros* species most closely related to cultivated persimmons, *D. oleifera,* may offer value as a model for studies of *D. kaki*. The phylogenetic tree based on chloroplast gene sequences also confirmed that *D.* 'Jinzaoshi' is a new species. In summary, the chloroplast genome represents an efficient and effective tool that can be used to explore phylogenetic relationships among *Diospyros*.

References

Anderberg AA, Rydin C, Källersjö M (2002) Phylogenetic relationships in the order Ericales s.l.: analyses of molecular data from five genes from the plastid and mitochondrial genomes. Am J Bot 89(4):677–687

Chumley TW, Palmer JD, Mower JP et al (2006) The complete chloroplast genome sequence of *Pelargonium* × *hortorum*: organization and evolution of the largest and most highly rearranged chloroplast genome of land plants. Mol Biol Evol 23(11):2175–2190

Daniell H, Ruiz ON, Dhingra A (2004) Chloroplast genetic engineering to improve agronomic traits. In: Transgenic plants: methods and protocols, vol. 286, pp 111–137

Du X, Zhang Q, Luo ZR (2009) Comparison of four molecular markers for genetic analysis in *Diospyros* L. (Ebenaceae). Plant Syst Evol 281(1–4):171–181

Fazekas AJ, Burgess KS, Kesanakurti PR et al (2008) Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. PLoS ONE 3(7):e2802

Fu J, Liang J, Wuyun T et al (2015) Sequence analysis of ITS regions and ndhA gene for determining phylogenetic relationship of *Diospyros kaki* (persimmon) with other related wild *Diospyros* (Ebenaceae) species. Bull Bot Res 35(4):515–520

Fu J, Liu H, Hu J et al (2016) Five complete chloroplast genome sequences from diospyros: Genome organization and comparative analysis. PLoS ONE 11(7): e0159566

Guisinger MM, Kuehl JV, Boore JL et al (2011) Extreme reconfiguration of plastid genomes in the angiosperm family Geraniaceae: rearrangements, repeats, and codon usage. Mol Biol Evol 28(1):583–600

Guo D, Luo Z (2011) Genetic relationships of the Japanese persimmon Diospyros kaki (Ebenaceae) and related species

Hu D, Zhang Q, Luo Z (2008) Phylogenetic analysis in some *Diospyros* spp. (Ebenaceae) and Japanese persimmon using chloroplast DNA PCR-RFLP markers. Sci Hortic 117(1):32–38

Huotari T, Korpelainen H (2012) Complete chloroplast genome sequence of Elodea canadensis and comparative analyses with other monocot plastid genomes. Gene 508(1):96–105

Ickert-Bond SM, Wen J (2006) Phylogeny and biogeography of Altingiaceae: evidence from combined analysis of five non-coding chloroplast regions. Mol Phylogenet Evol 39(2):512–528

Jansen RK, Raubeson LA, Boore JL et al (2005) Methods for obtaining and analyzing whole chloroplast genome sequences. Methods Enzymol 395:348–384

Kim YK, Park Cw, Kim KJ (2009) Complete chloroplast DNA sequence from a Korean endemic genus, *Megaleranthis saniculifolia*, and its evolutionary implications. Mol Cells 27(3):365–381

Lei W, Ni D, Wang Y et al (2016) Intraspecific and heteroplasmic variations, gene losses and inversions in the chloroplast genome of Astragalus membranaceus. Sci Rep 6

Liang Y, Han W, Sun P et al (2015) Genetic diversity among germplasms of *Diospyros kaki* based on SSR markers. Sci Hortic 186:180–189

Maréchal A, Brisson N (2010) Recombination and the maintenance of plant organelle genome stability. New Phytol 186(2):299–317

Parks M, Cronn R, Liston A (2009) Increasing phylogenetic resolution at low taxonomic levels using massively parallel sequencing of chloroplast genomes. BMC Biol 7(1):84

Shaw J, Lickey EB, Schilling EE et al (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Am J Bot 94 (3):275–288

Shaw J, Small RL (2005) Chloroplast DNA phylogeny and phylogeography of the North American plums (*Prunus subgenus Prunus* section Prunocerasus, Rosaceae). Am J Bot 92(12):2011–2030

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9):1312–1313

Tang D, Hu Y, Zhang Q et al (2014) Discriminant analysis of " Jinzaoshi " from persimmon (*Diospyros kaki* Thunb.; Ebenaceae): A comparative study conducted based on morphological as well as ITS and matK sequence analyses. Sci Hortic 168:168–174

Venkatasamy S, Khittoo G, Nowbuth P et al (2006) Phylogenetic relationships based on morphology among the *Diospyros* (Ebenaceae) species endemic to the Mascarene Islands. Bot J Linn Soc 150(3):307–313

Wang L, Wuyun Tn DuH et al (2016) Complete chloroplast genome sequences of *Eucommia ulmoides*: genome structure and evolution. Tree Genet Genomes 12(1):1–15

Wang RJ, Cheng CL, Chang CC et al (2008) Dynamics and evolution of the inverted repeat-large single copy junctions in the chloroplast genomes of monocots. BMC Evol Biol 8(1):36

Wills DM, Burke JM (2006) Chloroplast DNA variation confirms a single origin of domesticated sunflower (Helianthus annuus L.). J Heredity 97(4):403–408

Yang M, Zhang XM, Liu GM et al (2010) The complete chloroplast genome sequence of date palm (Phoenix dactylifera L.). PLoS ONE 5(9)

Yao XH, Tang P, Li ZZ et al (2015) The first complete chloroplast genome sequences in actinidiaceae: genome structure and comparative analysis. PloS ONE 10 (6)

Yi DK, Kim KJ (2012) Complete chloroplast genome sequences of important oilseed crop *Sesamum indicum* L. PLoS ONE 7(5):e35872

Yonemori K, Honsho C, Kanzaki S et al (2008) Sequence analyses of the ITS regions and the matK gene for determining phylogenetic relationships of *Diospyros kaki* (persimmon) with other wild *Diospyros* (Ebenaceae) species. Tree Genet Genomes 4(2):149–158

Sexual System and Its Evolution

8

Kanae Masuda and Takashi Akagi

Abstract

Sexual systems in tree crop species is often an important determinant of stable and efficient fruit production, and harnessing them is a key for both cultivation and breeding aspects. In contrast to animals, plants have various sexual systems to maintain their genetic diversities to fit new environments and to expand their habitats. Most of the *Diospyros* species are classified as dioecy (separated male and female individuals) controlled by the Y-encoded putatively single sex-determining gene, *OGI,* which encodes small-RNA repressing its autosomal target gene, *MeGI.* This mechanism would be specific to the *Diospyros* lineage, as these two sex-determining factors were derived from lineage-specific paleo-duplications, and thereafter, neofunctionalizations. Furthermore, some polyploid *Diospyros* species exhibit plastic sexuality mixed with male and female flowers or with occasional conversion from male to hermaphrodite flowers, which are determined by internal environmental conditions and epigenetic layers on the sex determinants. Although recent findings made some achievements for understanding the evolutionary paths into dioecy and the escape from that into plastic sexuality, many unclarified mechanisms have still remained. In this chapter, we introduce the sexual systems and their evolution in *Diospyros* species.

8.1 Introduction: Plant Sex

Separated sexuality is a fundamental strategy for maintaining genetic diversity and reproductive success in both animals and plants (Bawa 1980). Most flowering plants are hermaphroditism, which is thought to be the ancestral state of the sexual system (Ainsworth 2000). Some plants have independently and repeatedly evolved dioecy through internal sexuality (Westergaard 1958). Transitions between sexual systems may occur through several potential evolutionary pathways (Bachtrog et al. 2014), and in plants, they are thought to be beginning in hermaphroditism and ending in the rare state of dioecy with genetic sex determination (Goldberg et al. 2017). Although more than 100 years of research have been conducted on the genetic sex determination in plants, only a few sex determination factors have been discovered, and their regulatory mechanisms and evolutionary processes are mostly unexplained.

K. Masuda (✉) · T. Akagi
Graduate School of Environmental and Life Science, Okayama University, Okayama 700-8530, Japan
e-mail: masuda_kanae@s.okayama-u.ac.jp

T. Akagi
e-mail: takashia@okayama-u.ac.jp

T. Akagi
Japan Science and Technology Agency (JST), PRESTO, Kawaguchi-shi, Saitama 332-0012, Japan

R. Tao and Z. Luo (eds.), *The Persimmon Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-05584-3_8

Genetically separated sexuality in plants was first defined in the Cucurbitaceae genus *Bryonia dioica* (Correns 1903). Subsequently, sex chromosome was first identified in one of the basal angiosperm *Sphaerocarpos* called bottle liverworts (Allen 1917) and in angiosperms such as white campion (*Silene latifolia*), sorrel (*Rumex acetosa*), and hop (*Humulus lupulus*), and have been observed in more than 40 plants at least as of 2007 (Ming et al. 2007). Their sex determination systems are mainly classified into XY (heterogametic male) and ZW (heterogametic female) systems, and plants with separated sexuality were thought to be mostly determined by XY system. Previous studies on chromosome evolutions suggested that the structure of the Y chromosomes (W chromosome) in plants gradually collapse as represented in animals. The Y chromosome finally disappears (XO type) in some cases, in which sorrel was an XO system and its sexuality is determined by the X-to-autosome ratio (Ono 1935). In flowering plants, several evolution models for sex determination systems have been proposed based on the XY system, and the most famous model would be the "two-mutation model" proposed by Charlesworth and Charlesworth in 1978. In this model, the establishment of dioecy from hermaphroditism involves the following two events; 1. loss of function for maintaining male function (M factor), 2. acquisition of novel function for suppressor of feminization (SuF) on the Y chromosome (Charlesworth and Charlesworth 1978). Actually, genetic evidence supporting this model has been found in some plants. For example, hermaphrodite individuals appeared due to mutations in male individuals in white campion (Lardon et al. 1999) and papaya (*Carica papaya*) (Wang et al. 2012), and the two sex determinants were identified in asparagus (*Asparagus officinalis* L.) (Harkess et al. 2017) and kiwifruit (*Actinidia* spp.) (Akagi et al. 2018, 2019). On the other hand, another pathway in which dioecy was established by the acquisition of a single gene has been proposed (Goldberg et al. 2017). The precondition of this model is different from the above two-mutation model, and the transition into dioecy might begin in monoecy. In this model, sexuality is determined by internal environmental factors and genetic factors are selected for sex determination as adaptive evolution when the sexual balance is unstable (Charlesworth 2013). This single-factor model for sex determination was validated by an artificial evolution in *Cucurbitaceae* using TILLING collection (Boualem et al. 2015), and by the natural variations in persimmon (*Diospyros* species) (Akagi et al. 2014b, 2020) and in poplar (*Populus* species) (Müller et al. 2020). It would be worthy to note that the first finding of sex chromosome-encoded sex-determining gene in plants was made in the genus *Diospyros*. The small-RNA gene *OGI* encoded by the Y chromosome and autosomal gene *MeGI* as the suppressive target, genetically determine sexuality (Akagi et al. 2014b). The detailed molecular mechanism will be described later. More interestingly, some *Diospyros* species exhibit plastic sexuality shifted by environmental conditions, providing good materials for investigating transitions into and out of dioecy.

8.2 Floral Morphology Involving Sexuality in *Diospyros* species

Diospyros species are usually dioecious and occasionally exhibit plastic sexuality with polygamous (i.e., male, female, and hermaphrodite flowers in one individual) or monoecious (i.e., male and female flowers in one individual) systems (Duangjai et al. 2006). This plasticity tends to be found in some polyploids *Diospyros* species. For example, diploids *D. lotus*, *D. glaucifolia*, *D. dygina*, etc., are thought to be perfect dioecious, while the polyploids *D. kaki*, *D. virginiana*, *D. rhombifolia*, and *D. ebenum* often show plastic sexuality (Table 8.1) (Fig. 8.1). As an exception, the diploid *D. oleifera* might exhibit plastic sexuality mixed with male, female, and hermaphrodite flowers, so it may be also a good material to understand the plasticity (Suo et al. 2020).

Historically, the first study about flower morphology in *Diospyros* species was conducted by Hague (1911) and Yasui (1915), in American

Table 8.1 List of the sexual systems in representative *Diospyros* species

Species	Polyploidy	Sexual system	References
D. lotus	2× (N = 30)	Dioecy	Duangjai et al. (2006)
D. glaucifolia	2× (N = 30)	Dioecy	Duangjai et al. (2006)
D. digyna	2× (N = 30)	Dioecy	Ricker et al. (2000)
D. morrisiana	2× (N = 30)	Dioecy	He et al. (2021)
D. pentamera	2× (N = 30)	Dioecy	House (1992)
D. lasiocalyx (Mart.) B. Wall.		Dioecy	Aguiar et al. (2020)
D. oleifera	2× (N = 30)	Basically dioecy, with some reports for existence of monoecy and production of hermaphrodites	Suo et al. (2020)
D. ebenum	6× (N = 90)	Monoecy	Orwa et al. (2009) (Agroforestry Database 4.0)
D. kaki	6× (N = 90)	Polygamous (basically female andmonoecy), occasionally producinghermaphrodites	Yasui (1915)
			George et al. (1997)
			Yonemori et al. (1992)
			Li et al. (2019a; b)
			Missouri Botanical Garden web page
		Hypothetically androecious (or male)	Wang et al. (2018)
			Guan et al. (2020)
			Masuda et al. (2020b)
		Genetically female, with occasionalproduction of male flowers	Yakushiji et al. (1995)
			McDaniel (1973a; b)
			Masuda et al. (2020a)
D. virginiana	4× (N = 60)	Dioecy; occasionally producing hermaphrodites	Missouri Botanical Garden web page
	6× (N = 90)		
D. rhombifolia	4× (N = 60)	Dioecy; occasionally producinghermaphrodites	Personal communication

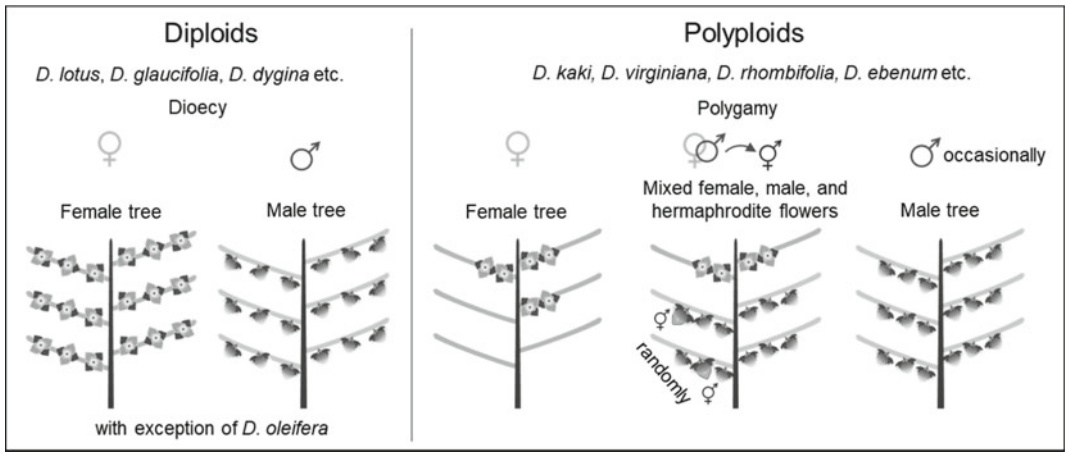

Fig. 8.1 Model of sexual expression in *Diospyros* species

persimmon (*D. virginiana*) and (Oriental) persimmon (*D. kaki*), respectively. From their first reports, *D. virginiana* is usually dioecious, with exception of male trees bearing hermaphrodite flowers and fruits (Hague 1911) and *D. kaki* is monoecious with occasionally producing hermaphrodite flowers (Yasui 1915). Their floral initiation cycles appear in the following order: a pair of bracts, sepal, petal, stamens, and lastly carpel. Their flower morphologies are almost the same. Male flowers grow in usually three-flowered cymes with 16 fertile stamens surrounding a sterile carpel, while female flowers grow individually with 8 sterile stamens and a carpel. Hermaphrodite flowers are observed as male flowers during the early developmental stage and develop sequential different sizes of pistils although their fruits are usually seedless. These observations are consistent with recent reports in *Diospyros* species (Yonemori et al. 1993; Akagi et al. 2014b, 2016a; Sun et al. 2017; Li et al. 2019a; Yang et al. 2019). More precise research showed that the developmental processes of male and female flowers are defined as three phases. First, the primordia of male and female flower organs are thought to be determined concurrently with the inflorescence structure formation (or flower numbers per inflorescence) in late June (typically in Japan, and potentially also in other temperate or subtropical Asia) during flower bud formation. Then, they go dormant from August to September, and androecium (male parts) and gynoecium (female parts) differentiation begin in late March (Hasegawa et al. 2003; Akagi et al. 2016a).

8.3 The Evolution of Dioecy in *Diospyros* species

As described, the first finding of the molecular mechanism and molecular evolution of dioecious sex-determining gene was made in *Diospyros* species (Akagi et al. 2014b). Dioecious diploid *D. lotus* was selected as a model to clarify the dioecious system because this species is a wild close relative of the cultivated hexaploid *D. kaki* which shows plastic sexuality as mentioned above. Amplified Fragment Length Polymorphism (AFLP) analysis in *D. lotus* has clarified a male heterogametic sex determination (or XY) system and found two molecular markers cosegregated with maleness, of which one was later converted into a SCAR marker (Akagi et al. 2014a). The fact that only two representative male-specific polymorphisms were identified from >300 primers trials in AFLP, potentially suggested small hemizygous regions specific to male (or potential homomorphic XY chromosome). These results suggested that it was not much difficult to identify the sex determination gene in *Diospyros* species with genetic or genomic approaches based on genetic recombinations in segregated population, in contrast to the plant species with large Y-specific regions (or heteromorphic XY chromosomes), such as *Silene latifolia*. Here, Illumina sequencing technology for application to a quite novel approach of k-mer cataloging successfully worked to identify the two sex determinants in *Diospyros*, a Y-encoded small-RNA gene *OGI* (*Oppressor of MeGI*), and its autosomal target *MeGI* (*Male Growth Inhibitor*), of which the names were derived from male and female trees in Japanese, respectively (Akagi et al. 2014b). *MeGI* is an HD-Zip1 type homeodomain gene and acts for both promoting gynoecium formation and suppressing androecium differentiation (Yang et al. 2019). Male flowers are specified by repression of the autosomal gene *MeGI* by transitive RNAi triggered by the expression of small-RNA pseudo-gene *OGI* (Akagi et al. 2014b). This mechanism is thought to be conserved among the genus *Diospyros* because the genetic regulation of dioecious by *OGI* and its specificity to male is consistent throughout the *Diospyros* species (Akagi et al. 2014b, 2016b; Zhang et al. 2016). While, this mechanism would be specific to the *Diospyros* lineage, as the duplication deriving *OGI* and *MeGI* from a single origin, would be specific to this genus (or the family Ebenaceae) (Akagi et al. 2014b, 2020). The draft genome sequences of *D. lotus* and their evolutionary interpretations for the establishment process of sex determination genes suggested the importance of the duplication events to derive three

paralogs, *OGI*, *MeGI*, and *SiMeGI*. After the putatively lineage-specific whole-genome duplication (named *Dd*-α) to derive *MeGI* and *SiMeGI* pair, the proto-*MeGI* underwent episodic positive selections, as an adaptive evolution, to acquire a novel function as a repressor of male organ development, while *SiMeGI* presumably maintained the original function (Akagi et al. 2020). Later, a segmental duplication event of *MeGI* established *OGI* encoding the palindrome sequence of *MeGI* (small-RNA) with the function as a suppressor of *MeGI*, and then initiated the formation of the Y-chromosome. These results exemplify how duplication events can provide good chances of the transition into dioecy. Consistent with this case of *Diospyros*, gene or genome-wide duplications have been often involved in the establishment of dioecy in other species, such as garden asparagus (Harkess et al. 2017), kiwifruit (Akagi et al. 2018), and also poplar (Müller et al. 2020), suggesting a potential commonality for genome evolution involving transitions between sexual systems. Although transitions into dioecy from ancestral hermaphrodite state are often explained by two mutations model (Charlesworth and Charlesworth 1978), the single factor of *OGI* may be physiologically explainable for the sex determination in *Diospyros*, as its target *MeGI* plays both roles of feminization and suppressor of masculinization in *Diospyros* species (Akagi et al. 2016a, b; Yang et al. 2019). For feminization, *MeGI* can directly regulate *KNOTTED1-like HOMEOBOX* (*KNOX*) and *OVATE FAMILY PROTEIN* (*OFP*) gene families related to the formation of gynoecium, while *SHORT VEGETATIVE PHASE* (*SVP*) is thought to play a main role to repress the androecium developing pathways, such as *PISTILLATA* (*PI*) (Yang et al. 2019; Fig. 8.2). On the other hand, in terms of the regulatory networks, genes reminiscent of various plant hormone responses, including cytokinin (CK), auxin, gibberellin, and ethylene, were differentially expressed in the comparison of male and female flower organs, throughout an annual cycle (Li et al. 2019a). In further late flower developmental stage, one of the major forms of CKs, zeatin, and ABA increase in female flowers in comparison to male flowers (Sun et al. 2017), which would be consistent with that the *KNOX* families can act for the promotion of CK synthesis and responses. Furthermore, programmed cell death (PCD) is proposed to be also involved in the process to arrest flower organ formation, leading to unisexual flowers (Wang et al. 2020). Future expression dissections with finer resolutions help to clarify the differentiation processes more in depth.

8.4 The Evolution of Plastic Sexuality: Environmental Sex Determination in *Diospyros* species

Plastic sexualities (or escape from dioecy) have been evolved mainly in highly polyploid *Diospyros* species as given in Table 8.1. Although dioecy (or separated sexuality) is often thought to be an irreversible state, called a "dead-end," in reproductive evolution (Heilbuth 2000), plant species have undergone frequent transitions into and out of dioecy, which have potentially acted as a key strategy to ensure the balance between genetic diversity and stable reproduction (Käfer et al. 2017). Hence, the genus *Diospyros*, which encloses frequent transitions from dioecy into monoecious/polygamous systems would be a good material for understanding the plant-specific plasticity. Although the sex-determining genes or candidates have been discovered in some plant species, few research focused on plastic sexualities in plants. Here, we introduce the evolutionary mechanisms in transitions from dioecy to monoecy and occasional reversion into hermaphroditism, in hexaploid polygamous *D. kaki*.

The flower sexuality in *D. kaki* is substantially influenced by environmental conditions such as tree age, previous crop load, nutrient status, phytohormones, bud position, and shoot type (George et al. 1997). The flower sexuality in monoecious cultivars would be predictable to some extent, based on the information of sexuality and length of the parental branch, or bud

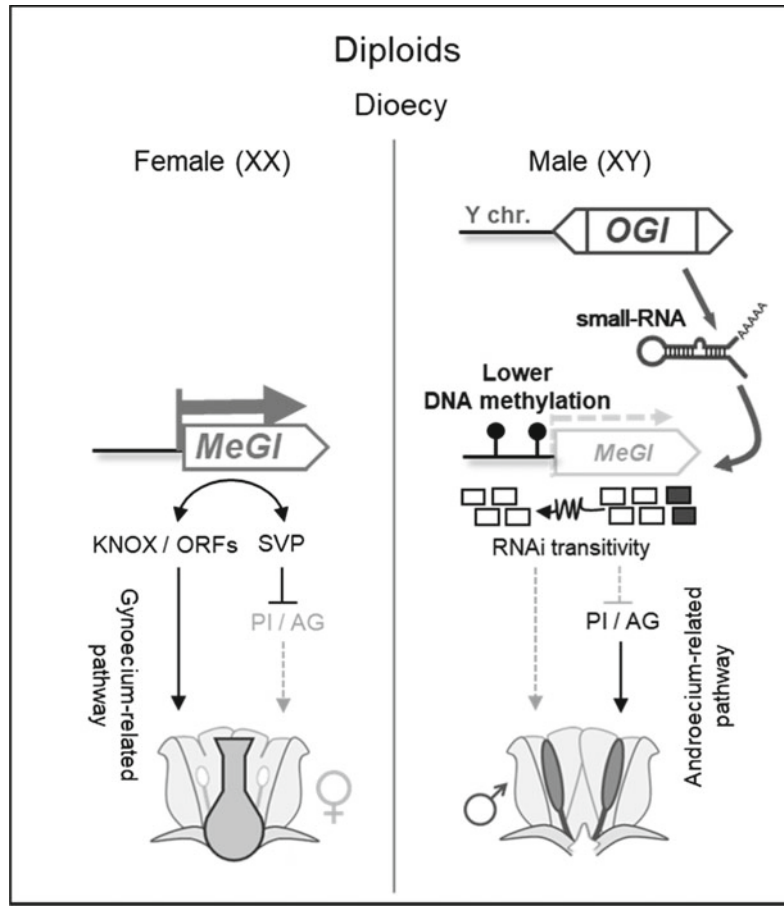

Fig. 8.2 Genetic sex determination of dioecy with *OGI* and *MeGI* in *Diospyros* species

positions (Yonemori et al. 1992; Akagi et al. 2016a). Briefly, female flowers tend to grow on maternal branches, while male flowers tend to on paternal ones. In addition, a few top buds in long maternal branches dominantly produce female flowers, while it depends on cultivars. Very occasional production of male flowers in genetically female (or *OGI*-null) cultivars has been also reported, in cv. Fuyu and cv. Jiro (Yakushiji et al. 1995), and in cv. Saijo (Esumi et al. 2015; Masuda et al. 2020a). Furthermore, some cultivars are reported to bear only male flowers. For instance, a tree bearing only male flowers for thousands of years was reported in China, which would be an effective genetic resource before female trees had been selected during potential domestication (Guan et al. 2020). Approximately, 120 predominantly androecious persimmons were collected in China, and 15 SSR loci associated with the androecious character have been developed (Wang et al. 2018). A chance seedling, cv. Kumemaru, bearing only male flowers over decades (Genebank Project, NARO, Japan; https://www.gene.affrc.go.jp/databases-plant_search_detail_en.php?jp=117219) have been found in Japan, of which the physiological mechanism has been partially unveiled, as described later (Masuda et al. 2020b).

Epigenetic regulations, as a kind of environmental factors, often involve sex expression in plants, such as in the genus *Populus/Salix* (Bräutigam et al. 2017; Müller et al. 2020), *Silene latiforlia* (Janoušek et al. 1996), or melon (Martin et al. 2009). Also in *D. kaki*, epigenetic regulation of the *MeGI* acts as a switch for flower sex conversion in monoecious sex-determination system. The basic regulatory network and the function of *OGI/MeGI* for sex determination are

conserved also in hexaploid *D. kaki*, while their expression patterns are quite distinct from the diploid relatives. In contrast to the stable annual expression pattern of *OGI* in *D. lotus* (Akagi et al. 2014b), the *OGI* expression in *D. kaki* is thought to be semi-silenced putatively due to the insertion of SINE-like retrotransposon sequences in the upstream region of *OGI* (Akagi et al. 2016a). The cytosine residues in this SINE-like structure, named *Kali*, are highly methylated, potentially contributing inactivation of the *OGI* expression. Although the timing of *OGI* activation in *D. kaki* remains to be solved, once small RNAs of *OGI* can access the *MeGI* transcript, it is thought to start transitive RNAi to repress the *MeGI* expression, as observed in *D. lotus*. However, in contrast to *D. lotus*, the transitive RNAi can induce maintainable substantial DNA methylation in the 5' promoter regions, which acts as an epigenetic switch to repress or release the *MeGI* expression to be male or female flowers, respectively (Fig. 8.3, Akagi et al. 2016a). Therefore, genetically male trees with *OGI* produce female flowers depending on the epigenetic status in the *MeGI* promoter. This situation corresponds to the monoecious sex-determination system in *D. kaki*. Thus, the individuals producing male flowers would definitely bear *OGI* gene in the genome, while the existence of *OGI* does not necessarily mean the production of male flowers, if *OGI* is highly silenced (Akagi et al. 2016a, b; Zhang et al. 2016). Still, the mechanisms for epigenetic regulation of *OGI/MeGI* have not fully been clarified. Genome-wide correlation/association analyses have detected some polysomic genetic factors conferring the frequency of male flowers, which could potentially act epigenetically for the activation of *OGI* (Masuda et al 2020c). The allele dosages in the male-specific regions of the Y-chromosome, including *OGI*, were also significantly correlated with the male flower frequency, suggesting that the *cis*-regulatory elements in *OGI* play an important role in the activation in a dosage-dependent manner. The epigenetic status is also thought to be involved in a non-canonical somaclonal sex conversion to male in cv. Saijo, of which the genome has no Y-chromosome and *OGI*. The genome-wide fluctuation of DNA methylation levels is associated with those in *MeGI*, and often resulted in hyper-methylation to be male (Masuda et al. 2020a). Although the relationship between polyploid and epigenetic control has been discussed empirically (Comai 2005; Song and Chen 2015), of which the mechanism has not been fully understood. Understanding the establishment of epigenetic regulation of *OGI/MeGI* in *D. kaki* will provide a good example of the evolution of plasticity activated by polyploidization.

Regarding the occasional production of hermaphrodite flowers, we would start to investigate phytohormones that usually affect sex expressions of not only *D. kaki* but also a number of other plant species. In *Cucurbitaceae*, the endogenous ethylene pathway induced female differentiation (Boualem et al. 2015), and brassinosteroid pathway is mainly involved in sex determination in maize (Hartwig et al. 2011). Treatment of cytokinin induced male differentiation in hemp (*Cannabis sativa* L.), while female differentiation in spinach (*Spinacia oleracea*), grape (*Vitis vinifera*), and also kiwifruit (Grant et al. 1994; Akagi et al. 2018). Auxin induced male differentiation in asparagus (*Asparagus officinalis*) and hop (*Humulus lupulus*), while female differentiation in hemp (Grant e al. 1994; Negi and Olmo 1966). These results suggested many ways to potentially invent sex-determination system in plants. Regarding *D. kaki*, consistent with grape or kiwifruit, treatment with cytokinin to male flowers in early flower developing stage results in the conversion into hermaphrodite flowers (Yonemori et al. 1990), which is consistent with the higher concentration of internal cytokinin in female and hermaphrodite flowers than in male (Sun et al. 2017; Li et al. 2021). A recent finding would provide a clue for the physiological mechanism to naturally produce hermaphrodite flowers from male. The signals involving stress responses and/or ABA, can participate in the activation of the hermaphroditism pathway, which is shared with the artificial hermaphrodite production with CK treatment. This would be consistent with the finding that CK and ABA contents are increased

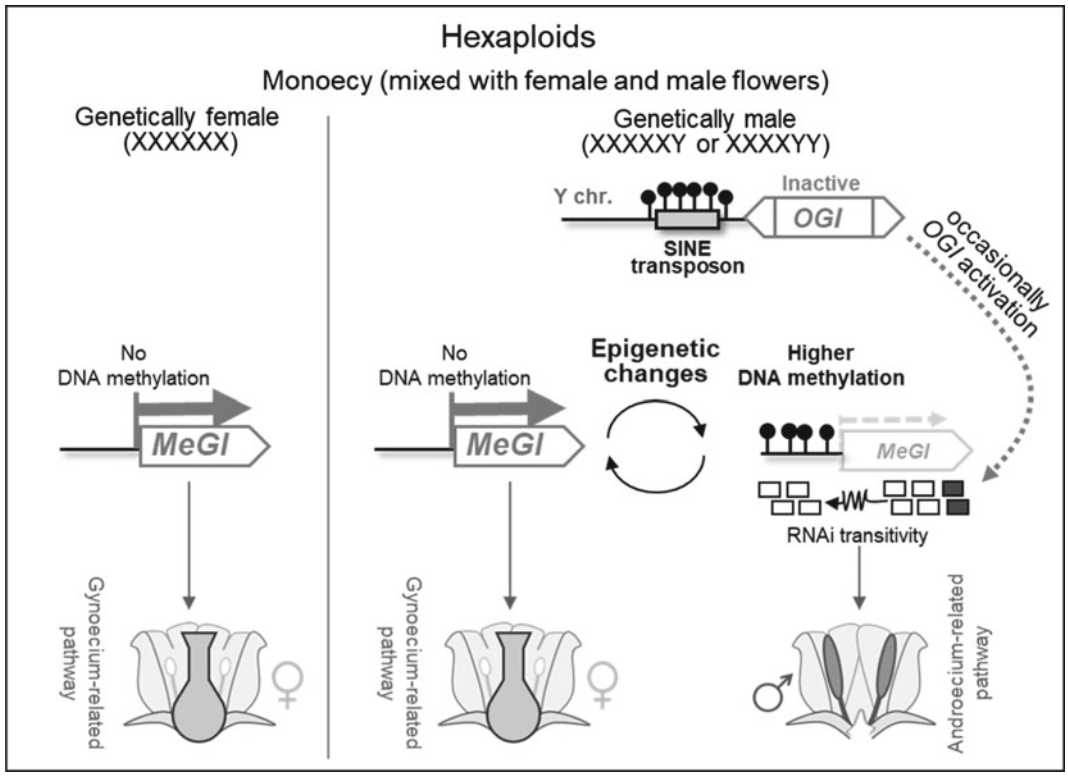

Fig. 8.3 Epigenetic regulation of *OGI* and *MeGI* system in monoecy in hexaploid *D. kaki*

in female (Sun et al. 2017). A small-Myb-like transcription factor, *RADIALIS*-like is supposed to integrate their antagonistic signals to promote gynoecium development in males (Masuda et al. 2022). Still, the fundamental mechanisms behind hermaphrodite flower formation only in highly polyploid *Diospyros* species are in a mystery.

8.5 Y-Chromosome Evolution and Sexual Dimorphisms

Transitions between sexual systems, particularly to dioecy, are often discussed together with sexual dimorphisms which are established via sex chromosome evolution. Sexual dimorphism, or sexual antagonism, means the traits specific to each male or female, which should be evolutionarily advantageous to each sexuality. A simple example in dioecious plants would be more flower numbers in males, although that depends on plant species (Barrett and Hough 2013). Based on a representative evolutionary framework, "two-mutation" model (Charlesworth and Charlesworth 1978), a transition to make maleness would require much more fitness specific to male individuals than that to make femaleness. Thus, conventional models suggested that recombination suppressions in sex (or Y) chromosomes, under "sexual selection," linked the determinant(s) and the genes advantageous to maleness, and have facilitated the sexual antagonisms between males and females, resulting in the rapid formation of long heteromorphic regions or MSY even in 10 million years (Ming et al. 2011; Barrett and Hough 2013; Charlesworth 2018). However, persimmon genomes (at least *D. lotus)* have very small MSY relative to the terms after the establishment of the Y origin, *OGI* gene, and the regions with recombination suppression include only a few canonical genes (see Chap. 6). This inconsistency has provided a clue for new insights into the expression of sexual dimorphisms and evolutionary context to

establish dioecy. The representative sexual dimorphism in persimmon would be the flower numbers per inflorescence, where male forms cyme-like structure, holding >3 flowers per inflorescence, while female produces a single flower. Importantly, this trend is conserved also in monoecious *D. kaki* cultivars with Y-chromosome, where the sex unit is defined not for individual, but for flower (Akagi et al. 2016a). This suggested the possibility that the flower numbers are not genetically regulated by the Y-chromosomal factors but under the control of the *MeGI* expression. Transformation of *Nicotiana tabacum*, as alternative ancestral hermaphroditism, with *MeGI* under the control of the native promoter, resulted in not only feminization but also the expression of representative sexual dimorphisms in female persimmon, such as fewer flower numbers per inflorescence and longer juvenile phase (Akagi et al. 2014b; Akagi and Charlesworth 2019). Further transformation with *OGI* resulted in the restoration of flower numbers (Akagi and Charlesworth 2019). In addition, the *MeGI*'s orthologs functions originally contribute to flower numbers or branching in barley (Komatsuda et al. 2007) or *Arabidopsis* (González-Grandío et al. 2017). These all implied that the proto-sex determinants originally contributed to the traits advantageous to each sexuality, followed by the positive acquisition of the function to determine sexes. This pleiotropy of the *OGI/MeGI* system can explain the reason that the MSY in persimmon is so small, and propose a new hypothesis that the current sexual dimorphisms in persimmon are not the resultant traits in sexual selections but the causal factor to promote separated sexuality, especially to male.

References

Aguiar BI, Sebbenn AM, Tarazi R, Vogado NO, Morellato LPC, Tambarussi EV, Moreno MA, Pereira LCSM, Montibeller C, Ferraz EM, Gandara FB, Kageyama PY (2020) Phenology, seed germination, and genetics explains the reproductive strategies of *Diospyros lasiocalyx* (Mart.) B. wall. Trop Plant Biol 13(1):23–35

Ainsworth C (2000) Boys and girls come out to play: the molecular biology of dioecious plants. Ann Bot 86 (2):211–221

Akagi T, Charlesworth D (2019) Pleiotropic effects of sex-determining genes in the evolution of dioecy in two plant species. Proc Roy Soc B 286:20191805

Akagi T, Kajita K, Kibe T, Morimura H, Tsujimoto T, Nishiyama S, Kawai T, Yamane H, Tao R (2014a) Development of molecular markers associated with sexuality in *Diospyros lotus* L. and their application in *D. kaki* Thunb. J Japan Soc Hort Sci CH-109

Akagi T, Henry IM, Tao R, Comai L (2014b) A Y-chromosome–encoded small RNA acts as a sex determinant in persimmons. Science 346(6209):646–650

Akagi T, Henry IM, Kawai T, Comai L, Tao R (2016a) Epigenetic regulation of the sex determination gene *MeGI* in polyploid persimmon. Plant Cell 28 (12):2905–2915

Akagi T, Kawai T, Tao R (2016b) A male determinant gene in diploid dioecious *Diospyros*, *OGI*, is required for male flower production in monoecious individuals of oriental persimmon (*D. kaki*). Sci Hortic 213:243–251

Akagi T, Henry IM, Ohtani H, Morimoto T, Beppu K, Kataoka I, Tao R (2018) A Y-encoded suppressor of feminization arose via lineage-specific duplication of a cytokinin response regulator in kiwifruit. Plant Cell 30 (4):780–795

Akagi T, Pilkington SM, Varkonyi-Gasic E, Henry IM, Sugano SS, Sonoda M, Firl A, McNeilage MA, Douglas MJ, Wang T, Rebstock VC, Datson P, Allan AC, Beppu K, Kataoka I, Tao R (2019) Two Y-chromosome-encoded genes determine sex in kiwifruit. Nat Plants 5(8):801–809

Akagi T, Shirasawa K, Nagasaki H, Hirakawa H, Tao R, Comai L, Henry IM (2020) The persimmon genome reveals clues to the evolution of a lineage-specific sex determination system in plants. PLoS Genet 16(2): e1008566

Allen CE (1917) A chromosome difference correlated with sex differences in *Sphaerocarpos*. Science 46 (1193):466–467

Bachtrog D, Mank JE, Peichel CL, Kirkpatrick M, Otto SP, Ashman TL, Hahn MW, Kitano J, Mayrose I, Ming R, Perrin N, Ross L, Valenzuela N, Vamosi JC, Tree of Sex Consortium (2014) Sex determination: why so many ways of doing it? PLoS Biol 12(7): e1001899

Barrett SC, Hough J (2013) Sexual dimorphism in flowering plants. J Exp Bot 64(1):67–82

Bawa KS (1980) Evolution of dioecy in flowering plants. Annu Rev Ecol Syst 11(1):15–39

Boualem A, Troadec C, Camps C, Lemhemdi A, Morin H, Sari MA, Fraenkel-Zagouri R, Kovalski I, Dogimont C, Perl-Treves R, Bendahmane A (2015) A cucurbit androecy gene reveals how unisexual flowers develop and dioecy emerges. Science 350(6261): 688–691

Bräutigam K, Soolanayakanahally R, Champigny M, Mansfield S, Douglas C, Campbell MM, Cronk Q (2017) Sexual epigenetics: gender-specific methylation of a gene in the sex determining region of *Populus balsamifera*. Sci Rep 7:1–8

Charlesworth D (2013) Plant sex chromosome evolution. J Exp Bot 64(2):405–420

Charlesworth D (2018) Does sexual dimorphism in plants promote sex chromosome evolution? Environ Exp Bot 146:5–12

Charlesworth B, Charlesworth D (1978) A model for the evolution of dioecy and gynodioecy. Am Nat 112 (988):975–997

Comai L (2005) The advantages and disadvantages of being polyploid. Nat Rev Genet 6(11):836–846

Correns C (1903) Über die dominierenden merkmale der bastarde. Ber Dtsch Bot Ges 21:133–147

Duangjai S, Wallnöfer B, Samuel R, Munzinger J, Chase MW (2006) Generic delimitation and relationships in Ebenaceae sensu lato: evidence from six plastid DNA regions. Am J Bot 93(12):1808–1827

Esumi T, Watanabe A, Kosugi Y, Ohata K, Itamura H (2015) Staminate flowers on "Saijo" persimmon (*Diospyros kaki* Thunb.). Bull Fac Life Env Sci Shimane Univ 20:3–8 (In Japanese with English summary)

George AP, Mowat AD, Collins RJ, Morley-Bunker M (1997) The pattern and control of reproductive development in non-astringent persimmon (*Diospyros kaki* L.): A review. Sci Hortic 70(2–3):93–122

Goldberg EE, Otto SP, Vamosi JC, Mayrose I, Sabath N, Ming R, Ashman TL (2017) Macroevolutionary synthesis of flowering plant sexual systems. Evolution 71(4):898–912

González-Grandío E, Pajoro A, Franco-Zorrilla JM, Tarancón C, Immink RG, Cubas P (2017) Abscisic acid signaling is controlled by a BRANCHED1/HD-ZIP I cascade in Arabidopsis axillary buds. Proc Natl Acad Sci USA 114(2):E245–E254

Grant S, Houben A, Vyskot B, Siroky J, Pan WH, Macas J, Saedler H (1994) Genetics of sex determination in flowering plants. Dev Genet 15(3):214–230

Guan C, Zhang P, Wu M, Zeng M, Chachar S, Ruan X, Wang R, Yang Y (2020) Discovery of a millennial androecious germplasm and its potential in persimmon (*Diospyros kaki* Thunb.) breeding. Sci Hortic 269: 109392

Hague SM (1911) A morphological study of Diospyros virginiana. Bot Gaz 52(1):34–44

Harkess A, Zhou J, Xu C, Bowers JE, Van der Hulst R, Ayyampalayam S, Mercati F, Riccardi P, McKain MR, Kakrana A, Tang H, Ray J, Groenendijk J, Arikit S, Mathioni SM, Nakano M, Shan H, Telgmann-Rauber A, Kanno A, Yue Z, Chen H, Li W, Chen Y, Xu X, Zhang Y, Luo S, Chen H, Gao J, Mao Z, Pires JC, Luo M, Kudrna D, Wing RA, Meyers BC, Yi K, Kong H, Lavrijsen P, Sunseri F, Falavigna A, Ye Y, Leebens-Mack JH, Chen G (2017) The asparagus genome sheds light on the origin and evolution of a young Y chromosome. Nat Commun 8 (1):1–10

Hartwig T, Chuck GS, Fujioka S, Klempien A, Weizbauer R, Potluri DPV, Choe S, Johal GS, Schulz B (2011) Brassinosteroid control of sex determination in maize. Proc Natl Acad Sci USA 108(49):19814–19819

Hasegawa K, Fukuta T, Nishio K, Kitajima A (2003) Male and female flower buds differentiation and development in Japanese persimmon "Nishimurawase", "Zenjimaru" and "Taisyu." Res Rep Kochi Univ Agric (japan) 52:1–12 (In Japanese with English summary)

He Y, Xu H, Liu H, Luo M, Chu C, Fang S (2021) Sexual competition and kin recognition co-shape the traits of neighboring dioecious *Diospyros morrisiana* seedlings. Hort Res 8(1):1–10

Heilbuth JC (2000) Lower species richness in dioecious clades. Am Nat 156(3):221–241

House SM (1992) Population density and fruit set in three dioecious tree species in Australian tropical rain forest. J Ecol 57–69

Janoušek B, Široký J, Vyskot B (1996) Epigenetic control of sexual phenotype in a dioecious plant, *Melandrium Album*. Mol General Genet 250(4):483–490

Käfer J, Marais GA, Pannell JR (2017) On the rarity of dioecy in flowering plants. Mol Ecol 26(5):1225–1241

Komatsuda T, Pourkheirandish M, He C, Azhaguvel P, Kanamori H, Perovic D, Stein N, Graner A, Wicker T, Tagiri A, Lundqvist U, Fujimura T, Matsuoka M, Matsumoto T, Yano M (2007) Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. Proc Natl Acad Sci USA 104(4):1424–1429

Lardon A, Georgiev S, Aghmir A, Le Merrer G, Negrutiu I (1999) Sexual dimorphism in white campion: complex control of carpel number is revealed by Y chromosome deletions. Genetics 151(3):1173–1185

Li S, Sun P, Du G, Wang L, Li H, Fu J, Suo Y, Han W, Diao S, Mai Y, Li F (2019a) Transcriptome sequencing and comparative analysis between male and female floral buds of the persimmon (*Diospyros kaki* Thunb.). Sci Hortic 246:987–997

Li H, Wang L, Sun P, Suo Y, Han W, Mai Y, Diao S, Yuan D, Fu J (2019b) Cytomorphological observation on development of pistil and stamen of male and hermaphrodite floral buds of diospyros kaki "Longyan Yeshi 1." Acta Hortic Sinica 46(10):1897–1906

Li H, Wang L, Mai Y, Han W, Suo Y, Diao S, Sun P, Fu J (2021) Phytohormone and integrated mRNA and miRNA transcriptome analyses and differentiation of male between hermaphroditic floral buds of andromonoecious *Diospyros kaki* Thunb. BMC Genom 22(1):1–19

Martin A, Troadec C, Boualem A, Rajab M, Fernandez R, Morin H, Pitrat M, Bendahmane A (2009) A transposon-induced epigenetic change leads to sex determination in melon. Nature 461(7267):1135-1138

Masuda K, Akagi T, Esumi T, Tao R (2020a) Epigenetic flexibility underlies somaclonal sex conversions in

hexaploid persimmon. Plant Cell Physiol 61(2):393–402

Masuda K, Fujita N, Yang HW, Ushijima K, Kubo Y, Tao R, Akagi T (2020b) Molecular mechanism underlying derepressed male production in hexaploid persimmon. Front Plant Sci 11

Masuda K, Yamamoto E, Shirasawa K, Onoue N, Kono A, Ushijima K, Kubo Y, Tao R, Henry IM, Akagi T (2020c) Genome-wide study on the polysomic genetic factors conferring plasticity of flower sexuality in hexaploid persimmon. DNA Res 27(3): dsaa012

Masuda K, Ikeda Y, Matsuura T, Kawakatsu T, Tao R, Kubo Y, Ushijima K, Henry IM, Akagi T (2022) Reinvention of hermaphroditism via activation of a RADIALIS-like gene in hexaploid persimmon. Nat Plants 8 (3):217–224

McDaniel JC (1973a) Persimmon cultivars for northern areas. Fruit Var Hortic Dig

McDaniel JC (1973b) American persimmon, on emerging horticultural crop. Fruit Var Hort Dig

Ming R, Wang J, Moore PH, Paterson AH (2007) Sex chromosomes in flowering plants. Am J Bot 94 (2):141–150

Ming R, Bendahmane A, Renner SS (2011) Sex chromosomes in land plants. Annu Rev Plant Biol 62:485–514

Müller NA, Kersten B, Montalvão APL, Mähler N, Bernhardsson C, Bräutigam K, Lorenzo ZC, Hoenicka H, Kumar V, Mader M, Pakull B, Robinson KM, Sabatti M, Vettori C, Ingvarsson PK, Cronk Q, Street NR, Fladung M (2020) A single gene underlies the dynamic evolution of poplar sex determination. Nat Plants 6(6):630–637

Negi SS, Olmo HP (1966) Sex conversion in a male *Vitis vinifera* L. by a kinin. Science 152(3729):1624–1624

Ono T (1935) Chromosomen und sexualital von Rumex acetosa. Sci Rep Tohoku Imp Univ Ser 4(10):4–210

Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A (2009) Agroforestree Database: a tree reference and selection guide. Version 4

Ricker M, Siebe C, Sánchez S, Shimada K, Larson BC, Martínez-Ramos M, Montagnini F (2000) Optimising seedling management: Pouteria sapota, *Diospyros digyna*, and Cedrela odorata in a Mexican rainforest. For Ecol Manag 139(1–3):63–77

Song Q, Chen ZJ (2015) Epigenetic and developmental regulation in plant polyploids. Curr Opin Plant Biol 24:101–109

Sun P, Li J, Du G, Han W, Fu J, Diao S, Suo Y, Zhang Y, Li F (2017) Endogenous phytohormone profiles in male and female floral buds of the persimmons (*Diospyros kaki* Thunb.) during development. Sci Hortic 218:213–221

Suo Y, Sun P, Cheng H, Han W, Diao S, Li H, Mai Y, Zhao X, Li F, Fu J (2020) A high-quality chromosomal genome assembly of *Diospyros oleifera* Cheng. GigaScience 9(1):giz164

Wang J, Na JK, Yu Q, Gschwend AR, Han J, Zeng F, Aryal R, VanBuren R, Murray JE, Zhang W, Navajas-Perez R, Feltus FA, Lemke C, Tong EJ, Chen C, Wai CM, Singh R, Wang M, Min XJ, Alam M, Charlesworth D, Moore PH, Jiang J, Paterson AH, Ming R (2012) Sequencing papaya X and Yh chromosomes reveals molecular basis of incipient sex chromosome evolution. Proc Natl Acad Sci USA 109(34):13710–13715

Wang L, Li H, Sun P, Fu J, Suo Y, Zhang J, Han W, Diao S, Li F, Mai Y (2018) Genetic diversity among wild androecious germplasms of *Diospyros kaki* in China based on SSR markers. Sci Hortic 242:1–9

Wang L, Li H, Suo Y, Han W, Diao S, Mai Y, Sun P, Li F, Fu J (2020) Programmed cell death facilitates the formation of unisexual male and female flowers in persimmon (*Diospyros kaki* Thunb.). Agronomy 10 (2):234

Westergaard M (1958) The mechanism of sex determination in dioecious flowering plants. Adv Genet 9:217–281

Yakushiji H, Yamada M, Yonemori K, Sato A, Kimura N (1995) Staminate flower production on shoots of 'Fuyu' and 'Jiro' persimmon (*Diospyros kaki* Thunb.). J Jpn Soc Hort Sci 64(1):41–46

Yang HW, Akagi T, Kawakatsu T, Tao R (2019) Gene networks orchestrated by *MeGI*: a single-factor mechanism underlying sex determination in persimmon. Plant J 98(1):97–111

Yasui K (1915) Studies of Diospyros kaki. I. Bot Gaz 60 (5):362–373

Yonemori K, Yomo Y, Sugiura A (1990) Sexuality in Japanese persimmons. 2. Induction of sex conversion in male flower by cytokinin treatment. J Jpn Soc Hort Sci 59:230–231

Yonemori K, Kameda K, Sugiura A (1992) Characteristics of sex expression in monoecious persimmons. J Jpn Soc Hort Sci 61(2):303–310

Yonemori K, Sugiura A, Tanaka K, Kameda K (1993) Floral ontogeny and sex determination in monoecious-type persimmons. J Am Soc Hortic Sci 118(2):293–297

Zhang PX, Yang SC, Liu YF, Zhang QL, Xu LQ, Luo ZR (2016) Validation of a male-linked gene locus (*OGI*) for sex identification in persimmon (*Diospyros kaki* Thunb.) and its application in F1 progeny. Plant Breed 135(6):721–727

9 Sex Expression in Chinese Persimmons

Peng Sun and Jianmin Fu

Abstract

Sexual types of *Diospyros* spp. are diverse. Here, monoecious, androgynomonoecious, androecious, and andromonoecious *Diospyros* spp. trees, which can bear male flowers, are collectively defined as male *D.* spp. germplasm resources (MDGR). In this chapter, the descriptors and data standards for the evaluation of MDGRs were firstly improved. Then 14 representative MDGRs were introduced in detail with both texts and pictures. Finally, the differentiation mechanism of hermaphroditic floral buds of a special andromonoecious *D. kaki* tree was highlighted. The third part uncovered that early (Stage 2) and mid-April (Stage 4) were critical morphological periods for sex differentiation of hermaphroditic floral buds compared with male floral buds. At both stages, *OGI* was differentially expressed in male and hermaphroditic buds, but *MeGI* was not. This was different from their expressions in dioecious and monoecious persimmons. High jasmonic acid (JA) levels at stage 4 and high zeatin (ZT) levels at stages 2 and 4 might have promoted hermaphroditic floral bud differentiation. In phytohormone biosynthesis and signaling pathways, 52 and 54 differential expression genes (including *Aux/IAA*, *ARFs*, *DELLA*, *AHP*, *A-ARR*, *B-ARR*, *CYP735A*, *CRE1*, *PP2C*, *JAZ*, *MYC2*, *COI1*, *CTR1*, *SIMKK*, *ACO*, and *MPK6*) were identified, respectively. During the development of male floral buds, five metacaspases genes might have been involved in pistil abortion. Noteworthy, miR169v_1, miR169e_3, miR319_1, and miR319 were predicted to contribute to phytohormone biosynthesis and signaling pathways and floral organogenesis and might also regulate hermaphroditic floral bud sex differentiation.

9.1 Introduction

Persimmon (*Diospyros kaki* Thunb.) is a hexaploid species and is an important temperate fruit tree species, which originated in East Asia. Persimmon cultivars were classified into two types:

P. Sun · J. Fu (✉)
Key Laboratory of Non-timber Forest Germplasm Enhancement and Utilization of State Administration of Forestry and Grassland, No. 3 Weiwu Road, Jinshui District 450003, Zhengzhou, China
e-mail: fjm371@163.com

P. Sun
e-mail: sunpeng1017@126.com

P. Sun · J. Fu
Research Institute of Non-timber Forestry, Chinese Academy of Forestry, No. 3 Weiwu Road, Jinshui District 450003, Zhengzhou, China

National Innovation Alliance of Persimmon Industry, No. 3 Weiwu Road, Jinshui District 450003, Zhengzhou, China

R. Tao and Z. Luo (eds.), *The Persimmon Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-05584-3_9

pollination-constant non-astringent (PCNA) and non-PCNA. The former includes Chinese PCNA (CPCNA) and Japanese PCNA (JPCNA). The latter includes pollination-variant non-astringent (PVNA), pollination-variant astringent (PVA), and pollination-constant astringent (PCA) types (Akagi et al. 2011; Xu et al. 2016).

Persimmon has been cultivated in China for a long history. The earliest documentation recording persimmon is "the Book of Rites · Nei Ze", written by Si Zi, grandson of Confucius, indicating persimmon was edible in China 2500 years ago.

In the long history of persimmon cultivation, a lot of representative cultivars were established in different areas in China, such as "Fu-ping-jian-shi", "Huo-jing-shi", "Ji-xin-huang-shi" in Shaanxi Province; "Bo-ai-ba-yue-huang-shi", "Xing-yang-shui-shi", "Yan-guo-hong-shi" in Henan Province; "Xiao-e-zi-shi", "Jin-ping-shi" in Shandong Province; "Luo-tian-tian-shi", "Gan-mao-kui" in Hubei Province; and "Mo-pan-shi" in Hebei Province. It is reported that there are more than 900 persimmon cultivars in China (Luo and Cai 1998). Most cultivars are gynoecious (bearing only female flowers), and a few cultivars are monoecious (bearing both female and male flowers) and androgynomonoecious (bearing female, male, and hermaphroditic flowers). Androecious (bearing only male flowers) and andromonoecious (bearing both hermaphroditic and male flowers) types were just occasionally found in wild persimmon germplasm resources (WPGR) (Fu et al. 2017). The WPGR are distributed in remote mountainous and high-elevation areas without domestication. Fruits of the WPGR were generally smaller than persimmon cultivars and always have more seeds. The flavor of the WPGR fruits is not as good as the cultivars (Flora Committee of China, Chinese Academy of Sciences 1987).

In this present chapter, monoecious, androgynomonoecious, androecious, and andromonoecious *Diospyros* spp. trees, which can bear male flowers, were collectively defined as male *D*. spp. germplasm resources (MDGR). The superior MDGR can be used as a pollen donor in crossing breeding, and are very valuable for the improvement of persimmon cultivars. The purpose of this chapter includes three aspects. Firstly, the improved descriptors and data standard for the evaluation of MDGRs were introduced. Then 14 representative MDGRs will be introduced in detail with both texts and pictures. Finally, the differentiation mechanism of hermaphroditic floral buds of a special andromonoecious *D. kaki* tree is highlighted.

9.2 Descriptors and Data Standard for MDGR

Twenty traits were recorded to describe the morphological characteristics of MDGRs. Traits 1 ∼ 10 were absolutely in accordance with that described in <Descriptors and Data Standard for Persimmon (*Diospyros* spp.)> (Yang et al. 2006), including 1 arrangement of corolla, 2 corolla shape before blooming, 3 uniformity of stamen, 4 connection status of base parts of stamen, 5 sepal number, 6 sepal color, 7 sepal posture, 8 uniformity of sepals, 9 sepal shape, and 10 connection status of sepals. The descriptors and data standard for the remaining ten traits were shown below.

11 Corolla tip color of male flowers before blooming

See Fig. 9.1.

Fig. 9.1 Corolla tip color of male flowers before blooming

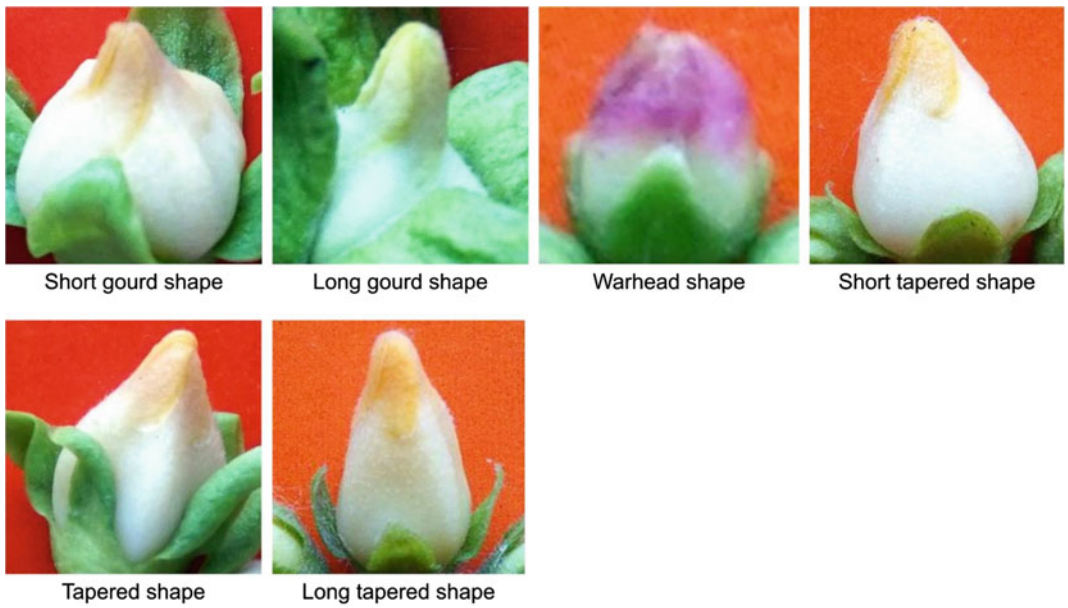

Fig. 9.2 Corolla tip shape of male flowers before blooming

12 Corolla tip shape of male flowers before blooming

See Fig. 9.2.

13 Color of middle and lower parts of corolla

See Fig. 9.3.

14 Color uniformity of middle and lower parts of corolla

See Fig. 9.4.

Fig. 9.3 Color of middle and lower parts of corolla

Fig. 9.4 Color uniformity of middle and lower parts of corolla

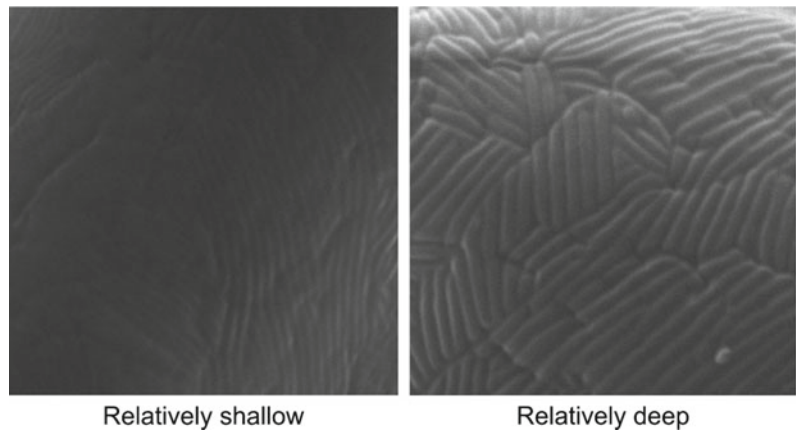

Fig. 9.5 Depth of stripes on pollen surface observed under a scanning electron microscope

15 Average stamen number inside a male flower

High level: 21 ~ 24; Relatively high level: 18 ~ 20; Medium level: 14 ~ 17; Low level: 10 ~ 13.

16 Depth of stripes on pollen surface observed under a scanning electron microscope

See Fig. 9.5.

17 Interleaved degree of the stripe on pollen surface

See Fig. 9.6.

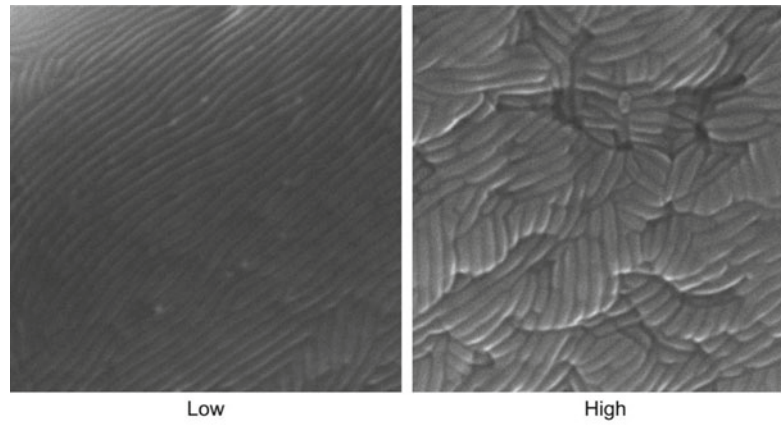

Fig. 9.6 Interleaved degree of the stripe on pollen surface

18 The number of male flowers on an inflorescence

19 Length of inflorescence stalks

Average length of inflorescence stalks: Long: 8.01 ~ 14.00 mm; Relatively long: 6.31 ~ 8.00 mm; Relatively short: 2.51 ~ 5.50 mm; Short: 1.00 ~ 2.50 mm.

20 Length of male flower stalks

Average length of male flower stalks: Long: 8.01 ~ 14.00 mm; Relatively long: 5.51 ~ 8.00 mm; Relatively short: 2.51 ~ 5.50 mm; Short: 0.50 ~ 2.50 mm.

9.3 Introduction of 14 Representative MDGRs

D. lotus No. 1

An androecious *D. lotus* tree named "*D. lotus* No. 1" was originated in Shaanxi Province. The scion was shared by the "National Field Genebank for Persimmon (NFGP)", Yangling, Shaanxi Province, and grafted in an experimental field in Yuanyang County, Henan Province, China (34° 55.30′–34° 56.45′ N, 113° 46.24′–113° 47.59′ E), which belongs to Non-timber Forestry Research and Development Center, Chinese Academy of Forestry. The corolla tip of male flower is purplish-red with a warhead shape. The middle and lower parts of corolla in the male flower are creamy white with uniform color, and slightly overlapped. The corolla is in a jar shape before blooming. Stamens inside a male flower grow neatly with the base parts slightly connected. The number of stamens in a male flower is at a medium level. Sepals are green with a heart shape. They are separately and neatly grown and straight in posture. Both male inflorescence and flower stalks are short. The pollen surface has relatively shallow stripes with a high interleaved degree (Fig. 9.7).

D. lotus No. 7

An androecious *D. lotus* tree named "*D. lotus* No. 7" was originated in Henan Province and deposited in the Yuanyang experimental field. The corolla tip of male flower is rosy with a warhead shape. The middle and lower parts of corolla in the male flower are creamy white with uniform color and slightly overlapped. The corolla is in a jar shape before blooming. Stamens inside a male flower grow neatly with the base parts slightly connected. The number of stamens in a male flower is at a medium level. Sepals are green with a heart shape. They are separately and neatly grown, and straight in posture. Both male inflorescence and flower stalks are short (Fig. 9.8).

D. glaucifolia No. 1

An androecious *D. glaucifolia* tree named "*D. glaucifolia* No. 1" was originated in Zhejiang Province. The scion was shared by the NFGP and grafted in the Yuanyang experimental field. The

Fig. 9.7 *D. lotus* No. 1

corolla tip of male flower is purplish red with a warhead shape. The middle and lower parts of corolla in the male flower is creamy white with uniform color and separated. The corolla is in a vase shape before blooming. Stamens inside a male flower grow neatly with the base parts slightly connected. The number of stamens in a male flower is at a medium level. Sepals are green with a heart shape. They are slightly connected and neatly grown and straight in posture. Both male inflorescence and flower stalks are short. The pollen surface has relatively shallow stripes with a high interleaved degree (Fig. 9.9).

D. oleifera No. 1

A monoecious *D. oleifera* tree named "*D. oleifera* No. 1" was originated in Zhejiang Province. The scion was shared by the NFGP and grafted in the Yuanyang experimental field. There are usually three male flowers that bear on an inflorescence, while occasionally 4, 5, 6, or 7 male flowers bear on an inflorescence. The corolla tip of male flower is yellow with a tapered shape. The middle and lower parts of corolla in the male flower are creamy white with uniform color and slightly overlapped. The corolla is in a cylinder shape with four arises before blooming.

Fig. 9.8 *D. lotus* No. 7

Stamens inside a male flower grow neatly with the base parts slightly connected. The number of stamens in a male flower is at a high level. Sepals are green with a heart shape. They are slightly connected and neatly grown and straight in posture. Male inflorescence stalks are long and flower stalks are short. The pollen surface has relatively shallow stripes with a high interleaved degree (Fig. 9.10).

Guangxi No. 51

A monoecious *D. oleifera* tree named "Guangxi No. 51" was originated in Guangxi Zhuang autonomous region. It is grafted and deposited in the Yuanyang experimental field. The corolla tip of male flower is light yellow with a tapered shape. The middle and lower parts of corolla in the male flower are creamy yellow with uniform color and separated. The corolla is in a cylinder shape with four arises before blooming. Stamens inside a male flower grow neatly with the base parts slightly connected. The number of stamens in a male flower is at a high level. There are usually four sepals in a male flower, while occasionally five or six sepals in a male flower. Sepals are green with a heart shape. They are slightly connected and neatly grown, and straight in posture. Male inflorescence stalks are relatively short and flower stalks are short (Fig. 9.11).

"Zhong-shi No. 4"

A new monoecious PCNA *D. kaki* cultivar named "Zhong-shi No. 4" was bred by Non-timber Forestry Research and Development Center, Chinese Academy of Forestry. It is grown in the Yuanyang experimental field. The tree is 1.9 m in height and with a diameter at the position of 1.2 m height is 2.7 cm. Female (pistillate) flowers of this tree are solitary and larger than male flowers (staminate), which are organized in a three-flower cyme. The corolla tip of male flower is light yellow with a long tapered shape. The middle and lower parts of corolla in

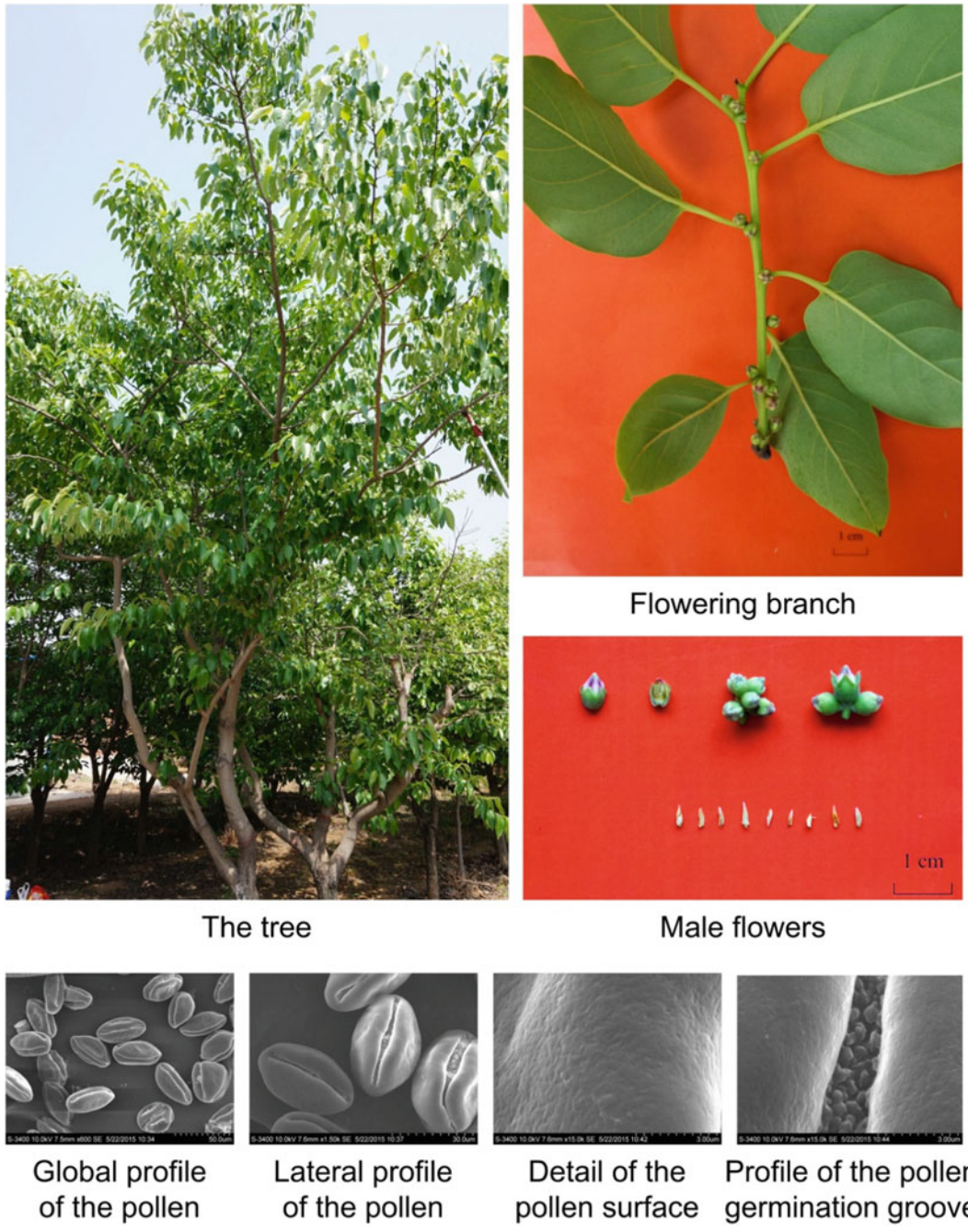

Fig. 9.9 *D. glaucifolia* No. 1

the male flower are creamy white with uniform color and slightly overlapped. The corolla is relatively larger than other MDGRs and in a bottle shape before blooming. Stamens inside a male flower grow neatly with the base parts slightly connected. The number of stamens in a male flower is at a medium level. Sepals are green with a flat heart shape. They are separately

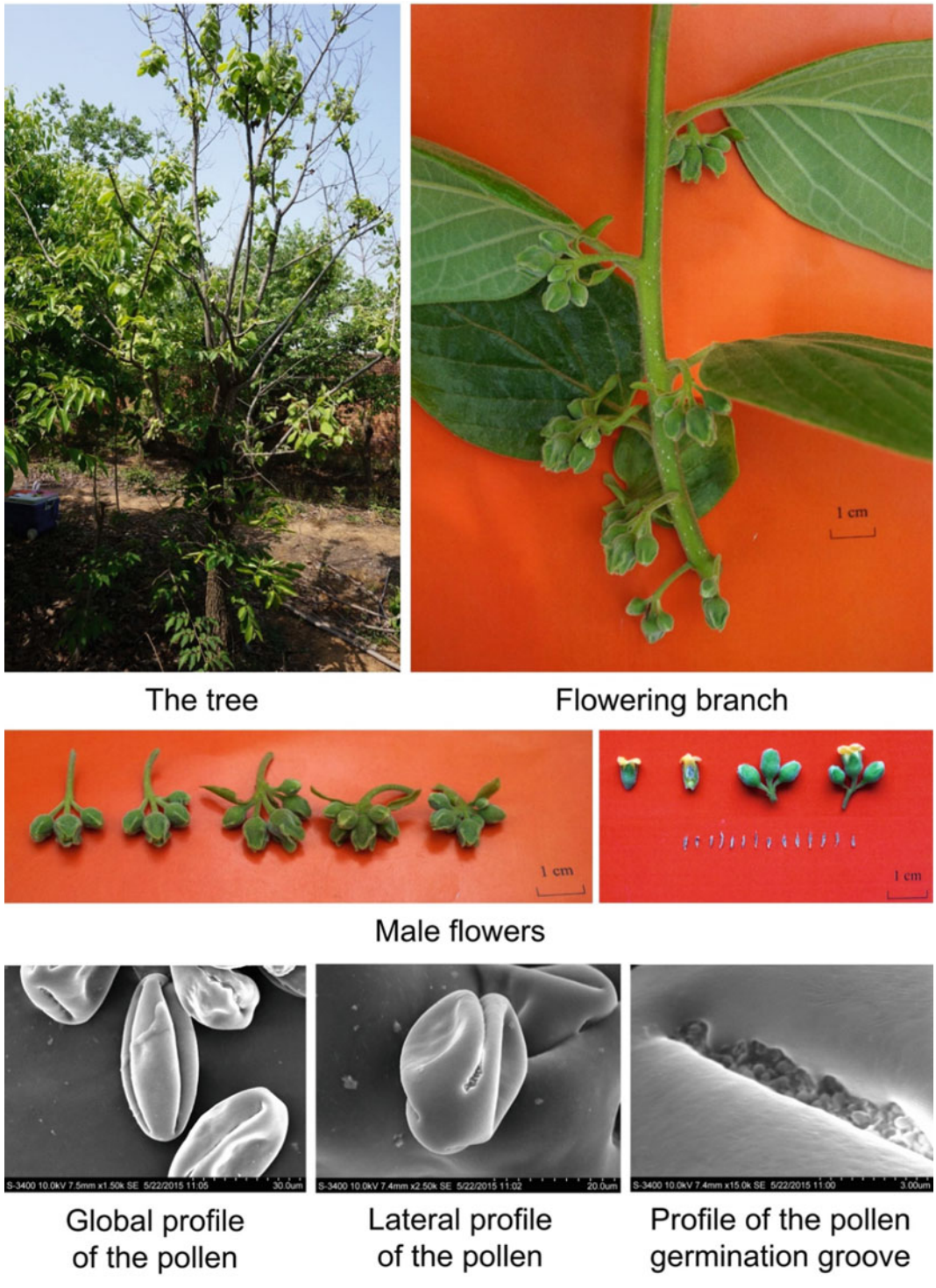

Fig. 9.10 *D. oleifera* No. 1

Fig. 9.11 *D. oleifera* "Guangxi No. 51"

and neatly grown and curled in posture. Both male inflorescence and flower stalks are relatively longer than other MDGRs. The pollen surface has deep stripes with a high interleaved degree. "Zhong-shi No. 4" is valuable to be used as both maternal and paternal parents for PCNA persimmon breeding (Fig. 9.12).

"Zhong-shi male No. 1"

A new andromonoecious PCNA *D. kaki* cultivar named "Zhong-shi male No. 1" was bred by Non-Timber Forestry Research and Development Center, Chinese Academy of Forestry. It was originally found in the Mulan mountain area, Huangpi District, Wuhan, Hubei Province, China. The tree is 3.2 m in height and with a diameter at the position of 1.2 m height is 3.5 cm. It was grafted and deposited in the Yuanyang experimental field. Flowers are usually organized in three-flower cymes and occasionally in four-flower cymes. The middle ones of the three-flower cymes are occasionally hermaphroditic. The corolla tip of male flower is light yellow with a tapered shape. The middle and lower parts of corolla in the male flower are creamy white with uniform color and slightly overlapped. The corolla is in a jar shape with four arises before blooming. Stamens inside a male flower grow neatly with the base parts slightly connected. The number of stamens in a male flower is at a medium level. Sepals are green with a long triangle shape. They are separately and neatly grown and curled in posture. Male inflorescence stalks are relatively long, and flower stalks are relatively short. The stripes on the pollen surface were shallower, less staggered, and more consistent in direction. "Zhong-shi male No. 1" contains an *RO2* gene, which potentially promotes the natural de-astringency ability in CPCNA. Thus, it is valuable to be used as a paternal parent for PCNA persimmon breeding (Fig. 9.13).

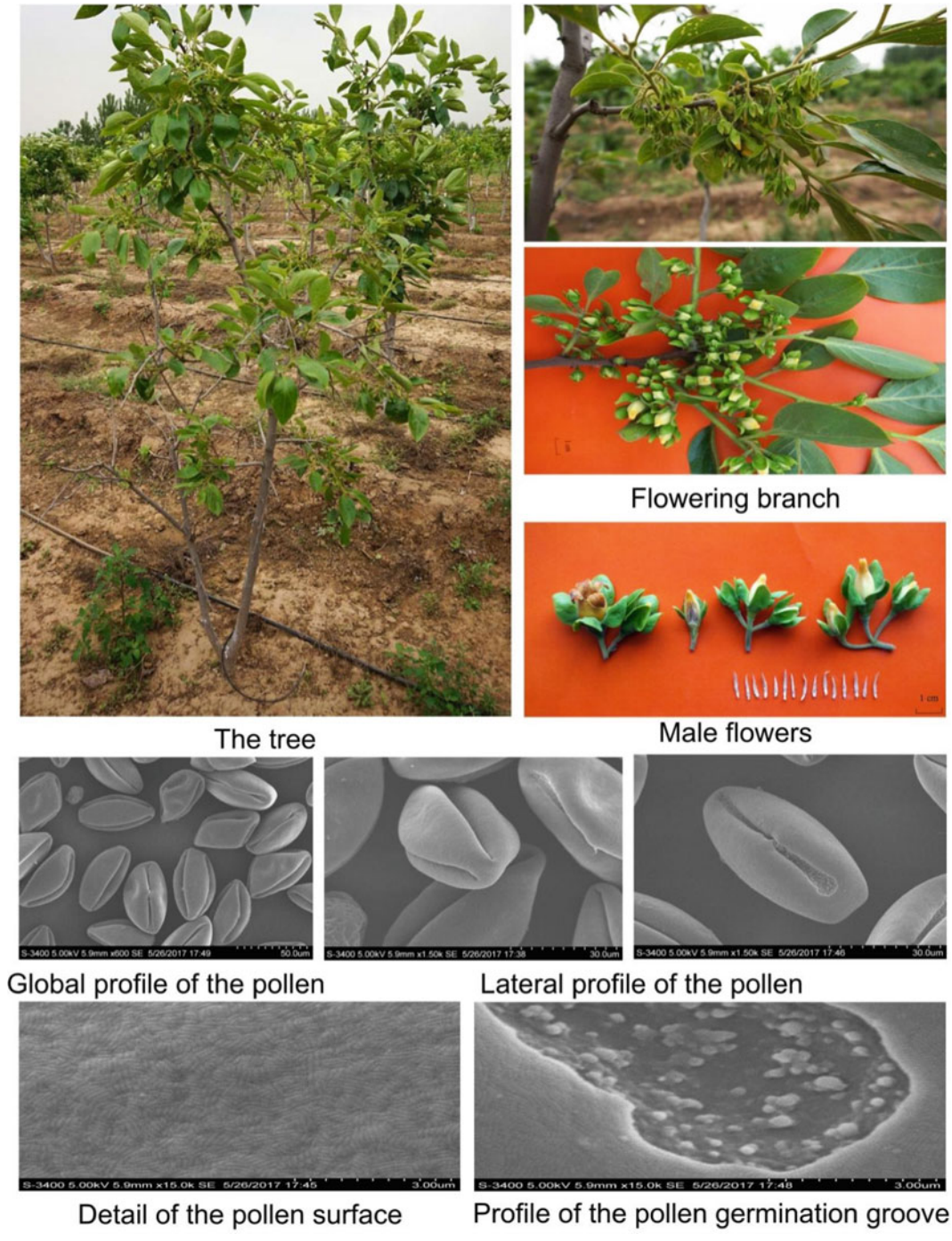

Fig. 9.12 *D. kaki* "Zhong-shi No. 4"

"Xiang-yang-niu-xin-shi"

A monoecious PCA *D. kaki* cultivar named "Xiang-yang-niu-xin-shi" was originated in Xiangyang City, Hubei Province. The scion was shared by the NFGP, and grafted in the Yuanyang experimental field. The corolla tip of male

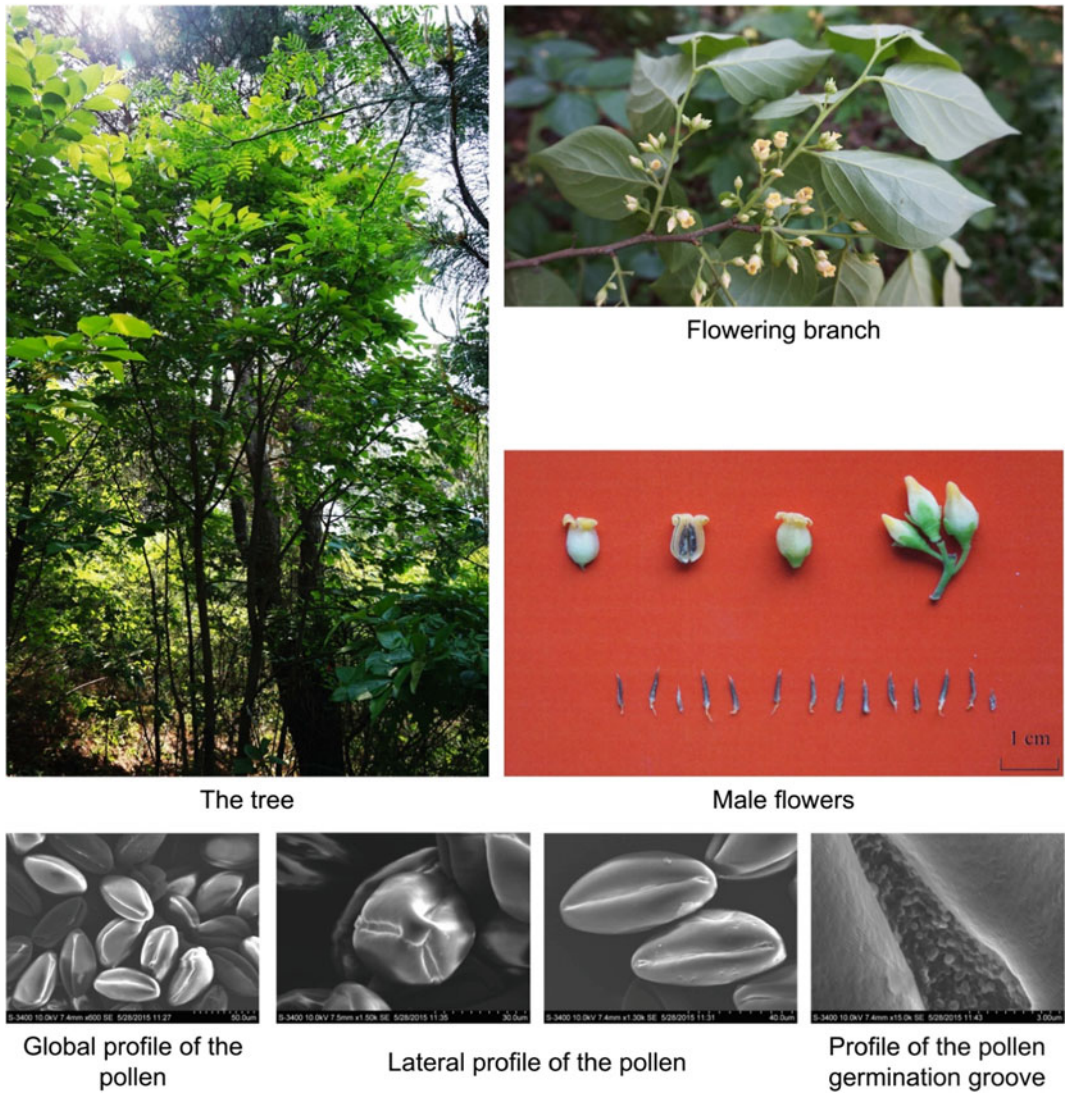

Fig. 9.13 *D. kaki* "Zhong-shi male No. 1"

flower is yellow with a long tapered shape. The middle and lower parts of corolla in the male flower are creamy white and the color is not uniform. The corollas are slightly overlapped. The corolla is in a jar shape with four arises before blooming. Stamens inside a male flower grow neatly with the base parts slightly connected. The number of stamens in a male flower is at a medium level. Sepals are green with a long triangle shape. They are separately and neatly grown, and reflexed in posture. Both male inflorescence and flower stalks are relatively short. The pollen surface has relatively deep stripes with a high interleaved degree (Fig. 9.14).

"Pan-xian-shui-shi"

A monoecious PCA *D. kaki* cultivar named "Pan-xian-shui-shi" was originated in Guizhou Province. The scion was shared by the NFGP and grafted in the Yuanyang experimental field. The corolla tip of male flower is yellow with a long tapered shape. The middle and lower parts of

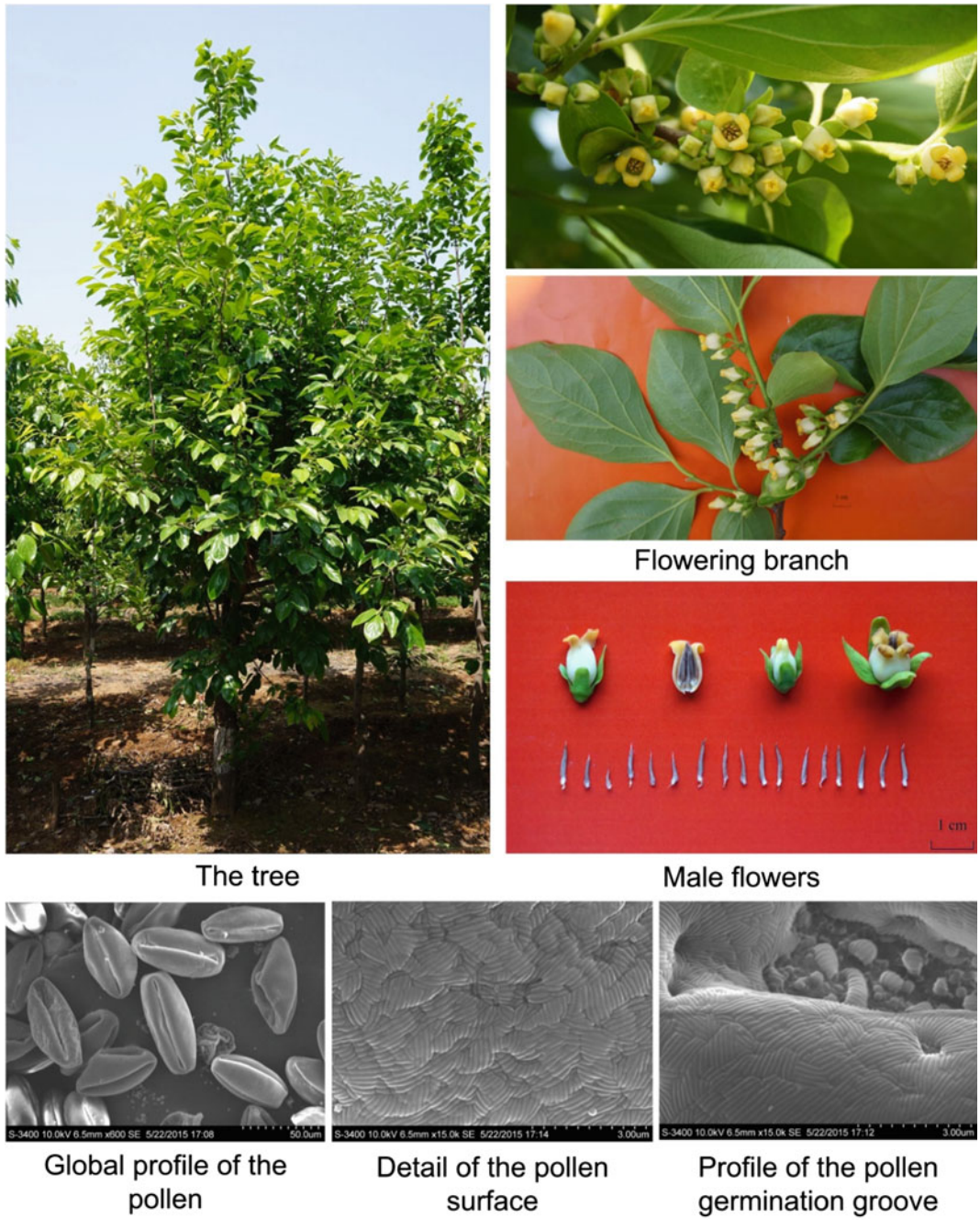

Fig. 9.14 *D. kaki* "Xiang-yang-niu-xin-shi"

corolla in the male flower are light pink and the color is not uniform. The corollas are slightly overlapped. The corolla is in a cylinder shape with four arises before blooming. Stamens inside a male flower grow neatly with the base parts slightly connected. The number of stamens in a male flower is at a medium level. Sepals are green with a long triangle shape. They are

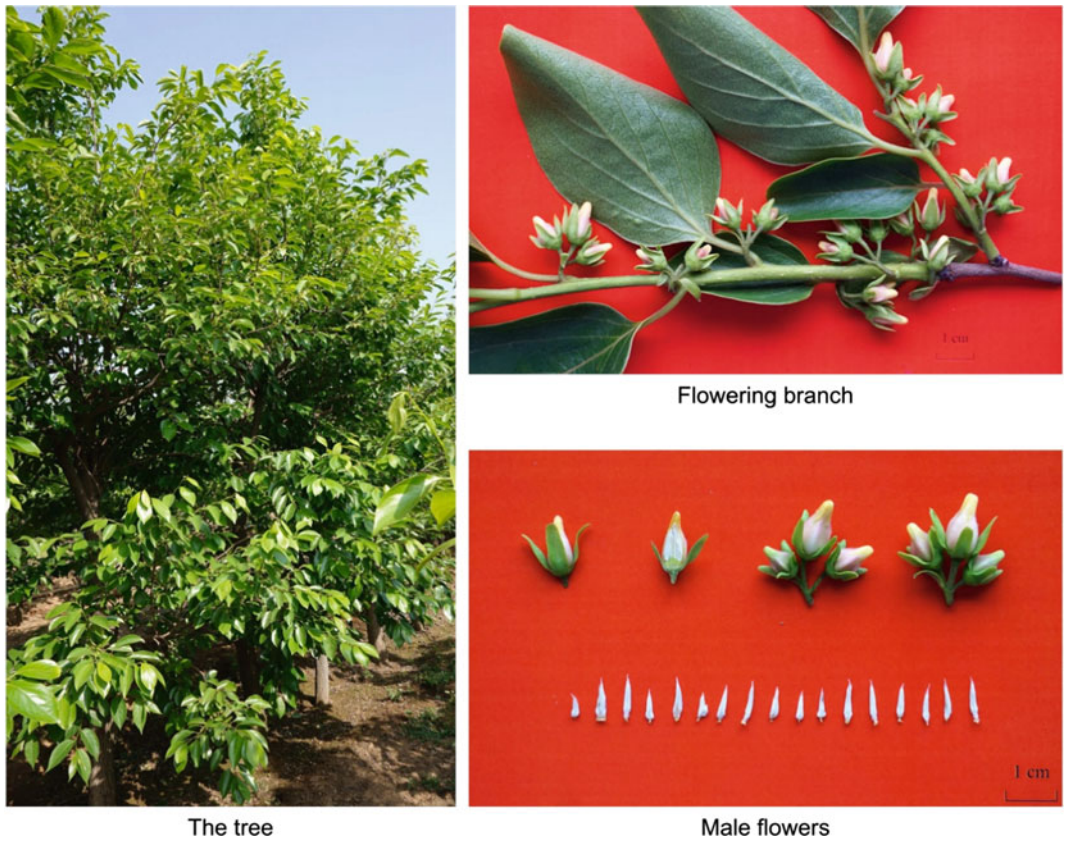

Fig. 9.15 *D. kaki* "Pan-xian-shui-shi"

separately and neatly grown and reflexed in posture. Male inflorescence stalks are relatively short and flower stalks are relatively long (Fig. 9.15).

"Tai-wan-zheng-shi"

A monoecious PCA *D. kaki* cultivar named "Tai-wan-zheng-shi" was originated in Taiwan Province. The scion was shared by the NFGP and grafted in the Yuanyang experimental field. The corolla tip of male flower is light yellow with a long gourd shape. The middle and lower parts of corolla in the male flower and creamy white with uniform color and slightly overlapped. The corolla is in a cylinder shape with four arises before blooming. Stamens inside a male flower grow neatly with the base parts slightly connected. The number of stamens in a male flower is at a medium level. Sepals are green with a long triangle shape. They are separately and not neatly grown, and reflexed in posture. Male inflorescence and flower stalks are relatively long. The pollen surface has relatively deep stripes with a high interleaved degree (Fig. 9.16).

"Yao-xian-wu-hua-shi"

An androgynomonoecious PCA *D. kaki* cultivar named "Yao-xian-wu-hua-shi" was originated in Shaanxi Province. The scion was shared by the NFGP, and grafted in the Yuanyang experimental field. The corolla tip of male flower is yellow with a long tapered shape. The middle and lower parts of corolla in the male flower are creamy white, and the color is not uniform. The corollas are slightly overlapped. The corolla is in a jar shape with four arises before blooming. Stamens inside a male flower grow neatly with the base parts slightly connected. The number of stamens in a male flower is at a medium level. Sepals are green with a heart shape. They are separately and

Fig. 9.16 *D. kaki* "Tai-wan-zheng-shi"

not neatly grown, and reflexed in posture. Male inflorescence and flower stalks are relatively long. The pollen surface has relatively deep stripes with a high interleaved degree (Fig. 9.17).

Jiangsu wild persimmon 2-9

An androecious *D. kaki* germplasm named "Jiangsu wild persimmon 2-9" was originated in Jiangsu Province. The tree is 1.3 m in height and with a diameter at the position of 1.2 m height is 1.7 cm. It is grafted and deposited in the Yuanyang experimental field. The corolla tip of male flower is light yellow with a tapered shape. The middle and lower parts of corolla in the male flower are creamy white with uniform color and separated. The corolla is relatively large and in a jar shape before blooming. Stamens inside a male flower grow neatly with the base parts slightly

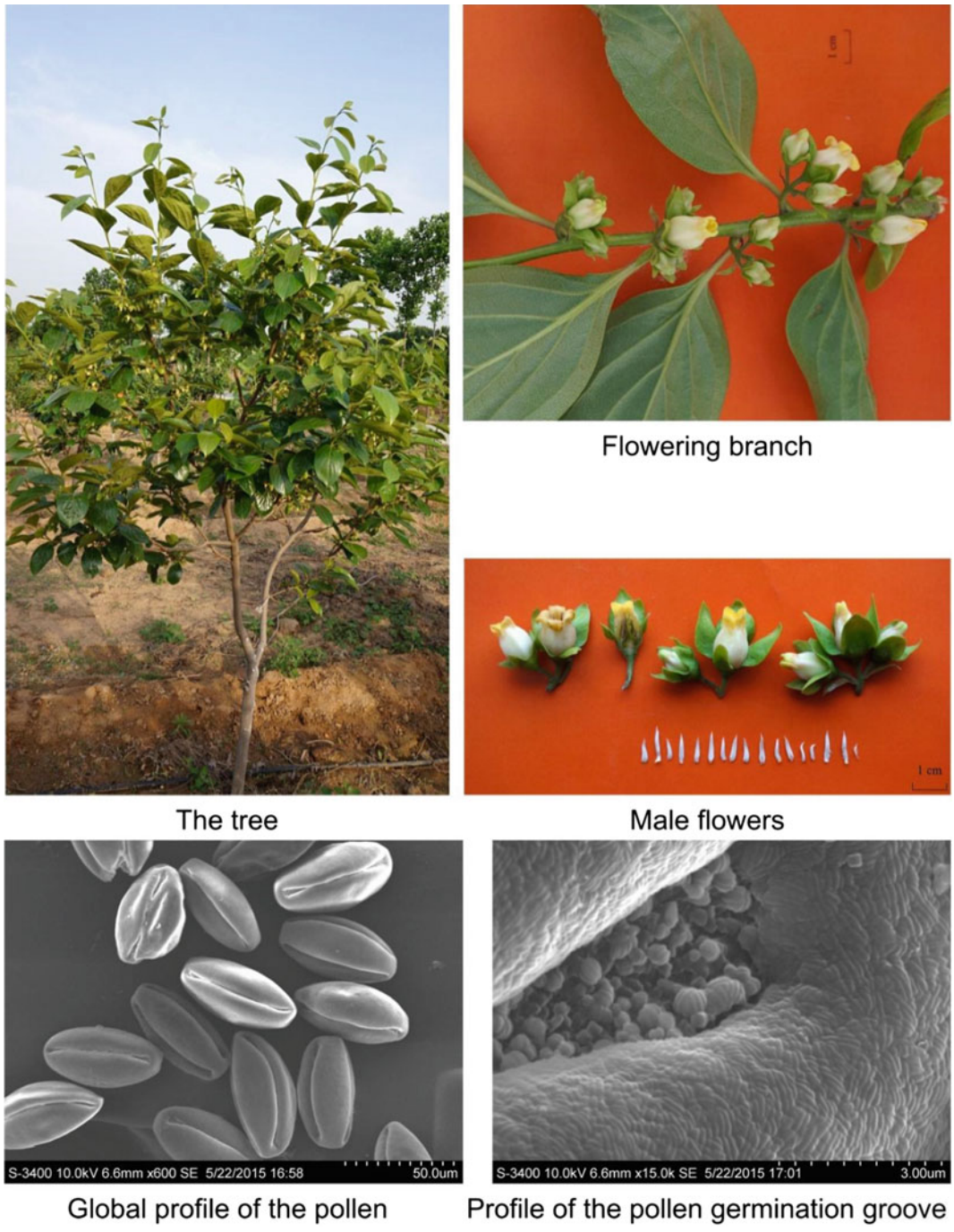

Fig. 9.17 *D. kaki* "Yao-xian-wu-hua-shi"

connected. The number of stamens in a male flower is at a relatively high level. Sepals are green with a long triangle shape. They are separately and neatly grown and straight in posture. Male inflorescence stalks are relatively short and flower stalks are relatively long. The pollen surface has relatively deep stripes with a high interleaved degree (Fig. 9.18).

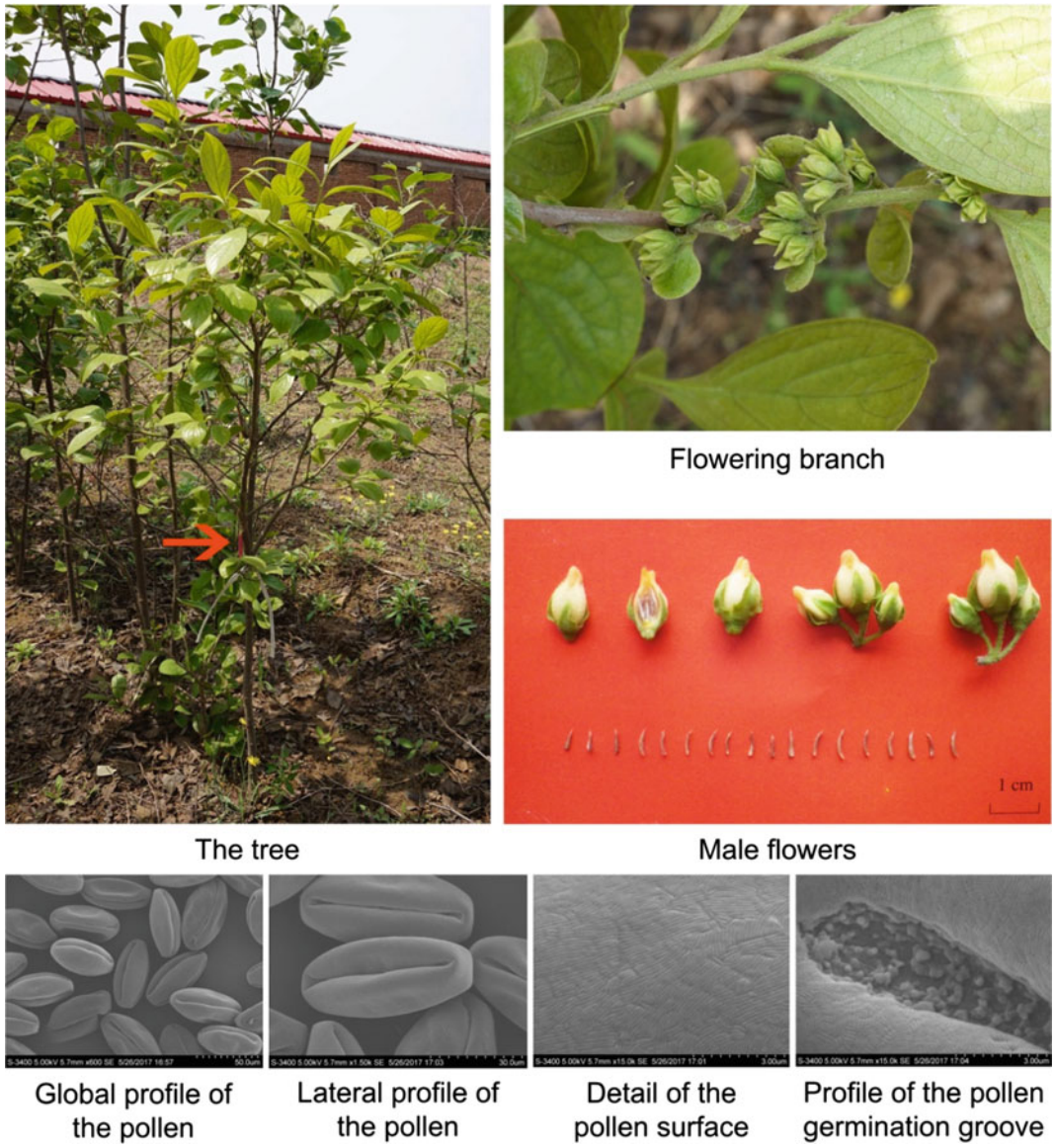

Fig. 9.18 *D. kaki* "Jiangsu wild persimmon 2-9"

Yunjiashan wild persimmon 20

An androecious *D. kaki* germplasm named "Yunjiashan wild persimmon 20" was originated in Luotian County, Hubei Province. The tree is 2.2 m in height and with a diameter at the position of 1.2 m height is 2.3 cm. It is grafted and deposited in the Yuanyang experimental field. The corolla tip of male flower is light yellow with a short tapered shape. The middle and lower parts of corolla in the male flower are creamy white with uniform color and slightly overlapped. The corolla is in a cylinder shape with four arises before blooming. Stamens inside a male flower grow neatly with the base parts slightly connected. The number of stamens in a male flower is at a medium level. Sepals are green with a long triangle shape. They are separately and neatly grown and straight in posture. Male inflorescence and flower stalks are relatively long (Fig. 9.19).

The tree

Flowering branch

Male flowers

Fig. 9.19 *D. kaki* "Yunjiashan wild persimmon 20"

Longyan wild persimmon No. 1

An andromonoecious PCA *D. kaki* "Longyan Yeshi No. 1" was a 6-year-old seedling growing in the Yuanyang experimental field, and it was cultivated from the seed of a wild *D. kaki* obtained in Longyan City, Fujian Province, Southeast China. Its floral buds were organized in three-flower cymes. The middle ones of the three-flower cymes were found being hermaphroditic types with a probability of over 80%, while the lateral two were absolutely found being male flowers. The tree is 3.6 m in height and with a diameter at the position of 1.2 m height is 6.5 cm. The corolla tip of male flower is yellow with a tapered shape. The middle and lower parts of corolla in the male flower are creamy white with uniform color, and slightly overlapped. The corolla is in a jar shape with four arises before blooming. Stamens inside a male flower grow neatly with the base parts slightly connected. The number of stamens in a male flower is at a medium level. Sepals are green with a long triangle shape. They are separately and neatly grown, and shrunk-and-rolled in posture. Male inflorescence stalks are relatively long and flower stalks are relatively short (Fig. 9.20).

The sexuality of this tree is special, and the differentiation mechanism of hermaphroditic floral buds of this tree is highlighted below.

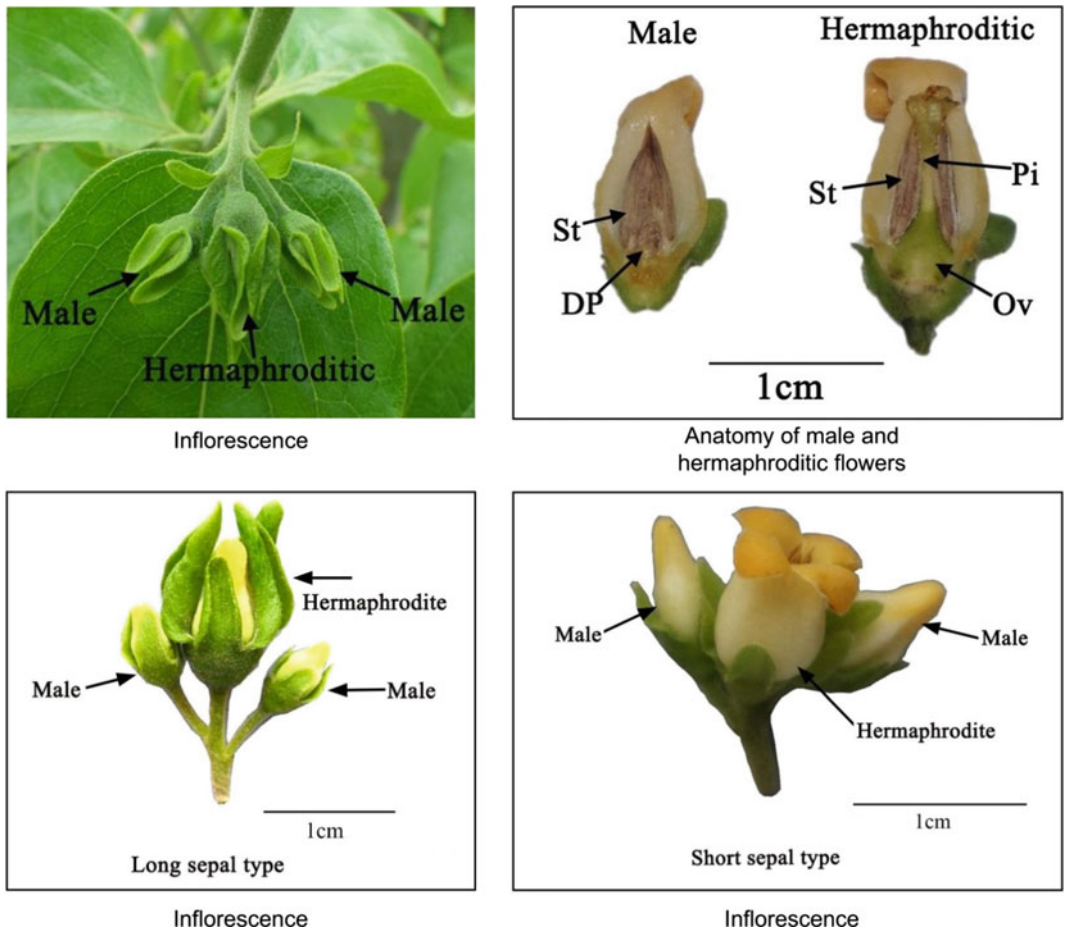

Fig. 9.20 *D. kaki* "Longyan wild persimmon No. 1"

9.4 Phytohormone and Integrated mRNA and miRNA Transcriptome Analyses of Regulatory Mechanisms Underlying Sexual Differentiation of Andromonoecious *Diospyros kaki* Thunb.

The "Longyanyeshi 1" persimmon (*Diospyros kaki*) tree was a 6-year-old seedling growing in the Yuanyang experimental field and it was cultivated from the seed of a wild *D. kaki* obtained in Longyan City, Fujian Province, Southeast China. The plant was an andromonoecious (with hermaphroditic and male flowers) persimmon found by Dr. Fu and her research team (Li et al. 2019a).

Several previous studies have elucidated sex differentiation in monoecious and dioecious persimmons (Akagi et al. 2014, 2016; Li et al. 2019b). Nevertheless, the sex regulation mechanism in the andromonoecious persimmon is unknown. Here, the male and hermaphroditic floral buds of "Longyanyeshi 1", which were in the critical morphological periods for sex differentiation, were used for phytohormone assays and mRNA and small RNA transcriptome analyses to identify the regulatory roles of phytohormone, candidate genes, and miRNAs in sex differentiation of andromonoecious persimmon.

The bud scales of the "Longyanyeshi 1" persimmon tree in Yuanyang County, Henan Province, loosened and turned green on March 28. As the floral buds grew and developed, three-flower cymes were fully exposed by April 6. The floral bud sepals everted by April 14. Between April 14 and April 20, the floral buds expanded and grew but did not change in appearance. On April 23, the sepals opened and yellow-white petals appeared. The floral buds bloomed on May 3 (Li et al. 2021).

Four representative stages were selected to describe the internal morphological differences between male and hermaphroditic floral buds. Stage 2 (April 1–6) and 4 (April 17–20) were crucial for the differentiation of male and hermaphroditic floral buds (Li et al. 2021). At stage 2, the ovary primordia and ovules were absent in the carpels in male floral buds, whereas they existed in hermaphroditic floral buds. At stage 4, the carpels aborted in male floral buds, which were distinct from hermaphroditic floral buds (Li et al. 2021).

Transcription factor (TF) *MeGI* was reported to promote the development of female floral buds and repress the male floral buds (Akagi et al. 2014, 2016) in *D. lotus* (Akagi et al. 2014) and *D. kaki* (Akagi et al. 2016). This gene was significantly upregulated in female floral buds compared with male in *D. kaki* in April (Li et al. 2019b). However, the expression levels of *MeGI* between hermaphroditic and male floral buds were not significantly different in both early (stage 2) and mid (stage 4)-April, indicating new mechanisms responsible for the sexuality of andromonoecious persimmon remained to be solved (Li et al. 2021). Furthermore, we found that (1) the upregulation of indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellin 3 (GA_3), and JA at stage 2 may have promoted male floral bud differentiation, whereas the upregulation of jasmonic acid (JA) at stage 4 and zeatin (ZT) at stages 2 and 4 may have promoted hermaphroditic floral bud differentiation; (2) in these phytohormone biosynthesis and signaling pathways, 52 and 54 differential expression genes (including *Aux/IAA*, *ARFs*, *DELLA*, *AHP*, *A-ARR*, *B-ARR*, *CYP735A*, *CRE1*, *PP2C*, *JAZ*, *MYC2*, *COL1*, *CTR*1, *SIMKK*, *ACO*, and *MPK*6) were identified between hermaphroditic and male floral buds at stages 2 and 4, respectively; (3) 95 and 183 TFs were differentially expressed at stages 2 and 4, respectively, and *MYB*, *FAR1*, *bHLH*, *WRKY*, and *MADS* might play important roles in persimmon floral bud sex differentiation; (4) five metacaspases might perform vital functions in "Longyanyeshi 1" male floral bud development by suppressing pistil primordia at stage 4; (5) integrated miRNA–mRNA analyses showed that several miRNAs involved in phytohormone biosynthesis and signaling pathways and floral organogenesis could regulate floral bud sex differentiation. This study laid an empirical

foundation for ongoing investigations of floral bud sex differentiation in andromonoecious persimmon (Li et al. 2021).

References

Akagi T, Katayama-Ikegami A, Yonemori K (2011) Proanthocyanidin biosynthesis of persimmon (*Diospyros kaki* Thunb.) fruit. Sci Hortic 30:373–380. https://doi.org/10.1016/j.scienta.2011.07.021

Akagi T, Henry IM, Tao R, Comail L (2014) A Y-chromosome–encoded small RNA acts as a sex determinant in persimmons. Science 346:646–650. https://doi.org/10.1126/science.1257225

Akagi T, Henry IM, Kawai T, Comail L, Tao R (2016) Epigenetic regulation of the sex determination gene *MeGI* in polyploid persimmon. Plant Cell 28:2905–2915. https://doi.org/10.1105/tpc.16.00532

Flora Committee of China, Chinese Academy of Sciences (1987) Flora of China 60(1):143–144. Science Press, Beijing

Fu JM, Sun P, Han WJ, Diao SF, Suo YJ, Li H, Li SZ, Deng XZ, Wang LY, Cheng JY, Zhang JJ, Zhang Y, Mo WJ, Luo Y (2017) Persimmon germplasm resources in male. China Forestry Publishing House, Beijing

Li H, Wang L, Sun P, Suo Y, Han W, Mai Y, Diao S, Yuan D, Fu J (2019a) Cytomorphological observation on development of pistil and stamen of male and hermaphrodite floral buds of *Diospyros kaki* 'Longyan Yeshi 1'. Acta Hortic Sin 46(10):1897–1906. https://doi.org/10.16420/j.issn.0513-353x.2018-0888. http://www.ahs.ac.cn

Li S, Sun P, Du G, Wang L, Li H, Fu J, Suo Y, Han W, Diao S, Mai Y, Fi F (2019b) Transcriptome sequencing and comparative analysis between male and female floral buds of the persimmon (*Diospyros kaki* Thunb.). Sci Hortic 246: 987–997. https://doi.org/10.1016/j.scienta.2018.11.073

Li H, Wang L, Mai Y, Han W, Suo Y, Diao S, Sun P, Fu J (2021) Phytohormone and integrated mRNA and miRNA transcriptome analyses and differentiation of male between hermaphroditic floral buds of andromonoecious *Diospyros kaki* Thunb. BMC Genomics 22:203. https://doi.org/10.1186/s12864-021-07514-4

Luo ZR, Cai LH (1998) Chinese persimmon and its research status. In: Proceedings of the first national symposium on dried fruit production and research progress. China Forestry Publishing House, Beijing, pp 12–16

Xu J, Zhang Q, Xu L, Guo D, Luo Z (2016) Recent developments in deastringency mechanism of persimmon fruit. Acta Hortic Sin 43:1653–1664 (in Chinese with English abstract). https://doi.org/10.16420/j.issn.0513-353x.2016-0312

Yang Y, Wang RZ, Li GC et al (2006) Descriptors and data standard for persimmon (*Diospyros* spp.). China Agriculture Press, Beijing

Chinese PCNA

10

Wenxing Chen and Zhengrong Luo

Abstract

Chinese PCNA (C-PCNA) refers to the pollination constant and non-astringent (PCNA)-type persimmon that originated in China. C-PCNA type mainly includes "Luotian-tianshi", "Eshi 1", "Xiaoguo-tianshi", "Baogai-tianshi", and "Sifang-tianshi", etc., which are mainly distributed in the Dabie Mountain area at the junction of Hubei, Henan, and Anhui Provinces in central China. Like Japanese PCNA (J-PCNA), C-PCNA has smaller tannin cells and lost their astringency during fruit development, but is later than that of J-PCNA. The mechanism of natural de-astringency in J-PCNA is mainly a result of the dilution of PAs due to the cessation of PA accumulation in the early stage of fruit development. However, this "dilution effect" was not sufficient to explain the loss of astringency in C-PCNA fruit, and the insolubilization of soluble PAs could be the other important effect for its natural de-astringency process. At present, the molecular marker linked to the natural de-astringency trait of C-PCNA has been developed and had made significant progress in identification and characterization of key genes involved in natural de-astringency in C-PCNA. Using C-PCNA cultivar as male parent and crossing with the main cultivar of J-PCNA or non-PCNA, and do early selection of the PCNA candidates assisted by molecular marker could improve the efficiency of PCNA breeding. In addition, with the deciphering of genome information of persimmon, identifying the key genes for natural de-astringency of C-PCNA, and using molecular breeding techniques such as genome editing and genetic transformation will become the major approach for PCNA germplasm innovation in the future.

10.1 Introduction

Persimmon (*Diospyros kaki* Thunb., $2n = 6x = 90$) is native to China and has been cultivated for more than 2,000 years (Luo and Wang 2008). Pollination constant and non-astringent (PCNA) is a mutant-type persimmon that has lost the ability to accumulate high levels of PAs in the fruit, and naturally lose its astringency during fruit development, whereas non-PCNA-type fruit is astringent and inedible without any artificial treatment. The non-PCNA type is further classified into pollination-variant and non-astringent (PVNA) types, pollination-variant and astringent (PVA) types, and pollination constant and

W. Chen · Z. Luo (✉)
Key Laboratory of Horticultural Plant Biology, Huazhong Agricultural University, Wuhan 430070, Hubei, China
e-mail: luozhr@mail.hzau.edu.cn

W. Chen
e-mail: chenwenxing@webmail.hzau.edu.cn

R. Tao and Z. Luo (eds.), *The Persimmon Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-05584-3_10

astringent (PCA) types according to whether or not loss of astringency is depending on the formation and accumulation of volatile compounds in flesh (Sugiura 1983). PCNA-type persimmon was thought to uniquely originate in Japan until a new PCNA type "Luotian-tianshi" was found in Luotian Country in Hubei Province (Wang 1983). This genotype has been confirmed to be a PCNA persimmon after it was introduced in the Institute of Fruit Tree and Tea Science, NARO, Japan (Yamada et al. 1993). Furthermore, it had a different genetic background from the Japanese PCNA (J-PCNA) type, since it appeared that also the astringent type of offspring is produced when crossing with J-PCNA type (Ikeda et al. 1985; Yamada and Sato 2002).

According to the records of *Luotian County Annals*, Chinese PCNA (C-PCNA) has been planted in 1,032, about 180 years earlier than the oldest PVNA cultivar "Zenji-maru" in Japan (Wang 1983). The survey of C-PCNA types in China began in 1958 (Pan et al. 2002). In 1990–1994, the Hubei Academy of Forestry Research and Luotian Institute of Forestry Research cooperated to investigate the resources of "Luotian-tianshi" and others, in order to ascertain its distribution and cultivation status and screened out ten excellent strains and five variation types of C-PCNA (Pan et al. 1994). In recent years, some androecious germplasms and one PVNA type "90-1-10" were found in the Dabie Mountains in central China (Luo et al. 2005), and the studies on their reproductive characteristics and genetic relationship (Xu 2008; Zhang et al. 2009), suggested that the germplasms obtained by survey may be the variation of seedlings of local C-PCNA.

It is generally believed that PCNA is the mutation of non-PNCA type, and these mutant individuals gradually become the current cultivar group in repeated natural hybridization with other genotypes (Yamada et al. 1993). In comparison with the astringency traits and tannin cell characteristics of C-PCNA and non-PCNA fruit, Wang (1983) speculated that "Luotian-tianshi" was originated from the persimmon germplasm with lower tannin (belong to condensed tannin, also known as proanthocyanidin, PA) content and high ethanol dehydrogenase activity in the Dabie Mountain. Using random amplified polymorphic DNA (RAPD) markers, Luo et al. (1999) showed a close relationship among "Luotian-tianshi" and persimmon resources in the Dabie Mountain, but a distant relationship was observed between "Luotian-tianshi" and the PCNA cultivars of Japanese origin. Amplified fragment length polymorphism (AFLP) analysis also showed that the PCNA cultivars of Chinese origin were distant from those of Japanese origin (Kanzaki et al. 2000). Using sequence-related amplified polymorphism (SRAP) (Guo and Luo 2006), inter-retrotransposon amplified polymorphism (IRAP), and retrotransposon–microsatellite amplified polymorphism (REMAP) markers (Guo et al. 2006) also showed that C-PCNA cultivars were distant from those of J-CPNCA cultivars, but cannot separate C-PCNA cultivars from non-PCNA. A similar result was also obtained by other markers, including mitochondrial DNA (mtDNA) noncoding region (Hu and Luo 2006) and chloroplast DNA (cpDNA) RFLP (Hu et al. 2008), sequence-specific amplification polymorphism (SSAP) (Du et al. 2009a), and inverse sequence-tagged repeats (ISTR) (Du et al. 2009b). These results indicated that C-PCNA may be derived from the variation of non-PCNA, and other new C-PCNA types, such as "Eshi 1" and "Baogai-tianshi" that are found in the Dabie Mountain may be derived from the offspring of "Mopanshi" and "Luotian-tianshi". Kanzaki (2016) indicated that *D. louts*, *D. oleifera*, and *D. glandulosa* may be the diploid ancestor of persimmon, and evolved to non-PCNA type after polyploidization from an unknown *Diospyros* species in China, finally evolved to C-PCNA and J-PCNA independently in China and Japan, respectively.

C-PCNA is mainly distributed in the Dabie Mountains (115° 06′–115° 46′ E, 30° 35′–31° 16′ N) at the junction of Hubei, Henan, and Anhui provinces (Pan et al. 1994), and no natural distribution in other regions has been reported so far. Luotian County in Hubei Province has the largest number of trees and the most abundant variations of C-PCNA and can find over 200 years old trees, while rarely find 100 years

old trees in Shangcheng, Henan Province, and Jinzhai, Anhui Province.

The C-PCNA are distributed in almost all towns in Luotian County, Hubei Province, mostly in the west and north. The trees over 100 years old are mainly distributed in the town of Hepu and Sanlifan, and a 350-year-old tree was found in Tangjiashan Village, Hepu town (Fig. 10.1) (Yan and Zhao 2006). From the perspective of vertical distribution, C-PCNA cultivars are distributed between 100 and 700 m altitude, with the most in the range of 300–500 m, and only a few *Diospyros* species were found above 700 m (Pan et al. 1994).

C-PCNA in the Dabie Mountains are usually growing together with non-PCNA, chestnuts (*Castanea mollissima*), tung trees (*Vernicia fordii*), and Chinese tallow tree (*Sapium sebiferum*), and most of them freely grow around the house and field. Moreover, the existing adult persimmon trees in China are mostly grafted, and graft unions are clearly identifiable. Before the 1980s, persimmon was usually grafted on high rootstocks of wild persimmon (*D. kaki* Thunb. var. *silvestris*) or oil persimmon (*D. oleifera* Cheng) with a diameter of 3–4 cm with cleft grafting. After that, wild persimmon or lotus (*D. lotus* L.) were gradually used for seedbed sowing and stock seedling breeding and produced grafted seedlings with cleft grafting or single bud cutting–grafting in the following spring (Pan et al. 1994).

Fig. 10.1 Old trees of "Luotian-tianshi" in Luotian, Hubei province

There are more than 20 genotypes of C-PCNA were reported, mainly including "Luotian-tianshi", "Eshi 1" (original name is Yinyangshi or Qiuyan-tianshi), "Baogai-tianshi", "Sifang-tianshi", "Xiaoguo-tianshi", and "Yesheng-tianshi" (Fig. 10.2) (Pan et al. 1994). In addition, there are other genotypes, including "Qiuhongyu" and "Qiuji" (Li 2003), "Baohua-tianshi" (Li 2004), "Tianbaogai" and "Baozhu-tianshi" (Li et al. 2006), "Wuhe-tianshi", "Longzhuashi", "Jinqiu", and "Mitangshi" (Yan and Zhao 2006). Among them, there may exist homonym or synonym phenomena. For example, "Tianbaogai" and "Baohua-tianshi" may be the other name of "Baogai-tianshi", and "Tianminshi" may be a type of "Xiaoguo-tianshi".

10.2 Natural De-astringency Characteristics of C-PCNA

The genetic behavior of the PCNA trait of C-PCNA differs from that of J-PCNA (Ikegami et al. 2004, 2006), its origin and evolution pathway are also different from those of J-PCNA (Luo et al. 1999; Kanzaki et al. 2000; Du et al. 2009a, b). All offspring from crosses among J-PCNA cultivars yield only PCNA phenotypes, and almost all of the offspring from crosses between J-PCNA and non-PCNA were non-PCNA types. Notably, about 50% of the offspring from crosses between "Luotian-tianshi" and non-PCNA cultivars had the PCNA phenotype, indicating that the PCNA trait of "Luotian-tianshi" is genetically dominant

Fig. 10.2 Fruit shape of Chinese pollination constant and non-astringent (C-PCNA) type

to the non-PCNA traits (Ikegami et al. 2006). Moreover, "Luotian-tianshi" does not have any *ast* alleles (Akagi et al. 2010a) and J-PCNA cultivars do not have any dominant *C-PCNA* alleles, since no PCNA offspring yield from crosses between J-PCNA and non-PCNA (Ikeda et al. 1985). These results indicate that C-PCNA is not closely related to J-PCNA, they are different PCNA traits.

10.2.1 The Development of Tannin Cells

Tannin cells are a kind of special cells that accumulate PAs in vacuoles. The stop of tannin cell augmentation in the number and the size at an early stage of fruit development is the main cause of the loss of astringency in J-PCNA-type fruits (Yonemori and Matsushima 1985, 1987). The tannin cell size is smaller in PCNA fruit rather than those in non-PCNA fruit, and large in C-PCNA type than those in J-PCNA type, due to the stopped development later in C-PCNA type (Ikegami et al. 2006). The area of tannin cells was less than 33×10^3 μm^2 in the PCNA-type fruits, whereas it ranged from 55×10^3 to 140×10^3 μm^2 in non-PCNA-type fruits (Ikegami et al. 2004). The tannin cell size of "Qiyan-tianshi" ("Eshi 1", C-PCNA type) is 43.0×10^3 μm^2, which is a little bit large than other PCNA cultivars and had a less astringent taste, but the area is much smaller than non-PNCA, and also thought to be PCNA type (Yonemori et al. 2005). Yonemori et al. (1985) found that PAs in J-PCNA-type fruits hardly coagulated, although at the time those in PVNA-type fruits completely changed into insoluble complexes. However, the coagulated tannin cell can be found in C-PCNA-type fruits. So, the development of the tannin cell in C-PCNA fruit differs from those in J-PCNA type.

10.2.2 PA Composition in Persimmon Fruit

The persimmon PA composition is complex and is thought to consist of two types of flavan-3-ol monomers: cis-flavan-3-ol and trans-flavan-3-ol. Including catechin (C), gallocatechin (GC), epicatechin (EC), and epigallocatechin (EGC), and their gallate ester forms, C-3-O-gallate (CG) and GC-3-O-gallate (GCG), EC-3-O-gallate (ECG) and EGC-3-O-gallate (EGCG) (Matsuo and Ito 1978; Akagi et al. 2009a, 2010b). In the fruit development stage, the main components of persimmon PAs include C, GC, EC, EGC, ECG, and EGCG at the developmental stage (Akagi et al. 2009a, 2010b). Most PA units were suggested to be either EGC or its gallate ester form (EGCG) in PCNA and non-PCNA types, and the composition was varied among fruit developmental (Akagi et al. 2009a, 2010b). In addition, EC and its gallate ester form (ECG) were detectable during fruit development; C and GC were detected at an early stage of fruit developmental but were barely detectable after the middle stage of fruit development (Akagi et al. 2009a). However, there are few studies on the PA composition of C-PCNA cultivars. Fei et al. (1999) found that the "Luotian-tianshi" has a higher content of C and gallic acid (GA), and lower content of GC. These results indicated that the tannin composition of C-PCNA is different from those of J-CPNCA, which may be related to the unique natural de-astringency characteristics of C-PCNA, but further studies were needed to clarify.

10.2.3 PA Accumulation Patterns

The PA content was decreased gradually with fruit development in both PCNA and non-PCNA fruits. The soluble PA levels were reduced to a much lower level and astringency was lost in the early stage of fruit development (earlier than 10 weeks after bloom, WAB) in J-PCNA, while still maintained at a higher level in C-PCNA until 20 WAB (Fig. 10.3) (Mo et al. 2016; Chen et al. 2017, 2021). A quick reduction of soluble PA in C-PCNA was observed from 20 WAB and almost lose their astringent at 25 WAB (soluble PA content < 0.2%), but non-PNCA cannot lose their astringency. Notably, the dilution of PAs in C-PCNA fruit is not sufficient to explain the loss

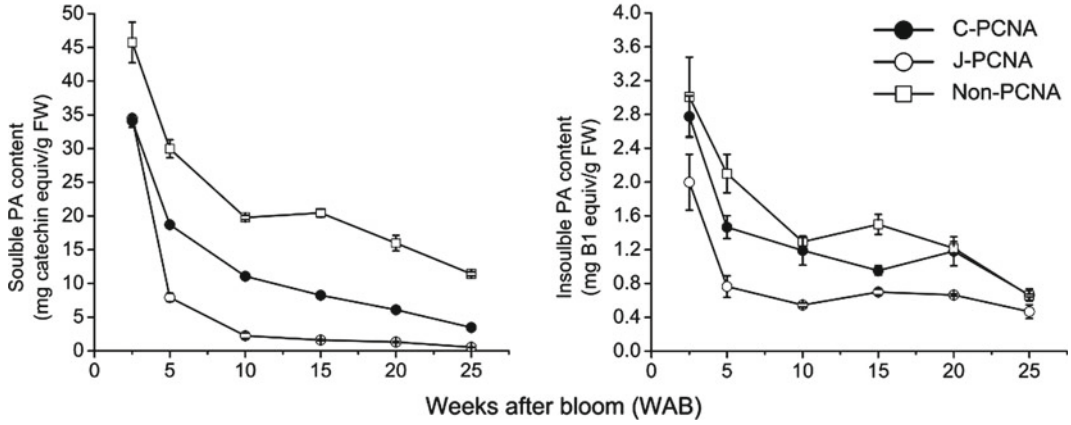

Fig. 10.3 Dynamic changes of soluble (left panel) and insoluble (right panel) proanthocyanidin (PA) levels among stages of fruit development. Error bars indicate SD (n = 3) (FW, fresh weight) (Chen et al. 2021)

of astringency. An increase in insoluble PAs was also observed during this period in C-PCNA fruit, which may be due to the insolubilization of soluble PAs at the later stage of fruit development (Guan et al. 2016, 2017; Mo et al. 2016; Chen et al. 2017, 2021).

10.2.4 Response to Artificial De-astringency Treatment

Removing astringency from persimmon fruits can be achieved by different methods, such as keeping the fruit under anaerobic conditions or being exposed to products that enhance anaerobic respiration (Pesis et al. 1986; Ben-Arie and Sonego 1993). The acetaldehyde produced by the fruit under these conditions is believed to cause the polymerization of tannin which leads to loss of astringency (Matsuo and Itoo 1982; Tanaka et al. 1994). However, treatment of young astringent fruits of J-PCNA with either ethanol or acetaldehyde was ineffective for the removal of their astringency, the tannins do not coagulate (Sugiura 1983; Zhang et al. 2013). Similar to non-PCNA fruit, the ethanol (Zhang et al. 2013; Luo et al. 2014), warm water (Guan et al. 2015; Chen et al. 2017) treatment can easily remove its astringency. This phenomenon is indicating that the molecular events of natural de-astringency in C-PCNA different from J-PCNA fruit but close to non-PNCA, which may cause by its specific PAs composition, but further studies are needed to be done to clarify this molecular basis.

10.3 Identification and Characterization of Natural De-astringency Associated Genes in C-PCNA Fruit

The PCNA trait in the J-PCNA type is controlled by a single recessive gene, while that in the C-PCNA type is controlled by a single dominant gene (Yonemori et al. 2000; Ikegami et al. 2006). The mechanism of natural de-astringency in J-PCNA is mainly a result of the dilution of PAs due to the cessation of PA accumulation in the early stage of fruit growth. Conversely, PA accumulation continues in non-PCNA persimmons until the late stages of fruit development. This process is mainly controlled by an MYB transcription factor, DkMyb4. A reduction in the expression of *DkMyb4* in J-PCNA fruit leads to the downregulation of PA biosynthesis during the early stages of fruit development and results in the non-astringency trait (Akagi et al. 2009b). Notably, the dilution of PAs in C-PCNA fruit is not sufficient to explain the loss of astringency. The content of insoluble PAs increased, while

that of soluble PAs decreased during the late stages of C-PCNA fruit development (Fig. 10.3). In recent years, a series of analyses, including genetic, transcriptome, and proteome were performed to identify the genes associated with nature astringency in C-PCNA.

10.3.1 Structural Genes

Early steps in the biosynthesis of PA from phenylpropanoids and malonyl-CoA into anthocyanidins are catalyzed by chalcone synthase (CHS), chalcone isomerase (CHI), flavonoid 3′-hydroxylase (F3′H), flavonoid 3′5′-hydroxylase (F3′5′H), dihydroflavonol 4-reductase (DFR), and anthocyanidin synthase (ANS). Anthocyanidin reductase (ANR) and leucoanthocyanidin reductase (LAR) are specific to the PA branch of the pathway and produce flavan-3-ols, typically (−)-epicatechin and (+)-catechin, respectively (Abrahams et al. 2003; Xie et al. 2003). Finally, the PA precursors are transported to and stored into the vacuole.

In persimmon, the PA biosynthetic pathway has also been well defined through homology cloning and RNA-seq analysis (Akagi et al. 2011; Chen et al. 2017). The genes involved in PA biosynthesis, such as phenylalanine ammonia-lyase (*PAL*), *CHS*, *CHI*, *F3H*, *F3′5′H*, *DFR*, *ANS*, *ANR*, and *LAR* have been isolated (Ikegami et al. 2005a, 2007; Akagi et al. 2009a). The PA transportation and polymerization-related genes, *DkMATE1* and *DkLAC* (homologs of TT12 and TT10, respectively) have also been isolated (Hu et al. 2013; Yang et al. 2016). In PCNA cultivars of Japanese origin, the PA biosynthetic-related genes were synchronously downregulated from 5 WAB and were almost below the detection limit after 7 WAB, and the accumulation of tannins was terminated in the early stage of fruit development, the dilution of PAs by fruit growth is the main reason for natural de-astringency (Yonemori and Matsushima 1985; Yonemori et al. 2003; Akagi et al. 2009b). However, the accumulation of soluble PA was terminated in the later development stage (20 WAB) of C-PCNA fruit (Mo et al. 2016), thus the "dilution effect" was not sufficient to cause C-PCNA fruit to lose its astringency. The expression pattern of genes related to PA biosynthesis between C-PCNA and J-PCNA fruits are quite different. Ikegami et al. (2005b) compared the expression patterns of PA biosynthesis genes in "Luotian-tianshi" (C-PCNA), "Suruga" (J-PCNA), and "Kuramitsu" (PCA, non-PCNA), and found that the C-PCNA cultivar behaves similar to PCA cultivar with regard to expression of the genes involved in PA biosynthesis except for *DkSCPL1*. Moreover, the PA branch-specific *ANR* was continuously expressed in C-PNCA after the termination of tannin cell development in J-PCNA cultivar (Ikegami et al. 2005b); another PA branch-specific gene *LAR*, whose expression in C-PCNA was also similar to the PCA cultivar (Wang et al. 2010). Besides the genes in the biosynthesis of PA precursors, the PA precursors transportation and polymerization associated genes *DkMATE1* and *DkLAC* have been also isolated from C-PCNA fruit (Hu et al. 2013; Yang et al. 2016). Chen et al. (2017) found that the transcription level of PA biosynthesis genes was consistent with the accumulation pattern of PA in different astringent-type cultivars, most of them are show high expression in C-PCNA than J-PCNA cultivar, but lower than in non-PCNA cultivars.

Soluble PA is the main reason that causes fruit astringency, which can interact with acetaldehyde under artificially astringency removal treatment. Pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) are two key enzymes that catalyzed pyruvate and ethanol into acetaldehyde, respectively, in plants (Yamada et al. 2002; Pesis 2005; Botondi et al. 2012). Luo et al. (2014) performed a genome-wide transcriptome analysis of "Luotian-tianshi" after ethanol treatment to remove fruit astringency and found that *PDC* gene was upregulated, and *aldehyde dehydrogenase family 2* (*ALDH2*) that reduce acetaldehyde accumulation by canalizing acetaldehyde to acetic acid was significantly downregulated after treatment. *DkADH1* and *DkPDC2* were suggested to be key genes involved in persimmon astringency removal

under high CO_2 treatment (Min et al. 2012), and may participate in natural de-astringency of C-PCNA fruit through a comprehensive analysis of gene expression patterns, seasonal change of tannins and acetaldehyde content of persimmon fruit flesh, and gene transient expression assay in persimmon leaves (Mo et al. 2016). Xu et al. (2017) cloned two *ALDH2* genes *DkALDH2a* and *DkALDH2b* from C-PCNA fruit and found that *DkALDH2a* and *DkALDH2b* are negatively correlated with natural de-astringency in C-PCNA persimmon. Moreover, *pyruvate kinases 1* and *8* (*PK1* and *PK8*), the upstream genes of pyruvate synthesis were suggested to be involved in natural de-astringency in C-PCNA by upregulating the *DkPDC* and *DkADH* expression (Guan et al. 2016, 2017).

10.3.2 Transcription Factors

The ternary complex comprising of MYB transcription factor, basic helix–loop–helix (bHLH) protein, and WD40 protein (MBW complex) have been reported in regulates the expression of PA structural genes (Koes et al. 2005; Ramsay and Glover 2005). In Arabidopsis, the complex is formed by at least four MBW complexes, namely TT2-TT8/GL3/EGL3-TTG1, and MYB5-TT8-TTG1, and activates the genes *DFR*, *ANS*, and *ANR*, the products of which cooperate to regulate the specific accumulation of PAs in the innermost cell layer of the seed coat (i.e., endothelium, chalaza, and micropyle) (Nesi et al. 2001; Debeaujon et al. 2003; Baudry et al. 2004).

In persimmon, some MYB-like genes have been identified from J-PCNA or non-PCNA cultivars (Akagi et al. 2009b), among them *DkMyb2* and *DkMyb4* were suggested to regulate PA biosynthesis in J-PCNA (Akagi et al. 2009b, 2010c). Reduced expression of *DkMyb4* leads to J-CPNA fruit-specific downregulation of PA biosynthesis at the early stage of fruit developmental and resultant non-astringent trait (Akagi et al. 2009b). *DkbZIP5* was found to respond to the seasonal abscisic acid signal and act as a *DkMyb4* regulator to modify PA accumulation in J-PCNA (Akagi et al. 2012). However, these factors are not the major factors that regulate PA accumulation in C-PNCA cultivars.

Su et al. (2012) cloned a bHLH factor, *VvMYC1*, the homolog of *AtTT8* from C-PCNA cultivar, and the expression pattern of *DkMYC1* was correlated with the PA accumulation in C-PCNA and non-PCNA cultivars. Guan et al. (2020) isolated two regulators of *DkPK1* genes, *DkWRY3* and *DkWRY15* from C-PCNA using yeast one-hybrid screen strategy. Transient overexpression of *DkWRKY3* and *DkWRKY15* in persimmon leaves reduced the soluble PA accumulation by activating acetaldehyde synthesis-related gene expression.

Recently, Chen et al. (2021) reported an MYB transcription factor, DkMYB14, which regulates the accumulation of PA in C-PCNA fruit flesh, is a bifunctional transcription factor that acts as a repressor in PA biosynthesis but becomes an activator when involved in acetaldehyde biosynthesis. Notably, both functions contribute to the elimination of astringency by decreasing PA biosynthesis and promoting its insolubilization. Furthermore, the amino acid Gly39 in the R2 domain and the EAR-like motif in the C-terminal are essential for the activities of DkMYB14, namely repressing PA biosynthesis and promoting PA insolubilization. *CPCNA* may regulate natural de-astringency through regulating *DkMYB14* expression, since the same sequence of *DkMYB14* among different cultivars (Chen unpublished data), but the underlying mechanisms are not clear.

10.3.3 microRNA

microRNAs (miRNAs) are small non-coding RNAs, with an average of 22 nucleotides in length, that functions in RNA silencing and post-transcriptional regulation of gene expression (Bartel 2018). Luo et al. (2015) performed a high-throughput small RNA sequencing on C-PNCA "Eshi 1" fruits at stages of 15 and 20 WABs, and a total of 236 known miRNAs and 33 novel miRNAs were identified. Among them, miRNA156j-5p, miRNA858b, miRNA385p-3p, miRNA2911a were suggested to have an

important role in the regulation of PA accumulation in persimmon. Yang et al. (2020) further conducted a functional analysis on miRNA858b and found that miRNA858b negatively regulating the PA accumulation by inhibiting the MYB transcription factors *DkMYB19* and *DkMYB20* expression.

In summary, the genes involved in acetaldehyde metabolism include *DkADH1*, *DkPDC2*, *DkALDH2*, *DkPK1/8*, and the regulators DkWRKY3/15 were thought to play an important role in natural de-astringency in C-PNCA fruits. In addition, miRNA858b and DkMYB14 regulate PA accumulation in C-PCNA fruits, but DkMYB14 plays an important role in natural de-astringency regulation in C-PCNA (Fig. 10.4).

10.4 Molecular Markers Related to *CPCNA* Trait

Ikegami et al. (2011) developed an effective SCAR marker, RO2, which specifically links to *C-PCNA* trait and can be used for selecting the C-PCNA type offspring in the early stage. Pei et al. (2013) verified the efficiency of RO2 marker in other F_1 populations that yield from the crossing with C-PNCA cultivar. Notably, the androecious persimmon genotypes, namely Male strains No. 1, No. 2, No. 3, No. 6, No. 7, and No. 8 are also carrying the RO2 marker and some of the offspring generated from crossing combination "Huashi 1" (PVA) × Male strain No. 3 also find carrying RO2 marker. These results indicated that the androecious persimmon genotypes have *CPCNA* locus and can be used from PCNA breeding.

10.5 Future Prospects

PCNA is the most desirable type since the fruit is edible without any de-astringency treatment; therefore, breeding PCNA type is one of the most important goals for persimmon breeding. Since the very narrow genetic variability of J-PCNA-type cultivars, using only J-PCNA cultivars causes a serious problem for breeding new cultivars because of inbreeding depression in breeding population. However, the genetic

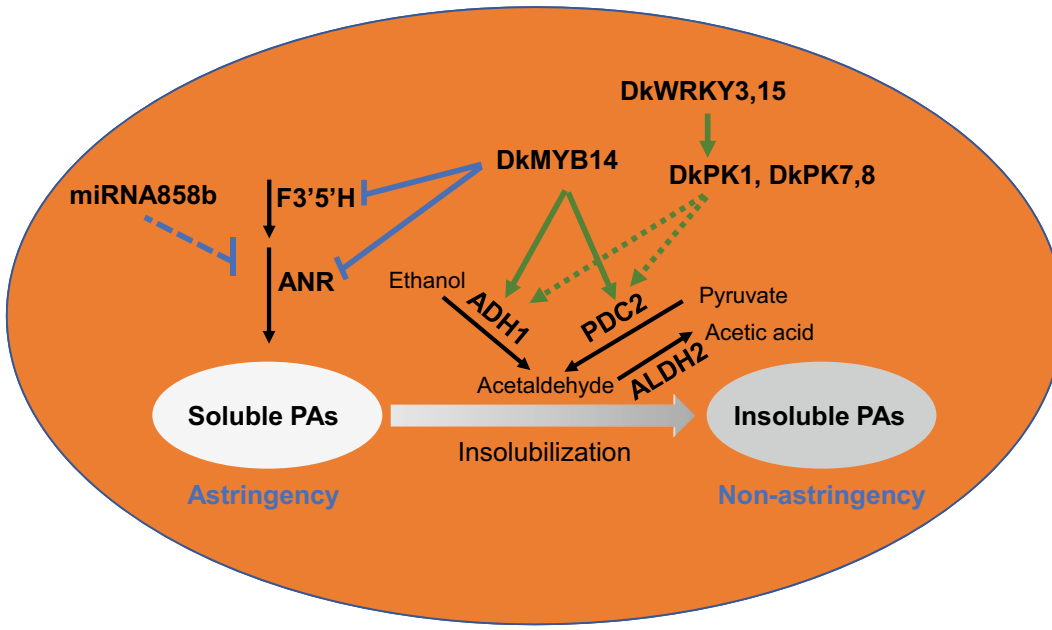

Fig. 10.4 Hypothetical model of the regulation network of proanthocyanidin (PA) accumulation in Chinese pollination constant and non-astringent (C-PCNA) fruit

behavior of the PCNA trait of C-PCNA differs from that of J-PCNA cultivar, and yields 50% of the PCNA offspring from a cross with C-PCNA cultivar. Using C-PCNA as male parent and crossing with the main cultivar of J-PCNA or non-PCNA, and early selection of the PCNA candidates assisted by molecular marker could improve the efficiency of PCNA breeding. In addition, with the release of genome information of persimmon, identification of the key genes for natural de-astringency of C-PCNA, and using molecular breeding techniques such as genome editing and genetic transformation will become the major approach for PCNA germplasm innovation in the future.

References

Abrahams S, Lee E, Walker AR, Tanner GJ, Larkin PJ, Ashton AR (2003) The Arabidopsis *TDS4* gene encodes leucoanthocyanidin dioxygenase (LDOX) and is essential for proanthocyanidin synthesis and vacuole development. Plant J 35:624–636

Akagi T, Ikegami A, Suzuki Y, Yoshida J, Yamada M, Sato A, Yonemori K (2009a) Expression balances of structural genes in shikimate and flavonoid biosynthesis cause a difference in proanthocyanidin accumulation in persimmon (*Diospyros kaki* Thunb.) fruit. Planta 230:899–915

Akagi T, Ikegami A, Tsujimoto T, Kobayashi S, Sato A, Kono A, Yonemori K (2009b) DkMyb4 is a Myb transcription factor involved in proanthocyanidin biosynthesis in persimmon fruit. Plant Physiol 151:2028–2045

Akagi T, Takeda Y, Ikegami A, Kono A, Yamada M, Kanzaki S, Yonemori K (2010a) Quantitative genotyping for astringency locus in hexaploid persimmon cultivars using quantitative real-time PCR. J Am Soc Hortic Sci 135:59–66

Akagi T, Suzuki Y, Ikegami A, Kamitakahara H, Takano T, Nakatsubo F, Yonemori K (2010b) Condensed tannin composition analysis in persimmon (*Diospyros kaki* Thunb.) fruit by acid catalysis in the presence of excess phloroglucinol. J Jpn Soc Hortic Sci 79:275–281

Akagi T, Ikegami A, Yonemori K (2010c) DkMyb2 wound-induced transcription factor of persimmon (*Diospyros kaki* Thunb.), contributes to proanthocyanidin regulation. Planta 232:1045–1059

Akagi T, Katayama-Ikegami A, Yonemori K (2011) Proanthocyanidin biosynthesis of persimmon (*Diospyros kaki* Thunb.) fruit. Sci Hortic 130:373–380

Akagi T, Katayama-Ikegami A, Kobayashi S, Sato A, Kono A, Yonemori K (2012) Seasonal abscisic acid signal and a basic leucine zipper transcription factor, DkbZIP5, regulate proanthocyanidin biosynthesis in persimmon fruit. Plant Physiol 158:1089–1102

Bartel DP (2018) Metazoan MicroRNAs. Cell 173:20–51

Baudry A, Heim MA, Dubreucq B, Caboche M, Weisshaar B, Lepiniec L (2004) TT2, TT8, and TTG1 synergistically specify the expression of *BANYULS* and proanthocyanidin biosynthesis in *Arabidopsis thaliana*. Plant J 39:366–380

Ben-Arie R, Sonego L (1993) Temperature affects astringency removal and recurrence in persimmon. J Food Sci 58:1397–1400

Botondi R, Russo V, Mencarelli F (2012) Anaerobic metabolism during short and long term storage of kiwifruit. Postharvest Biol Technol 64:83–90

Chen WX, Xiong YL, Xu LQ, Zhang QL, Luo ZR (2017) An integrated analysis based on transcriptome and proteome reveals deastringency-related genes in CPCNA persimmon. Sci Rep 7:44671

Chen WX, Zheng QY, Li JW, Liu Y, Xu LQ, Zhang QL, Luo ZR (2021) DkMYB14 is a bifunctional transcription factor that regulates proanthocyanidin accumulation in persimmon fruit. Plant J 106:1708–1727

Debeaujon I, Nesi N, Perez P, Devic M, Grandjean O, Caboche M, Lepiniec L (2003) Proanthocyanidin-accumulating cells in Arabidopsis testa: regulation of differentiation and role in seed development. Plant Cell 15:2514–2531

Du XY, Zhang QL, Luo ZR (2009a) Development of retrotransposon primers and their utilization for germplasm identification in *Diospyros* spp. (Ebenaceae). Tree Genet Genomes 5:235–245

Du XY, Zhang QL, Luo ZR (2009b) Comparison of four molecular markers for genetic analysis in *Diospyros* L. (Ebenaceae). Plant Syst Evol 281:171–181

Fei XQ, Zhou LH, Gong BC (1999) Differences of the components of tannin among three types of persimmon fruits and characteristics of tannin from 'Luotian Tianshi.' J For Res 12:369–373 (in Chinese)

Guan CF, Chen L, Chen WX, Mo RL, Zhang QL, Du XY, Liu JH, Luo ZR (2015) SSAP analysis reveals candidate genes associated with de-astringency in persimmon (*Diospyros kaki* Thunb.) treated with 40 ° C water. Tree Genet Genomes 11:20

Guan CF, Chen WX, Mo RL, Du XY, Zhang QL, Luo ZR (2016) Isolation and characterization of *DkPK* genes associated with natural de-astringency in C-PCNA persimmon. Front Plant Sci 7:156

Guan CF, Du XY, Zhang QL, Ma FW, Luo ZR, Yang YY (2017) *DkPK* genes promote natural de-astringency in C-PCNA persimmon by up-regulating *DkPDC* and *DkADH* expression. Front Plant Sci 8:149

Guan CF, Wang MK, Zhang YF, Ruan XF, Zhang QL, Luo ZR, Yang Y (2020) DkWRKY interacts with pyruvate kinase gene *DkPK1* and promotes natural de-astringency in C-PCNA persimmon. Plant Sci 290: 110285

Guo DL, Luo ZR (2006) Genetic relationships of some PCNA persimmons (*Diospyros kaki* Thunb.) from

China and Japan revealed by SRAP analysis. Genet Resour Crop Evol 53:1597–1603

Guo DL, Zhang HQ, Luo ZR (2006) Genetic relationships of *Diospyros kaki* Thunb. and related species revealed by IRAP and REMAP analysis. Plant Sci 170:528–533

Hu DC, Luo ZR (2006) Polymorphisms of amplified mitochondrial DNA non-coding regions in *Diospyros* spp. Sci Hortic 109:275–281

Hu DC, Zhang QL, Luo ZR (2008) Phylogenetic analysis in some *Diospyros* spp. (Ebenaceae) and Japanese persimmon using chloroplast DNA PCR-RFLP markers. Sci Hortic 117:32–38

Hu QN, Luo C, Zhang QL, Luo ZR (2013) Isolation and characterization of a *Laccase* gene potentially involved in proanthocyanidin polymerization in oriental persimmon (*Diospyros kaki* Thunb.) fruit. Mol Biol Rep 40:2809–2820

Ikeda I, Yamada M, Kurihara A, Nishida T (1985) Inheritance of astringency in Japanese persimmon. J Jpn Soc Hortic Sci 54:39–45 (in Japanese with English summary)

Ikegami A, Yonemori K, Sugiura A, Sato A, Yamada M (2004) Segregation of astringency in F_1 progenies derived from crosses between pollination-constant, nonastringent persimmon cultivars. HortScience 39:371–374

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

Ikegami A, Sato A, Yamada M, Kitajima A, Yonemori K (2005b) Expression of genes involved in proanthocyanidin biosynthesis during fruit development in a Chinese pollination-constant, nonastringent (PCNA) persimmon, 'Luo Tian Tian Shi'. J Am Soc Hortic Sci 130:830–835

Ikegami A, Eguchi S, Yonemori K, Yamada M, Sato A, Mitani N, Kitajima A (2006) Segregations of astringent progenies in the F_1 populations derived from crosses between a Chinese pollination-constant nonastringent (PCNA) 'Luo Tian Tian Shi', and Japanese PCNA and pollination-constant astringent (PCA) cultivars of Japanese origin. HortScience 41:561–563

Ikegami A, Eguchi S, Kitajima A, Inoue K, Yonemori K (2007) Identification of genes involved in proanthocyanidin biosynthesis of persimmon (*Diospyros kaki*) fruit. Plant Sci 172:1037–1047

Ikegami A, Eguchi S, Akagi T, Sato A, Yamada M, Kanzaki S, Kitajima A, Yonemori K (2011) Development of molecular markers linked to the allele associated with the non-astringent trait of the Chinese persimmon (*Diospyros kaki* Thunb.). J Jpn Soc Hortic Sci 80:150–155

Kanzaki S (2016) The origin and cultivar development of Japanese persimmon (*Diospyros kaki* Thunb.). J Jpn Soc Food Sci 63:328–330

Kanzaki S, Yonemori K, Sato A, Yamada M, Sugiura A (2000) Analysis of the genetic relationships among pollination-constant and non-astringent (PCNA) cultivars of persimmon (*Diospyros kaki* Thunb.) from Japan and China using amplified fragment length polymorphism (AFLP). J Jpn Soc Hortic Sci 69:665–670

Koes R, Verweij W, Quattrocchio F (2005) Flavonoids: a colorful model for the regulation and evolution of biochemical pathways. Trends Plant Sci 10:236–242

Li XM (2003) Resources investigation of 'Luo Tian Tian Shi.' South China Fruits 32:69–70 (in Chinese)

Li XM (2004) 'Baohua'—an early maturing variety of pollination constant non-astringent persimmon native to China. Guangxi Hortic 15:22–23 (in Chinese)

Li GC, Yang Y, Wang RZ (2006) Germplasm resources in persimmon coming of China. China Seed Ind 4:52–53 (in Chinese)

Luo ZR, Wang RZ (2008) Persimmon in China: domestication and traditional utilizations of genetic resources. Adv Hortic Sci 22:239–243

Luo ZR, Li FF, Cai LH (1999) Molecular systematics of China native non-astringent persimmon based on random amplified polymorphic DNA. Acta Hortic Sin 26:297–301 (in Chinese with English summary)

Luo ZR, Zhang QL, Guo DY, Gu QQ (2005) General situation on science and industry of persimmon in China mainland. Acta Hortic 685:29–36

Luo C, Zhang QL, Luo ZR (2014) Genome-wide transcriptome analysis of Chinese pollination constant nonastringent persimmon fruit treated with ethanol. BMC Genom 15:112

Luo YJ, Zhang XN, Luo ZR, Zhang QL, Liu JH (2015) Identification and characterization of microRNAs from Chinese pollination constant non-astringent persimmon using high-throughput sequencing. BMC Plant Biol 15:11

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

Matsuo T, Itoo S (1982) A model experiment for de-astringency of persimmon fruit with high carbon dioxide treatment: in vitro gelation of kaki-tannin by reacting with acetaldehyde. Agric Biol Chem 46:683–689

Min T, Yin XR, Shi YN, Luo ZR, Yao YC, Grierson D, Ferguson IB, Chen KS (2012) Ethylene-responsive transcription factors interact with promoters of *ADH* and *PDC* involved in persimmon (*Diospyros kaki*) fruit de-astringency. J Exp Bot 63:6393–6405

Mo RL, Yang SC, Huang YM, Chen WX, Zhang QL, Luo ZR (2016) *ADH* and *PDC* genes involved in tannins coagulation leading to natural de-astringency in Chinese pollination constant non-astringency persimmon (*Diospyros kaki* Thunb.). Tree Genet Genomes 12:17

Nesi N, Jond C, Debeaujon I, Caboche M, Lepiniec L (2001) The Arabidopsis *TT2* gene encodes an R2R3 MYB domain protein that acts as a key determinant for proanthocyanidin accumulation in developing seed. Plant Cell 13:2099–2114

Pan DS, Ma YP, Yu QY, Yi ZW, Zhang SH (1994) Survey the resources of 'Luotian-tianshi' and selection

of excellent strains. Hubei For Sci Technol 2:24–28 (in Chinese)

Pan DS, Ouyang SX, Wang XQ, Chen CF, Deng XZ (2002) Suggestions to rename 'Luotian-tianshi' to 'China PCNA persimmon.' Hubei For Sci Technol 2:27–28 (in Chinese)

Pei X, Zhang QL, Guo DY, Luo ZR (2013) Effectiveness of the RO2 marker for the identification of non-astringency trait in Chinese PCNA persimmon and its possible segregation ratio in hybrid F_1 population. Sci Hortic 150:227–231

Pesis E (2005) The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. Postharvest Biol Technol 37:1–19

Pesis E, Levi A, Ben-Arie R (1986) De-astringency of persimmon fruits by creating a modified atmosphere in polyethylene bags. J Food Sci 51:1014–1016

Ramsay NA, Glover BJ (2005) MYB-bHLH-WD40 protein complex and the evolution of cellular diversity. Trends Plant Sci 10:63–70

Su FY, Hu J, Zhang QL, Luo ZR (2012) Isolation and characterization of a basic helix-loop-helix transcription factor gene potentially involved in proanthocyanidin biosynthesis regulation in persimmon (*Diospyros kaki* Thunb.). Sci Hortic 136:115–121

Sugiura A (1983) Relationships of ethanol production by seeds of different types of Japanese persimmon and their tannin content. HortScience 8:319–321

Tanaka T, Takahashi R, Kouno I, Nonaka G-I (1994) Chemical evidence for the de-astringency (insolubilization of tannins) of persimmon fruit. J Chem Soc Perkin Trans 1:3013–3022

Wang RZ (1983) The origin of 'Luo Tian Tian Shi.' Chin Fruit Tree 2:16–19 (in Chinese)

Wang Y, Zhang QL, Luo ZR (2010) Isolation and expression of gene encoding leucoanthocyanidin reductase (LAR) from Japanese persimmon (*Diospyros kaki* Thunb.) during fruit development. Biol Plant 54:707–710

Xie DY, Sharma SB, Paiva NL, Ferreira D, Dixon RA (2003) Role of anthocyanidin reductase, encoded by *BANYULS* in plant flavonoid biosynthesis. Science 299:396–399

Xu LQ (2008) Studies on the biological characters and new germplasm creation of some staminate germplasm in *Diospyros* L. native to China. Ph.D. dissertation, Huazhong Agricultural University, Wuhan (in Chinese with English summary)

Xu JC, Ding JG, Gan JP, Mo RL, Xu LQ, Zhang QL, Luo ZR (2017) *ALDH2* genes are negatively correlated with natural deastringency in Chinese PCNA persimmon (*Diospyros kaki* Thunb.). Tree Genet Genomes 13:122

Yamada M, Sato A (2002) Segregation for fruit astringency type in progenies derived from crosses of 'Nishimura-wase' and pollination constant non-astringent genotypes in oriental persimmon (*Diospyros kaki* Thunb.). Sci Hortic 92:107–111

Yamada M, Sato A, Yakushiji H, Yoshinaga K, Yamane H, Endo M (1993) Characteristics of 'Luo Tian Tian Shi', a non-astringent cultivar of oriental persimmon (*Diospyros kaki* Thunb.) of Chinese origin in relation to non-astringent cultivars of Japanese origin. Bull Fruit Tree Res Sta 25:19–32 (in Japanese with English summary)

Yamada M, Taira S, Ohtsuki M, Sato A, Iwanami H, Yakushiji H, Wang RZ, Yang Y, Li GC (2002) Varietal differences in the ease of astringency removal by carbon dioxide gas and ethanol vapor treatments among Oriental astringent persimmons of Japanese and Chinese origin. Sci Hortic 94:63–72

Yan HY, Zhao HQ (2006) Non-astringent persimmon. China Agriculture Press, Beijing (in Chinese)

Yang SC, Jiang Y, Xu LQ, Shiratake K, Luo ZR, Zhang QL (2016) Molecular cloning and functional characterization of DkMATE1 involved in proanthocyanidin precursor transport in persimmon (*Diospyros kaki* Thunb.) fruit. Plant Physiol Biochem 108:241–250

Yang SC, Zhang M, Xu LQ, Luo ZR, Zhang QL (2020) MiR858b inhibits proanthocyanidin accumulation by the repression of *DkMYB19* and *DkMYB20* in persimmon. Front Plant Sci 11:576378

Yonemori K, Matsushima J (1985) Property of development of the tannin cells from non-astringent and astringent type fruits of Japanese persimmon (*Diospyros kaki*) and its relationship to natural astringency. J Jpn Soc Hortic Sci 54:201–208 (in Japanese with English summary)

Yonemori K, Mastushima J (1987) Changes in tannin cell morphology with growth and development of Japanese persimmon fruit. J Am Soc Hortic Sci 112:818–821

Yonemori K, Yamada M, Sugiura A (2000) Persimmon genetics and breeding. Plant Breed Rev 19:191–225

Yonemori K, Ikegami A, Kanzaki S, Sugiura A (2003) Unique features of tannin cells in fruit of pollination constant non-astringent persimmons. Acta Hortic 601:31–35

Yonemori K, Ikegami A, Kitajima A, Luo ZR, Kanzaki S, Sato A, Yamada M, Yang Y, Wang RZ (2005) Existence of several pollination constant non-astringent type persimmons in China. Acta Hortic 685:77–84

Zhang QL, Guo DY, Luo ZR (2009) Identification and taxonomic status of Chinese *Diospyros* spp. (Ebenaceae) androecious germplasms. Acta Hortic 833:91-96F

Zhang QL, Chen DM, Luo ZR (2013) Natural astringency loss property of a pollination-constant non-astringent persimmon newly found in central China. Acta Hortic 996:207–211

Japanese PCNA

11

Soichiro Nishiyama and Keizo Yonemori

Abstract

Proanthocyanidin (PA) accumulation is a unique feature of persimmon fruit physiology. Unlike many species bearing fleshy fruit, persimmon progressively accumulates PAs during very long period of fruit development, resulting in a strong astringent taste at physiological maturation. Pollination constant and non-astringent (PCNA)-type cultivars stop accumulating PAs in the early stages of fruit development, and the mature fruit is edible and can be commercialized without an artificial deastringency treatment. Two distinct loci, *ASTRINGENCY* (*AST*) for Japanese PCNA and *CPCNA* for Chinese PCNA, are independently responsible for the PCNA trait, and *AST*, in which the recessive allele confers the Japanese PCNA trait, is currently the major locus used in PCNA fruit production and modern breeding. Here, we review findings on the PAs in persimmon with a focus on the Japanese PCNA trait. We propose that the unique characteristic of PA accumulation in persimmon fruit may be used to analyze biological processes that cannot be clarified in model plants. The available genetic information collectively shows a hypothesis regarding the process of establishing the recessive trait under polyploid conditions that would be difficult to achieve in nature. Future directions for PA research and its application to fruit production, in combination with available reference genomes, are discussed.

11.1 Introduction

Persimmon (*Diospyros kaki* Thunb.) is a temperate deciduous fruit tree originating from East Asia and is now cultivated in many parts of the world, especially in a temperate climate. One unique property of persimmon is the accumulation of large amounts of proanthocyanidins (PAs) in the fruit throughout the growth period. PAs are important determinants of astringency and bitterness in cultivated plants, including persimmon, and thus are important components that determine the production quality of many crops (Dixon et al. 2005; Aron and Kennedy 2008). The strong astringent taste of persimmon fruit is attributed to the presence of PAs. They also have strong free-radical scavenging capabilities; consequently, they have attracted attention as functional components in clinical nutrition (Manach et al. 2005; Bagchi et al. 2014). PAs are found in many plants, probably

S. Nishiyama (✉)
Graduate School of Agriculture, Kyoto University, Kyoto, Japan
e-mail: nishiyama.soichiro.8e@kyoto-u.ac.jp

K. Yonemori
Ryukoku Extension Center (REC), Ryukoku University, Otsu, Japan
e-mail: yonemori.keizo.78r@st.kyoto-u.ac.jp

R. Tao and Z. Luo (eds.), *The Persimmon Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-05584-3_11

functioning as defensive substances, but PAs in persimmon fruit have a particularly high degree of polymerization (average degree of polymerization: 10–40; Akagi et al. 2009a, 2011a). The continuous accumulation of a large amount of high-molecular-weight PAs in the edible plant part is unique compared with other cultivated plants.

Hume (1914) found that flesh coloration in matured persimmon fruit depends on the presence or absence of seeds and classified persimmon cultivars into pollination-constant and pollination-variant (Fig. 11.1a, b). Because flesh color is related to astringency, persimmon cultivars are now classified into four groups on the basis of the PA content and the effects of seed formation: (1) pollination-constant and non-astringent (PCNA), which is non-astringent regardless of seed formation, (2) pollination-variant and non-astringent (PVNA), which is non-astringent only when seeds are formed, (3) pollination-variant and astringent (PVA), which is astringent but the astringency around seeds disappears as they form, and (4) pollination-constant and astringent (PCA), which is astringent regardless of seed formation (Kajiura 1946; Yonemori et al. 2010). Among them, PCA, PVA, and PVNA are quantitative traits in which the astringency loss is determined by the differences in the amounts of volatiles generated by the seeds in the fruit (Sugiura and Tomana 1983). In contrast, PCNA-type cultivars exhibit non-astringency by a different mechanism: the synthesis and accumulation of PA cease early during fruit development, and the subsequent fruit enlargement results in a decrease in the PA concentration, leading to non-astringent fruit (Yonemori and Matsushima 1985). In the PCNA-type group, some cultivars, such as ‘Fuyu’, produce volatiles equivalent to the pollination-variant, whereas some cultivars, such as ‘Okugosho’, do not, indicating that PCNA and pollination-constant/variant are independent traits (Sugiura and Tomana 1983; Ikeda et al. 1985).

Among the cultivars, the PCNA group is currently the most economically important. Some PCA and PVA cultivars have excellent fruit quality, but to make them edible, the PAs must be insolubilized by some means such as ethanol and carbon dioxide. In addition, persimmon fruit subjected to artificial deastringency treatments generally have poor shelf lives. On the other hand, PCNA and PVNA fruit are naturally non-astringent, thus, they can be consumed without deastringency treatments. In particular, the PCNA trait has been widely adopted in economic cultivars because such cultivars stably produce non-astringent fruit regardless of the success of pollination. For these reasons, current persimmon breeding is aimed at producing superior PCNA cultivars that are in high demand for production (Sato and Yamada 2016).

11.2 Inheritance and Breeding

11.2.1 Japanese- and Chinese-PCNA

PCNA cultivars are classified into Japanese- and Chinese-PCNAs (J-PCNA and C-PCNA) on the basis of their genetic regulatory mechanisms (Ikegami et al. 2004; Akagi et al. 2011a; Sato and Yamada 2016). J-PCNA is a recessive trait controlled by a single locus (Ikeda et al. 1985; Yamada and Sato 2002), *ASTRINGENCY* (*AST*), and a recessive homozygous genotype is required for J-PCNA trait expression. J-PCNA was originally found in Japan and is now distributed and cultivated worldwide. On the contrary, C-PCNA was originally found in China and represents a dominant trait controlled by a single locus, *CPCNA* (Wang 1982; Ikegami et al. 2004, 2006). J-PCNA is generally preferable in production because the fruit has less PA than the fruit of available C-PCNA cultivars; however, the dominant nature of the C-PCNA trait may be a valuable resource for future PCNA breeding (Sato and Yamada 2016). A direct functional comparison between J-PCNA and C-PCNA may be meaningful but has not been performed to date. In this chapter, we summarize the J-PCNA trait. Please refer to Chap. 10 for a summarization of the C-PCNA trait.

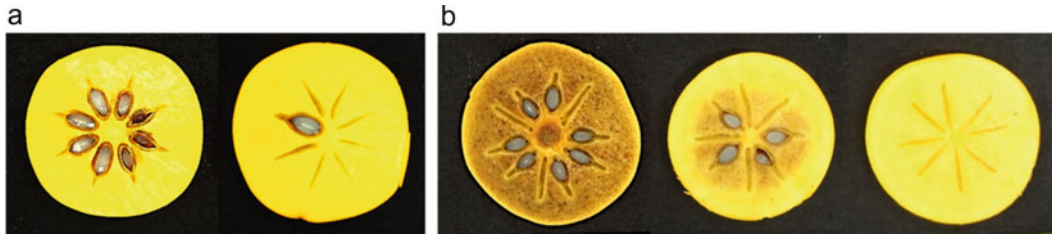

Fig. 11.1 Effects of the seed formation on the flesh color phenotype. **a** 'Okugosho' (PCNA), **b** 'Zenjimaru' (PVNA)

11.2.2 Origin of J-PCNA

'Gosho', which is believed to be the first J-PCNA, was first mentioned in literature written in the seventeenth century (Kikuchi 1948; Yamada et al. 2012). The J-PCNA group has been developing for <400 years and, thus, it has not yet been highly differentiated within persimmon. In fact, of the 600 cultivars planted at the Grape and Persimmon Research Station, NIFTS (Hiroshima, Japan), the number of indigenous J-PCNA cultivars, excluding synonyms and bud-sports, is reported to be 18. Thus, it represents a very small percentage (Yamada 2005).

The allelic dose of the *AST* gene in polyploid persimmon is significant to the development of J-PCNA. Owing to the recessive nature of the J-PCNA trait, crossing with individuals having more than three counts of the astringent allele (quadruplex, pentaplex, and hexaplex for the dominant astringent allele), which are predominant in the germplasm (Akagi et al. 2010), does not produce any J-PCNA offspring, when assuming no double reduction. Even in the case of crossing individuals with duplex or triplex for the astringent allele, the frequency of the J-PCNA progeny in the next generation is quite low, implying a low possibility of producing J-PCNA individuals by a cross between J-PCNA and non-PCNA (Ikeda et al. 1985; Onoue et al. 2018). Consequently, it is believed that the current PCNA population was formed mainly by crosses among a small number of ancestral PCNA individuals, and thus, the PCNA group has limited genetic diversity (Sato and Yamada 2016). Several genetic analyses using molecular markers supported this idea (Yonemori et al. 2008b; Naval et al. 2010; Parfitt et al. 2015).

The recessive nature of the J-PCNA trait raises the question of how the initial J-PCNA was established in polyploid persimmon. The circumstantial evidence points to the natural accumulation of the mutated non-astringent allele. As mentioned above, the allele frequency of the non-astringent allele is quite low compared with the astringent allele (Akagi et al. 2010; Onoue et al. 2018). The predominance of hexasomic inheritance at this locus has been suggested (Akagi et al. 2012b), implying the absence of any special inheritance that facilitates the accumulation of the mutated non-astringent allele. It should be noted that the mutated non-astringent allele in the *AST*-linked marker region is highly polymorphic in diverse cultivars (Kono et al. 2016; Onoue et al. 2018), suggesting a considerably early origin of the non-astringent allele. Future analyses of the diversity in the *AST* region may help to clarify the origin of J-PCNA.

11.2.3 Breeding

Consequently, to expand the genetic diversity, J-PCNA from crosses between PCNA and non-PCNA, but not from crosses among PCNAs, have been selected in recent new PCNA breeding programs (Sato and Yamada 2016) (see Chap. 3). Because persimmon exhibits a long juvenile phase, its crossbreeding has both high labor and financial costs. Kanzaki et al. (2010) developed a practical PCR marker that determines the *AST* genotype at the seedling stage, and through its use, marker-assisted selection has been actively

conducted in persimmon breeding (Sato and Yamada 2016). As mentioned earlier, parental allelic information on the *AST* gene is important for efficiently obtaining J-PCNA plants through J-PCNA and non-PCNA crosses, and the putative gene dose has been characterized through the use of a highly polymorphic site that is amplified by the PCR marker (Kono et al. 2016; Onoue et al. 2018). Although the marker region is very tightly linked to the *AST* gene, a few genotype–phenotype inconsistencies have been reported (Mitani et al. 2014), suggesting a small genetic distance between the marker region and the *AST* gene.

11.2.4 Genetic Mapping of *AST*

As with most polyploid species, genetic studies, including the map-based cloning of *AST*, have been severely hampered by the complexity of allelic segregation and heterozygosity in the hexaploid background. In particular, for persimmon, the limited availability of *Diospyros* sequence resources was a major barrier to the success of genetic analyses. To date, the PCNA trait is found only in polyploid *D. kaki* and has not been reported in the other diploid relatives, implying that direct analysis of polyploid species is required to uncover the genetic regulation of the PCNA trait. Nishiyama et al. (2018a) applied a "shuttle mapping" approach (Le Cunff et al. 2008), by referencing the corresponding genomic region of *D. lotus*, to identify the *AST* locus region (Fig. 11.2). *D. lotus* is one of the closest diploid relatives of *D. kaki* (Duangjai et al. 2006; Yonemori et al. 2008a), and it shows similar PA accumulation patterns as non-PCNA cultivars (Fig. 11.3a, b), allowing its use as a genomic reference for the mapping of *AST*. A genetic analysis of *D. kaki* and *D. lotus* F_1 populations using haplotype-specific markers delimited the *AST* region, which spans over an approximately 915-kb region in the *D. lotus* genome (Nishiyama et al. 2018a). Despite these efforts, the underlying gene has yet to be identified.

11.3 Molecular and Biochemical Control

11.3.1 PA Biosynthesis Pathway

The PA biosynthetic pathway and its regulatory mechanisms have been elucidated mainly using *Arabidopsis* and *Medicago* as models. PAs are synthesized through the shikimate pathway and the subsequent main flavonoid pathway starting from phenylalanine. The components for PA biosynthesis are shared with many flavonoids until the last steps of the reactions producing flavan-3-ols through leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR) (Saito et al. 2013; Dixon and Sarnala 2020). The functions of many genes, including those encoding enzymes involved in biosynthetic pathways and vacuolar trafficking, as well as their expression regulators, including the MYB–bHLH–WD40 (MBW) complex, have been identified and characterized (Lepiniec et al. 2006; Saito et al. 2013; Xu et al. 2015). Not only genetic control but also the environmental control mechanisms of the PA regulatory MBW complexes are being actively studied (Li 2014; Xu et al. 2015; Allan and Espley 2018) because the accumulation levels of many flavonoids fluctuate under environmental conditions, including abiotic stresses (Jaakola and Hohtola 2010; Fini et al. 2011; Xu et al. 2015).

11.3.2 Gene Expression Dynamics Controlled by *AST*

Because PA is biosynthesized in diverse plants, PAs in persimmon fruit are thought to be also synthesized through the PA pathway, as in other plant species (Akagi et al. 2011a). In the J-PCNA fruit, genes encoding flavonoids/PA biosynthesis enzymes, such as *Flavonoid 3',5'-Hydroxylase* (*F3'5'H*), *phenylalanine ammonia-lyase* (*PAL*), and *ANR* are downregulated (Ikegami et al. 2005a; Akagi et al. 2009a; Gil-Muñoz et al. 2020). These changes in expression levels have also been observed at the end of the first quarter

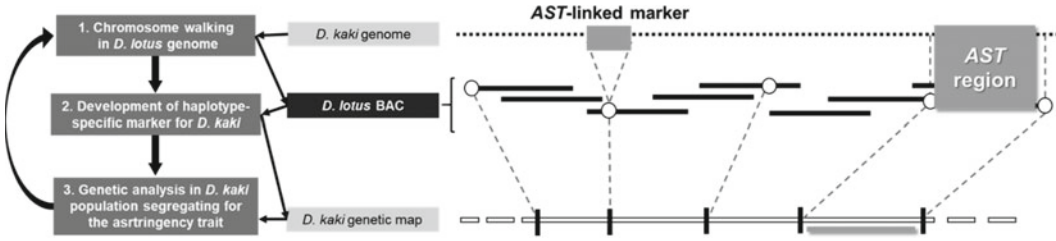

Fig. 11.2 Schematic representation of comparative genome mapping of *AST* in Nishiyama et al. (2018b). Chromosome walking, starting from the *AST*-linked marker region (Kanzaki et al. 2010), was performed in diploid persimmon. The process of "shuttling" (Le Cunff et al. 2008) between chromosome walking in the diploid *D. lotus* and the genetic analysis of hexaploid *D. kaki* using a segregating population for the J-PCNA trait was repeated until the *AST* locus region was identified

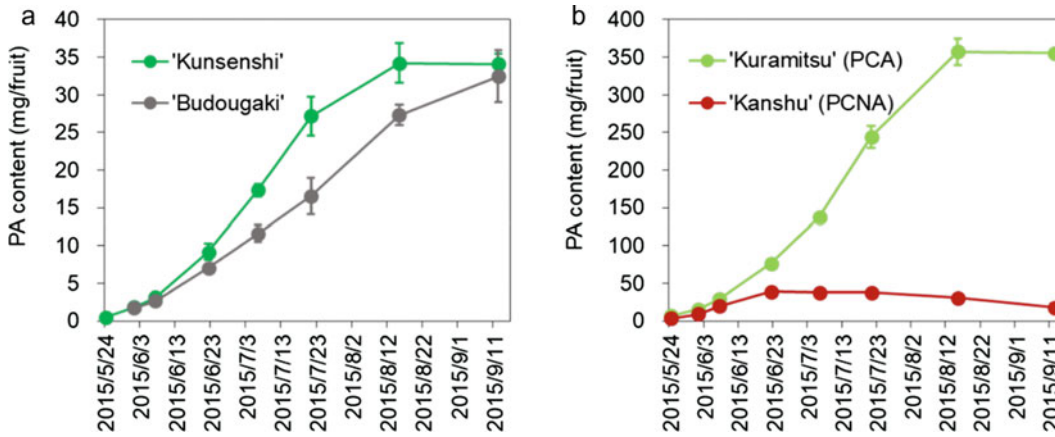

Fig. 11.3 Proanthocyanidin (PA) accumulation patterns of **a** *D. lotus* and **b** *D. kaki*. Plants in the experimental orchard of the Kyoto Farmstead of the Experimental Farm, Kyoto University were analyzed. The PA content was determined using the *p*-dimethylaminocinnamaldehyde (DMACA) method (Li et al. 1996). Error bars represent standard errors ($n = 3$)

of fruit development, indicating that *AST* functions at an early stage of fruit growth (Ikegami et al. 2005a; Akagi et al. 2009a; Nishiyama et al. 2018b).

The PCNA traits are associated with decreased expression levels of genes encoding enzymes not only in the main flavonoid pathway but also in the upstream shikimate pathway (Akagi et al. 2009a; Nishiyama et al. 2018b). In the shikimate pathway, phosphoenolpyruvate and erythrose-4-phosphate are converted to chorismate, which is a precursor of phenylalanine (Herrmann and Weaver 1999), at the start of the flavonoid main pathway. Hierarchical clustering using transcriptomic data obtained from the developing fruit of PCNA/non-PCNA F_1 individuals showed that key enzymes in the shikimate pathway are strongly correlated with PA accumulation (Nishiyama et al. 2018b). The coordinated expression of the shikimate and flavonoid main pathways may be attributed to the high carbon flow requirement for the PA pathway that would allow for high PA accumulation in persimmon fruit.

Important transcription factors that regulate PA accumulation in persimmon have been identified. *DkMYB4* is an MYB transcription factor that functions in PA regulation, and its overexpression in persimmon and kiwifruit (*Actinidia deliciosa*) calli results in increased PA

accumulations (Akagi et al. 2009b). *DkMYB4* is reported to be an important regulator of the J-PCNA trait, and its expression patterns in J-PCNA and non-PCNA developing fruit are strongly correlated with PA accumulation in the fruit (Akagi et al. 2009b; Nishiyama et al. 2018b). *DkMYC1* is a bHLH transcription factor with an unknown function. However, its expression pattern in persimmon fruit is highly correlated with PA accumulation (Su et al. 2012), and it physically binds to DkMYB4 (Naval et al. 2016). *DkWDR1*, a WD40-repeat gene, which binds to DkMYB4–DkMYC1, has also been identified previously (Naval et al. 2016). Nishiyama et al. (2018b) reported that a very limited number of candidate regulators, including DkMYB4 and DkMYC1, belong to the same coexpression module as the PA pathway genes, as assessed by a fruit transcriptome analysis of a population segregating for the J-PCNA trait. These results highlight the importance of DkMYB4 and DkMYC1 in the J-PCNA trait. The expression variation between J-PCNA/non-PCNA is very similar to that altered by overexpression of VvMYBPA1, a grape (*Vitis vinifera*) ortholog of DkMYB4, suggesting the wide conservation of its molecular function in PA accumulation (Terrier et al. 2008).

Another molecular genetic feature of PA regulation in persimmon fruit is the marked expressional variations of chloroplast-related genes (Nishiyama et al. 2018b). In particular, the expression levels of genes functioning in Photosystem I are strongly associated with PA accumulation (Nishiyama et al. 2018b). While the cause of this is unclear, it may relate to the hypothesis that PA biosynthesis occurs in chloroplast thylakoids and that PAs are trapped in a special organelle, the tannosome, arising from thylakoid membranes, for trafficking towards vacuoles (Brillouet et al. 2013). However, this hypothesis appears inconsistent with existing biochemical and genetic evidence (Zhao 2015; Dixon and Sarnala 2020). In addition, because *AST* has not been cloned, we cannot exclude the possibility that a dynamic change in chloroplast signals has direct/indirect effects on *AST* or other genes located near *AST*. Future molecular genetic research in persimmon may provide new insights into organelle dynamics associated with PA accumulation.

11.3.3 Polymerization

The PAs in persimmon fruit have extremely high degrees of polymerization (Matsuo and Ito 1978; Akagi et al. 2011a), which are essential for the making of fermentation products, such as "Kaki-Shibu" in Japan, that have been used for various purposes since ancient times and are still used as clarifying agents for Japanese sake (Iwamoto 2013). As a mechanism to control the degree of polymerization, recent genetics, and biochemical studies using *Medicago truncatula* as a model have revealed the role of LAR in the determination of molecular weight (Dixon and Sarnala 2020). A *LAR* loss of function results in increased levels of insoluble higher molecular weight PAs in *M. truncatula* (Liu et al. 2016). Additionally, a genetic analysis of grapevine revealed the genetic linkage of polymorphisms on *LAR* gene body to PA polymer chain length (Huang et al. 2012; Liu et al. 2016), suggesting the wide conservation of the regulatory functions of *LAR* in chain length. In persimmon, the molecular weight of PAs may be lower in J-PCNA fruit than in non-PCNA fruit (Ikegami et al. 2005b; Akagi et al. 2009a). Akagi et al. (2009a) showed a higher expression of *LAR* in the developing J-PCNA fruit than in non-PCNA fruit, which is consistent with the findings shown in *M. truncatula* (Liu et al. 2016).

11.3.4 Environment

The non-PCNA cultivars are now grown in various climates, but there are limits to the J-PCNA cultivation region. When J-PCNA cultivars are grown in cold regions, astringency accidentally arises in the fruit even at harvest time depending on the climate of the year; thereby limiting the PCNA cultivation (Chujo 1982; Harada et al. 1990; Mowat et al. 1998). The low temperatures in cold climates may delay the reduction of the soluble PA concentration in the late stages of

fruit growth in some cultivars (Chujo 1982; Harada et al. 1990). A low-temperature treatment was suggested to modulate the expression levels of PA-related genes, resulting in an increase in the PA contents of persimmon fruit (Akagi et al. 2011b).

The regulatory roles of phytohormones in PA accumulation in persimmon fruit are not clear. Akagi et al. (2012a) reported a regulatory role for abscisic acid (ABA) in the control of *DkMYB4* expression through the transcription factor DkbZIP5. However, continuous applications of ABA in developing J-PCNA fruit have only faint effects on PA accumulation (Nishiyama et al. 2014).

11.4 Fruit Physiology

11.4.1 Tannin Cells

Plants usually avoid phenolic-associated cytotoxicity by transporting them into vacuoles. In addition to this intracellular compartmentalization of PA, persimmon forms PA-accumulating idioblasts, so-called "tannin cells", which represent another remarkable feature of PA accumulation in persimmon fruit (Howard 1906; Tokugawa and Yuasa 1936). These cells have been observed in several plants (Rhee and Iwata 1982; Karwatzki et al. 1993), but we believe the best-characterized example is persimmon. The tannin cells are scattered throughout the persimmon fruit flesh and accumulate PAs in vacuoles as the surrounding parenchyma cells enlarge.

The development of tannin cells is closely related to the J-PCNA trait (Yonemori and Matsushima 1985). In non-PCNA fruit, tannin cells continue to enlarge as the fruit develops, but in PCNA fruit, the enlargement ends in the early stages of fruit development. The timing is quite consistent with the pattern of PA accumulation, and there is a marked difference in tannin cell size between PCNA and non-PCNA fruits (Fig. 11.4a, b).

Several interesting histological characteristics of tannin cells have been observed. In model plants, several processes, such as vesicle trafficking, membrane transporter, and glutathione *S*-transferase-mediated transport, were proposed to play roles in the vacuolar sequestration of flavonoids (Zhao 2015; Dixon and Sarnala 2020). In persimmon, in particular, the development of many vesicular structures was observed during the early stages of PA accumulation (Yonemori et al. 1997; Tessmer et al. 2014). In addition, tannin cells in fruit tissues are not randomly distributed. They are arranged in close proximity and may, in fact, be connected (Yonemori and Suzuki 2009). The presence of pores in the cell walls of tannin cells has also been reported, and their opening closely coincides with PA accumulation (Yonemori and Matsushima 1987a, b; Tessmer et al. 2014). These observations collectively suggest the significance of cell-to-cell communications in PA accumulation, which had been largely overlooked in the flavonoid-related research conducted in model plants. In this context, persimmon may be a good resource for studying the molecular genetics of PA accumulation. Additionally, the expression levels of the vacuolar membrane-localized transporter *DkMATE1* and other putative transporters correlate with PA accumulation (Yang et al. 2016; Nishiyama et al. 2018b), making them prime targets for molecular genetic research on PA transport and tannin cells in persimmon.

11.4.2 PA Insolubilization

Soluble PAs may become insolubilized naturally or artificially. Insolubilized PAs generally do not produce an astringent taste upon consumption, making this process important for agricultural production. In persimmon, PA insolubilization is a fundamental mechanism of artificial deastringency (Matsuo and Itoo 1982; Tanaka et al. 1994). In addition, the differences among the PCA, PVA, and PVNA groups are attributed to the extent of insolubilization, which may be controlled by the level of volatile compound emissions from seeds (Sugiura and Tomana 1983). Furthermore, PAs in the PCNA fruit are less reactive to the insolubilization process than

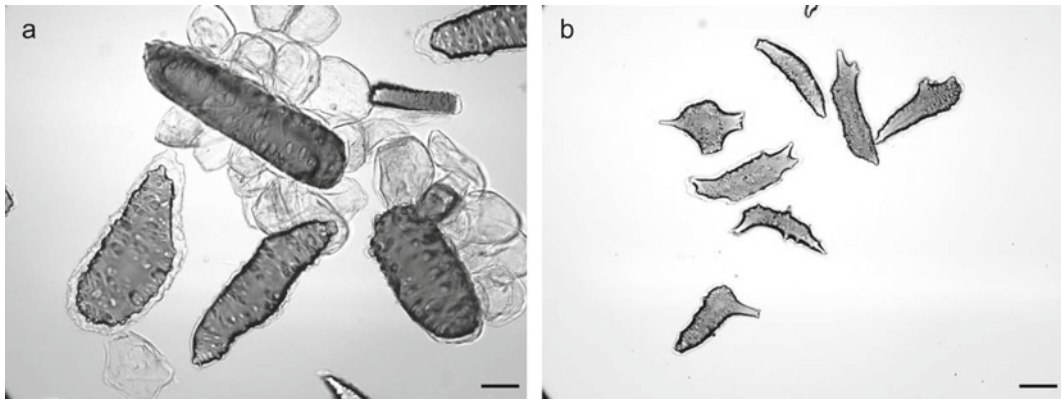

Fig. 11.4 Microscopic view of extracted tannin cells. Developing fruit tissue at the middle of the fruit development (August) was fixed, and tannin cells were isolated as described in Ikegami et al. (2006). **a** Non-PCNA and **b** PCNA tannin cells from a segregating population for the J-PCNA trait. Scale bars indicate 100 μm

those in the non-PCNA fruit, but they may be naturally insolubilized through the fruit development, resulting in the production of the non-astringent fruit (Yonemori and Matsushima 1985). Dixon and Sarnala (2020) mentioned that the insoluble PAs are quantified by conversion to anthocyanidins by heating in acidic butanol. However, in persimmon, several studies reported different degrees of re-solubility of the insolubilized PAs in an acid solvent (Oshida et al. 1996; Taira et al. 1998), indicating potential physicochemical diversity during the insolubilization process.

11.4.3 Pleiotropic Effects

Many J-PCNA individuals commonly possess several shared and often undesirable traits, such as fruit cracking, flat-shaped fruit, small fruit size, and weak tree vigor (Yamada et al. 1986, 1994; Sato and Yamada 2016). Most of the traits are considered to be the outcome of the low genetic diversity level of J-PCNA and the repeated use of individuals with similar genetic backgrounds for the J-PCNA group development (Sato and Yamada 2016). However, some of the traits, such as fruit size, maybe genetically associated with the J-PCNA trait (Sato et al. 2013). Uncovering the pleiotropic effects of *AST* is important with respect to both molecular regulatory mechanisms and horticultural development, and they should be studied after *AST* is cloned.

11.5 Conclusions and Future Perspectives

Here, we summarized the genetics, physiology, and applications of the J-PCNA trait. PAs are common metabolites of defense responses found in diverse plants, and large amounts of PAs are generally stored in fruit. PA synthesis in fruit is active during the early stages of fruit development in many plants, but PA synthesis actively continues throughout fruit development in the astringent persimmon. The accumulation patterns of PAs in mutant J-PCNA fruit are more similar to the patterns in most plants; therefore, the *AST* gene should be closely related to an evolutionary driver responsible for the distinct PA accumulation in persimmon.

The most important issue that needs to be resolved in persimmon PA research is the cloning of *AST*. Identifying the *AST* gene will facilitate our understanding of the distinct accumulation of PAs in persimmon. It will allow for more efficient PCNA breeding and the analysis of pleiotropic effects, which are important to the persimmon industry. The genetic interval for the *AST* gene has been delimited and corresponds

to a 915-kb region in *D. lotus* (Nishiyama et al. 2018a). However, no promising candidate genes/mutations have been identified using the *D. lotus* and *D. oleifera* genomes as a reference (Nishiyama et al. unpublished). Future analyses of the polyploid genome may allow the identification of the determinant gene.

We believe that the recently published diploid *Diospyros* (*D. lotus* and *D. oleifera*) genomes (Zhu et al. 2019; Suo et al. 2020; Akagi et al. 2020) will highly facilitate PA research in persimmon. Both species accumulate vast amounts of PAs in fruit, and the available *Agrobacterium*-mediated transformation system (Li et al. 2018; Tao et al. unpublished), in combination with their reference genomes, may allow the efficient cloning of PA-related genes. Because vesicle-like structures and cell-to-cell communications have been observed (see Sect. 11.4.1), persimmon research may potentially contribute to our basic understanding of PA biology, as well as the agricultural significance of PA accumulation control.

References

Akagi T, Ikegami A, Suzuki Y et al (2009a) Expression balances of structural genes in shikimate and flavonoid biosynthesis cause a difference in proanthocyanidin accumulation in persimmon (*Diospyros kaki* Thunb.) fruit. Planta 230:899–915

Akagi T, Ikegami A, Tsujimoto T et al (2009b) DkMyb4 is a Myb transcription factor involved in proanthocyanidin biosynthesis in persimmon fruit. Plant Physiol 151:2028–2045

Akagi T, Takeda Y, Yonemori K et al (2010) Quantitative genotyping for the astringency locus in hexaploid persimmon cultivars using quantitative real-time PCR. J Am Soc Hortic Sci 135:59–66

Akagi T, Katayama-Ikegami A, Yonemori K (2011a) Proanthocyanidin biosynthesis of persimmon (*Diospyros kaki* Thunb.) fruit. Sci Hortic 130:373–380

Akagi T, Tsujimoto T, Ikegami A, Yonemori K (2011b) Effects of seasonal temperature changes on DkMyb4 expression involved in proanthocyanidin regulation in two genotypes of persimmon (*Diospyros kaki* Thunb.) fruit. Planta 233:883–894

Akagi T, Katayama-Ikegami A, Kobayashi S et al (2012a) Seasonal abscisic acid signal and a basic leucine zipper transcription factor, DkbZIP5, regulate proanthocyanidin biosynthesis in persimmon fruit. Plant Physiol 158:1089–1102

Akagi T, Tao R, Tsujimoto T et al (2012b) Fine genotyping of a highly polymorphic *ASTRINGENCY*-linked locus reveals variable hexasomic inheritance in persimmon (*Diospyros kaki* Thunb.) cultivars. Tree Genet Genomes 8:195–204

Akagi T, Shirasawa K, Nagasaki H et al (2020) The persimmon genome reveals clues to the evolution of a lineage-specific sex determination system in plants. PLoS Genet 16:e1008566

Allan AC, Espley RV (2018) MYBs drive novel consumer traits in fruits and vegetables. Trends Plant Sci 23:693–705

Aron PM, Kennedy JA (2008) Flavan-3-ols: nature, occurrence and biological activity. Mol Nutr Food Res 52:79–104

Bagchi D, Swaroop A, Preuss HG, Bagchi M (2014) Free radical scavenging, antioxidant and cancer chemoprevention by grape seed proanthocyanidin: an overview. Mutat Res 768:69–73

Brillouet J-M, Romieu C, Schoefs B et al (2013) The tannosome is an organelle forming condensed tannins in the chlorophyllous organs of Tracheophyta. Ann Bot 112:1003–1014

Chujo T (1982) Studies on the effects of thermal conditions on the growth and quality of fruits of Fuyu kaki (in Japanese). Mem Fac Agric Kagawa Univ 37:1–63

Dixon RA, Sarnala S (2020) Proanthocyanidin biosynthesis—a matter of protection. Plant Physiol 184:579–591

Dixon RA, Xie DY, Sharma SB (2005) Proanthocyanidins—a final frontier in flavonoid research? New Phytol 165:9–28

Duangjai S, Wallnöfer B, Samuel R et al (2006) Generic delimitation and relationships in Ebenaceae sensu lato: evidence from six plastid DNA regions. Am J Bot 93:1808–1827

Fini A, Brunetti C, Di Ferdinando M et al (2011) Stress-induced flavonoid biosynthesis and the antioxidant machinery of plants. Plant Signal Behav 6:709–711

Gil-Muñoz F, Sánchez-Navarro JA, Besada C et al (2020) MBW complexes impinge on anthocyanidin reductase gene regulation for proanthocyanidin biosynthesis in persimmon fruit. Sci Rep 10:3543

Harada H, Itamura H, Taira S, Zheng G-H (1990) Natural removal of astringency in PCNA persimmon fruit cv. 'Jiro' grown in some different districts of Japan. J Jpn Soc Hortic Sci 58:807–811

Herrmann KM, Weaver LM (1999) The shikimate pathway. Annu Rev Plant Physiol Plant Mol Biol 50:473–503

Howard BJ (1906) Tannin cells of persimmons. Bull Torrey Bot Club 33:567–576

Huang Y-F, Doligez A, Fournier-Level A et al (2012) Dissecting genetic architecture of grape proanthocyanidin composition through quantitative trait locus mapping. BMC Plant Biol 12:30

Hume HH (1914) A kaki classification. J Hered 5:400–406

Ikeda I, Yamada M, Kurihara A, Nishida T (1985) Inheritance of astringency in Japanese persimmon. J Jpn Soc Hotic Sci 54:39–45

Ikegami A, Yonemori K, Sugiura A et al (2004) Segregation of astringency in F1 progenies derived from crosses between pollination-constant, nonastringent persimmon cultivars. HortScience 39:371–374

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

Ikegami A, Sato A, Yamada M et al (2005b) Molecular size profiles of tannins in persimmon fruits of Japanese and Chinese pollination-constant non-astringent (PCNA)-type cultivars and their offspring revealed by size-exclusion chromatography. J Jpn Soc Hortic Sci 74:437–443

Ikegami A, Eguchi S, Yonemori K et al (2006) Segregations of astringent progenies in the F1 populations derived from crosses between a Chinese pollination-constant nonastringent (PCNA) 'Luo Tian Tian Shi', and Japanese PCNA and pollination-constant astringent (PCA) cultivars of Japanese origin. HortScience 41:561–563

Iwamoto K (2013) The historical background and the application for "sake" of clarifier "Kaki-Shibu." J Brew Soc Japan 108:318–325

Jaakola L, Hohtola A (2010) Effect of latitude on flavonoid biosynthesis in plants. Plant Cell Environ 33:1239–1247

Kajiura M (1946) Persimmon cultivars and their improvement. 2. Breed Hortic 1:175–182

Kanzaki S, Akagi T, Masuko T et al (2010) SCAR markers for practical application of marker-assisted selection in persimmon (*Diospyros kaki* Thunb.) breeding. J Jpn Soc Hotic Sci 79:150–155

Karwatzki B, Herget A, Beerhues L, Wiermann R (1993) In situ localization of chalcone synthase in tannin-containing plants. Phytochemistry 32:585–590

Kikuchi A (1948) Pomology-part I (in Japanese). Yokendo, Tokyo

Kono A, Kobayashi S, Onoue N, Sato A (2016) Characterization of a highly polymorphic region closely linked to the *AST* locus and its potential use in breeding of hexaploid persimmon (*Diospyros kaki* Thunb.). Mol Breed 36. https://doi.org/10.1007/s11032-016-0480-0

Le Cunff L, Garsmeur O, Raboin LM et al (2008) Diploid/polyploid syntenic shuttle mapping and haplotype-specific chromosome walking toward a rust resistance gene (Bru1) in highly polyploid sugarcane (2n ~ 12x ~ 115). Genetics 180:649–660

Lepiniec L, Debeaujon I, Routaboul J-M et al (2006) Genetics and biochemistry of seed flavonoids. Annu Rev Plant Biol 57:405–430

Li S (2014) Transcriptional control of flavonoid biosynthesis: fine-tuning of the MYB-bHLH-WD40 (MBW) complex. Plant Signal Behav 8:1–7

Li YG, Tanner G, Larkin P (1996) The DMACA–HCl protocol and the threshold proanthocyanidin content for bloat safety in forage legumes. J Sci Food Agric 70:89–101

Li X, Jiang Z, Shen Y et al (2018) In vitro regeneration and Agrobacterium tumefaciens-mediated genetic transformation of *D. lotus* (*Diospyros lotus* L.). Sci Hortic 236:229–237

Liu C, Wang X, Shulaev V, Dixon RA (2016) A role for leucoanthocyanidin reductase in the extension of proanthocyanidins. Nat Plants 2:1–7

Manach C, Williamson G, Morand C et al (2005) Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr 81:230S-242S

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

Matsuo T, Itoo S (1982) A model experiment for de-astringency of persimmon fruit with high carbon dioxide treatment: in vitro gelation of kaki-tannin by reacting with acetaldehyde. Agric Biol Chem 46:683–689

Mitani N, Kono A, Yamada M et al (2014) Practical marker-assisted selection using two SCAR markers for fruit astringency type in crosses of 'Taiten' × PCNA cultivars in persimmon breeding. Sci Hortic 170:219–223

Mowat AD, Poole PR, Whitaker G, Edwards C (1998) Tannin cell content and distribution in persimmon (*Diospyros kaki* L.) as a cause of residual astringency. Acta Hortic 482–482

Naval M del M, Zuriaga E, Pecchioli S et al (2010) Analysis of genetic diversity among persimmon cultivars using microsatellite markers. Tree Genet Genomes 6:677–687

Naval M del M, Gil-Muñoz F, Lloret A et al (2016) A WD40-repeat protein from persimmon interacts with the regulators of proanthocyanidin biosynthesis DkMYB2 and DkMYB4. Tree Genet Genomes 12:1–11

Nishiyama S, Yonemori K, Ueda J (2014) Effect of ABA treatment on tannin accumulation at an early stage of fruit development in persimmon. Acta Hortic 217–222

Nishiyama S, Onoue N, Kono A et al (2018a) Comparative mapping of the *ASTRINGENCY* locus controlling fruit astringency in hexaploid persimmon (*Diospyros kaki* Thunb.) with the diploid *D. lotus* reference genome. Hortic J 87:315–323

Nishiyama S, Onoue N, Kono A et al (2018b) Characterization of a gene regulatory network underlying astringency loss in persimmon fruit. Planta 247:733–743

Onoue N, Kobayashi S, Kono A, Sato A (2018) SSR-based molecular profiling of 237 persimmon (*Diospyros kaki* Thunb.) germplasms using an *ASTRINGENCY*-linked marker. Tree Genet Genomes 14:28

Oshida M, Yonemori K, Sugiura A (1996) On the nature of coagulated tannins in astringent-type persimmon fruit after an artificial treatment of astringency removal. Postharvest Biol Technol 8:317–327

Parfitt DE, Yonemori K, Honsho C et al (2015) Relationships among Asian persimmon cultivars, astringent and non-astringent types. Tree Genet Genomes 11:24

Rhee JK, Iwata M (1982) Histological observations on the chilling injury of taro tubers during cold storage. J Jpn Soc Hortic Sci 51:362–368

Saito K, Yonekura-Sakakibara K, Nakabayashi R et al (2013) The flavonoid biosynthetic pathway in Arabidopsis: structural and genetic diversity. Plant Physiol Biochem 72:21–34

Sato A, Yamada M (2016) Persimmon breeding in Japan for pollination-constant non-astringent (PCNA) type with marker-assisted selection. Breed Sci 66:60–68

Sato A, Kono A, Mitani N et al (2013) Comparison of fruit traits between pollination constant non-astringent (PCNA) and non-PCNA offspring derived from two backcrosses (non-PCNA × PCNA) × PCNA. Acta Hortic 123–126

Su F, Hu J, Zhang Q, Luo Z (2012) Isolation and characterization of a basic helix-loop-helix transcription factor gene potentially involved in proanthocyanidin biosynthesis regulation in persimmon (*Diospyros kaki* Thunb.). Sci Hortic 136:115–121

Sugiura A, Tomana T (1983) Relationships of ethanol production by seeds of different types of Japanese persimmons and their tannin content. HortScience 18:319–321

Suo Y, Sun P, Cheng H et al (2020) A high-quality chromosomal genome assembly of *Diospyros oleifera* Cheng. Gigascience 9. https://doi.org/10.1093/gigascience/giz164

Taira S, Matsumoto N, Ono M (1998) Accumulation of soluble and insoluble tannins during fruit development in nonastringent and astringent persimmon. J Jpn Soc Hortic Sci 67:572–576

Tanaka T, Takahashi R, Kouno I, Nonaka GI (1994) Chemical evidence for the de-astringency (insolubilization of tannins) of persimmon fruit. J Chem Soc Perkin Trans 3013–3022

Terrier N, Torregrosa L, Ageorges A et al (2008) Ectopic expression of VvMybPA2 promotes proanthocyanidin biosynthesis in grapevine and suggests additional targets in the pathway. Plant Physiol 149:1028–1041

Tessmer MA, Kluge RA, Appezzato-da-Glória B (2014) The accumulation of tannins during the development of 'Giombo' and 'Fuyu' persimmon fruits. Sci Hortic 172:292–299

Tokugawa Y, Yuasa A (1936) Notes on the tannin cells of persimmon. Shokubutsugaku Zasshi 50:277–283

Wang R (1982) The origin of 'Luo Tian Tian Shi.' Chin Fruit Tree 2:16–19

Xu W, Dubos C, Lepiniec L (2015) Transcriptional control of flavonoid biosynthesis by MYB-bHLH-WDR complexes. Trends Plant Sci 20:176–185

Yamada M (2005) Persimmon genetic resources and breeding in Japan. Acta Hortic 55–64

Yamada M, Sato A (2002) Segregation for fruit astringency type in progenies derived from crosses of 'Nishimurawase' × pollination constant non-astringent genotypes in oriental persimmon (*Diospyros kaki* Thunb.). Sci Hortic 92:107–111

Yamada M, Ikeda I, Yamane H, Hirabayashi T (1986) Inheritance of fruit cracking persimmon at the calyx end and stylar in Japanese persimmon. J Jpn Soc Hotic Sci 57:8–16

Yamada M, Yamane H, Ukai Y (1994) Genetic analysis of Japanese persimmon fruit weight. J Am Soc Hortic Sci 119:1298–1302

Yamada M, Giordani E, Yonemori K (2012) Persimmon. In: Badenes ML, Byrne DH (eds) Fruit breeding. Springer US, Boston, MA, pp 663–693

Yang S, Jiang Y, Xu L et al (2016) Molecular cloning and functional characterization of DkMATE1 involved in proanthocyanidin precursor transport in persimmon (*Diospyros kaki* Thunb.) fruit. Plant Physiol Biochem 108:241–250

Yonemori K, Matsushima J (1985) Property of development of tannin cells in non-astringent type fruits of Japanese persimmon (*Diospyros kaki*) and its relationship to natural deastringency. J Jpn Soc Hotic Sci 54:201–208

Yonemori K, Matsushima J (1987a) Changes in tannin cell morphology with growth and development of Japanese persimmon fruit. J Am Soc Hortic Sci 112:818–821

Yonemori K, Matsushima J (1987b) Morphological characteristics of tannin cells in Japanese persimmon fruit. J Am Soc Hortic Sci 112:812–817

Yonemori K, Suzuki Y (2009) Differences in three-dimensional distribution of tannin cells in flesh tissue between astringent and non-astringent type persimmon. Acta Hortic 119–124

Yonemori K, Oshida M, Sugiura A (1997) Fine structure of tannin cells in fruit and callus tissues of persimmon. Acta Hortic 403–416

Yonemori K, Honsho C, Kanzaki S et al (2008a) Sequence analyses of the ITS regions and the matK gene for determining phylogenetic relationships of *Diospyros kaki* (persimmon) with other wild *Diospyros* (Ebenaceae) species. Tree Genet Genomes 4:149–158

Yonemori K, Honsho C, Kitajima A et al (2008b) Relationship of European persimmon (*Diospyros kaki* Thunb.) cultivars to Asian cultivars, characterized using AFLPs. Genet Resour Crop Evol 55:81–89

Yonemori K, Sugiura A, Yamada M (2010) Persimmon genetics and breeding. In: Plant breeding reviews. Wiley, Oxford, pp 191–225

Zhao J (2015) Flavonoid transport mechanisms: how to go, and with whom. Trends Plant Sci 20:576–585

Zhu Q-G, Xu Y, Yang Y et al (2019) The persimmon (*Diospyros oleifera* Cheng) genome provides new insights into the inheritance of astringency and ancestral evolution. Hortic Res 6:138

Fruit-Size Control

12

Soichiro Nishiyama and Hisayo Yamane

Abstract

Fruit size is an important trait of fruit tree crops that determines the agricultural performance and commercial value of fruit. Elucidating the molecular, genetic and physiological bases of fruit-size control provides basic knowledge required for the breeding and cultivation of desirably sized fruit. Cultivated persimmon shows a great genetic variation in fruit size, and, despite the importance, very little information is available on the genetic control of fruit size and the related molecular mechanisms. Here, we summarize the accumulated knowledge on fruit-size control in persimmon. The effects of phytohormones on fruit-size regulation have been intensively analyzed over the years, and cytokinins are the predominant and most-effective hormones that control fruit size in persimmon. Despite the paucity of information at the molecular and genetic levels, we discuss two topics significantly related to persimmon fruit size: inbreeding depression effects and small fruit mutations. Recently developed reference genomes should further advance genetic and physiological studies on persimmon fruit-size control.

12.1 Introduction

How plants control the size of fleshy fruit is a universal question of significance to scientists, fruit growers and consumers. Fruit size is a commercially important trait that influences production outcomes and consumer preferences, and large fruit size is generally preferred in the market. However, from the perspective of plant reproduction, large fruit size is not always advantageous for efficient seed dispersal in many wild populations (Mazer and Wheelwright 1993). In other words, a large fruit size reflects the outcome of human selection during cultivation and, at least in part, has evolved along with human consumption, as a 'domestication trait'. Understanding the genetic, environmental and internal control of fruit size aids in detangling the complex history of crop evolution, as well as targeted fruit-size breeding and production.

Fruit size is a quantitative polygenic trait that is highly variable under the influences of environmental factors. Genetic effects generally have greater impacts, and even minor genetic changes can drastically influence fruit development and thus, final fruit size. However, understanding the genetics underlying the continuous phenotypic variations is difficult because it is hard to identify

S. Nishiyama · H. Yamane (✉)
Graduate School of Agriculture, Kyoto University, Kyoto, Japan
e-mail: yamane.hisayo.6n@kyoto-u.ac.jp

S. Nishiyama
e-mail: nishiyama.soichiro.8e@kyoto-u.ac.jp

R. Tao and Z. Luo (eds.), *The Persimmon Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-05584-3_12

the genes that exert significant effects among the many alleles of multiple loci.

Persimmon (*Diospyros kaki*), originating from East Asia, shows a very large genetic diversity in fruit size. Large variations in fruit weights (FWs), ranging from less than 50 g to over 350 g, have been reported in germplasm collections in Japan and China (Yamada et al. 1995). Recent breeding efforts resulted in the development of a cultivar, 'Taiten', bearing extremely large-sized fruit of 506 g on average (Yamada et al. 2012b), with fruit exceeding 700 g available in the market. In contrast, 'Totsutanenashi' (TTN), which bears extremely small-sized fruits, averaging 40–50 g, has been marketed under a special brand as 'baby persimmon' to meet the recent market demand in Japan (Tao et al. 2013). Despite these breeding efforts and outstanding genetic variation, most of the physiological and genetic bases for controlling fruit size in persimmon remain to be elucidated.

Organ-size diversity is primarily a function of cell size and cell number. Therefore, the molecular network that regulates cell division and cell expansion is the major driving force of growth and a great subject of fruit-size control research. In this chapter, we first present an overview of early efforts on understanding the general properties of fruit growth and its modulation in persimmon. The major developmental drivers—phytohormones—are introduced with particular emphasis on understanding their physiological roles in fruit development and their applications. Next, we summarize critical works that have analyzed genetic diversity in persimmon fruit size, with a special focus on advances in model plants. Finally, future directions for persimmon fruit-size research and breeding, in combination with recently developed reference genomes, are discussed.

12.2 Fruit Growth Patterns

Fruit growth is an important physiological process in various fruit trees that determines the commercial value of the agricultural output and thus, the details of this process have been investigated for decades. In many species, cell division and cell enlargement are the factors that drive fruit growth. Cell division functions from the early differentiation stage of the floral organ to the very beginning of fruit development, followed by cell enlargement during fruit development. Cell division and enlargement integratively determine and control fruit growth. Fruit growth is represented by growth curves, which can be classified into two types: sigmoid and double sigmoid (Crane 1964). Apple and pear fruit grow in a sigmoid fashion, with vigorous mid-growth enlargement. However, grapes and peaches show growth stagnation in the mid-growth stage, and fruit growth fits a double sigmoid shape consisting of growth stages I, II and III (Connors 1919; Nitsch et al. 1960). This growth stagnation generally corresponds to the period of active seed development, and while it has been considered primarily due to competition within the fruit, many aspects remain unclear. Persimmon fruit growth exhibits the double sigmoid shape with a distinct stagnation stage (Nii 1980; Candir et al. 2009). The growth stagnation period in persimmon clearly coincides with the period of active seed development. However, a growth suspension period also exists in the seedless 'Hiratanenashi' (HTN) that is equivalent to that of the seeded cultivars (Zheng et al. 1990). A low temperature during growth stage II shortens the stagnation period and accelerates fruit enlargement (Zheng et al. 1990).

Cultivation experiments have revealed several important factors that determine persimmon fruit size. The position of the fruit within the canopy generally affects fruit size in many fruit crops. For persimmons, fruit produced on the upper side of the canopy have higher sugar contents and better coloration; however, there is no significant difference in FW based on the fruit location (Hasegawa and Nakajima 1990). Additionally, under cultivation conditions in which multiple fruits are produced on a single fruiting branch, the FW at the base of the branch is significantly less than the FW at the center or top of the branch, suggesting a directional nature to the competition among fruits (Kitajima et al. 1993). Flowering time also affects fruit size. Some

persimmon cultivars bear delayed flowers that bloom approximately 10 days later than normal flowers. The resulting fruits from the delayed flowers are smaller than the normal fruits and generally ripen later (Hasegawa 1983; Hasegawa and Nakajima 1990). However, Choi et al. (2014) investigated the delayed flowering of 'Fuyu' and found that the fruits were slightly smaller, but there was no delay in ripening and the fruits could be harvested at the same time as normal fruits. The delayed flowers may be produced by the differentiation of new flower buds after bud break, which occurs later than normal flowers that differentiate during the growth of the previous year (Hasegawa 1983). Collectively, developmental differences are critical for determining fruit size in persimmon. According to a survey of genetic resources in Japan, approximately half of all plants produce delayed flowers (Fruit Tree Experiment Station of Hiroshima Prefecture 1973), but no genetic analysis has been performed to date.

A noteworthy aspect of persimmon fruit physiology is the critical role of calyxes in fruit development (Nakamura 1967). The persimmon calyx has many stomata and is the 'gas exchange organ' of persimmon fruit (Kitagawa and Glucina 1984; Nakano et al. 2003). Removing the calyx lobe in the middle of growth stage I or at the beginning of stage II severely inhibits fruit growth (Yonemori et al. 1995). Additionally, the persimmon calyx may not contribute to photosynthesis, but rather plays a role in the gas exchange that allows the active respiration of the developing fruit (Yonemori et al. 1996; Nakano et al. 1997a).

Owing to nutritional competition among fruits, the effects of fruit thinning techniques on fruit-size control have been extensively studied (Kishimoto 1975; George et al. 1995; Choi et al. 2002). In Japan, fruit/flower thinning is performed by hand from the flower bud stage until 20 days after full bloom to ensure high-quality production and avoid alternate bearing (George et al. 1997). Competition among fruit, or between fruit and other organs, is particularly apparent in persimmon (Kitajima et al. 1993; George et al. 1995; Park 2011), and future research may provide useful insights into fruit production by clarifying the physiological and genetic bases of this characteristic.

12.3 Phytohormones

Phytohormones are essential for regulating fruit development and maturation; therefore, they can be determinants of final fruit size. Phytohormones are generally effective at low doses and have been intensively applied in agricultural practices in many crops (Šimura et al. 2018; Jiang and Asami 2018), including persimmon. Here, we summarize the general characteristics and previous efforts to understand and modulate fruit-size control using phytohormones in persimmon, with a focus on auxin, gibberellin and cytokinin.

The importance of auxin and gibberellin in fruit growth has been established across multiple plant species. Auxin is the primary hormone regulating fruit size in some fruit species, such as apple (Devoghalaere et al. 2012; Fenn and Giovannoni 2021). In persimmon, endogenous auxin levels are highest at the flowering stage (Sobajima et al. 1969; Kojima et al. 1999; Reig et al. 2018). According to descriptive analyses of seedless cultivars, the auxin levels may be involved in physiological fruit abscission and the growth of young developing fruit (Kojima et al. 1999; Reig et al. 2018). The required balancing of the auxin level in developing flowers appears to be critical, and auxin applications to the apical bud or whole tree induce severe fruit drop (Suzuki et al. 1988; Agustí et al. 2004). However, physiological fruit drop induced by girdling and calyx lobe removal may be rescued by the direct application of 1-naphthaleneacetic acid (NAA) on fruit (Suzuki et al. 1988). The effects of auxin applications on fruit size have been reported to be very small or absent (Suzuki et al. 1988; Agustí et al. 2004).

The effects of gibberellins on persimmon fruit development are, however, more significant than those of auxin and have been well characterized. Gibberellins in plants generally act as growth-promoting substances by regulating cell size. Endogenous gibberellins exist at high levels at

flowering in persimmon (Kojima et al. 1999; Reig et al. 2018), and gibberellin applications near flowering time effectively reduce early fruit abscission (Yamamura et al. 1989; Shaya et al. 2019). In contrast, five gibberellic acid (GA) applications during growth stage II inhibit fruit growth owing to the reduction in the fruit respiration rate (Nakano et al. 1997b). Exogenous applications of gibberellin at the flowering stage appear to have slightly negative, or no, effects on fruit size in persimmon (Hasegawa and Nakajima 1990; Krisanapook et al. 1998).

Cytokinins generally promote cell division, and they represent the most significant phytohormones that have been tested for affecting fruit-size control in persimmon. Endogenous cytokinins are abundant in developing female flower buds (Sun et al. 2017), and the cytokinin activity in 'Fuyu' fruit is markedly high up to 30 days after full bloom (Sobajima et al. 1974). Exogenous applications of cytokinins have remarkable effects on fruit size, especially transverse growth (Fig. 12.1). Itai et al. (1995) reported that the application of 100 ppm of the synthetic cytokinin 4PU-30 one week before full bloom causes high-level transverse growth in the oblong shape cultivar 'Saijo', resulting in ball-shaped fruit. The effects of cytokinins on transverse growth are larger after a prebloom treatment than after a postbloom treatment (Itai et al. 1995). The greatest size increase-related effect occurred when synthetic cytokinin was sprayed 10 days after full bloom; however, the longitudinal growth was predominant instead of the transverse growth (Hasegawa et al. 1991), suggesting that the effects on fruit size and shape vary greatly depending on the application stage being before and after flowering. Exogenous applications of synthetic cytokinins may promote cell expansion, as well as cell division, by extending the fruit-growth period (Hamada et al. 2007).

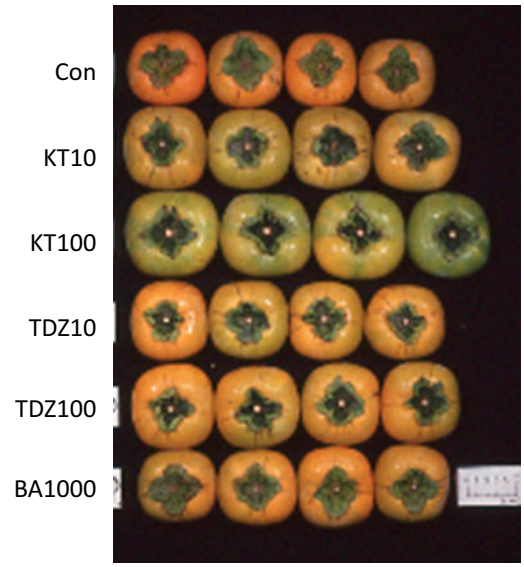

Fig. 12.1 Effects of the synthetic cytokinin-like compounds, N-(2-Chloro-4-pyridyl)-N′-phenylurea (known as CPPU) (named KT here), thidiazuron (TDZ) and benzyl adenine (BA) on Japanese persimmon fruit size at harvest. 10, 100 and 1000 refer to 10, 100 and 1000 ppm, respectively. Photo was kindly provided by Prof. Akihiro Itai, Kyoto Prefectural University

12.4 Genetic and Molecular Control

As described above, despite physiological studies and detailed descriptions of persimmon fruit development, little is known regarding the genetic and molecular mechanisms underlying fruit development. In this section, we first summarize the molecular genetic findings on fruit-size control obtained from model plants. Then, two genetic studies that analyzed the fruit-size control in persimmon are introduced.

12.4.1 Knowledge from Model Plants

Tomato (*Solanum lycopersicum*), with a short life cycle and high seed production, has become a broadly used model plant for research on fleshy fruit physiology and development that cannot be uncovered using the dry fruit-producing model plant *Arabidopsis thaliana* (Mauxion et al. 2021). By taking advantage of the remarkable genetic diversity among wild and cultivated tomatoes, a number of genes involved in fruit development have been cloned. Remarkably, the genome editing of domestication genes has enabled the de novo domestication of wild relatives, resulting in up to a 200% increase in fruit size compared with the wild tomato *Solanum*

pimpinellifolium (Zsögön et al. 2018). Thus, targeted molecular breeding is now possible when precise genetic knowledge and molecular genetic technology are available. Here, we present an overview of molecular genetic knowledge from model plants that is potentially applicable in persimmon.

The quantitative trait loci (QTL) *fw2.2* has a significant effect on tomato fruit size, contributing up to 30% of the trait variation (Alpert and Tanksley 1996). *SlFW2.2*, the gene responsible for the QTL locus, belongs to the Cell Number Regulator (CNR) family, which functions in the determination of organ size by regulating cell number (Guo and Simmons 2011). The expression of *SlFW2.2* may be responsible for controlling fruit size in tomato, and *SlFW2.2* may act as a negative regulator of cell division (Frary et al. 2000; Cong et al. 2002). Although the CNR family of genes in *Diospyros* has not been analyzed in detail, the similarity between the CNR family and the mammalian placenta-specific PLAC8 proteins has been highlighted, suggesting that each plant *FW2.2* gene may function in a unique tissue or cellular location, but carry out similar biochemical functions (Libault and Stacey 2010). The organ size-determination function of the maize *FW2.2* ortholog further suggests functional conservation across diverse plants (Guo et al. 2010).

In tomato, the locule number has evolved during domestication, with increased locule numbers resulting in large flat-shaped fruit (Cong et al. 2008; Rodríguez et al. 2011). The *WUSCHEL* (*SlWUS*) and *CLAVATA3* (*SlCLV3*) complex acts as the main control system for locule number in tomato (Liu et al. 2011; Xu et al. 2015) by determining the size of the stem cell population (Chu et al. 2019). The Arabidopsis orthologs are involved in the maintenance of stem cell identity within the shoot apical meristem (Laux et al. 1996; Ohyama et al. 2009). There is diversity in the locule numbers in persimmon, and it is affected by genetic and environmental factors (Maeda et al. 2018). Future research may determine the involvement of the locule-controlling genes in the fruit-size diversity of persimmon.

The OVATE protein family is another notable gene family characterized in tomato. *Ovate* is a QTL that controls fruit elongation but does not control FW (Liu et al. 2002; Mauxion et al. 2021). Notably, the expression of a putative ortholog of the *OVATE* gene in persimmon is highly correlated with a gene module associated with the shape index in diverse persimmons (Maeda et al. 2019). Thus, the gene regulatory network underlying fruit shape may be at least partially conserved between tomato and persimmon, which suggests that the genetic regulatory mechanisms of fruit shape in other model plants may be good references for future molecular genetic studies on fruit shape in persimmon.

12.4.2 Insights from Breeding Practices

Fruit size is an important commercial trait in persimmon, and it has been intensively targeted in modern breeding. In breeding practices in Japan, fruit size is represented by FW, which is a quantitative characteristic with high broad-sense heritability in persimmon (Yamada et al. 1993), and many studies have been made to predict the distribution of genotypic values controlling FW in a progeny population (Yamada and Yamane 1997). Yamada et al. (1994b) showed good-fitting multiple regressions based on the inbreeding coefficient and mid-parental value for the prediction of FW, which enabled accurate predictions of crossing outcomes in breeding. It should be emphasized that FW is influenced more by the inbreeding coefficient than by the mid-parental value and thus, inbreeding greatly reduces FW (Yamada et al. 1994b; Sato and Yamada 2016). In Japan, there is a history of intensive inbreeding of persimmon associated with the recessive pollination-constant non-astringent (PCNA) trait within a narrow genetic diversity of PCNA individuals (see Chap. 3 in this book), which has hindered breeding aimed at producing offspring with large FWs (Sato and Yamada 2016). In particular, early ripening cultivars have been developed by repeated crossings over generations with the narrow PCNA genetic

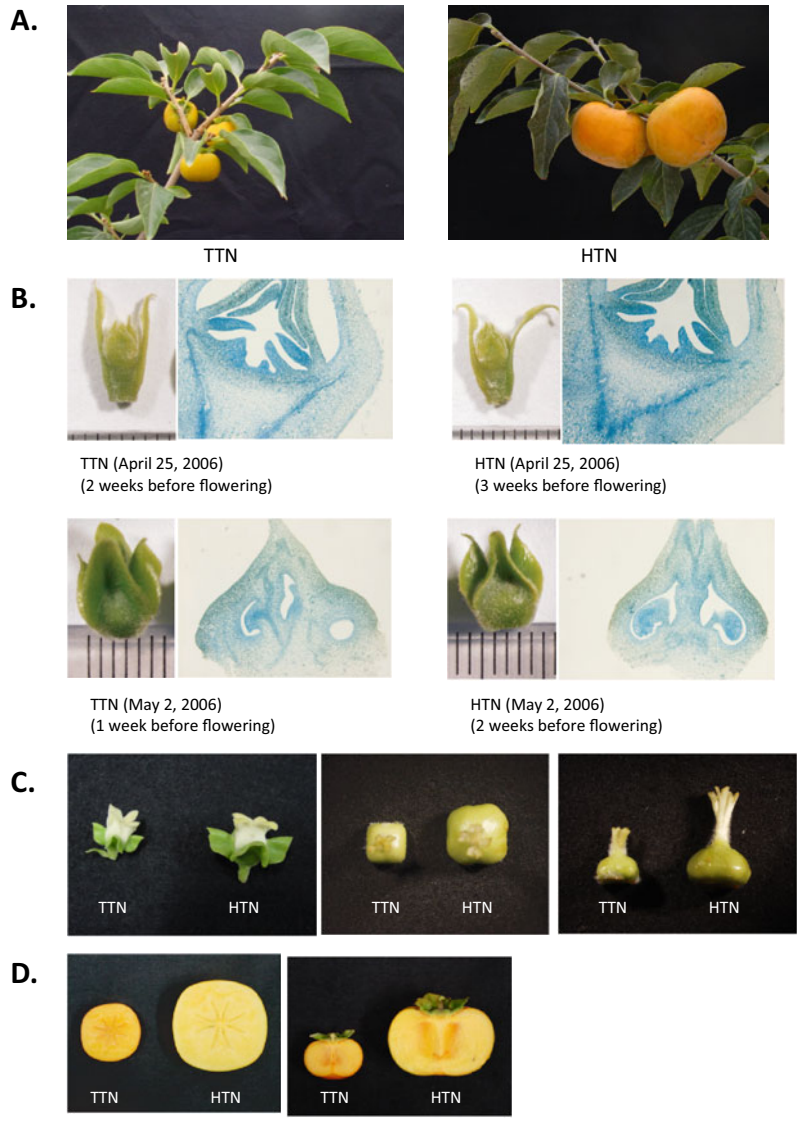

Fig. 12.2 The photographs of 'Hiratanenashi' (HTN) and 'Totsutanenashi' (TTN) reproductive organs. **a** Fruit on the pot-grown trees. **b** Microscopic observations of flower bud and floral organ development. In each section, the left photograph shows the whole-flower bud, and the right photograph shows the toluidine blue-stained flower primordia in the flower bud. **c** Flowers and ovaries at flowering. The left photograph shows flowers, and the center and right photographs show top and side views of ovaries, respectively, dissected from flowers at flowering. **d** Fruit at the commercial harvest period. Left and right photographs show cross and longitudinal fruit sections, respectively. From Yamane et al. (2008)

resources, which are thus highly inbred (Sato and Yamada 2016). In conclusion, fruit-size control in diverse persimmon germplasms, especially in a group of modern elite cultivars, appears to be polygenic in nature. Practically, utilizing genetically distinct individuals, such as non-PCNA individuals, for crossings to avoid inbreeding may be required to obtain larger fruit size (Yamada et al. 2012a), although clarifying the complex genetic basis of fruit-size control in diverse germplasms may further facilitate the breeding of cultivars of desirable sizes.

12.4.3 Small-Fruit Mutant TTN

A persimmon bud sport mutant, TTN, has been the focus of much attention in persimmon fruit-size research. Bud sports, mutated branches or vegetatively propagated plants that occur during cultivation are common sources of trait variation among horticultural crops (Foster and Aranzana 2018). The small-fruit mutant of persimmon, TTN, is a bud sport that originated from HTN, a major persimmon cultivar in Japan (Yamane et al. 2008). Owing to the extreme difference in

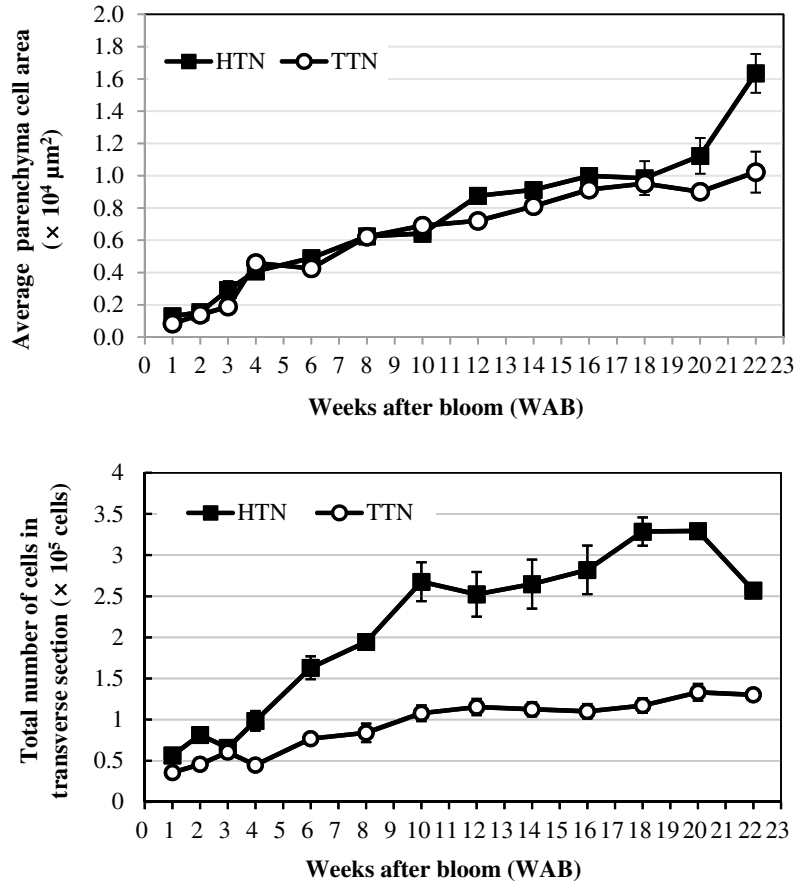

Fig. 12.3 The seasonal changes in the average parenchymal cell area (**a**) and the total numbers of cells (**b**) in transverse sections of 'Hiratanenashi' (HTN) and 'Totsutanenashi' (TTN) fruit in 2005. Vertical bars represent SEs (N = 3). From Habu et al. (2016)

fruit size between the mutant TTN and the original HTN, TTN has been used as an excellent resource for understanding the control of, and domestication effects on, persimmon fruit size.

Throughout the fruit developmental stages, from the very beginning of fruit development, TTN is significantly smaller than HTN (Yamane et al. 2008) (Fig. 12.2a–d). Histological observations indicate that the lower number of cells in TTN fruit is the main factor causing the fruit-size difference between these cultivars (Habu et al. 2016) (Fig. 12.3a, b). The TTN mutation has pleiotropic effects on vegetative growth and fruit development; TTN has shorter internode lengths and thus, a more compact tree shape (Yamane et al. 2008). Furthermore, TTN fruit maturation occurs much earlier than that of the original HTN, and it is one of the earliest among the germplasms. Transcriptome analyses of HTN and TTN fruit revealed differences in the expression levels of cell cycle-related genes, such as *D3-type cyclin* and *MAP-kinase kinase kinase* (*MAP-KKK*) (Habu et al. 2016), whereas exact genetic determinants remain to be elucidated. Interestingly, when synthetic cytokinins are sprayed on TTN flowers one and two weeks after full bloom, the fruit growth is comparable to that of the original HTN (Habu et al. 2016). Additionally, the cytokinin levels and the expression levels of related genes are higher in HTN than in TTN (Naito et al. 2018). Collectively, these results suggest that a decrease in cytokinin levels in the early fruit developmental stages, and a concomitant decrease in cell division activity, may result in the smaller fruits of TTN.

Another striking feature of TTN is the frequent occurrence of reversion events. During TTN cultivation, several reverse-mutated branches have been found on TTN trees that

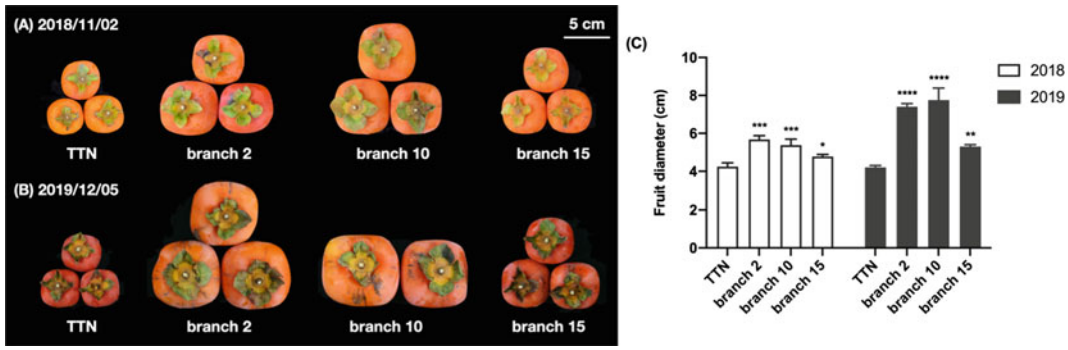

Fig. 12.4 'Totsutanenashi' (TTN) and revertant fruit on specific branches during the 2018 (**a**) and 2019 (**b**) harvesting seasons. **c** Comparisons of fruit diameters. Asterisks indicate significant differences between TTN and revertant fruits (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, one way ANOVA). Each bar represents the mean ± SD of two or three biological replicates. From Wang et al. (2021)

produced fruits of a size equivalent to those of HTN (Wang et al. 2021). The vegetative growth of the reverted plants is also comparable to that of HTN, suggesting that genetic modifications occurred in the system controlling the differences between HTN and TTN (Wang et al. 2021). Additionally, a large branch-dependent variation in fruit size, from TTN size to HTN size, was observed in a TTN tree, and it was stable for two consecutive years on the same branch (Wang et al. 2021) (Fig. 12.4a–c). Epigenetic control may explain this situation, and further analyses of the mechanisms behind size variation may yield genes having large effects, providing a good example of the combined genetic and epigenetic control of fruit size.

12.5 Conclusions and Future Prospects

Here, we summarized the accumulated knowledge, and other relevant information, on fruit-size control in persimmon. Although no single genetic determinant responsible for persimmon fruit-size variation has been cloned to date, the recently published diploid reference genomes (Zhu et al. 2019; Suo et al. 2020; Akagi et al. 2020), and the genomes of cultivated polyploid persimmons that should become available in the future, may facilitate our understanding of the genetic size control in persimmon. Specifically, the large variation in fruit size of cultivated polyploid persimmons (Yamada et al. 1994a, 1995) could be a great resource for understanding the domestication of this polyploid crop and interactions with human culture, as well as the molecular and application aspects of fruit-size control mechanisms. In this context, polyploid genomes, as well as diploid relatives, need to be analyzed more deeply in the future.

References

Agustí M, Juan M, Mesejo C et al (2004) Persimmon fruit size and climacteric encouraged by 3,5,6-trichloro-2-pyridyloxyacetic acid. J Hortic Sci Biotechnol 79:171–174

Akagi T, Shirasawa K, Nagasaki H et al (2020) The persimmon genome reveals clues to the evolution of a lineage-specific sex determination system in plants. PLoS Genet 16:e1008566

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

Candir EE, Ozdemir AE, Kaplankiran M, Toplu C (2009) Physico-chemical changes during growth of persimmon fruits in the East Mediterranean climate region. Sci Hortic 121:42–48

Choi S-T, Kang S-M, Park D-S et al (2002) Thinning effect on fruit characteristics and reserve accumulation of persimmon trees defoliated in early autumn. J Korean Soc Hortic Sci 43:660–665

Choi S-T, Park D-S, Kim E-S et al (2014) Seasonal growth and characteristics of fruits developed from

delayed flowers of 'Fuyu' persimmon. Sci Hortic 177:108–111
Chu Y-H, Jang J-C, Huang Z, van der Knaap E (2019) Tomato locule number and fruit size controlled by natural alleles of lc and fas. Plant Direct 3:e00142
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 99:13606–13611
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
Connors CH (1919) Growth of fruits of peach. New Jersey Agric Exp Stn Annu Rep 40:82–88
Crane JC (1964) Growth substances in fruit setting and development. Annu Rev Plant Physiol 15:303–326
Devoghalaere F, Doucen T, Guitton B et al (2012) A genomics approach to understanding the role of auxin in apple (*Malus* × *domestica*) fruit size control. BMC Plant Biol 12:7
Fenn MA, Giovannoni JJ (2021) Phytohormones in fruit development and maturation. Plant J 105:446–458
Foster TM, Aranzana MJ (2018) Attention sports fans! The far-reaching contributions of bud sport mutants to horticulture and plant biology. Hortic Res 5:44
Frary A, Nesbitt TC, Grandillo S et al (2000) fw2.2: a quantitative trait locus key to the evolution of tomato fruit size. Science 289:85–88
Fruit Tree Experiment Station of Hiroshima Prefecture (1973) Showa 53-nendo Shubyo-tokusei-bunrui-chosa-hokokusho (Kaki). Fruit Tree Experiment Station of Hiroshima Prefecture, Akitsu, Hiroshima
George AP, Nissen RJ, Collins RJ, Rasmussen TS (1995) Effects of fruit thinning, pollination and paclobutrazol on fruit set and size of persimmon (*Diospyros kaki* L.) in subtropical Australia. J Hortic Sci 70:477–484
George AP, Mowat AD, Collins RJ, Morley-Bunker M (1997) The pattern and control of reproductive development in non-astringent persimmon (*Diospyros kaki* L.): a review. Sci Hortic 70:93–122
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 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
Habu T, Yamane H, Naito I et al (2016) Differences in physiological characteristics and gene expression levels in fruits between Japanese persimmon (*Diospyros kaki* Thunb.) 'Hiratanenashi' and its small fruit mutant 'Totsutanenashi.' Hortic J 85:306–314
Hamada K, Hasegawa K, Ogata T (2007) Strapping and a synthetic cytokinin promote cell enlargement in 'Hiratanenashi' Japanese persimmon. Plant Growth Regul 54:225
Hasegawa K (1983) Studies on the flower-buds formation of Japanese persimmon: reference to the alternate bearing. Mem Fac Agric Kochi Univ 41:1–96
Hasegawa K, Nakajima Y (1990) Effects of bloom date, seediness, GA treatment and location of fruits in the foliar canopy on the fruit quality of persimmon *Diospyros kaki* Thunb. J Jpn Soc Hortic Sci 59:263–270
Hasegawa K, Kuge N, Mimura T, Nakajima Y (1991) Effects of KT-30 and GA3 on the fruit set and the fruit growth of persimmon cvs. Saijo and Hiratanenashi. J Jpn Soc Hortic Sci 60:19–29
Itai A, Tanabe K, Tamura F et al (1995) Synthetic cytokinins control persimmon fruit shape, size and quality. J Hortic Sci 70:867–873
Jiang K, Asami T (2018) Chemical regulators of plant hormones and their applications in basic research and agriculture. Biosci Biotechnol Biochem 82:1265–1300
Kishimoto O (1975) Estimations of optimum range of degree of fruit thinning and desirable fruit weight in Japanese pear and persimmon tree. J Jpn Soc Hortic Sci 43:368–376
Kitagawa H, Glucina PG (1984) Persimmon culture in New Zealand. Science Information Publishing Centre
Kitajima A, Kuramoto Y, Ohoka K et al (1993) Influence of fruiting position and co-existence of pollinated fruit on a shoot on parthenocarpic fruit set of kaki cvs. Fuyu and Hiratanenashi (*Diospyros kaki* L. f.). J Jpn Soc Hortic Sci 62:317–325
Kojima K, Shiozaki K, Koshita Y, Ishida M (1999) Changes of endogenous levels of ABA, IAA and GA-like substances in fruitlets of parthenocarpic persimmon. J Jpn Soc Hortic Sci 68:242–247
Krisanapook K, Subhadrabandhu S, Saleeto S et al (1998) Increasing fruit size in persimmon CV. Xichu by plant bioregulators and girdling. Acta Hortic 463:419–424
Laux T, Mayer KF, Berger J, Jürgens G (1996) The WUSCHEL gene is required for shoot and floral meristem integrity in Arabidopsis. Development 122:87–96
Libault M, Stacey G (2010) Evolution of FW2.2-like (FWL) and PLAC8 genes in eukaryotes. Plant Signal Behav 5:1226–1228
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, Kim YJ, Müller R et al (2011) AGAMOUS terminates floral stem cell maintenance in Arabidopsis by directly repressing WUSCHEL through recruitment of polycomb group proteins. Plant Cell 23:3654–3670
Maeda H, Akagi T, Tao R (2018) Quantitative characterization of fruit shape and its differentiation pattern in diverse persimmon (*Diospyros kaki*) cultivars. Sci Hortic 228:41–48
Maeda H, Akagi T, Onoue N et al (2019) Evolution of lineage-specific gene networks underlying the considerable fruit shape diversity in persimmon. Plant Cell Physiol 60:2464–2477
Mauxion J-P, Chevalier C, Gonzalez N (2021) Complex cellular and molecular events determining fruit size. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2021.05.008

Mazer SJ, Wheelwright NT (1993) Fruit size and shape: allometry at different taxonomic levels in bird-dispersed plants. Evol Ecol 7:556–575

Naito I, Nishiyama S, Gao-Takai M et al (2018) Changes in cytokinin metabolism in a small fruit mutant of 'Hiratanenashi' persimmon (*Diospyros kaki* Thunb.). Acta Hortic 1208:245–250

Nakamura M (1967) Physiological and ecological studies on the calyx of the Japanese persimmon fruit. Bull Facul Agric Gifu Univ 23:1–62

Nakano R, Yonemori K, Sugiura A (1997a) Photosynthesis by calyx lobes has no contribution to early fruit development in persimmon. Acta Hortic 345–354

Nakano R, Yonemori K, Sugiura A, Kataoka I (1997b) Effect of gibberellic acid and abscisic acid on fruit respiration in relation to final swell and maturation in persimmon. Acta Hortic 203–214

Nakano R, Ogura E, Kubo Y, Inaba A (2003) Ethylene biosynthesis in detached young persimmon fruit is initiated in calyx and modulated by water loss from the fruit. Plant Physiol 131:276–286

Nii N (1980) Seasonal changes of fruit growth in Japanese persimmon, *Diospyros kaki* cv. Fuyu, in relation to vascular tissue development in the fruit stalk. J Jpn Soc Hortic Sci 49:160–170

Nitsch JP, Pratt C, Nitsch C, Shaulis NJ (1960) Natural growth substances in concord and concord seedless grapes in relation to berry development. Am J Bot 47:566–576

Ohyama K, Shinohara H, Ogawa-Ohnishi M, Matsubayashi Y (2009) A glycopeptide regulating stem cell fate in *Arabidopsis thaliana*. Nat Chem Biol 5:578–580

Park SJ (2011) Dry weight and carbohydrate distribution in different tree parts as affected by various fruit-loads of young persimmon and their effect on new growth in the next season. Sci Hortic 130:732–736

Reig C, Martínez-Fuentes A, Mesejo C, Agustí M (2018) Hormonal control of parthenocarpic fruit set in 'Rojo Brillante' persimmon (*Diospyros kaki* Thunb.). J Plant Physiol 231:96–104

Rodríguez GR, Muños S, Anderson C et al (2011) Distribution of SUN, OVATE, LC, and FAS in the tomato germplasm and the relationship to fruit shape diversity. Plant Physiol 156:275–285

Sato A, Yamada M (2016) Persimmon breeding in Japan for pollination-constant non-astringent (PCNA) type with marker-assisted selection. Breed Sci 66:60–68

Shaya F, David I, Yitzhak Y, Izhaki A (2019) Hormonal interactions during early physiological partenocarpic fruitlet abscission in persimmon (*Diospyros kaki* Thunb.) 'Triumph' and 'Shinshu' cultivars. Sci Hortic 243:575–582

Šimura J, Antoniadi I, Široká J et al (2018) Plant hormonomics: multiple phytohormone profiling by targeted metabolomics. Plant Physiol 177:476–489

Sobajima Y, Ishida M, Kiyokawa I, Sakiyama M (1969) Investigations on the cause and control of physiological dropping in the Japanese persimmon fruits II: effects of pallination and gibberelin treatment on the physiological dropping of fruits, and changes of auxin in the fruits [in Japanese with English summary]. Sci Rep Kyoto Pref Univ Agric 21:12–23

Sobajima Y, Ishida M, Inaba A, Horiguchi H (1974) Studies on the fruit development of Japanese persimmon (*Diospyros Kaki* L.). J Jpn Soc Hortic Sci 43:224–228

Sun P, Li J, Du G et al (2017) Endogenous phytohormone profiles in male and female floral buds of the persimmons (*Diospyros kaki* Thunb.) during development. Sci Hortic 218:213–221

Suo Y, Sun P, Cheng H et al (2020) A high-quality chromosomal genome assembly of *Diospyros oleifera* Cheng. Gigascience 9:giz164

Suzuki A, Murakami Y, Maotani T (1988) Physiological studies on physiological fruit drop of persimmon, *Diospyros kaki* Thunb., 4: effect of fruit growth on physiological fruit drop of persimmon. Bull Fruit Tree Res 15:41–49

Tao R, Kawai T, Yamane H (2013) Small-fruit mutant of 'Hiratanenashi' persimmon (*Diospyros kak*i Thunb.). Acta Hortic 996:127–132

Wang S, Tam CL, Naito I et al (2021) Frequent reversion and continuous size variation in the small fruit mutant 'Totsutanenashi' persimmon. Acta Hortic 117–122

Xu C, Liberatore KL, MacAlister CA et al (2015) A cascade of arabinosyltransferases controls shoot meristem size in tomato. Nat Genet 47:784–792

Yamada M, Yamane H (1997) Relationship between the observed and predicted distribution of offspring for fruit ripening time and fruit weight in Japanese persimmon. Sci Hortic 69:157–167

Yamada M, Yamane H, Yoshinaga K, Ukai Y (1993) Optimal spatial and temporal measurement repetition for selection in Japanese persimmon breeding. HortScience 28:838–841

Yamada M, Yamane H, Sato A et al (1994a) Variations in fruit ripening time, fruit weight and soluble solids content of oriental persimmon cultivars native to Japan. J Jpn Soc Hortic Sci 63:485–491

Yamada M, Yamane H, Ukai Y (1994b) Genetic analysis of Japanese persimmon fruit weight. J Am Soc Hortic Sci 119:1298–1302

Yamada M, Wang R, Yamane H et al (1995) Comparisons in the variations in fruit maturing time, fruit weight, and soluble solids content of oriental persimmon cultivars of Chinese and Japanese origin. J Jpn Soc Hortic Sci 64:227–233

Yamada M, Giordani E, Yonemori K (2012a) Persimmon. In: Badenes ML, Byrne DH (eds) Fruit breeding. Springer US, Boston, MA, pp 663–693

Yamada M, Sato A, Yamane H, et al (2012b) New Japanese persimmon cultivar 'Taiten'. Bull Nat Inst Fruit Tree Sci 14:39–52

Yamamura H, Matsui K, Matsumoto T (1989) Effects of gibberellins on fruit set and flower-bud formation in unpollinated persimmons (*Diospyros kaki*). Sci Hortic 38:77–86

Yamane H, Ichiki M, Tao R et al (2008) Growth characteristics of a small-fruit dwarf mutant arising

from bud sport mutation in Japanese persimmon (*Diospyros kaki* Thunb.). HortScience 43:1726–1730

Yonemori K, Hirano K, Sugiura A (1995) Growth inhibition of persimmon fruit caused by calyx lobe removal and possible involvement of endogenous hormones. Sci Hortic 61:37–45

Yonemori K, Itai A, Nakano R, Sugiura A (1996) Role of calyx lobes in gas exchange and development of persimmon fruit. J Am Soc Hortic Sci 121:676–679

Zheng GH, Taira S, Yonemori K, Sugiura A (1990) Fruit growth and ripening of Japanese persimmons (*Diospyros kaki* Thunb.) grown at two locations with different temperature conditions. J Jpn Soc Hortic Sci 59:471–477

Zhu Q-G, Xu Y, Yang Y et al (2019) The persimmon (*Diospyros oleifera* Cheng) genome provides new insights into the inheritance of astringency and ancestral evolution. Hortic Res 6:138

Zsögön A, Čermák T, Naves ER et al (2018) De novo domestication of wild tomato using genome editing. Nat Biotechnol 36:1211–1216

Transcriptomics During Artificial Deastringency Treatment

13

Qing-Gang Zhu, Wei Wu, and Xue -Ren Yin

Abstract

Persimmon fruit accumulated the large amount of proanthocyanidins (PAs) which caused astringency in most cultivars during development. Many artificial deastringency treatments, including high CO_2, ethylene, hot water and ethanol, were applied to remove astringency. The physiological and biochemical mechanisms had been explored during the past few decades. In recent years, the molecular mechanisms of artificial deastringency treatments in persimmon fruit appealed much more focus. With the development of high-throughput sequencing technologies, the transcriptomics analyses were applied to investigate the key genes involved in persimmon fruit artificial deastringency treatment. The fruits treated with different treatments, like high CO_2 treatment, ethylene treatment and so on, were applied in transcriptome sequencing analysis. Results showed that the anaerobic respiration-related enzymes and the transcription factors, including ERFs, WRKYs, NACs and zinc finger family, played important roles in persimmon fruit during the artificial deastringency treatment.

13.1 Introduction

Persimmon fruit is unique for accumulating proanthocyanidins, also known as condensed tannins (CTs) which caused the fruit astringency with the high contents. According to the content of soluble condensed tannins (SCTs) at maturity stage, persimmon fruit can be divided into astringent and non-astringent types. Most of the native cultivars in East Asia are astringent types (Yamada et al. 1994; Wang et al. 1997) which tend to be rejected by consumers since the high SCTs contents. Thus, astringency removal technologies and mechanisms in persimmon fruits have attracted many focuses. Many artificial treatments, including treatments with high concentration of CO_2, N_2, ethylene and ethanol, have been developed to remove astringency in persimmon fruits (Ikegami et al. 2007; Salvador et al. 2007; Min et al. 2012; Zhu et al. 2018). Among these treatments, high concentration of CO_2 application had proved to be the most effective one (Min et al. 2012; Yin et al. 2012; Zhu et al. 2019). The physiological and biochemical

Q.-G. Zhu (✉)
College of Horticulture, Northwest A&F University, Yangling 712100, China
e-mail: qinggangzhu@nwafu.edu.cn

W. Wu · X. -RenYin (✉)
Department of Horticulture, Zhejiang University, Hangzhou 310058, China
e-mail: xuerenyin@zju.edu.cn

W. Wu · X. -RenYin
Zhejiang Provincial Key Laboratory of Integrative Biology of Horticultural Plants, Hangzhou 310058, China

R. Tao and Z. Luo (eds.), *The Persimmon Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-05584-3_13

mechanisms of CO_2-driven deastringency are much clearer than others. A high concentration of CO_2 leads to anaerobic fermentation by triggering acetaldehyde metabolism (Matsuo and Ito 1977; Pesis and Ben-Arie 1984; Min et al. 2012). Acetaldehyde reacts with SCTs to convert them to insoluble condensed tannins (InSCTs), which causes the decrease of SCTs content and quickly leads to persimmon fruit deastringency (Tanaka et al. 1994; Taira et al. 2001; Min et al. 2012). During this process, the enzyme activities of alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) increased (Tamura et al. 1999; Liu et al. 2008; Min et al. 2012). ADH and PDC enzymes are both encoded by gene families which have been studied in various plant species (Strommer 2011). In recent years, the exploration of molecular mechanisms of persimmon fruit in response to deastringency treatments (CO_2 and ethylene) appealed much more attentions. And the gene expression levels of ADH and PDC were up-regulated in response to deastringency treatments (Tamura et al. 1999; Min et al. 2012), which indicated that the transcriptional regulation is an important mechanism involved in persimmon fruit postharvest deastringency and the transcriptomics is the best way to explore this mechanism.

13.2 Effects of Artificial Treatments on Persimmon Fruit Astringency

Although the different treatments have different effects, many artificial treatments play roles in persimmon fruit astringency removal, where the high concentration CO_2 treatment was the most effective (Yin et al. 2012). Exogenous ethylene treatment (2 days) could strongly reduce the SCTs concentration and increase the InSCTs concentration (Fig. 13.1) (Yin et al. 2012), and the same results could also be observed in Park et al. (2019). While CO_2 treatment (95%) promoted persimmon fruit deastringency and caused a rapid decrease in the concentration of SCTs as early as 1 d. Furthermore, the concentrations of acetaldehyde and ethanol, the products of anaerobic respiration, were highly increased during this process (Fig. 13.2). Luo et al. (2014) found that ethanol could successfully remove astringency in the treated CPCNA persimmon fruit (Fig. 13.3). In the hot water (40 °C) treated CPCNA fruit, the soluble tannin content was sharply decreased after 12 h, company with insoluble tannin content increased, while hot air treatment was invalid (Fig. 13.4) (Chen et al. 2017).

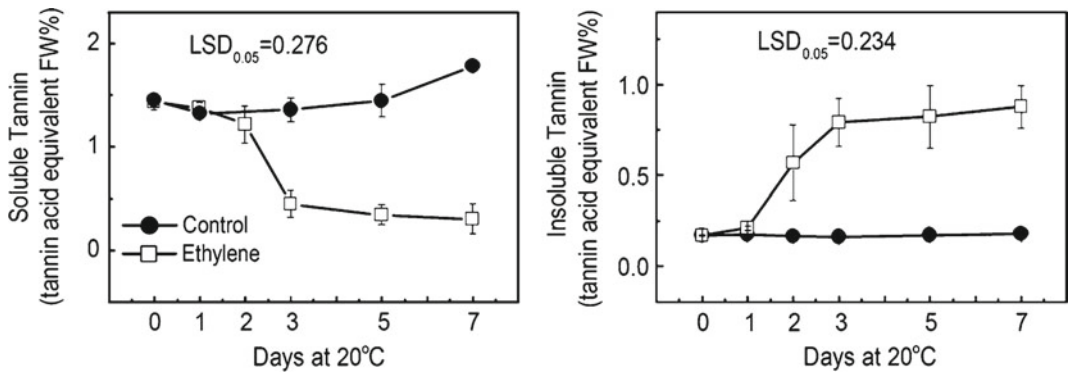

Fig. 13.1 Effects of exogenous ethylene treatment on soluble tannins and insoluble tannins in 'Mopan' persimmon fruit held at 20 °C. Mature fruit was treated with ethylene (100 μl L^{-1}, 2 days) (Yin et al. 2012)

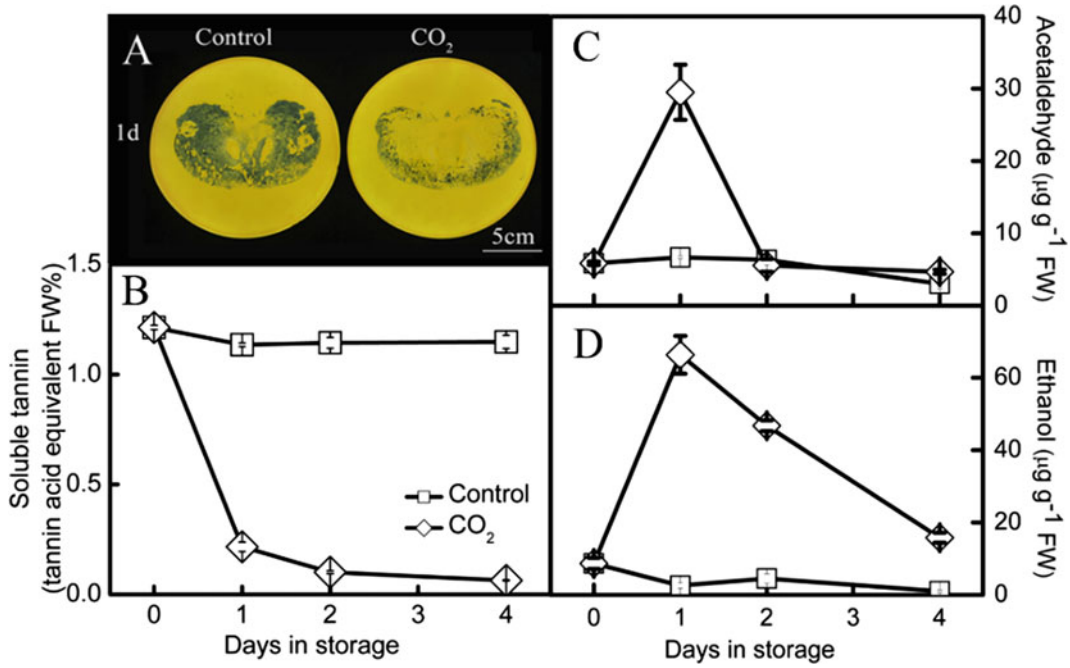

Fig. 13.2 Effects of high CO_2 treatment (95% CO_2, 1 d) on 'Mopanshi' persimmon fruit postharvest deastringency at 20 °C. **a** Astringency was indicated by soluble tannin content, using the tannin printing method. The black colour indicates soluble tannins and the intensity of black reflects the soluble tannin content. **b–d** The concentrations of soluble tannin, acetaldehyde and ethanol, respectively (Zhu et al. 2018)

13.3 Genome-Wide RNA-seq Analysis of Astringency Removal in Persimmon Fruit by Artificial Treatments

High-throughput sequencing technologies developed from the first-generation sequencing (Sanger sequencing method), the next generation sequencing (NGS) and the third-generation sequencing (Pacbio or Nanopore) have provided convenient ways of establishing a rapid and efficient molecular research platform (Wee et al. 2019). In recent years, large-scale transcriptome sequencing has been applied in illustrating the molecular mechanisms of persimmon fruit deastringency.

Min et al. (2012) characterized DkERF9 and DkERF10 which played important roles in persimmon fruit deastringency through transactivating the transcription of *DkADH1* and *DkPDC2* during high CO_2 (95%) treatment. Further analysis showed that another eight transcription factors including *DkbHLH1*, *DkMYB5-11*, *DkRH2-1*, *DkGT3-1*, *DkAN1-1* and *DkHSF1* were responsive to high CO_2 treatment, where the DkMYB6 and DkMYB10 were verified to involved in persimmon fruit deastringency (Fang et al. 2016; Zhu et al. 2018).

Luo et al. (2014) used 454 platform sequencing for the de novo transcriptome assembly of persimmon fruit treated with (Tr library) /without (Co library) 5% ethanol to investigate the genes involved in tannin coagulation. A total of 83,898 unigenes were identified and 54,719 unigenes among them were annotated based on similarity searches with known proteins. There were 3639 unigenes differentially expressed between the Tr and Co libraries, where 1560 were up-regulated and 2079 were down-regulated in the treated fruit. This work presented the first de novo transcriptome sequencing analysis of the CPCNA persimmon fruit using the 454 platform and provided the differentially

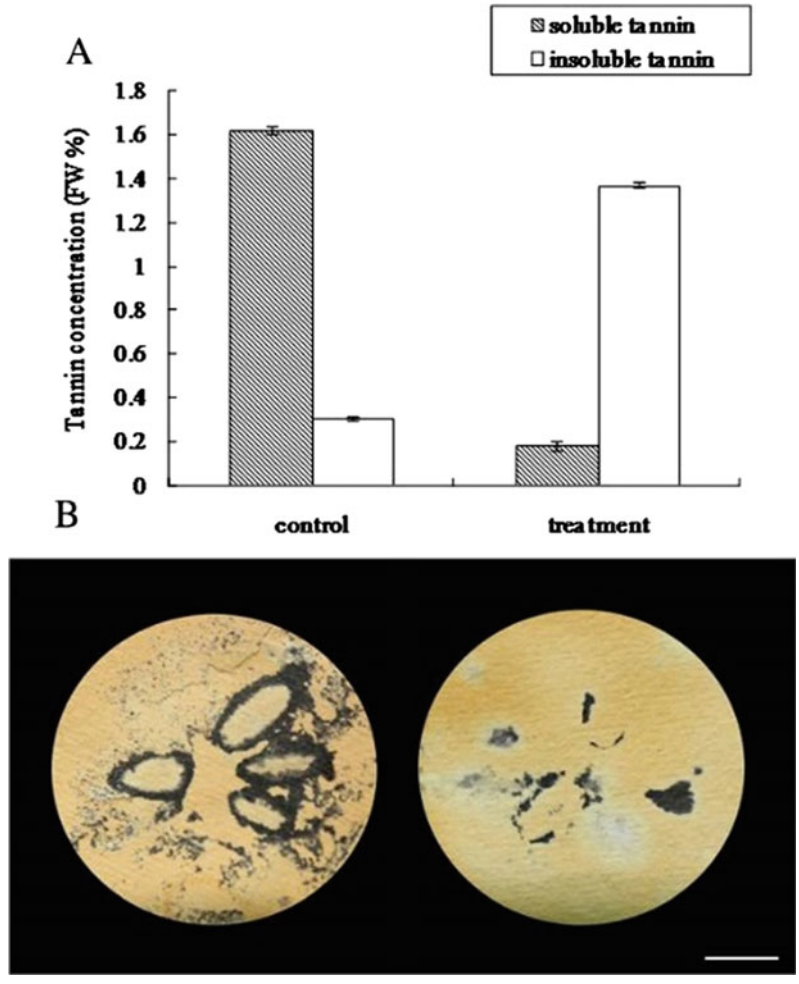

Fig. 13.3 Effect of ethanol treatment on deastringency. **a** Effect of ethanol treatment on soluble tannin content and insoluble tannin content of 'Luotian-tianshi'. **b** Analysis of soluble tannin content by printing method (Luo et al. 2014)

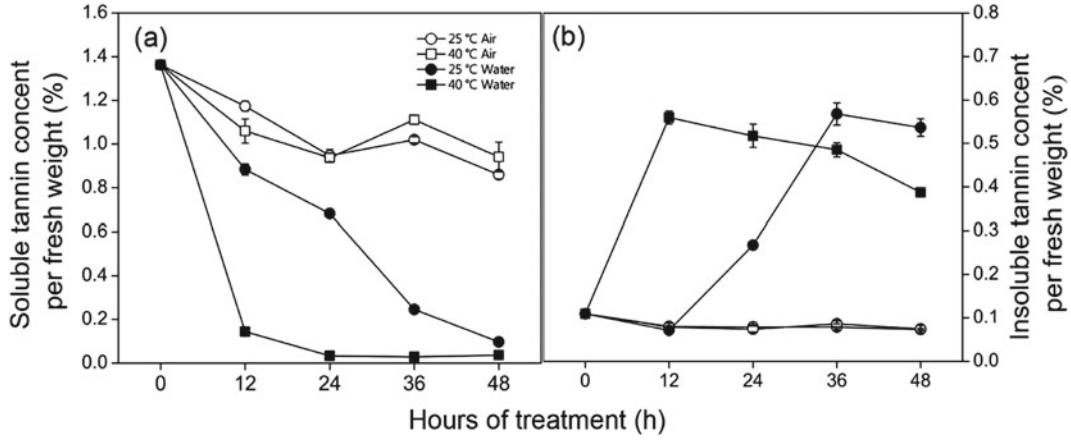

Fig. 13.4 Effects of hot treatments (hot air and hot water) on persimmon fruit deastringency. **a**–**b** The concentrations of soluble tannin and insoluble tannin, respectively

expressed unigenes involved in persimmon fruit deastringency. ADH, PDC and ALDH2 were found to have important functions in tannin coagulation (Luo et al. 2014).

Furthermore, in 2017, another research of transcriptome to reveal deastringency-related genes in CPCNA persimmon from the same lab was reported. Persimmon fruit treated with hot water and at two key stages of natural astringency removal was selected to perform the transcriptome sequencing on an Illumina Hiseq™ 2000 platform (Chen et al. 2017). The differential gene expression analysis suggested that a total of 3818 unigenes were differentially expressed in natural deastringency according to comparing the fruit at 20 weeks after bloom to 10 weeks. The number of DEGs increased to 15,597 unigenes between the fruit treated with 40 °C water at 12 h versus the untreated one, and it was more than the natural deastringency group. The water treatment strongly triggered glycolysis/acetaldehyde metabolism and decreased the expression of genes involved in PA biosynthetic pathway in persimmon fruit. The similar but weaker trends were found during the natural astringency removal process. Further analysis suggested that several transcription factors (TFs) were related to persimmon fruit deastringency, where ten TFs including four ERFs, three NACs, two WRKYs and one zinc finger shared up-regulated in two deastringency process, while sixteen TFs including two bHLHs, one bZIP, three ERFs, two WRKYs and eight zinc fingers shared down-regulated in two deastringency process. Taken together, a hypothesis was proposed for the natural deastringency of CPCNA fruit based on these results (Fig. 13.5) (Chen et al. 2017).

Although persimmon fruit is classified either as non-climacteric or climacteric fruit depending on the literature, ripe fruit seems to be a typical climacteric type fruit because it is sensitive to ethylene. Ethylene treatment promoted the deastringency of persimmon fruit and meanwhile

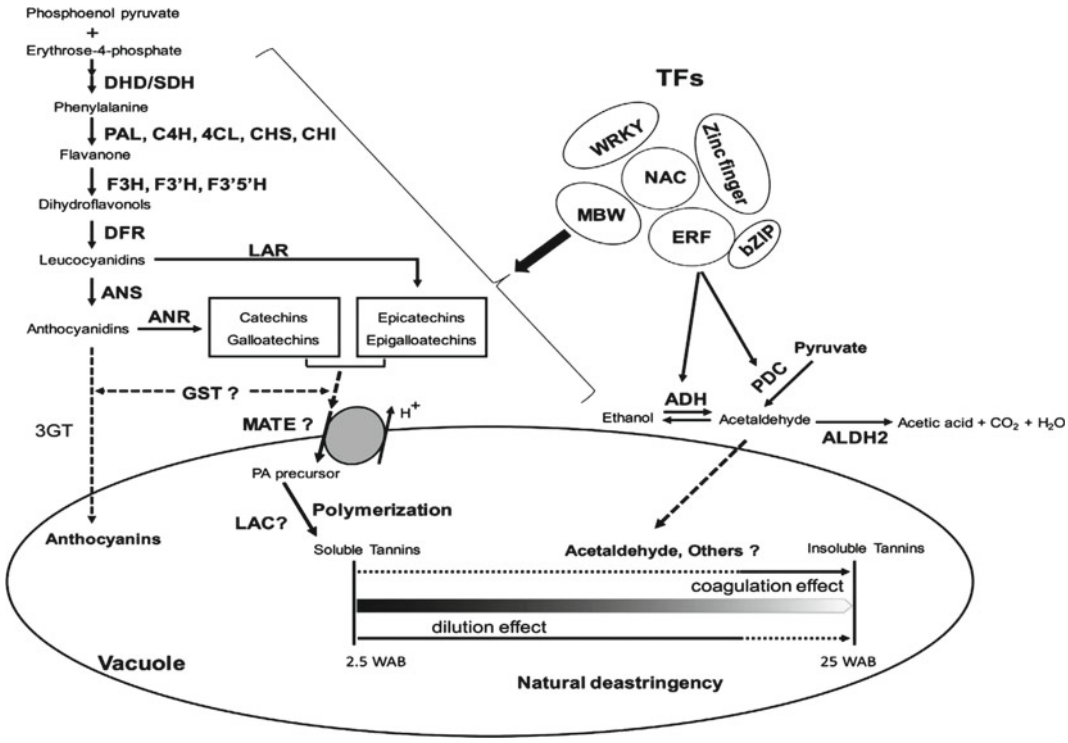

Fig. 13.5 A hypothesis for PA biosynthesis and natural deastringency of CPCNA fruit (Chen et al. 2017)

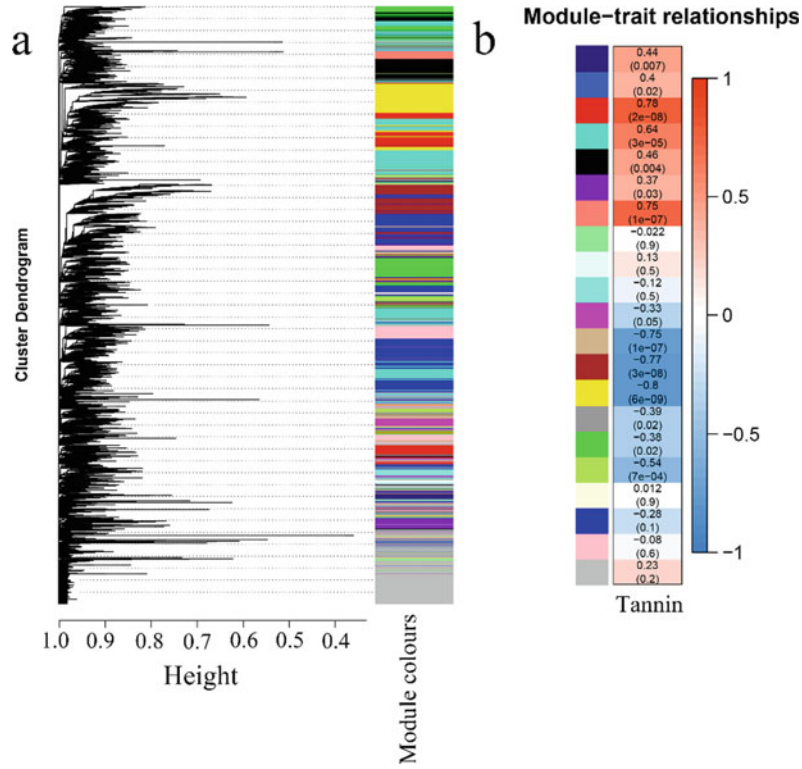

Fig. 13.6 RNA-seq analysis through WGCNA. **a** WGCNA dendrogram indicating the expression of different gene modules in all persimmon samples. **b** Trait and module relationship analysis. Different colours represent different modules

led to the rapid softening of them. The molecular mechanism of astringency removal and fruit ripening has been studied in astringent 'Cheongdo-Bansi' persimmon fruit (Park et al. 2019). Fruits treated with/without ethylene were used to test the differentially expressed genes through Illumina high-throughput sequencing platform. It suggested that a total of 12,374 unigenes were differentially expressed between the ethylene versus control groups, where 6072 unigenes were up-regulated and 6302 ones were down-regulated. Among these DEGs, 38 genes showed significantly different expression, 26 of them were up-regulated and the rest 12 genes were down-regulated. These identified genes were categorized as genes related to astringency removal, softening and other ripening-related changes (Park et al. 2019). Because of the absence of a systematic genome database in persimmon fruit, many of the candidate genes involved in astringency removal were undiscovered. In 2019, Zhu et al. established the persimmon (*Diospyros oleifera* Cheng) genome database. According to this genome database, four representative cultivars of persimmon were selected to uncover the mechanism of astringency removal systematically through RNA-seq analysis. Based on the quantitative soluble tannin analysis, two of the four representative cultivars showed rapid deastringency after high CO_2 treatment (*D. kaki* cv. Sigoushi, and cv. Luoyangfangtianshengshi) and two showed slow deastringency (*D. kaki* cv. Laopige, and cv. Shijiazhuanglianhuashi). DEGs between these four cultivars were clustered into 21 co-expression modules through WGCNA (weighted gene co-expression network analysis), where the tan (−0.75), brown (−0.77) and yellow (−0.64) modules showed a significantly negative correlation with the tannin contents (Fig. 13.6). Further analysis suggested that the genes located in these three modules included anaerobic respiration related-enzymes genes (i.e. *DkPDC1*, *DkADH1*, *DkPK1* and *DkPDC2*), transcription

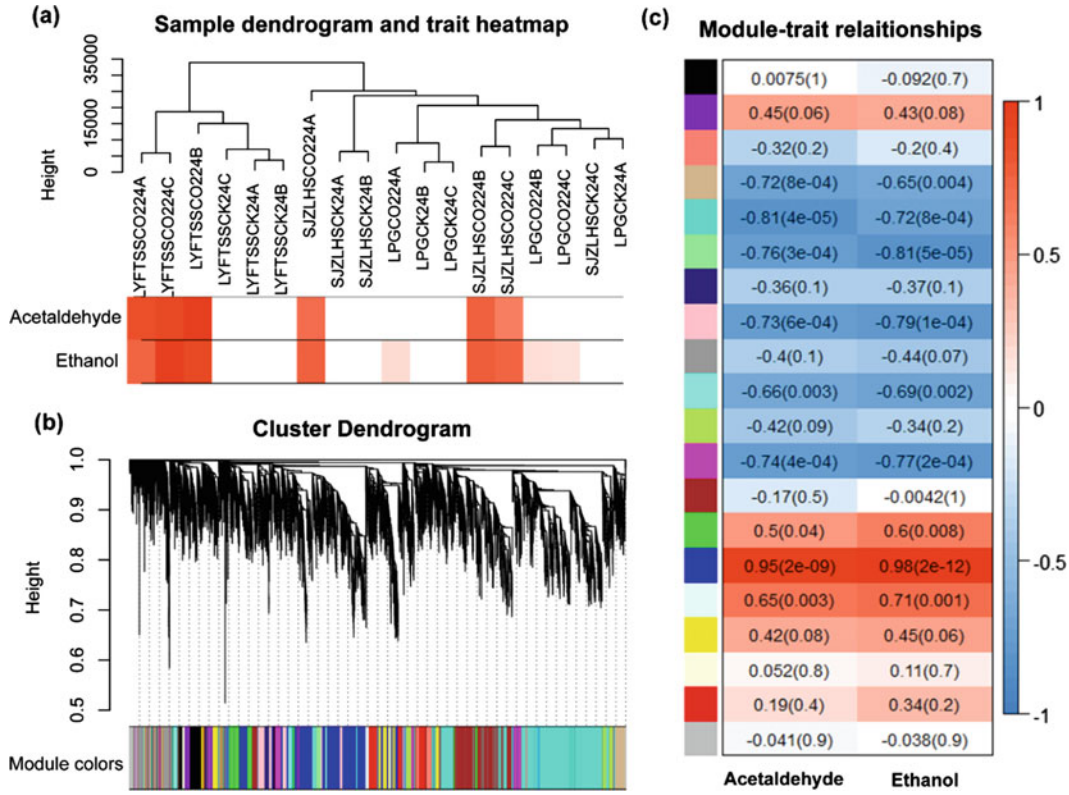

Fig. 13.7 Sample cluster analysis and construction of the co-expression modules by WGCNA base on RNA-seq data and physiological data. **a** Sample dendrogram and trait heatmap based on the gene expression data and physiological data. **b** Clustering dendrograms of genes, with dissimilarity based on the topological overlap, together with assigned module colours. **c** Module-sample association, module-sample correlations and corresponding *P* values

factors and others. As expected, genome-wide transcriptomic analysis provided a more comprehensive overview of astringency removal (Zhu et al. 2019). Moreover, the candidate genes especially related to acetaldehyde and ethanol which were the metabolites in de-astringency process were also uncovered by means of WGCNA. The module which comprised of 1773 unigenes showed significantly correlated with the contents of acetaldehyde and ethanol ($P < 0.001$). Among these unigenes, 440 genes were annotated into the KEGG database and were mainly distributed in metabolism and genetic information processing. Carbon metabolism, plant hormone signal transduction and glycolysis were three of the most significant pathways in metabolism classification (Kou et al. 2021) (Fig. 13.7).

13.4 Conclusions

All these transcriptomic analyses provided insights into the molecular basis of persimmon fruit artificial deastringency treatments, including high-CO_2 treatment, ethylene treatment, hot water treatment and ethanol treatment. And these studies will also deliver important resources for further study of the genes related to astringency removal for persimmon breeding and improvement.

References

Chen WX, Xiong YL, Xu LQ, Zhang QL, Luo ZR (2017) An integrated analysis based on transcriptome and proteome reveals deastringency-related genes in CPCNA persimmon. Sci Rep 7:44671

Fang F, Wang MM, Zhu QG, Min T, Grierson D, Yin XR, Chen KS (2016) *DkMYB6* is involved in persimmon fruit deastringency, via transcriptional activation on both *DkPDC* and *DkERF*. Postharvest Biol Technol 111:161–167

Ikegami A, Eguchi S, Kitajima A, Inoue K, Yonemori K (2007) Identification of genes involved in proanthocyanidin biosynthesis of persimmon (*Diospyros kaki*) fruit. Plant Sci 172:1037–1047

Kou SM, Jin R, Wu YY, Huang JW, Zhang QY, Sun NJ, Yang Y, Guan CF, Wang WQ, Zhu CQ, Zhu QG, Yin XR (2021) Transcriptome analysis revealed the roles of carbohydrate metabolism on differential acetaldehyde production capacity in persimmon fruit in response to high-CO_2 treatment. J Agric Food Chem 69:836–845

Liu CP, Zheng ZM, Liang YN, Li B (2008) Relationship between activity of alcohol dehydrogenase and soluble tannin in persimmon. Acta Hortic Sin 35:741–746 (in Chinese, with English abstract)

Luo C, Zhang QL, Luo ZR (2014) Genome-wide transcriptome analysis of Chinese pollination-constant nonastringent persimmon fruit treated with ethanol. BMC Genomics 15:112

Matsuo T, Ito S (1977) On mechanisms of removing astringency in persimmon fruits by carbon dioxide treatment. I. Some properties of the two processes in the de-astringency. Plant Cell Physiol 18:17–26

Min T, Yin XR, Shi YN, Luo ZR, Yao YC, Grierson D, Ferguson IB, Chen KS (2012) Ethylene-responsive transcription factors interact with promoters of *ADH* and *PDC* involved in persimmon (*Diospyros kaki*) fruit de-astringency. J Exp Bot 63:6393–6405

Park DS, Tilahun S, Park KC, Choi IY, Jeong CS (2019) Transcriptome analysis of astringent 'Cheongdo-Bansi' persimmon fruit treated with ethylene for removal of astringency. Postharvest Biol Technol 150:52–59

Pesis E, Ben-Arie R (1984) Involvement of acetaldehyde and ethanol accumulation during induced deastringency of persimmon fruits. J Food Sci 49:896–899

Salvador A, Arnal L, Besada C, Larrea V, Quiles A, Pérez-Munuera I (2007) Physiological and structural changes during ripening and deastringency treatment of persimmon fruit cv. 'Rojo Brillante'. Postharvest Biol Technol 46:181–188

Strommer J (2011) The plant *ADH* gene family. Plant J 66:128–142

Taira S, Ikeda K, Ohkawa K (2001) Comparison of insolubility of tannins induced by acetaldehyde vapor in fruit of three types of astringent persimmon. J Jpn Soc Hortic Sci 48:684–687

Tamura F, Tanabe K, Itai A, Hasegawa M (1999) Characteristics of acetaldehyde accumulation and removal of astringency with ethanol and carbon dioxide treatments in 'Saijo' persimmon fruit. J Jpn Soc Hortic Sci 68:1178–1183

Tanaka T, Takshashi R, Kouno I, Nonaka G (1994) Chemical evidence for the de-astringency (insolubilization of tannins) of persimmon fruit. J Chem Soc Perkin Trans 20:3013–3022

Wang RZ, Yang Y, Li GC (1997) Chinese persimmon germplasm resources. Acta Hortic 436:43–50

Wee Y, Bhyan SB, Liu YN, Lu JC, Li XY, Zhao M (2019) The bioinformatics tools for the genome assembly and analysis based on third-generation sequencing. Brief Funct Genomics 18:1–12

Yamada M, Yamane H, Sato A, Hirakawa N, Wang RZ (1994) Variations in fruit ripening time, fruit weight and soluble solids content of oriental persimmon (*Diospyros kaki*) cultivars native to Japan. J Jpn Soc Hortic Sci 63:485–491

Yin XR, Shi YN, Min T, Luo ZR, Yao YC, Xu Q, Ferguson IB, Chen KS (2012) Expression of ethylene response genes during persimmon fruit astringency removal. Planta 235:895–906

Zhu QG, Gong ZY, Wang MM, Li X, Grierson D, Yin XR, Chen KS (2018) A transcription factor network responsive to high CO_2/hypoxia is involved in deastringency in persimmon fruit. J Exp Bot 69:2061–2070

Zhu QG, Xu Y, Yang Y, Guan CF, Zhang QY, Huang JW, Grierson D, Chen KS, Gong BC, Yin XR (2019) The persimmon (*Diospyros oleifera* Cheng) genome provides new insights into the inheritance of astringency and ancestral evolution. Hortic Res 6:138

Future Prospects

Ryutaro Tao and Zhengrong Luo

In this persimmon genome book, several commercially important *Diospyros* species of the Ebenaceae family are introduced; however, undoubtedly *Diospyros kaki* is the most important fruit tree species in the genus *Diospyros*. China, Japan, Korea, and Brazil are the traditional persimmon producing countries. In recent decades, other countries in Asia, as well as Europe and the United States, also produce persimmon fruit. Among these countries, the industrial scale in Spain has grown very rapidly, with annual yield surpassing Korea and Japan to become the second-largest producer in the world. Other countries, including Azerbaijan, Uzbekistan, Italy, Israel, Iran, and New Zealand, also produce considerable amounts of persimmon fruit. In terms of scientific research and industrial technology development, Japan is still at the highest level in the world. Based on the industrial scale, China is undoubtedly the world's largest producer, and this state is supposed not to change in the short term. Since persimmons are grown worldwide and become well-known fruit, we can consider persimmon not an Asian exotic fruit but one of the common fruits like apples and pears.

One of the most unique characteristics of persimmon is fruit astringency. Based on the fruit astringency loss character, persimmon cultivars can be divided into the pollination constant and non-astringent (PCNA) type and the non-PCNA type. Since the PCNA type is regarded as commercially most important type, genetic and molecular basis of this character has been extensively studied. It is now known that there are two distinct types of PCNA, the Chinese (C-PCNA) and Japanese (J-PCNA) types. The former is dominant to non-PCNA, while the latter is recessive. Although genes controlling these are yet to be identified, information obtained from genomic studies described in this book will make speed up the identification process.

Current cultivar improvement in persimmon is mainly focusing on the development of early or late ripening PCNA varieties with good appearance and internal quality, high and stable yields, excellent storage capacity, good environmental adaptability. Although transgenic and mutation breeding is conducted, crossbreeding is still the main way of genetic improvement. Japan has achieved the greatest success in crossbreeding. To date, almost all of the main cultivars of PCNA have been developed from Japan. Korea, China, and Spain have their own genetic improvement programs and have made some achievements. Although persimmon crossbreeding is very difficult because of the long breeding cycle, high genetic heterozygosity, and polyploidy, a breakthrough technology should be developed based on genomics, transgenic, and gene editing technologies.

The accurate evaluation and effective utilization of germplasm resources depend on a full understanding of the genome information along with their phenotype descriptions. Recently, several whole genome sequence information of diploid *Diospyros* have been reported from the several research teams for persimmon genome sequencing in China and Japan. Combining all information together, we could further utilize the information for genetic studies and breeding. Furthermore, the whole genome sequencing of

R. Tao and Z. Luo (eds.), *The Persimmon Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-05584-3

tetraploid and hexaploid *Diospyros* species is underway. With the further application of multi-omics technologies such as genetics, transcriptomics, proteomics, metabolomics, and phonemics as well as the establishment of technologies such as genetic transformation and gene editing, the genetic improvement of persimmon will enter the genome-wide information-based molecular breeding stage.

We hope that this book on persimmon genome will contribute to future persimmon research and breeding to address all aspects of persimmon researches, and that persimmon fruit will be consumed by more people around the world.