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Edited by Jaya R. Soneji and Madhugiri Nageswara-Rao

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http://dx.doi.org/10.5772/intechopen.69915
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Contributors

Ayzin B. Küden, Songül Çömlekçioğlu, Burhanettin İmrak, Ali Küden, Kadir Sarier, Sally Elnawasany, Juan C. Castro, J. Dylan Maddox, Marianela Cobos, Sixto A. Imán, Jianjun Chen, ChihCheng T. Chao, Xiangying Wei, Chengjiang Ruan, Jian Ding, Susan Mopper, Turgut Yesiloglu, Berken Cimen, Meral Incesu, Bilge Yilmaz, Boris Krška

Notice

First published in London, United Kingdom, 2018 by IntechOpen
eBook (PDF) Published by IntechOpen, 2019
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Breeding and Health Benefits of Fruit and Nut Crops
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p. cm.

Print ISBN 978-1-78923-272-1

Online ISBN 978-1-78923-273-8

eBook (PDF) ISBN 978-1-83881-439-7

Meet the editors

Jaya R. Soneji, PhD, works in the areas of plant breeding, genomics, bioenergy, genetic engineering, population, and eco-evolutionary genetics. She is the author of peer-reviewed research articles, book chapters, and popular articles and has guest-edited special issues for journals, edited books, and newsletters. She was an adjunct faculty at Polk State College, USA. Her work has been broadcasted on Fox News, USA. She was invited by CBC Radio, Canada, to speak on air. She has served in the "Executive Committee" of GII. She was recognized as "Young Scientists" by Bioclues in 'Member in Spotlight' of GII and featured in ASPB News. The University of Florida's International Programs appraised her contribution in "International Focus." She has peer-reviewed manuscripts for prominent international journals and grant proposals for international institutions. Dr. Soneji obtained her BSc degree (rank second) and MSc degree (rank second) from the University of Mumbai and her PhD degree from BARC, India. She has received many scholarships and awards for her academic achievements. She taught plant genetics, physiology, tissue culture, and genetic engineering at Ramnarain Ruia College, India.

Madhugiri Nageswara-Rao, PhD, works in the areas of plant breeding, genomics, bioenergy, genetic engineering, disease diagnostics, population, and eco-evolutionary genetics. He is the author of peer-reviewed research articles, book chapters, and popular articles, and has guest-edited special issues for journals, edited books, and newsletters. He was an adjunct faculty at Polk State College, USA. His work has been broadcasted on Fox News, USA. He was invited by CBC Radio, Canada, to speak on air. He has served in the "Executive Committee" of GII. He was recognized as 'Young Scientists' by Bioclues, in "Member in Spotlight" of GII and featured in ASPB News. The University of Florida's International Programs appraised his contribution in "International Focus." He has peer-reviewed manuscripts for prominent international journals and grant proposals for international institutions. Dr. Rao obtained his BSc and MSc degrees from Bangalore University and his PhD degree from FRI, India. He was featured in "Tomorrow's Principal Investigators: Rising Young Investigators" by Genome Technology, USA. He secured a "silver award" as a team member from the American Museum of Natural History, USA. He was also selected for AAAS/Science Program for Excellence in Science. He has served on the editorial boards of six journals.

Contents

Preface

The fruit and nut crops are laden with health benefits. As people are becoming more conscious about their health and nutritional uptake, the worldwide demand and consumption of fruit and nut crops are steadily increasing. This has made it hard to keep pace between the rate of fruit and nut production and its consumption. To meet this increasing demand, there is a need to produce improved, better yielding, and high-quality fruit and nut crops. This book intends to provide the reader with a comprehensive overview of the current status and future prospects of fruit and nut crops.

Over the past decade, the standard of living has greatly improved and has led to an increased demand for fruits and nuts. It has led to an interest in consuming fruits and nuts with health-promoting phytochemicals. A lot of work is being done in the field of fruit and nut breeding, biotechnology, and genomics to produce varieties with traits of interest. Breeding of fruits and nuts has been enhanced by advanced technologies such as marker-assisted selection, genome-wide association studies, and genomic selection.

The chapters of this book discuss details of the botanical characteristics, origin and domestication, genetic diversity, genetic resources, breeding, genetic improvement, biotechnology, genomics, effects of health-promoting phytochemicals, and edible oils or clinical applications of the fruit and nut crops. Such comprehensive information covered in this book will directly enhance both basic and applied research in fruit and nut crops and will particularly be useful for students, scientists, researchers, teachers, breeders, policy-makers, and growers.

Dr. Jaya R. Soneji
Department of Entomology, Plant Pathology, and Weed Science
New Mexico State University
Las Cruces, USA

Dr. Madhugiri Nageswara-Rao
Department of Biology
New Mexico State University
Las Cruces, USA

Section 1

Breeding and Genetics

Chapter 1

Gojiberry Breeding: Current Status and Future Prospects

Jianjun Chen, ChihCheng T. Chao and Xiangying Wei

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76388

Abstract

Goji, gojiberry, or wolfberry is the fruit of *Lycium barbarum* L., *L. chinense* Mill., or *L. ruthenicum* Murr. in the family Solanaceae Juss. The fruit is bright orange-red or black and is edible with a sweet and tangy flavor. Gojiberry is rich in polysaccharides, flavonoids, carotenoids, betaine, kukoamine A, sitosterol, and other compounds which have antioxidant, anti-inflammatory, and anti-neoplastic properties and have been used for the treatment of various blood circulation disorders and diabetes. Recently, there is an increased demand for high-quality gojiberry and its products because they are considered a superfruit. China is the main producer and supplier of gojiberry in the world. Thus far, limited information is available about genetic resources, breeding activities, and major cultivars of gojiberry. This chapter is intended to review the current knowledge on gojiberry germplasm resources and their relationships as well as to describe gojiberry breeding activities. Future prospects on gojiberry cultivar development are also discussed.

Keywords: gojiberry, *Lycium barbarum*, *Lycium barbarum* polysaccharides (LBPs), *Lycium chinense*, *Lycium ruthenicum*, Solanaceae, wolfberry

1. Introduction

Gojiberry generally includes *Lycium barbarum* L., *L. chinense* Mill., and *L. ruthenicum* Murr. (**Figure 1**). They are deciduous woody shrubs, often thorny, spiny, and growing from 1 m to 4 m in height [1]. Stems are slender, erect or spreading, often scrambling. The leaves are gray green, fleshy, ovate, lanceolate, or subcylindric shaped and are alternately arranged,

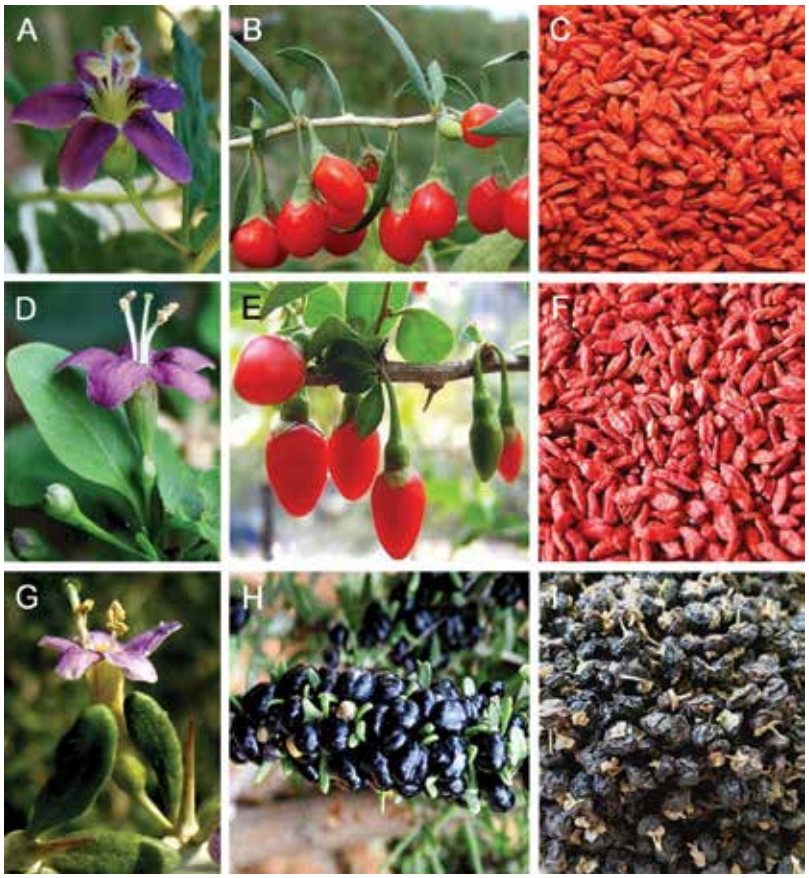

Figure 1. Flower, fruit, and dried fruit appearance of *Lycium barbarum* L., *L. chinense* Mill., and *L. ruthenicum* Murr. *L. barbarum* bears royal purple flower (A) with lanceolate leaves and produces bright red berries (B) which resemble red raisins after they have been dried (C). The flower of *L. chinense* is purple (D), leaves are ovate, fresh (E) and dried (F) berries are orange-red. *L. ruthenicum* bears purple flowers that are lighter than *L. chinense* (G), leaves are subsessile and succulent in a linear shape (G). This species produces dark colored berries (H) that are harvested with peduncle and sepal; thus individual dried black berries often have peduncle and sepal (I).

sometimes in fascicles. Petioles are short. Flowers are solitary or clustered in leaf axils. The corolla is funnel or bell shaped in white, green, or purple. Five stamens are structured with filaments longer than the anthers. Anthers dehisce longitudinally. The fruit is a two-chambered, usually fleshy and juicy berry and typically orange-red or black (*L. ruthenicum*). Seeds are few or many, most with over 10. In the Northern Hemisphere, flowering occurs from June to September, and berry maturation starts from August to October.

Gojiberry has been consumed as food and used as medicine for more than 4000 years in China [2]. The first mentioned "Gou Qi" was in the ancient classic "Shen Nong Ben Cao Jing" (The Classic of Herbal Medicine), a Chinese book on agriculture and medicinal plants written between 200 and 250 AD. The fruit of *L. barbarum*, *L. chinense*, and *L. ruthenicum* are commonly called "Ningxia Gou Qi," "Gou Qi," and "Black Gou Qi," respectively. The fruit is a

renowned Yin strengthening agent, and the root bark, known as "Di Gu Pi," is a cooling agent [3]. Traditional English vernacular names include boxthorn, fructus lycii, wolfberry, Chinese wolfberry, or matrimony vine [4]. Since the beginning of the century, the plant has been commonly called Goji, an appellation derived from the Chinese name "Gou Qi."

2. Nutraceutical and pharmaceutical values

The berries harvested from August to October are eaten as fresh fruit, dehydrated to make dried fruit or soaked in liquor to produce *Lycium* juice. As a medicinal food, it is used as a condiment with steamed rice. Young soft leaves can also be used as a vegetable. Gojiberry has been used for its anti-aging activities, tranquilizing and thirst-quenching effects, and its ability to increase stamina [5]. Consuming gojiberry has been shown to improve health-related problems, such as diabetes, hyperlipidemia, cancer, hepatitis, immune disorders, thrombosis, and male infertility and also benefit vision, kidney, and liver function [6–8].

2.1. Nutraceutical value

Scientific analysis of gojiberry constituents started in the 1970s. Qian et al. [9] reviewed 142 publications from 1975 to 2016 and summarized that at least 355 compounds occur in different species of *Lycium*, which were categorized as alkaloids at 20%, sterols, steroids, and their derivatives 16%, terpenoids 11%, amides 10%, flavonoids 9%, organic acid 9%, phenylpropanoids 9%, glycerogalactolipids 6%, ligans 4%, coumarins 3%, antraquinones 1%, peptides 1%, and others 1%.

The most intensively studied components are a group of water-soluble glycoconjugates, which are identified as arabinogalactan-proteins, commonly known as *L. barbarum* polysaccharides or LBPs. It is estimated that dried fruits comprise 5–8% of LBPs [10, 11]. Chinese Pharmacopeia [12] recommended using LBPs as an indicator for evaluation of gojiberry quality. Molecular weights of the LBPs range from 24 to 241 kDa, and they are mainly composed of six types of monosaccharides: arabinose, glucose, galactose, mannose, xylose, and rhamnose [13]. LBPs also contain galacturonic acid and 18 amino acids and share a glycan-o-ser glycopeptide structure [14]. The main chains of the glycan backbones of LBPs have been reported to be either alpha-(1–N6)-D-glucans or alpha-(1–N4)-D polygalacturonans [3, 15].

A second major group of metabolites in fruit is carotenoids [3], the content of which increases during the fruit ripening process. A total of 11 free carotenoids and 7 carotenoid esters were detected from unsaponified and saponified *L. barbarum* extracts [16]. Zeaxanthin dipalmitate was found to account for 80% of the total carotenoids [17]. β-Cryptoxanthin palmitate, zeaxanthin monopalmitate, small amounts of free zeaxanthin and β-carotene are also present [3]. Seeds contain zeaxanthin (83%), β-cryptoxanthin (7%), β-carotene (0.9%), and mutatoxanthin (1.4%) [13].

The fruits also consist of vitamins including ascorbic acid (vitamin C), riboflavin, and thiamin. The content of vitamin C (42 mg/100 g) is comparable to that of fresh lemon fruits [3].

Flavonoids are another important group of compounds. Total flavonoids of *L. barbarum* var. aurauticarpum, a yellow fruit variety, reach up to 2.0 mg/g which is four times higher than red-fruited *L. barbarum*. Fruits of *L. ruthenicum* contain oligomeric proanthocyanidins (OPC) at 3690 mg/100 g, which surpasses blueberries at 3380 mg/100 g [18]. Furthermore, aglycones myricetin, quercetin, and kaempferol were identified after hydrolysis [19].

Gojiberry fruits contain 1–2.7% free amino acids, of which proline is the major constituent [3]. Non-proteinogenic amino acids include taurine, γ-aminobutyric acid, and betaine (trimethylglycine) [20, 21]. Some miscellaneous compounds, such as β-sitosterol and its glucoside daucosterol, scopoletin, p-coumaric acid, the dopamine derivative lyciumide A, and L-monomethyl succinate occur in fruits [22–24].

2.2. Pharmaceutical value

Consuming gojiberry has been shown to improve general well-being, anti-myelosuppression, and sleep quality. Five randomized clinical studies conducted in the US [25–28] and China [29, 30] indicated that daily consumption of standardized *L. barbarum* fruit juice [GoChi juice (Goji juice) 120 ml = equivalent to 150 g of fresh fruit] for 14 or 30 days improved general well-being including neurological and psychological status, cardiovascular, joint, and muscle functions as well as gastrointestinal regularity without any adverse effects.

LBPs from *L. barbarum* have anti-aging and neuroprotective activities [31]. An arabinogalactan-protein (LBP-III) isolated from LBPs exhibited cytoprotective effects against stress. The protective role was mediated by reducing the phosphorylation of double-stranded RNA-dependent protein kinase (PKR) and also by decreasing the dithiothreitol (DTT)-induced LDH release and caspase-3 activity [32]. The reduction of PKR was caused by beta-amyloid peptide. It is well known that the phosphorylation state of PKR increases with age, the reduction of phosphorylation triggered by beta-amyloid peptide suggests that LBP-III from gojiberry could be a potential neuroprotective agent [33, 34]. *L. barbarum* intake was effective to control waist circumference in humans and may reduce the risks of metabolic syndrome [28].

Several experimental and clinical studies showed that *L. barbarum* exhibited anti-diabetic effects. *L. barbarum* reduced oxidation in patients with retinopathy [35]. In a randomized diabetic retinopathy study, the intake of fruit of *L. barbarum* for 3 months was shown to increase the contents of vitamin C by 61% and the activities of SOD by 87% and also to reduce serum content of lipid peroxide by about 20% compared to a control group [36]. *L. barbarum* was also effective when used for improving immunologic function of red blood cells in patients with diabetic retinopathy [36]. In a study with 44 patients with diabetic retinopathy, RBC C_3b receptor rosette (RBC-C_3bRR) in the patients treated with *L. barbarum* for 3 months decreased [37]. LBPs were shown to have immunomodulatory effects on patients with type-2 diabetes by reducing T8 and interleukin 6 (IL-6) by 23% and increasing T4/T8 and IL-2 by 30 and 62% compared to the normal level, respectively [38].

Gojiberry has been considered to be the richest source for zeaxanthin [39, 40] varying from 1.18 to 2.41 mg/g dried fruit [11]. Gojiberry is, thus, a natural pill for eye health. Drinking goji berry juice daily as a dietary supplementation for 90 days was reported to increase plasma

zeaxanthin and antioxidant levels significantly, which protected eyes from hypopigmentation and accumulation of oxidative stress compounds that can damage the macula [41].

There are several reviewed articles regarding chemical constituents and nutraceutical value of gojiberry as well as pharmaceutical effects of gojiberry consumption. The reader is referred to these reviews [3, 9, 11, 16, 31] for more detailed information.

2.3. Uses

Gojiberry has been commercialized with different names depending on the origin of plants and product types. "Ningxia Goji berry," produced from Ningxia (Ningxia Hui Autonomous Region, which is equivalent to a Province, located in Northwestern China) and harvested primarily from *L. barbarum*, is considered to be the authentic origin. "Black Wolfberry" is the berry harvested from *L. ruthenicum*. "Himalayan Goji berry" or "Tibetan Goji berry" are also on the global functional food market; these two names are used by health food promoters for a nomenclatural marketing advantage, though commercial cultivation of the crop does not occur in those regions [42]. In addition to being an important traditional Chinese medicinal herb, commercialized products vary from dried berries in various sized bags to juices, beers, and wines. Gojiberry is also found in cookies, chocolate candies, muesli, sausages, and snack bars. Gojiberry products have been marketed online since 2002 and are termed as a "super-food" [43].

Gojiberry and its products are sold as food or food supplements in the US and in Europe [16]. These products, however, are not allowed to be promoted as drugs, and therapeutic claims are prohibited thus far. In the US, the Food and Drug Administration (FDA) warned about some Gojiberry juice distributors using marketing claims which violated the Food Drug and Cosmetic Act [3]. In the United Kingdom (UK), the Food Standards Agency in 2007 concluded that there were sufficient records of alimentary use of Gojiberry in the UK before 1997 and thus the fruit does not fall under the Novel Food legislation. In the US, however, Gojiberry is not listed on the generally regarded as safe (GRAS) list by the FDA [3].

3. Gojiberry production

China is the main producer and supplier of gojiberry in the world. The majority of commercially produced gojiberry comes from Ningxia with gojiberry plantations typically ranging from 40 and 400 ha. Gojiberry, primarily *L. barbarum*, has been produced along the fertile floodplains of the Yellow River for more than 700 years. Fresh fruits are uniformly orange-red, and dried fruit has a sweet-and-tangy flavor. Ningxia has earned a reputation for premium quality gojiberries. As of 2015, more than 66 million hectares were planted with gojiberry in Ningxia. The region is recognized as the largest annual harvest in China, accounting for 45% of the nation's total yield of gojiberries. Fresh red or black berries are harvested starting in August. The harvested fruits are immediately washed and then preserved by drying them directly under full sun or through dry machines. Gojiberries are celebrated each August in Ningxia with an annual festival coinciding with the berry harvest. The celebration was originally held in Ningxia's capital, Yinchuan City;

the festival has been moved since 2000 to Zhongning County. Furthermore, gojiberries are drought-tolerant plants. As Ningxia's borders merge with three deserts, gojiberries are also planted to control erosion and reclaim irrigable soils from desertification. Commercial volumes of gojiberry also grow in other provinces of China including Xingjiang, Hebei, Inner Mongolia, Qinghai, Gansu, and Shaanxi. Jinghe County in Xingjiang, Julu County in Hebei, and Huangjinhou in Inner Mongolia also produce high quality gojiberries

Gojiberry has also been commercially produced in the other countries including India, Korea, Japan, and other Asian countries. During the first decade of the twenty-first century, farmers in Canada and the US began cultivating gojiberry on a commercial scale to meet potential markets for fresh berries, juice, and processed products. Gojiberry has been propagated by tissue culture by Agri-starts, Inc. in Apopka, Florida, US and tissue-cultured liners are sold nationwide for production in the US and Canada. Goji farm USA celebrated the harvest of gojiberry from 8000 plants grown in Sonoma Valley of the moon region in California, US (GojiFarmUSA.com)

4. Current concerns on gojiberry production

The demand for quality gojiberry fruit has become much greater than the supply. A variety of factors are implicated in the shortage of supply, which includes the availability of reliable cultivars, the lack of corresponding production protocols, disease and pest control information, appropriate methods for processing fruits, quality control, and food safety issues. Among these factors, the availability of new and reliable cultivars is a key factor. China is the only country with gojiberry breeding programs. Current breeding efforts have been largely limited to *L. barbarum*, *L. chinense*, and *L. ruthenicum*, and only a few cultivars have been released and utilized. Some of the cultivars are unstable in fruit setting, fruit size, and final yield due to seed propagation or inappropriate planting of comparable cultivars adjacently for pollination. Additionally, the evaluation of gojiberry germplasm has remained in the descriptive stage; their potential has not been fully exploited. Thus, a better understanding of the current status of gojiberry breeding is important for future development of new cultivars and for increasing its production.

5. Genetic improvement of gojiberry

It has been well documented that naturally occurring variation among wild relatives of cultivated crops is an underexploited resource in plant breeding [44]. The genus *Lycium*, a member of the family Solanaceae, comprises about 80 species [2]. Most of them remain in the wild, and their reproduction systems have been studied, but their agronomic traits and nutraceutical and pharmaceutical value have barely been exploited.

5.1. Genetic resources

Lycium species are distributed in temperate and subtropical regions worldwide, but are absent in both the Old and New World tropics. Areas of greatest species richness are in South

America, primarily in Argentina and Chile, with more than 30 species. There are about 21 species in Southwestern and North America, approximately 17 species in southern Africa [45], and about 10 species in Eurasia [46]. Based on the analysis of chloroplast DNA sequences, Fukuda et al. [46] proposed that *Lycium* originated from the New World. All species in southern Africa, Australia, and Eurasia have a common progenitor from the New World. Australian and Eurasian species originated once from a southern African progenitor, and *L. sandwicense* differentiated from the New World species. As a result, phylogenetic analysis showed that gojiberries (*L. barbarum*, *L. chinense*, and *L. ruthenicum*) are clustered with *L. europaceum* L. as they belong to Eurasian species. The Eurasian species are closely related to species from Australia such as *L. australe* F. Muell. and also those from southern Africa, such as *L. afrum* L., *L. cinereum* Thumb., *L. ferocissimum* Miers, *L. pilifolium* C. H. Wright, *L. prunus-spinosa* Dunal, *L. schizocalyx* C. H. Wright, and *L. villosum* Schinz. Species from North or South America as well as Pacific Island were clustered together [46].

Most *Lycium* species have perfect flowers and are bisexual or hermaphrodites. However, like some others in the family Solanaceae, *Lycium* species are generally considered to be outcrossed due to gametophytic self-incompatibility [47]. For example, allelic diversity at the self-incompatibility (*S*) gene in *L. andersonii* was estimated to have more than 35 alleles, and coalescence analysis showed that the *S*-allele lineages in this species are older than the genus as a whole, indicating that self-incompatibility is the basal condition for *Lycium* [48]. Most species are diploid with chromosome number of 2n = 2x = 24. However, Miller and Venable [49] reported that three North American species, *L. californicum* Nutt. Ex Gray, *L. exsertum* A. Gray, and *L. fremontii* A. Gray are polyploids and display functional dioecy. In addition, seven species in Africa have separate male and female plants [50, 51]. Levin and Miller [52] believed that gender dimorphism (the presence of two sexual morphs in a population) evolved twice among North American *Lycium* and probably three times in Africa. Thus, gender dimorphism is more common among African *Lycium*, occurring in 7 of the 27 African species (26%) compared to only 3 of 50 American species (6%). Furthermore, gender dimorphism has been shown to be uniformly associated with polyploidy, which resulted in a proposition that polyploidy disrupts self-incompatibility of North America diploid *Lycium* species and resultant self-compatible polyploids are then subject to invasion by male sterile plants [47].

It is unknown when and how *Lycium* species were dispersed to Eurasian regions. Among the 10 Eurasian species, there are 7 species that have been naturalized in China including *L. barbarum*, *L. chinense*, *L. cylindricum* Kuang, *L. dasystemum* Pojark., *L. ruthenicum*, *L. truncatum* Y.C. Wang, and *L. yunnanense* Kuang [53], of which *L. barbarum*, *L. chinense*, and *L. ruthenicum* are the most popular species. *L. barbarum*, *L. dasystemum*, and *L. ruthenicum* are primarily distributed in northwest China including Ningxia, Gansu, Inner Mongolia, Qinghai, Xinjiang, Shaanxi, and Shanxi. *L. chinense* is largely dispensed in central and east China. *L. cylindricum* is spread in Gansu, Inner Mongolia, Ningxia, Qinghai, North Shaanxi, Xinjiang, Tibet as well as Afghanistan, Kazakhstan, Kyrgyzstan, Mongolia, Pakistan, Russia, Tajikistan, Turkmenistan, and Uzbekistan. *L. truncatum* is distributed in dry regions with altitude ranging from 800 to 1500 m including Gansu, Inner Mongolia, Ningxia, Shanxi, and Xinjiang. *L. yunnanense* is mainly situated in Yunnan, southwest China.

5.2. Domestication of *L. barbarum*

How *L. barbarum* became naturalized in northwest China and centralized in Ningxia, particularly Zhongning County, is unclear. One possible reason could be due to the geographical location of Zhongning, as berries of *L. barbarum* produced in this county had the highest quality. An important irrigation project during Qin (221–206 BC), Han (206 BC–220 AD), and Tang (618–907 AD) dynasties channeled water from the Yellow River to irrigate farmland on the Yinchuan Plain in Ningxia, where Zhongning County was the gateway of the irrigation project. This project created favorable conditions for *L. barbarum* production. During Ming (1368–1644) and Qing (1644–1912) dynasties as well as the China Republic (1912–1949) periods, *L. barbarum* production was largely concentrated in Zhongning County with production acreage of about 200 hectares. Growers might have consciously harvested large fruits from individual plants. The harvested seeds might have been combined or kept separately by trees and planted the next year. Such conscious practices or domestication over thousands of years might have resulted in plants differing from their wild progenitors in several morphophysiological traits. Improved traits could be associated with seed retention and germination, growth habit, fruit size and coloration, and fruit edibility and taste. The domestication resulted in the establishment of landraces. No attention was given to the number of landraces in Zhongning County until the 1960s when Mr. Guofeng Qin conducted a survey in the County and identified 10 landraces, which included "Damaye," "Xiaomaye," "Heyemaye," and "Baitiaogouqi" [54]. "Damaye" was found in Mr. Zhuohan Zhang's garden who was a gojiberry grower. He probably never realized what important role this landrace has played in gojiberry cultivar development. "Damaye" grew to a height of 1.5 m with a canopy diameter of 1.7 m in 6 years. The fruit is spheroid-shaped, and 1000 fresh fruits weigh from 450 to 510 g. A single plant produces 7–8 kg fresh fruit. "Damaye" has been considered to be the most reliable landrace as it produces large, uniform fruits with little variation over the years.

5.3. Individual plant selection

An organized breeding effort on gojiberry was initiated in the 1970s at the Ningxia Research Center of Wolfberry Engineering Technology, which was renamed as National Wolfberry Engineering Research Center in 2011. The breeding center is located in Yinchuan City, Ningxia, China. This is the only federally sponsored institute devoted to gojiberry research. Initial breeding efforts were primarily focused on the selection of individual plants with vigorous growth rates, larger fruit, and higher fruit yield. Mr. Shenyuan Zhong volunteered to work in Ningxia after graduation from the Northwest Agricultural College and stayed in Zhongning County from 1965 to 1985. Mr. Zhong started individual plant selection from "Damaye" populations. A large number of individual plants were selected from "Damaye." Seeds were harvested from each selected individual plant, and individual plant's progenies were evaluated separately. If progenies from selected plants were variable, cloning propagation, such as rooting of cuttings, was used to stabilize the phenotypes. Using this selection method, Mr. Zhong selected 12 lines. After large-scale progeny testing, he released cultivars: "Ningqi 1" and "Ningqi 2" [54, 55]. Through mass selection, Mr. Zhong and his

associates released "Ningqi 3" in 2005. Another scientist, Mr. Zhongqin Hu at the Zhongning Wolfberry Industry Bureau made selections from "Damaye" and released "Ningqi 4" in 2005 [55]. Using "Damaye" and "Ningqi 1" as primary resources, subsequent selections resulted in the release of "Ningqi 5," "Ningqi 6," "Ningqi 7," "Ningqi 8," and "Ningqi 9." "Ningqi 5" is a male sterile cultivar, "Ningqi 6" and "Ningqi 8" require outcrossing, while "Ningqi 7" is self-compatible. "Ningqi 9" is a tetraploid. Both "Ningqi 5" and "Ningqi 9" were selected from "Ningqi 1."

Among the Ningqi series, "Ningqi 1" has been highly successful. It has a similar growth rate and canopy shape to "Damaye," but it produces ellipsoid-shaped fruits with 1000 fresh fruits weighing more than 586 g, which is 15–30% greater than "Damaye." "Ningqi 1" is particularly stable in production and is able to adapt to a wide range of environments. Its production was quickly expanded in the entire Ningxia region, subsequently Xinjiang, Gansu, Inner Mongolia, Hubei, and Shanxi, moving from northwest China to Central China in over 20 provinces. Its production acreage totaled 88,000 ha. Mr. Shenyuan Zhong, the inventor of "Ningqi 1," "Ningqi 2," and "Ningqi 3" was considered the father of Ningxia gojiberry. He received the National Science and Technology Progress Second Prize in China in 2005. Mr. Zhong passed away in 2012, but production of his cultivars, particularly "Ningqi 1," has rapidly increased in China.

The success of "Ningqi 1" is closely related to the reproduction mode. As mentioned earlier, *Lycium* species are self-incompatible. Recent studies of "Damaye" and "Ningqi 1" showed that the two are self-compatible [56], which ensures the stability of agronomic traits when propagated through seed. The selection scheme used by Mr. Zhong was similar to the application of the pure-line theory [57] with a modification by vegetative propagation to fix a phenotype if needed. The finding of self-compatibility in "Damaye" and "Ningqi 1" was somewhat unexpected because *L. barbarum* has been considered to be an outcrossing species. The finding provides explanation as to why "Damaye" was the most valuable landrace and also why "Ningqi 1" was the most popular cultivar.

5.4. Hybridization

The performance of selected Ningqi series in the field suggested that several reproduction modes occurred in *L. barbarum.* Some are self-compatible, such as "Ningqi 1," and "Ningqi 7." Some were self-incompatible, such as "Ningqi 3," "Ningqi 4," "Ningqi 6," and "Ningqi 8." Whereas "Ningqi 5" showed high male sterility, and "Ningqi 9" was a tetraploid. As a result, intraspecific hybridizations have been carried out for breeding of new gojiberry cultivars. The hybridizations within *L. barbarum* included the use of self-incompatibility and male sterility and the crosses between Ningqi series cultivars with cultivars from the other regions [58]. For the use of the self-incompatible cultivars, one cultivar was planted in a row with another in a 1:1 ratio. The percentage of fruit set, 1000 fresh fruit weight, and total fruit yield per tree were evaluated for identifying the best combinations. For example, "Ningqi 6" should be planted with "Ningqi 8" in a 1:1 ratio for high fruit set. "Ningqi 5," the male sterility line, should be planted with "Ningqi 1" or "Ningqi 4" in a 1:1 or 1:2 ratio for maximizing fruit set. Ningqi cultivars were also hybridized with cultivars from Xinjiang and other provinces. An et al. [59]

made crosses between "Ningqi 1" and "Ningqi 9" (a tetraploid) and selected a triploid cultivar, which produced fruit that had no or little seeds, contained more sugar and amino acids with better taste compared to "Ningqi 1," and was resistant to aphid infestation.

Interspecific hybridization has also been used for developing new cultivars. Crosses between "Ningqi 1" with a wild species by Li et al. [60] resulted in the release of "Ningqi Cai 1." It produces green, tender shoots and leaves, which is used as a vegetable, not for fruit production. This unique cultivar contains 352 g/kg protein, 18 amino acids with vitamin C at 135 mg/kg, and has been widely grown in China as a vegetable crop. Reciprocal crosses were made between either "Ningqi 1" or "Ningqi 4" with cultivars of *L. ruthenicum*, and results showed that they were highly compatible, resulting in the fruit set rate ranging from 52.38 to 91.43% [58]. Furthermore, Wang et al. [61] crossed *L. barbarum* with tomato (*Solanum lycopersicum* L.). Seven lines were selected from 21 cross combinations, and two of them flowered and produced fruit, suggesting *L. barbarum* has a wide range of crossability within the family Solanaceae.

5.5. Breeding through chromosome manipulation

Chromosome manipulation has been another approach for improving *L. barbarum* cultivars. In vitro culture of *L. barbarum* anthers produced haploid plants (2n = 12), and subsequent culture of hypocotyls of the haploids caused simultaneous doubling, resulting in homozygous diploid plants [62]. In vitro culture of endosperm of *L. barbarum* by Wang et al. [63] and Gu et al. [64] resulted in the isolation of triploid plants from mixoploid populations. Colchicine treatment of in vitro cultured meristems [65] or in vitro culture of ovary [66] produced tetraploid *L. barbarum*. In general, fruits produced from triploid and tetraploid plants were larger than diploid plants. Additionally, polyploid plants had larger flowers and thicker fruit pulp than the diploid plants.

5.6. Biotechnological approaches

Biotechnological approaches for improving gojiberry have been limited thus far. Methods for in vitro culture of existing meristems, anthers, embryos, and endosperm have been reported, and some of the methods have become well established. In vitro cultured materials were also used for inducing mutation, and some progress has been made. For example, a breeding line for resistance to *Fusarium graminearum* was produced from in vitro cultured embryonic calluses treated by ^{60}Co-γ ray under the selection pressure of crude toxin of *Fusarium graminearum* [67]. Selection of EMS-treated embryonic calluses in the presence of 1.5% NaCl resulted in the development of salt tolerant plants [68].

Agrobacterium tumefaciens-mediated genetic transformation was established in *L. barbarum* [69]. In attempts to improve *L. barbarum* resistance to aphids, transgenic plants containing the gene encoding snowdrop lectin (*Galanthus nivalis* agglutinin, GNA) were generated [70]. The transgenic plants showed aphid resistance and also increased fruit weight and total sugar content, but seed count decreased. Because GNA was under the control of constitutive and phloem-specific promoters, the transgene product was only detected in leaves and young

shoots, not in the fruit. Additionally, field test of the transgenic plants showed that rhizosphere microorganisms were not affected by the expression of the transgene [71].

In a study of genetic engineering of targeted genes, five carotenogenic genes from *L. barbarum*: geranylgeranyl diphosphate synthase, phytoene synthase and delta-carotene desaturase gene, lycopene beta-cyclase, and lycopene epsilon-cyclase were functionally analyzed in transgenic tobacco (*Nicotiana tabacum* L.) plants. Results showed that all transgenic tobacco plants constitutively expressing these genes and beta-carotene contents in their leaves and flowers increased [72]. These results imply that such genes could be used for improving *L. barbarum* in beta-carotene biosynthesis.

6. Retrospect and prospect

There is a growing interest in gojiberry around the world, but some questions about *Lycium* species are still unanswered. *Lycium* species originated in South and North America, but how and when they were dispersed to Africa and Eurasian regions remain unclear. How has *L. sandwicense* been distributed across different island archipelagos? Why has *L. barbarum* been domesticated mainly in northwest China, and how has it become an important medicinal plant known as Ningxia gojiberry? A total of 355 compounds have been identified thus far [9], do any other compounds remain to be discovered? Nutraceutical and pharmaceutical value of important compounds require further investigation.

A fundamental question from a breeding point of view, however, is the reproduction modes of *Lycium* species. Almost all reports in the literature described that diploid *Lycium* species are outcrossed due to their gametophytic self-incompatibility. Miller and Venable [47] used North American species to show that gender dimorphism has evolved in polyploid, self-compatible taxa from co-sexual, self-incompatible diploids. They proposed that polyploidy is a trigger of unrecognized importance for the evolution of gender dimorphism, which operates by disrupting self-incompatibility and leading to inbreeding depression. Subsequently, male sterile mutants invade and increase because they are unable to inbreed. Research results from China, however, showed that *L. barbarum* is diploid, no occurrence of dimorphism, and landraces range from self-compatible to self-incompatible. The primary reason for the dominance of "Damaye" is its self-compatibility. The most popular cultivar selected from "Damaye," "Ningqi 1," is also widely produced and reproduced due to its self-compatibility. Was *L. barbarum* originally a self-compatible species being dispensed to Eurasian regions? Baker's Law [73–75] stated that self-compatible species are more likely to be successful island colonizers than obligate out-crossers that require pollen transfer between plants (i.e., self-incompatible species). In support of this law, a higher frequency of self-compatibility, as opposed to self-incompatibility, has been documented in island flora. Although the region where *L. barbarum* naturalized is not an island, its surroundings may be similar to an isolated region. Another possibility could be that *L. barbarum* is a self-incompatible species, and adaptation to northwest China caused switching to self-compatibility. The transition from self-incompatibility to self-compatibility has occurred often in the history of Solanaceae [76]. Thus, the reproduction

modes in *L. barbarum* should be further investigated as the modes are fundamentally important for new cultivar development as documented in this article.

Significant progress has been made in *L. barbarum* breeding compared to other emerging fruit crops, such as pawpaw (*Asimina triloba* Dunal.), quince (*Cydonia oblonga* Mill.), or blue honeysuckle (*Lonicera caerulea* L.) [42]. The *Lycium* story is that a South and North America species was naturalized in northwest China and the domestication of this species produced more than 10 landraces. Selection from the landraces resulted in the release of a series of cultivars. Its reproduction modes, cross-ability, self-incompatibility, male sterility, and phylogenetic relationships with other species have been revealed. Regeneration and transformation have been developed. From a local, traditional medicinal plant, it has now received increasing attention as an important nutraceutical and pharmaceutical crop. However, *L. barbarum* and its relatives in the genus require further attention:

1. Genetic potential of other species should be exploited. Current research has been largely focused on *L. barbarum*, and to a certain extent on *L. chinense* and *L. ruthenicum*. Attention should be expanded to species from South and North America as well as Africa. Fruit constitutes of those species should be analyzed and those producing valuable compounds should be used for breeding purposes. Their reproduction modes, cross-ability, and corresponding breeding schemes should be developed. Since *L. barbarum* has been shown to be able to cross with tomato, it is believed that *L. barbarum* could be easily crossed with other *Lycium* species. With the use of other species, it is anticipated that new cultivars with more desirable traits could be developed for commercial production.
2. Breeding objectives should include not only fruit size and yield but also disease and pest resistance, valuable compound content, and adaptability. Early maturity should also be important. Constitutes of fruits or leaves should be systematically analyzed, and compounds with beneficial functions or negative effects should be clearly identified. Breeding schemes should be designed to maximize production of beneficial compounds and minimize those with negative effects.
3. Effective methods for breeding of *Lycium* species should be developed. Current methods are based on individual plant selection and to some extent the use of self-incompatibility or male sterility. Individual plant selection continues to be useful but it should be accompanied with *in vitro* shoot culture to produce a large number of clones for commercial production. Pure lines derived from simultaneous doubling of anther cultured haploids should be tested to determine if homozygosity is an option for improving gojiberry productivity. If not, methods for maximizing heterozygosity should be evaluated to increase fruit production. Furthermore, molecular marker technologies should be incorporated into breeding schemes to increase breeding efficiency.

With the increased recognition of their roles as functional foods, plants in the genus *Lycium* will draw more attention for systematic research. It is anticipated that the potential of this small fruit crop will be fully exploited and valuable products that benefit human health and well-being will be developed and utilized.

Author details

Jianjun Chen[1]*, ChihCheng T. Chao[2] and Xiangying Wei[1,3]

*Address all correspondence to: jjchen@ufl.edu

1 Institute of Food and Agricultural Sciences, Mid-Florida Research and Education Center, University of Florida, Apopka, FL, USA

2 USDA-ARS, Plant Genetic Resources Unit, Geneva, NY, USA

3 College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, China

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Chapter 2

Genetic Diversity and Breeding of Persimmon

Turgut Yesiloglu, Berken Cimen, Meral Incesu and Bilge Yilmaz

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.74977

Abstract

The genus *Diospyros,* which is distributed in tropical and subtropical regions of the world, contains hundreds of plant species. However, four species of them have commercial importance. *D. kaki* Thunb. is the most widely cultivated species of the *Diospyros* genus. Persimmon (*D. kaki* Thunb.) is grown in many parts of the world that display subtropical climate conditions. In recent years, the cultivation of persimmon has found renewed interest in various countries of the Mediterranean basin. In China (which is the origin of persimmon) and in Japan and Korea (where it is grown widely), persimmons were selected from some well-known old varieties. Recently in countries such as Italy, Spain, USA, Brazil, Turkey and Israel, persimmons were selected from new cultivars. Currently China, Japan and Korea have the big persimmon germplasm collections with a large number of varieties and other *Diospyros* species. Also, Italy, Spain, USA, Brazil, Turkey, Israel, Azerbaijan, Uzbekistan and Pakistan have constituted the germplasms by high commercial value cultivars and/or local varieties. In this chapter, we tried to provide an overview of the genetic diversity and breeding of persimmon by combining literature with our studies.

Keywords: *Diospyros*, genetic resources, selection, hybridization, breeding

1. Introduction

Persimmon is fleshy fibrous subtropical and tropical, deciduous fruit belonging to *Ebenaceae* family. The Oriental persimmon (*Diospyros kaki* Thunb.) is an exotic fruit rich in vitamins, nutrients and antioxidants vital for optimum health with various medicinal and chemical uses of fruits and leaves. Its fruit is usually consumed as a fresh or dried fruit. It is believed to have originated in the mountain area of southern China and has been cultivated as an important fruit crop in China, Korea as well as in Japan for centuries [1]. It is commonly

cultivated in warm regions of the world including China, Korea, Japan, Brazil, Spain, Turkey, Italy and Israel. The world's total persimmon production was low between 1961 (990,079 tons) and 1993 (1,290,971 tons). However, persimmon production has increased since 1995. Total persimmon production of the world was 5,190,624 tons in 2014 (**Table 1**). Similarly, while persimmon acreage was low from 1961 to 1993, the acreage expanded rapidly increasing from 262,039 ha in 1993 to 1,028,051 ha in 2014 [2].

As represented in **Table 2**, China is the highest producer with a production of about 3,730,800 tonnes. This amount of production accounts for about 71.88% of the world's production. It is followed by Korea (428,363 tonnes), Spain (245,000 tonnes), Japan (240,600 tonnes), Brazil (182,290 tonnes), Azerbaijan (140,405 tonnes), Taiwan (72,674 tonnes), Uzbekistan (66,000 tonnes), Italy (39,149 tonnes), Israel (36,592 tonnes) and Turkey (33,470 tonnes) [2, 3]. The variation of production amounts of the countries differed by years. As is presented in **Table 2**, China's persimmon production has steadily increased from 495,000 tonnes in 1961 to 3,730,800 tonnes in 2014. Similarly, Korea's production increased by 32.28 times and has reached 428,363 tonnes. Spain has shown the maximum rate of increase with the production increasing from 591 tonnes in 1991 to 245,000 tonnes in 2014. In Spain, about 20 years ago, persimmon was grown for local consumption. The selection of cultivar 'Rojo Brillante' (PVA) having high fruit yield and quality and the application of the technique for removing astringency without losing fruit firmness led to the expansion of the persimmon culture in the 1990s [4]. Israel and Turkey that have lower production have significantly increased their production (respectively, 36,592 and 33,470 tonnes). On the other hands, while Japan's and Italy's production were 393,500 tonnes and 70,740 tonnes in 1961, they have dramatically decreased to 240,600 and 39,149 tonnes in 2014.

About 5% of the world's total persimmon production is exported as fresh fruit. The rest of persimmon is consumed in the internal market, and a good part of production is dried or processed. Although Azerbaijan is the sixth persimmon producer country, it is the first fresh fruit persimmon exporting country in the world. In 2013, Azerbaijan exported 95,118 tonnes of persimmon fruits. It is followed by Spain (40,121 tonnes), China mainland (35,799 tonnes), Israel (13,084 tonnes) and Poland (12,142 tonnes) (**Table 3**). Further, Russia (114,596 tonnes), Kazakhstan (58,464 tonnes) and Germany (30,233 tonnes) are the largest persimmon-importing countries in the World (**Table 4**).

Persimmons have proved to be highly adaptable to a wide range of climate conditions, ranging from subtropical coastal regions to mild coastal areas and warm inland temperate areas. Persimmons do best in areas that have moderate winters and relatively mild summers. Generally, astringent varieties prefer cooler climatic conditions than non-astringent varieties. Non-astringent cultivars require warmer growing conditions. If the cultivars of non-astringent types are grown in cooler regions, the fruit flesh may not lose all of its astringency and have lower sugar content at harvest. They can tolerate temperatures of −18°C when fully dormant. Persimmons need only a short chilling period (about 100–400 h below 7.2°C). The chilling requirement of non-astringent varieties is lower than that of astringent varieties. If the dormancy is broken or the chilling requirement of the variety has been supplied early by the warming climate, the new shoots can be damaged by a late spring frost. The leaves are killed by −3.3°C when growing. However, persimmons generally bloom late in the spring

Year	China	Korea	Spain	Japan	Brazil	Azerbaijan	Taiwan	Uzbekistan	Italy	Israel	Turkey*	World
1961	495,000	13,271	—	393,500	15,298	—	2250	—	70,740	—	—	990,079
1962	440,000	16,594	—	322,500	16,005	—	2109	—	72,830	—	—	870,058
1963	475,000	14,114	—	383,500	16,239	—	1804	—	75,200	—	—	965,877
1964	465,000	23,602	—	464,000	17,198	—	2340	—	73,500	—	—	1,045,660
1965	515,000	23,510	—	346,400	19,988	—	1997	—	72,000	—	—	978,915
1966	495,000	22,075	—	419,300	19,586	—	2101	—	71,400	—	—	1,029,482
1967	475,000	23,609	—	504,400	20,037	—	2203	—	73,600	—	—	1,098,869
1968	470,000	34,579	—	450,100	20,427	—	2333	—	73,300	—	—	1,050,764
1969	414,000	33,854	—	444,100	20,849	—	2152	—	74,000	—	—	988,980
1970	455,000	30,310	—	342,700	21,659	—	2341	—	59,600	—	—	911,635
1971	427,000	22,887	—	303,200	21,558	—	2411	—	59,200	—	—	836,286
1972	480,000	31,115	—	306,900	25,188	—	2359	—	61,700	—	—	907,292
1973	535,000	32,284	—	347,200	19,374	—	2515	—	59,000	—	—	995,403
1974	538,000	41,928	—	283,600	20,446	—	2745	—	63,500	—	—	950,249
1975	528,000	20,890	—	274,700	22,114	—	2843	—	60,000	—	—	908,607
1976	530,000	16,946	—	264,100	22,364	—	3826	—	60,690	1700	—	899,686
1977	525,000	30,138	—	275,400	23,801	—	4422	—	62,100	2400	—	923,321
1978	570,000	29,984	—	287,000	36,340	—	4463	—	57,800	1900	—	987,547
1979	506,500	33,386	—	263,900	40,385	—	5267	—	58,500	2500	—	910,503
1980	560,400	31,837	—	265,200	39,958	—	6238	—	61,100	3400	—	968,198
1981	506,000	39,254	—	260,500	37,598	—	7313	—	62,800	5600	—	919,207

http://dx.doi.org/10.5772/intechopen.74977

Year	China	Korea	Spain	Japan	Brazil	Azerbaijan	Taiwan	Uzbekistan	Italy	Israel	Turkey*	World
1982	482,000	57,807	—	333,700	38,396	—	6621	—	65,400	8100	—	992,416
1983	553,000	91,052	—	309,900	45,298	—	7850	—	76,700	12,700	—	1,096,858
1984	608,000	68,812	—	297,400	41,915	—	9525	—	78,400	13,900	—	1,118,477
1985	680,000	97,031	—	289,700	43,658	—	10,945	—	56,200	8100	—	1,186,165
1986	750,000	98,906	—	291,300	43,488	—	10,626	—	69,700	11,900	—	1,277,032
1987	820,000	75,677	—	290,200	45,000	—	11,811	—	69,820	19,200	—	1,332,767
1988	732,921	98,337	—	287,600	45,745	—	15,694	—	69,170	7000	—	1,257,684
1989	650,283	113,403	—	268,100	46,836	—	16,404	—	72,920	16,000	—	1,185,538
1990	624,773	95,758	0	285,700	46,712	—	15,457	—	68,770	17,200	—	1,157,241
1991	641,576	109,722	591	248,800	47,662	—	14,935	—	61,810	16,200	10,000	1,144,666
1992	724,329	155,111	591	307,700	46,611	—	14,636	—	66,546	17,390	10,000	1,336,383
1993	789,113	116,070	2963	241,900	48,086	—	16,380	—	56,753	15,780	10,000	1,290,971
1994	826,870	167,471	6895	302,200	55,406	—	15,100	—	48,999	13,800	9300	1,440,701
1995	969,363	194,585	10,826	254,100	51,685	—	16,440	—	61,300	11,000	9200	1,573,421
1996	1,025,219	210,766	14,757	240,500	52,534	—	15,811	—	67,800	18,200	9400	1,649,285
1997	1,075,417	239,570	18,688	301,700	52,198	—	19,018	—	59,800	16,800	10,000	1,787,234
1998	1,314,136	260,671	21,842	260,100	60,423	47,900	18,962	—	62,000	17,400	10,500	2,068,143
1999	1,481,327	273,846	29,469	286,000	64,096	60,300	25,754	—	40,769	14,000	11,500	2,280,753
2000	1,591,906	287,847	33,000	278,800	63,300	70,300	23,891	16,000	42,450	14,186	12,000	2,427,646
2001	1,584,660	270,338	34,500	281,800	105,000	86,000	26,169	16,000	48,240	14,900	13,500	2,473,149
2002	1,740,591	281,143	36,050	269,300	141,364	104,800	34,747	16,500	54,170	35,700	15,000	2,720,098

Year	China	Korea	Spain	Japan	Brazil	Azerbaijan	Taiwan	Uzbekistan	Italy	Israel	Turkey*	World
2003	1,795,110	249,207	45,200	265,000	158,131	114,899	38,247	17,000	47,000	40,100	15,000	2,776,510
2004	1,998,214	299,046	45,800	232,500	162,288	48,089	36,177	19,000	57,110	38,700	17,000	2,943,525
2005	2,185,041	363,822	51,950	285,900	164,849	108,965	27,111	21,000	51,332	48,000	18,000	3,314,731
2006	2,320,346	352,822	63,000	232,700	168,274	124,485	26,395	27,213	53,100	24,606	19,297	3,399,970
2007	2,574,143	395,614	67,000	244,800	159,851	128,407	32,962	28,000	52,500	37,347	23,713	3,727,432
2008	2,710,988	430,521	95,400	266,600	173,297	132,179	33,899	31,000	51,600	45,350	24,302	3,977,744
2009	2,834,165	416,705	100,200	258,000	171,555	135,549	37,032	40,000	51,593	32,291	25,281	4,084,050
2010	2,875,600	390,630	125,280	189,400	167,215	142,188	58,401	38,000	48,165	28,201	26,277	4,070,375
2011	3,187,239	390,820	159,400	207,500	154,625	146,084	90,100	53,400	50,347	29,271	28,295	4,476,946
2012	3,417,586	401,049	212,300	253,800	158,241	140,082	81,894	56,000	51,165	31,292	32,392	4,813,100
2013	3,538,823	351,990	242,800	214,700	173,169	143,106	63,694	75,000	41,858	35,692	33,232	4,890,000
2014	**3,730,800**	**428,363**	**245,000**	**240,600**	**182,290**	**140,405**	**72,674**	**66,000**	**39,149**	**36,592**	**33,470**	5,190,624

Source: FAO, 2017. * Represents data from Turkish Statistical Institute (TUIK), 2017.

Table 1. World persimmon production amount (tonnes).

http://dx.doi.org/10.5772/intechopen.74977

Rank	Country	Production in tonnes	Area (ha)	Yield (kg/ha)
1	China, mainland	3,730,800	931,907	4003.4
2	Republic of Korea	428,363	27,988	15,305.2
3	Spain	245,000	13,370	18,324.6
4	Japan	240,600	21,300	11,295.8
5	Brazil	182,290	8323	21,902.0
6	Azerbaijan	140,405	8712	16,116.3
7	China, Taiwan Province of	72,764	5263	13,825.6
8	Uzbekistan	66,000	4218	15,647.0
9	Italy	39,149	2531	15,467.8
10	Israel	36,592	1374	26,631.7
11	Turkey*	33,470	2062	16,232.6
12	New Zealand	2600	164	15,853.7
13	Iran (Islamic Republic of)	2452	275	8926.6
14	Nepal	1918	288	6667.4
15	Australia	715	86	8272.1
16	Slovenia	801	70	11,442.9
17	Mexico	175	18	9722.2
18	Chile	—	102	—
	World total production	**5,190,624**	**1,028,051**	**5049.5**

FAO stat [2]. http://www.faostat.com.*TUİK [3]. Turkish Statistic Council records.

Table 2. Persimmon production amount (ton), planted area (ha) and yield (kg ha^{-1}) in 2014.

(mid-April) to avoid spring frosts. On the other hand, persimmon has been damaged by early-autumn frosts. Early-autumn frosts can lead to skin blemishes on fruit and early defoliation. Persimmon does not tolerate wind. It does not provide a good fruit yield and quality, if strong winds occur during the growing season. Fruit is also prone to wind rub from leaves and branches causing skin blemish on fruit. Windbreakers can be used to reduce the wind speed.

Full sun with some air movement is recommended for persimmon trees in inland areas, although they will tolerate some partial shade. But trees do not produce well in the high summer heat of desert regions, which sunburn the bark.

Kaki persimmons are drought tolerant. Persimmon trees can withstand drought, but fruit yield and quality (especially size) are reduced. Also, adequate moisture in the soil is required to produce sufficient shoot growth and formation of flower buds for next year's crop. The trees should be irrigated during dry periods.

The persimmons trees can grow well on a wide range of soil types but do best in deep, well-drained loam soils with a good supply of organic matter. Heavy clay loam soils that are prone

Rank	Country	Amount (tonnes)
1	Azerbaijan	95,118
2	Spain	40,121
3	China, mainland	35,799
4	Israel	13,084
5	Poland	12,142
6	Lithuania	9057
7	Republic of Korea	7379
8	Netherlands	6957
9	Georgia	6781
10	South Africa	5809
	World total	**232,247**

Table 3. Top 10 countries with highest persimmon exports in 2013.

Rank	Country	Amount (tonnes)
1	Russian Federation	114,596
2	Kazakhstan	58,464
3	Germany	30,233
4	Belarus	14,788
5	France	12,929
6	Italy	11,427
7	Lithuania	10,177
8	Poland	9715
9	Thailand	6997
10	Canada	6407
	World total	**323,858**

Table 4. Top 10 countries with highest persimmon imports in 2013.

to water-logging should be avoided. The preferred soil pH for optimum tree growth is in the range of 6.0–7.5. However, persimmon trees can tolerate a wider variety of conditions than most fruit trees.

Pest and disease problems: protection of fruits from bats and birds are required. Fruit flies are the potential problem as are aphids and mealybugs. Persimmon trees are also susceptible to collar rot, thus keeping mulch clear of the trunk is required.

2. Origin and history

Zeven and Zhukovsky [5] suggested that persimmon (*D. kaki*) has a primary center of genetic origin in the mountains of central China and a secondary center in Japan. Persimmon cultivation in China began more than 2000 years ago, and it is also scientifically known as *D. chinensis*. In China, it is found wild at altitudes up to 6000–8000 ft. [6]. It spread from China to Korea and to Japan many years ago. Since from prehistoric times, permission is consumed as food source in these countries. There are some trees that are 400–500 years old. It was imported in Europe (South France) for the first time in 1760. Thereafter it spread to the Mediterranean coast (Italy, Spain, Greece, Turkey and Algeria). The persimmon plant was introduced in North America (California, Florida), South America (Brazil) and Australia in the mid-1800s. Early in the fourteenth century, the explorer Marco Polo recorded the Chinese trade in persimmons [7]. Its cultivation has recent traditions in western countries where it is present only since the second half of the nineteenth century. Currently, persimmon is one of the most important fruit crops in Asian countries and, there is also steady increase in its production in some European countries.

3. Botany of persimmon

The genus *Diospyros* contains hundreds of plant species and are distributed in the tropical and subtropical regions of the world. Four species of them have commercial importance. *D. kaki* L. is the most widely cultivated species of the *Diospyros* genus. *D. kaki* is also known as the persimmon, Japanese persimmon, Oriental persimmon, Japanese persimmon, Kaki, Asian persimmon. It has been reported that wild type *D. kaki* exists in the forests of China [8, 9]. The other species are *D. lotus* L. (the date plum), *D. virginiaina* L. (native American persimmon) and *D. oleifera* Cheng [10].

The origin of *D. kaki* and its relationship to other *Diospyros* species is not well understood. The persimmon culture was known to occur in the fifth or sixth century in China [9]. In addition to *D. kaki*, *D. lotus* and *D. oleifera* also have been cultivated as fruit crop. *D. lotus* has been consumed as a fresh as well as dried fruit, and it is a source for tannin [9, 10]. Another important species known as a fruit crop is *D. virginiana*, of the eastern United States origin. This species which is consumed as fresh and processed is grown on a much smaller scale and is not yet considered a commercial crop [11]. These species are quite important as horticultural crops among the *Diospyros* species of temperate origin. On the other hands, *D. rhombifolia* originated from China is an ornamental plant which bears tiny attractive-colored fruit on a dwarf tree [9]. There are other species such as *D. digyna* (black sapote), *D. discolor* and *D. decandra* that have originated in the tropics and subtropics and produce edible fruits.

In the genus *Diospyros,* there are species and varieties having diploid (2n = 2x = 30), tetraploid (2n = 4x = 60), hexaploid (2n = 6x = 90), nonaploid *(2n* = 9x =135) and dodecaploid (2n = 12x = 180) chromosome number. Therefore, it is thought that the basic chromosome number of the genus *Diospyros* is 15 [9, 12]. The chromosome numbers of some wild species of genus *Diospyros*

(*D. oleifera*, *D. glandulosa*, *D. confertiflora*, *D. discolorare*, *D. ehretioides*, *D. lycioides*, *D. mollis*, *D. rhodocalyx* and *D. sumatrana*) are 2n = 30, except for 2n = 60 for *D. rhombifolia* and 2n = 90 for *D. ebenum* [13–15]. *D. kaki* L. is a hexaploid (2n = 6x = 90). However, octoploid (2n = 8x = 120) cultivars such as Hasshu and nonaploid (2n = 9x =135) cultivars such as 'Hiratanenashi' and 'Tonewase' have also been reported [14–16]. On the other hand, *D. virginiana* has two karyotypes with 2n = 60 and 90 [15, 17], while *D. lotus* is diploid (2n = 2x =30) [14].

4. Pomological classification

Persimmon fruit is highly astringent due to soluble tannins in the vacuoles of the fruit flesh. However, some cultivars lose astringency naturally on the tree as fruit ripens, while others retain astringency until maturity. Therefore, persimmons are classified into two major groups (based on the presence or absence of astringency in the fruit at maturity) as astringent (A) and non-astringent or sweet (NA) cultivars. Water-soluble tannins which cause astringency in the flesh of astringent types decrease as the fruit softens and becomes edible. However, astringency can be removed by various chemical treatments. Carbon dioxide gas or alcohol can be used to remove astringency, while the fruit remains firm. If ethylene is used for removing astringency, the fruit softens very quickly. Fruit of the non-astringent types naturally loses astringency, while the fruit is still firm. Thus, the fruit of non-astringent types is edible either the flesh is firm or soft [10, 12, 18].

Each group can be further subdivided, based on their response to pollination [18]. The amount of dark flesh coloration around the seeds varies in cultivars and changes in flesh color are related to seed formation, not pollination. In pollination variant types (PV), the flesh is dark and streaked around the seeds, but clear orange when seedless. When pollination is poor and only one or several seeds are formed, a dark area develops around the seeds, but the remaining flesh is light colored. The pollination variant types include cultivars that are astringent when they have several seeds or seedless (PVA), as well as partially or totally non-astringent when they have only one or a few seeds (PVNA). Also, in astringent cultivars of the pollination variant type, fruit which has a great degree of the dark flesh is non-astringent even when the fruit flesh is firm [10, 18].

Pollination constant (PC) types lack the dark streaking regardless of seed formation. The flesh color of pollination constant astringent (PCA) cultivars is not influenced by pollination and it does not develop dark flesh around the seeds. Pollination constant non-astringent (PCNA) persimmons are always edible when still firm, regardless of whether or not pollination has occurred.

PVA types can vary to either PCA or PVNA depending on several situations. If PVA type does not have any seed for some reason or when PVA persimmon varieties are cultivated without pollinators, the fruit has clear orange flesh and remains astringent (PCA) such as the Spainish variety 'Rojo Brillante' and Japanese variety 'Hiratanenashi'. Similarly, when PVA type has enough seeds (usually four or five) after pollination, the fruit has a great degree of dark flesh and loses astringency (PVNA) such as 'Nishimura Wase'.

5. Commercial and recently improved persimmon varieties

The fruits of two species (*D. kaki* L. and *D. virginiana*) in the genus *Diospyros* have commercial importance. In China, using native persimmon germplasm, several common persimmon varieties were developed. However, they all are PCA with the exception of 'Luo Tian Shi' [19, 20]. Persimmon is the main species cultured for edible fruit production in northern China. Recently, 'Jirou', 'Youhou', 'Taishuu' and 'Fuyu' among PCNA cultivars are gaining popularity. Persimmon growing regions are also spreading widely in Japan and Korea, thus some old well-known Persimmon varieties which were still being produced were selected from these countries. Persimmon has been a major fruit crop in Japan for many years [21] and for Japanese persimmon commercial production, 'Fuyu' (PCNA), 'Hiratanenashi' (PVA) and 'Tonewase' have been the three important cultivars. About 57% of the total area is devoted to these varieties [22]. Other varieties growing in Japan are 'Kosyu Hyakume', 'Matsumotowase Fuyu', 'Early ripening Jiro', 'Ichidagaki', 'Jiro Dojohachiya' and 'Taishu'. However, newly released cultivars such as 'Reigyoku' and 'Taiho' are also available. In Korea, non-astringent varieties have increased, while astringent varieties have decreased. In the recent years, amount of production of non-astringent varieties are higher than those of astringent varieties. Major cultivar of non-astringent type is 'Fuyu', which accounts for almost 82% in total production of persimmon, and 'Jiro' with 9.8% [23].

In Taiwan, 'Suzchou', 'Niouhsin' and 'Shihshih' local PCA are the major commercial varieties used. 'Fuyu' and' Jiro' are the main PCNA varieties [24]. Countries such as Azerbaijan and Uzbekistan focused on local astringent cultivars. In Brazil, the most cultivated persimmon cultivars include 'Rama Forte' and 'Giombo', which belong to the PVA group, 'Taubate' which is continually astringent with yellow flesh either with or without seeds (PCA), and 'Fuyu', which belongs to the PCNA group [25, 26].

'Fuyu', 'Hana Fuyu' and 'Ichikikei Jiro' cultivars which are PCNA and 'Hachiya' (PCA) are commonly produced in the USA [10, 18]. In new persimmon growing countries such as New Zealand and Australia, most of the cultivation area is devoted to 'Fuyu' [27]. In Spain, the most produced cultivars are 'Rojo Brillante' (**Figure 1**) and 'Triumph' which can be stored for

Figure 1. Fruits and tree of 'Rojo Brillante' cultivar.

Figure 2. Fruits and tree of 'Triumph' cultivar.

a long time [28]. In Italy, almost 90% of the persimmon production is Kaki Tipo (PVNA), the rest of production is other PVNA varieties (Vainiglia, Mercatelli and Moro) and PCNA cultivars such as Hana Fuyu, Jiro and Gosho [29].

Israel has its own cultivar, Triumph (**Figure 2**) which is sold under the name of Sharon fruit, and it is planted on 95% of the total area devoted to persimmon [27]. Also, persimmon production in South Africa is based on Triumph [30]. In Turkey, a great amount of production is PCA and PVNA varieties, which are selected from Turkey. However, recently introduced PCNA cultivars such as Fuyu, Hana Fuyu, Jiro and Izu have become popular with the growers. The new orchards with 'Fuyu' and 'Hachiya' cultivars have been established.

6. Persimmon germplasm resources

Persimmon originated from China, but it has been cultivated and produced mostly in Japan [31]. Persimmon has limited amount of production in the rest of the World. However, Spain, Italy, Israel and Brazil are now producing important amounts and these countries have developed their own cultivars such as 'Rojo Brillante' in Spain, 'Kaki Tipo' in Italy, 'Triumph' in Israel and 'Lama Forte' in Brazil. Recently, Australia and New Zealand have started to produce persimmon mainly for export, and the USA is also producing persimmon on a small scale.

Greene and Morris [32] indicated that germplasm collections are a source of genetic diversity to support crop improvement and botanical research as well as to support conservation efforts [33]. For the specific breeding objectives, these variations can either be created spontaneously or artificially by budwood mutations or cross breeding. The importance of germplasm can be explained by the variation of plant material. Therefore, recording and registration of genetic resources is critical for breeders in terms of improving new varieties.

Currently, more than 950 cultivars of persimmon exist from the subtropical to temperate regions of China [34]. There is only 1 genus and 63 species in persimmon family and most of them are distributed in tropic and sub-tropic regions of Hainan, Yunnan, Guangdong, Guangxi and Fujian provinces in China. The 63 species originated from this genus in China. Among these species *D. kaki* Thunb., *D. oleifera* Cheng., *D. lobata* L., *D. discilir* Willd., *D. potingensis* Merr. et Chun., *D. lotus* Linn., *D. glaucifolia* Metc., *D. rhombifolia* Hemsl. and *D. morrisiana* Hance. have been cultivated as fruit crops [35]. There are 550 accessions including most cultivars native to China and some native to Japan and Korea.

Aside from this exceptional existence of a PCNA type cultivar in China, almost all non-astringent type cultivars were developed in Japan. Historical records show that 'Zenjimaru', known to be the oldest PVNA type cultivar, was found in the beginning of the twentieth century, and that 'Gosho', was the first PCNA type cultivar, which was recorded in the seventeenth century [36]. In the beginning of the nineteenth century, 'Fuyu' and 'Jiro' were recorded as the most popular PCNA type cultivars. According to a nationwide survey on persimmon cultivars in Japan (Agricultural Research Station 1912), there were only 6 PCNA type cultivars in contrast to 401 PVNA type cultivars among more than 1000 cultivars collected from all over Japan. This means that in addition to its more recent appearance, the PCNA type probably has very narrow genetic variability. A total of 40 PCNA cultivars, including bud sports, which may cover almost all PCNA type cultivars currently existing in Japan, are now preserved at the National Institute of the Fruit Tree Science (NIFTS) in Akitsu, Hiroshima [9]. There are many astringent and PVNA local cultivars throughout Japan. The current conservation in Japan consists of approx. 600 genotypes [37].

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. In addition, a breeding program for obtaining new PCNA cultivars was started in 1995 by crosses among PCNA cultivars that were introduced from Japan. The breeding objectives in Korea are focused on obtaining superior PCNA cultivars with good eating qualities, large fruit and early ripening characteristics [9].

In Europe, persimmon is considered a secondary fruit tree species; only few countries, located in the Mediterranean area, are interested in a large-scale production.

Persimmon was introduced in Italy at the end of the ninteenth century. Later in Tuscany, the interest for this new species was increased and the genotypes were collected together with exotic and local varieties of fruit tree species (citrus, peaches and plums among others). As early as 1940, the University of Florence collected 11 accessions from the USA and France, or as local varieties and characterized them. The persimmon collection of Florence consisted of 52 cultivars and was totally destroyed by winter frost in 1985. Then a new germplasm orchard was established by introducing new accessions from Japan. A French germplasm was recorded at the beginning of the twentieth century. The Spanish collection was created in 1993 with material from Italy (54.2% of accessions) and from Spanish institutions and nurseries (45.8%) [38].

Persimmon was introduced into Brazil's São Paulo state in 1890. However, its cultivation expanded around 1920 with Japanese immigration. São Paulo is the main persimmon producing state. Rio Grande do Sul state has the second largest persimmon production of Brazil. In recent years, the persimmon acreage has increased and the trend is to continue crop expansion. 'Fuyu', 'Rama Forte', 'Giombo' and 'Taubaté' are the cultivars grown in Brazil. In Azerbaijan, persimmon production is widely spread since 1998, although its history has deep roots [2]. In terms of persimmon genetic resources in Israel, only high commercial value cultivars are collected.

Although the exact date of the introduction of persimmon to Anatolia is unknown, it is clear that it dates back to rather old times [39, 40]. Persimmon was introduced to Turkey from Russia via the Black Sea region. Turkey has main persimmon species (*D. kaki*, *D. lotus* and *D. oleifera*). *D. oleifera* can be seen only in the Mediterranean region of Turkey, while *D. lotus* grows as wild in Northern Anatolia and is used as dried fruits in this region. *D. kaki* and *D. oleifera* have been introduced from other countries at least 200 years ago. During this time, continuous propagation of persimmon by its seeds resulted in genetic diversity in *D. kaki* trees due to the high heterozygosity. Therefore, in the northeastern part of Turkey, persimmon trees differ from another in terms of fruit productivity, yield, shape, size, astringency and plant growth. This diversity in persimmon population in Turkey provided a great opportunity to the breeders for selection programs. As a result, the breeders were able to identify many promising clones in different parts of Turkey. A germplasm collection in the Black Sea region in Turkey with selected promising genotypes has been established.

First studies on persimmon in Turkey were started to introduce the foreign cultivars by the Ministry of Agriculture in 1967. Then, some selection studies were done in different parts of Turkey. After 1989, the total number of the known cultivars and types reached up to 74. Most of these varieties were introduced from Italy and some of them were from Israel, Japan, France and Pakistan especially after the attempts made by the Cukurova University, in Eastern Mediterranean, Turkey.

Selection of different genotypes was started by the Department of Horticulture of Cukurova University, by Department of Horticulture of Ondokuzmayıs University in Black Sea and by Citrus Research Institute of Antalya belonging to the Ministry of Agriculture in Western Mediterranean regions. Recently, the wider selections have been carried out especially on Black Sea coast by Citrus Research Institute of Antalya for 1 year and they presently have 43 promising candidate clones. Most even totality of the selections is astringent type. It seems to be rather difficult to find non-astringent types in Turkey. Yilmaz et al. [41] established a characterization study on persimmon genetic resources collected from Turkey. These germplasms were preserved with commercial cultivars in an *ex situ* germplasm preservation orchard located at the Cukurova University, Turkey. Persimmon genotypes were characterized based on their morphological traits. The collection comprising traditional genotypes, local accessions and also global varieties were collected from five different provinces of the Mediterranean region of Turkey where persimmon is widely produced. A total of 48 persimmon genotypes and cultivars were morphologically characterized, using 59 morphological and agronomic traits (**Table 5**).

No	Cultivar and selections	Scientific Name	Origin	Type of astringency
1	Shakoku	*Diospyros kaki* L.	France	PCA
2	*Diospyros lotus*	*Diospyros lotus*	France	PCA
3	*Diospyros virginiana*	*Diospyros virginiana*	Israel	PCA
4	Seedless Mardan	*Diospyros kaki* L.	Pakistan	PCA
5	Yesil Hurma	*Diospyros oleifera*	Selection from Adana-ME-Turkey	PCA
6	07 TH 13	*Diospyros kaki* L.	Selection from Antalya-ME-Turkey	PCA
7	07 TH 14	*Diospyros kaki* L.	Selection from Antalya-ME-Turkey	PVA
8	07 TH 17	*Diospyros kaki* L.	Selection from Antalya-ME-Turkey	PVA
9	07 TH 18	*Diospyros kaki* L.	Selection from Antalya-ME-Turkey	PVA
10	31 TH 01	*Diospyros kaki* L.	Selection from Hatay-ME-Turkey	PVA
11	31 TH 03	*Diospyros kaki* L.	Selection from Hatay-ME-Turkey	PCA
12	55 TH 05	*Diospyros kaki* L.	Selection from Samsun-BS-Turkey	PVA
13	Fatsa-1	*Diospyros kaki* L.	Selection from Ordu-BS-Turkey	PVA
14	Sarı Yenen	*Diospyros kaki* L.	Selection from Istanbul-MR-Turkey	PCA
15	Cekirdekli	*Diospyros kaki* L.	Selection from Adana-ME-Turkey	PVA
16	Saijo	*Diospyros kaki* L.	Israel	PCA
17	Hachiya	*Diospyros kaki* L.	Italy	PCA
18	Guilbecky	*Diospyros kaki* L.	Italy	PCA
19	BST-29	*Diospyros kaki* L.	Italy	PCA
20	Fennio	*Diospyros kaki* L.	Italy	PCA
21	Lycopersicon	*Diospyros kaki* L.	Italy	PCA
22	Farmacista Honorati	*Diospyros kaki* L.	Italy	PCA
23	Fujiwara O'Gosho	*Diospyros kaki* L.	USA	PCNA
24	Triumph	*Diospyros kaki* L.	Israel	PCA
25	Vainiglia	*Diospyros kaki* L.	Pakistan	PVNA
26	Aman Kaki-1	*Diospyros kaki* L.	Pakistan	PVNA
27	Sirin Hurma	*Diospyros kaki* L.	Iran	PVA
28	Nishimura wase	*Diospyros kaki* L.	Italy	PVA
29	Mikatani O'Gosho	*Diospyros kaki* L.	Italy	PVNA
30	Mandarino	*Diospyros kaki* L.	Italy	PVNA
31	Bruniquel	*Diospyros kaki* L.	Italy	PVNA
32	Aman Kaki-2	*Diospyros kaki* L.	Italy	PVNA
33	Koshu Hyakume	*Diospyros kaki* L.	Japan	PVA
34	Mizushima O'Gosho	*Diospyros kaki* L.	Italy	PVNA
35	Chienting	*Diospyros kaki* L.	USA	PVA

No	Cultivar and selections	Scientific Name	Origin	Type of astringency
36	Jiro C – 24,276	*Diospyros kaki* L.	Italy	PCNA
37	Kawabata O'Gosho	*Diospyros kaki* L.	Italy	PCNA
38	Giant Fuyu	*Diospyros kaki* L.	Israel	PCNA
39	Tipo Kaki	*Diospyros kaki* L.	Pakistan	PVNA
40	Shogatsu	*Diospyros kaki* L.	Italy	PVNA
41	Giboshi	*Diospyros kaki* L.	Italy	PVNA
42	Thiene	*Diospyros kaki* L.	Italy	PVNA
43	Moro	*Diospyros kaki* L.	Italy	PVNA
44	Brazzale	*Diospyros kaki* L.	Italy	PVNA
45	Kirakaki	*Diospyros kaki* L.	Italy	PVNA
46	Akouman Kaki	*Diospyros kaki* L.	Italy	PVNA
47	Kurokuma	*Diospyros kaki* L.	Italy	PVNA
48	Hyakume	*Diospyros kaki* L.	Italy	PVNA

Table 5. Origins of persimmon accessions.

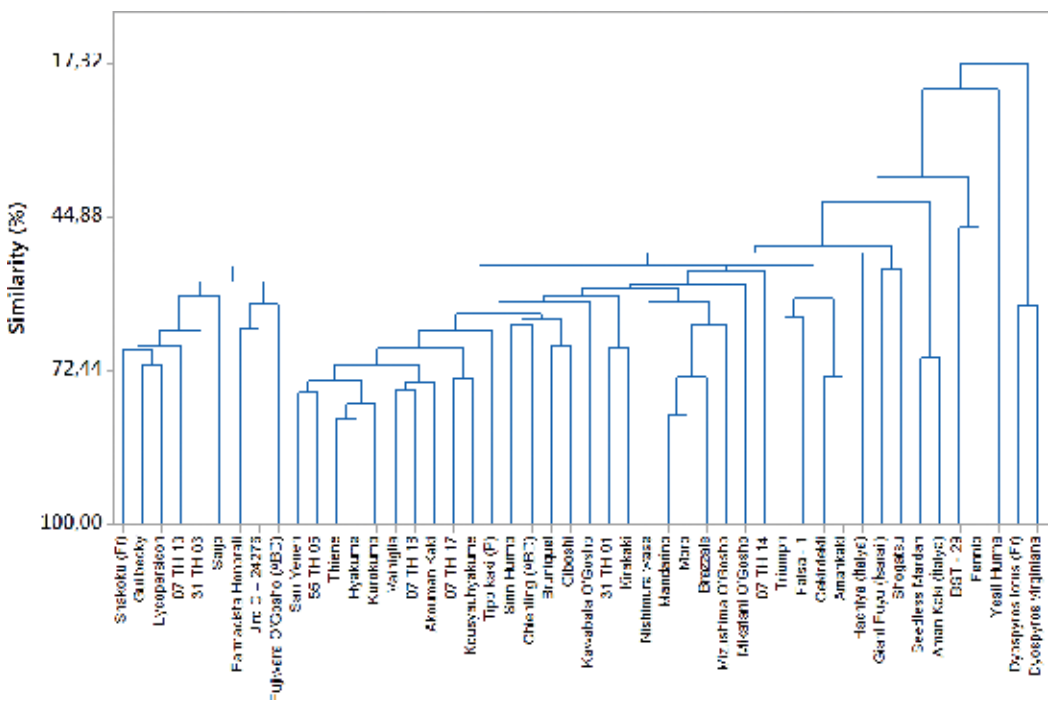

Figure 3. Dendrogram of persimmon accessions collected from Turkey obtained from cluster analysis of 59 agro-morphological traits using average method.

From them, 9 traits were related with plant growth, 5 with leaves, 7 with flowers, 32 with fruits and 6 with seeds. As a result of the morphological characterization, persimmon varieties and types were classified by PCA, PVA, PCNA and PVNA. Besides, data obtained by characterization were subjected to similarity coefficient, principal components and cluster analyses to study phenotypic relationships among these genotypes. As a result of their study, the 12 factor scores represented 74.75% of the total multivariate variation, and cluster analysis indicated that the similarity index of the population consisting of the investigated genotypes ranged between 81.09 and 17.32% (**Figure 3**).

7. Persimmon breeding and genetic improvement

Breeding aims for persimmon emphasized on enhanced fruit quality such as fruit weight, shape, color, soluble solids content, fruit cracking, fruit ripening time, high productivity, long shelf life, parthenocarpy and sex expression. Selection breeding is the most common breeding technique in persimmon due to the fact that persimmon breeding is mainly hindered by its high ploidy level and by its complex sex expression [42]. Because somatic and bud sport mutations affect the fruit traits, new lines of persimmon are frequently improved by bud mutations [43, 44]. Also, there are many seed propagated populations in persimmon growing countries of the world especially in native Asian countries. Many established cultivars are chance seedlings selected by growers or researchers in Japan, China and Korea. There are also many selections from introduced genotypes or seedling populations in the USA, Israel, India, Australia, New Zealand, Taiwan, Malaysia and some other countries. 'Kaki Tipo' in Italy, 'Lama Forte' in Brazil, 'Triumph' in Israel and 'Rojo Brillante' in Spain are cultivars that developed from bud sports [45]. 'Fuyu', 'Hachiya', 'Hiratatenashi', 'Izu', 'Jiro' and 'Saijo' cultivars were selected from shoot of bud sports in Japan and these selected cultivars are extensively growing all over the world. Early ripening bud sports of 'Fuyu' (Matsumotowase-Fuyu) and 'Jiro' (Maekawa-Jiro) were also found in a farmer's orchard. Early ripening Matsumotowase-Fuyu showed fruit cracking tendency as 'Fuyu'. Recently, a small fruit mutant, 'Totsutanenashi' (TTN), was discovered in Japan as a bud sport mutant of the leading cultivar Hiratanenashi (HTN) [44]. Following cultivars were also obtained by bud sports in Japan: 'Uenishiwase' (PCNA) and 'Kyi-joh' (PCNA) are bud mutation of 'Matsumotowase-Fuyu'; 'Sunami' (PCNA) and 'Tanbawase-Fuyu' (PCNA) are bud mutation of 'Fuyu'; 'Aisyuhou' (PCNA) is bud mutation of 'Maekawa-Jiro'; 'Tonewase' (PVA), 'Ohtanenashi' (PVA) and 'Kohshimaru' (PVA) are bud mutation of 'Hiratanenashi' [42]. 'Nantongxiaofangshi' variety having dwarfness character is a persimmon that has been found in Nantong. 'Nantong small persimmon' (*D. kaki* Linn. cv. Nantongxiaofangshi) is a rare and dwarf variety of persimmon found in 1982 in Jiangsu Province Nantong City Fruit resource survey.

The height of the adult tree is only about 2 m, which is approximately equal to 60% that of the standard type growing under the same conditions [46]. 'Hasshu' persimmon (*D. kaki* Thunb.) is a dwarf cultivar originated by a bud sport from the leading persimmon cultivar 'Hiratanenashi' in Hiroshima prefecture, Japan in 2005. Its somatic polyploidy (2n = 120 = 8x) was confirmed by flow cytometric analysis and chromosome observation. Although non-aploid 'Hiratanenashi' and some of its bud sports are known to be seedless, 'Hasshu' produces regular seeds with the ability to germinate [16].

Main objective of persimmon breeding has been to produce commercially attractive cultivars of the PCNA type which can be eaten without any postharvest treatment [46]. Therefore, PCNA fruit are the most desirable for fresh consumption because it is not necessary to apply any postharvest treatment in order to remove the astringency. Hence, the breeding of new PCNA cultivars is the most popular objective in the entire persimmon growing countries.

Although persimmon is produced in Brazil, Israel, Italy, Spain, Azerbaijan, Uzbekistan, New Zealand and Australia, new persimmon cultivars developed by cross breeding have been released only in Japan and Korea [21] and also in these countries persimmon cultivars have been selected over time for commercial production. Hybridization method can also be used in persimmon breeding. In Japan, hybridization method has been used for fruit ripening time, crack-resistance and large fruit size in persimmon.

Persimmon breeding is complex, and results are not always as expected, especially when working on the PCNA group [42]. Researchers found that fruit ripening time is under additive and quantitative control. The tendencies of persimmon fruit are quantitatively inherited traits, the non-cracking cultivars are homozygous, whereas cultivars with cracking are heterozygous. Also, it has been claimed that fruit weight is a quantitative characteristic with high broad-sense heritability [21]. Many crosses performed using large and small fruit size parents indicated that small fruit alleles were dominant to large size alleles [48]. Complete loss of astringency is important for commercial persimmon production. Generally, a little astringency remains in PCNA fruit at maturity in cooler regions, so that they are commercially produced in warm regions. Incomplete loss of astringency results from not only environmental factors but also genetic factors [21]. According to the criteria established for persimmon cultivars, persimmon can be categorized into two major groups, PCNA type consisting of two subcategories, Chinese PCNA (CPCNA) and Japanese PCNA (J-PCNA). The second group is non-PCNA type consisting of three subcategories: PCA, PVNA and PVA [49]. Japanese PCNA cultivars are based on a recessive character and their genetic resources are very few. Repeated crossings within the narrow gene pool cause inbreeding depression, which hinders tree vigor, fruit yield and size. Therefore, studies have been ongoing to obtain new cultivars through the backcross (PCNA × non-PCNA) × PCNA since 1990. In 2007, 'Taiten' and 'Taigetsu', which are PVA cultivars, were derived from the cross of 'Kurokuma' (a local PVNA cultivar in Japan) × PCNA cultivar 'Taishu'. Parthenocarpy in 'Taigetsu' is high [19]. The trait of natural astringency loss is dominant and controlled by the single locus *CPCNA* in Chinese PCNA persimmon [50]. At the end of the hybridization studies in Japan, PCNA cultivars 'Shinshuu', 'Soshu', 'Kanshu' and 'Kishu' were released as early ripening cultivars while 'Suruga', 'Youhou', 'Taishuu' and 'Yubeni' were released as medium to late ripening cultivars [21]. Other persimmon hybrids are 'Fuyuhana' and 'Ito'. 'Fuyuhana' were developed from a 'Fuyu' × 'Hanogosho' cross as an alternative to 'Fuyu' and 'Jiro'. Ito is another hybrid obtained by crossing 'Fuyu' × 'Oku-Ogosho' [51].

In persimmon, different genus can pollinate each other. Native American persimmon (*D. virgininiana*) and Japanese persimmon (*D. kaki*) hybridization would set a goal of stabilizing and improving the variable flavors and cold hardiness of the native American persimmon. *D. kaki* and *D. virginiana* are apparently cross-incompatible; however a hybrid 'Rossiyanka' has been developed through embryo culture technique [51]. Rossiyanka is cold hardy, nearly seedless and it is smooth textured with Asian persimmon flavor [52]. Nikita's gift hybrid persimmon is unique hybrid of Asian and American persimmon; the fruit is sweet and flavorful [53].

In persimmon breeding programs, mutation breeding technique has also used. The main objectives of persimmon mutation breeding were focused on obtaining new cultivars with the positive agronomic features but with more diversity in ripening date, astringency and fruit characteristics from the PCNA types. However, obtaining PCNA type varieties is difficult due to the dominant inheritance of astringency, the limited number of cultivars which bear male flowers and the hexaploid inheritance of basic persimmon cultivars. Therefore, the PCNA type cultivars have low genetic diversity and crossing among these generally result in negative effects of inbreeding. Mutation breeding has been used as an alternative method for generating diversity in persimmon [54]. Some researchers studied to determine which gamma ray doses can be used in persimmon. Ray [51] claimed that 5–10 kR gamma doses obtained widest range of viability on cuttings, seeds and pollen of persimmon. In Spain, shoot buds of the persimmon 'Rojo Brillante' were subjected to various doses of gamma rays, 15 and 20 gray from a ^{60}Co source. In this study, Naval et al. [55] found that the most favorable gamma irradiation dose combining survival and mutation induction was 20 gray. Two new varieties with similar fruit quality to 'Rojo Brillante', that allow to enlarge the persimmon harvest season in Spain, were selected [56].

8. Biotechnology and genomics

Biotechnology refers to the use of living organisms or their components to provide useful products in its broadest sense. Using biotechnology in plant breeding has become the most attractive method due to increasing knowledge in plant biotechnology and genomics. Improvements in the field of genomics have resulted in the development of huge quantities of useful new knowledge that greatly assists scientific plant breeding. Also, improvements in biotechnological techniques like plant tissue culture provided new methods for rapid production of high-quality, disease-free and true to type planting material.

In persimmon, biotechnological advances and molecular biology have been used for the classification of *Diospyros* species, *in vitro* propagation, regeneration from callus, root, protoplast and endosperm, ploidy manipulations, agrobacterium-mediated genetic transformation and marker-assisted selection. Molecular markers have been widely used for investigating the genetic relationships among persimmon genotypes. Akbulut et al. [57] compared persimmon genotypes by using random amplified polymorphic DNA (RAPD) and fatty acid methyl esters (FAME) data. The results showed that RAPD analyses could differentiate the relationship of persimmon (*D. kaki* Thunb.) genotypes used in their study. The authors suggested that more cultivars were needed as plant materials in terms of determining the degree of relationships of RAPD and FAME data which could help delimiting taxonomic classes within persimmon. Raddová et al. [58] indicated that RAPD and inter-primer binding site (i-PBS) were reliable enough to detect differences between the genetically close cultivars of persimmon. In addition, Badenes et al. [45] studied the genetic diversity of introduced and local Spanish persimmon cultivars as revealed by RAPD markers. The authors suggested that a correct identification of germplasm material from persimmon collections should be the first step in projects related to breeding or management of cultivar aimed at improving the crop. They also indicated that RAPD technology is adequate for fingerprinting persimmon. Yonemori et al. [59] studied the relationship between the European persimmon (*D. kaki* Thunb.) cultivars and Asian cultivars using AFLPs. The authors indicated

that the placement of several Japanese cultivars within the European cultivar group suggests that European cultivars were developed from Japanese germplasm relatively recent and differences among cultivars are much greater than differences among cultivar groups regarding AFLP markers. In addition, Guo and Luo [60] indicated that SSR markers are a valuable tool for the estimation of genetic diversity and divergence in *Diospyros*.

The main *in vitro* tissue culture techniques developed for persimmon deal with direct regeneration (from dormant buds and root tips) and indirect regeneration through callus from dormant buds, apexes and leaves. Kochanová et al. [61] indicated that in the genus *Diospyros* L., biotechnological researches focused on quality improvement and preservation of the cultivars that has been economically cultivated. The authors also remarked that the genetic variability had been lost as only those limited cultivars that are popular among growers are grown. In recent years, studies were conducted on *in vitro* micro-propagation of persimmon [62], especially on Jiro [63] and Rojo Brillante [64]. Choi et al. [65] recorded an efficient and simple plant regeneration via organogenesis from leaf segment cultures of persimmon (*D. kaki* Thunb.). The authors indicated that the frequencies of adventitious shoot regeneration by 'Nishimurawase' and 'Fuyu' reached up to 100% and the regenerated shoots rooted successfully with over 80% efficiency. Yokoyama et al. [66] suggested that the meristematic nodule is a promising material for propagation and long-term conservation of 'Fuyu' variety. Naval et al. [67] recorded a protocol for plant regeneration of *D. kaki* Thunb. cv. 'Rojo Brillante' via organogenesis from leaf explants by using combined phytohormones and dosages. In addition, Naval et al. [67] studied somaclonal variation of 'Rojo Brillante' as a breeding tool by using various combinations of cytokinin (Z or BA) with different auxins (IAA or NAA). Furthermore, Palla et al. [68] studied *in vitro* culture and rooting of *D. virginiana* L. from nodal root explants by using several phytohormones and culture media. The authors indicated that the presence of auxins was not essential but slightly accelerated the organogenic callus formation and organogenesis. Cryopreservation is recognized as having the distinctive advantage of allowing long-term conservation with minimum space and maintenance [69]. Matsumoto et al. [70] studied cryopreservation of persimmon (*D. kaki* Thunb.) by vitrification of dormant shoot tips. The authors recorded that using dormant shoot tips was promising as a routine method for the cryopreservation of *Diospyros* germplasm.

After the great progress in *in vitro* regeneration of plants from protoplasts, several researches focused on plant somatic hybridization which allows combining protoplasts from different cultivars, species or genera for variety improvement [71]. Tao et al. [72] reported plant regeneration from callus protoplast of *D. kaki*. They used callus as the protoplast source derived from leaf primordia excised from dormant winter buds of adult Japanese persimmon (*D. kaki* L. cv. Jiro) for plant regeneration. Tamura et al. [73] studied protoplast culture and plant regeneration of *D. kaki* L. and reported that plantlets could be obtained from the protoplast-derived calli. Tamura et al. [74] indicated that somatic hybrids of Japanese persimmon (*D. kaki* L.) were obtained by electrofusion of protoplasts. Callus protoplasts of Jiro and Suruga were fused electrically and cultured in modified KM8p medium using agarose-bead culture. The authors recorded that the fused products had the dodecaploid chromosome number of around 2n = 180, which is twice the number of parental plants (2n = 90x = 15). In addition, Tamura et al. [75] recorded interspecific somatic hybrids between *D. glandulosa* (2n = 2x = 30) and *D. kaki* cv. Jiro (2n = 6x = 90) produced by electrofusion of protoplasts. Colchicine treatment of actively dividing cells can induce chromosome doubling and has been used to make plants with

doubled chromosome number. Colchicine treatment to a protoplast at the very beginning of its division could be one method to overcome the problem because plants can be regenerated from a single cell with doubled chromosome number. Tamura et al. [74] reported production of dodecaploid plants of Japanese persimmon by colchicine treatment of protoplasts.

Improved genomic research and resources, in recent years, have resulted in the development of screening tools via marker-assisted selection (MAS). Using MAS has led to more efficient selections and has increased the efficiency in persimmon breeding programs hastening the release of new cultivar. In order to obtain PCNA offspring in breeding programs, the parental materials considered for choosing the cross combinations have to be PCNA type regarding the inheritance of astringency. However, repeated crosses among PCNA cultivars/selections has led to inbreeding depression for tree vigor, productivity and fruit weight [36]. In these situations, marker-assisted selection should be developed for selecting PCNA offspring efficiently. Recently, Kanzaki et al. [47] have developed molecular markers associated with the trait of natural astringency loss in persimmon fruit and the markers are practically useful in persimmon breeding programs. In addition, Mitani et al. [76] studied if the SCAR markers could reliably distinguish PCNA and non-PCNA genotypes in a large number of offspring derived from backcross between 'Taigetsu' and PCNA 'Kanshu'. The authors indicated that PCNA offspring can be selected by two PCR primers in the progeny derived from 'Taigetsu' × 'Kanshu'. Yonemori et al. [77] reported molecular marker for selecting PCNA type persimmon progenies at the juvenile stage. Yonemori et al. [77] constructed a reliable PCR marker for selecting PCNA type offspring among breeding population of persimmon. In addition Kanzaki et al. [47] and Mitani et al. [76] reported that SCAR markers can practically be used in application of marker-assisted selection in persimmon breeding.

Genetic transformation is also an alternative technique for persimmon genetic improvement. Transgenic persimmon cultivars thus produced have potential for commercial success and grower acceptance because the unique genetic constitution of the cultivars has not been disturbed. Tao et al. [78] reported genetic transformation of persimmon by A*agrobacterium rhizogenes*. Phenotypic alterations such as dwarfness and decrease in rooting ability were observed in the transformants. In addition, Gao et al. [79] transformed 'Jiro' persimmon with Arabidopsis FT gene (*AtFT*) and *PmTFL1* gene, a *Prunus mume* ortholog of Arabidopsis *TFL1* gene. The authors indicated that the PmTFL1 transgenic *in vitro* shoots did not show a different appearance compared with non-transformed 'Jiro' shoots, however, the AtFT transgenic shoots indicated a 'bushy' phenotype having the short internodes.

9. Conclusions

Persimmon can adapt to a wide range of climatic conditions. Production in many countries having subtropical and tropical climates satisfies domestic demand and creates new export opportunities. Increasing the world persimmon production has been very successful since 1995. Recently, the applications of the technique for removing astringency without losing fruit firmness have been significantly promoted to increase the production. It is expected that the production will significantly increase over the next few decades.

Selection breeding is the most common breeding technique in persimmon because persimmon breeding is mainly hindered by its high ploidy and by its complex sex expression. Fuyu, Hachiya, Hiratatenashi, Izu, Jiro and Saijo cultivars which are extensively growing all over the world were selected from shoots of bud sports in Japan. We should continue the screening of plants coming from spontaneous mutations. Hybridization method can also be used in persimmon breeding. Hybridization studies among *D. kaki* in Japan have led to the release of a lot of PCNA cultivars which ripen at different times. There is also a unique hybrid of Asian and American persimmon. In Spain, studies on induced mutation have also led to the development of new cultivars.

There are a number of collections including many accessions in institutions of the various persimmon growing countries. The morphological and molecular characterization of all the persimmon accessions needs to be achieved. The information developed from this will be highly beneficial for screening against biotic and abiotic stress factors. Genomics and transcriptomic resources need to be developed for persimmon. It will also lead to the development of new and improved cultivars of persimmon.

Author details

Turgut Yesiloglu*, Berken Cimen, Meral Incesu and Bilge Yilmaz

*Address all correspondence to: tyesil@cu.edu.tr

Faculty of Agriculture, Department of Horticulture, Cukurova University, Turkey

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Chapter 3

Peach Breeding Studies in Turkey and the Evaluation of Peach and Nectarine Hybrids

Ayzin Baykam Kuden, Songul Comlekcioglu,
Kadir Sarier, Burhanettin Imrak and Ali Kuden

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73440

Abstract

Peach (*Prunus persica* [L.] Batsch) is widely cultivated due to its easy adaptability to different ecological conditions, early fruit set and a long period of harvest. Peach cultivation extends along 30–45° North and South parallels of latitude. Around 21,638,953 tons of peaches are produced in an area of 1538,174 ha across the world. Turkey ranks sixth with a production of 637,573 tons/year in 29,092 ha area. Early fruiting habit and correlative characteristics of peaches encouraged fruit breeders to study on this fruit species. The main aims of the study are new cultivar or rootstock breeding, resistance to diseases, late ripening season, fruit quality improvement, fruit shape changes, new tree shapes, and low chilling cultivars. Breeding studies have been carried out at the Department of Horticulture at the University of Cukurova since 1990s. In these, peach and nectarine breeding programs with different aims such as late ripening, quality improvement, Sharka resistance, and low chilling cultivars were studied. In this chapter, some of the results on late ripening peach and nectarine breeding program have also been presented.

Keywords: molecular markers, embryo rescue, low and high chilling cultivars, Sharka resistance, fruit quality characteristics

1. Introduction

Iwata et al. [1] stated that in genomic analysis technologies, many new advancements promote the efficiency of plant breeding. They also determined that genome-wide association studies (GWAS) and genomic selection (GS) are very helpful, especially in various fruit tree breeding research programs. Breeding of fruit crops is not so easy, as it takes a very long time to get the quality fruits with good size of a tree, to overcome the juvenility period as well as to obtain

desired fruits according to the aim of the breeding program [1]. Whole-genome sequences have been recently released for apple, peach, strawberry, Japanese apricot, and Chinese and European pear. After these developments, in breeding programs, marker-assisted selection has been used in wide genomics studies. These studies have caused to develop genome scale SNPs and SSR markers and to place reference linkage maps in Rosacea family to allow the identification of evolutionary relationships, which can be found in Genome Database in Rosaceae website. Yamamoto and Terakami [2] reviewed the recent advances in genomic studies and their practical applications for Rosaceae fruit trees, particularly in pear, apple, peach, and cherry.

Peach (*Prunus persica* [L.] Batsch) and nectarine (*P. persica* var. nectarine Maxim) belong to the *Rosaceae* family. Peach is widely cultivated due to its easy adaptability to different ecological conditions, early fruit set and a long period of harvest. Peach cultivation extends along 30–45° north and south parallels of latitude. At higher elevations, low winter temperatures and late spring frosts are limiting factors for peaches [3]. Around 21,638,953 tons of peaches are produced in an area of 1,538,174 ha across the world. Turkey ranks number 6, with a production of 637,573 tons/year in 29,092 ha area, following China (11,924,085 tons), Italy (1,401,795 tons), Spain (1,329,800 tons), the USA (964,890 tons), and Greece (666,200 tons) [4].

Fruit breeders preferred to work on peach breeding for its early fruiting habit and correlative characteristics [5]. In cultivar or rootstock breeding programs, disease tolerant genotypes, early or late ripening habit, improvement of fruit quality characteristics such as shape, aroma, flesh firmness, especially low chilling requirements and to improve tree shape were aimed [6]. Important characteristics on peach breeding have already been determined to be; white fruit flesh is dominant to yellow fruit flesh; pubescence to smooth skin; soft fruit flesh to firm and freestone to clingstone. In crossbreeding of peaches and nectarines, since pubescence characteristics are controlled by single gene, generally heterozygote variation of 3:1 is seen. In addition, several correlations between fruit flesh color and receptacle and the color of leaves are seen. These correlative characteristics reveal that early selection is available in peach breeding [7, 8].

Classical plant breeding depends on the phenotypic selection of superior genotypes obtained as a result of crossbreeding. However, genotype × environment interaction causes time consumption and is quite difficult. Moreover, phenotypic selection is expensive and most of the time is not feasible for some characteristics like tolerance to abiotic stress conditions. Marker-assisted selection is an approach developed as an alternative to these problems faced in classical plant breeding [9]. Usually, fruit demand of the consumers in peach is large weight and big-sized fruits, which are quantitative characters (QTLs) affected by several genes and various environmental conditions. Linge et al. [10] carried out an experiment on these characteristics and determined a genetic map of an F2 progeny with 117 individuals of PI91459 ('NJ Weeping') 9 'Bounty' with SNP markers. The fruit quality characters such as fruit weight, height, width, and depth of the progeny and parents were determined in 2011 and in 2012 and were compared with SNP markers. They found a positive correlation between characteristics of fruit weight and characteristics of fruit size. They also constructed a SNP map obtained from 1148 markers distributed across more than eight linkage groups. With this study, they identified 28 QTLs for these characters in which 11 of them were stable in both2011 and 2012 [10].

Various regions of Turkey are suitable to grow peaches under different ecological conditions. The Marmara region can grow high chilling requiring peaches, but the ripening time of the latest peach cultivars is about mid-September. A peach breeding study was carried out by Eroglu [11] in this important peach growing area. In this project, five foreign cultivars (Rio Oso Gem, Fortuna, Monroe, Jungerman and Vivian) and four local types (Bayramiç Tüysüzü, Alyanak Hulu, Sarı Papa and Takunyacı I) were hybridized to obtain fresh market peaches and two foreign and one local type peaches were hybridized for processing peaches. Eroglu et al. [12] stated the fruit quality performances of 121 peach genotypes for fresh market and 35 genotypes for processing. Adana province in Cukurova plain at the Mediterranean region has a subtropical climate. It is located very close to the Mediterranean Sea, thus this condition is suitable to grow only low or midseason chilling requiring peach cultivars. Within 1 h distance to this area, in Taurus Mountains (1100 m elevation) region, high chilling requiring cultivars could be grown efficiently [5].

a. The first peach breeding study in Turkey was conducted by Tanriver and Kuden [13] on 'Ustun', a very late ripening (in mid-October) peach cultivar from the beginning of the 1990s. This study was carried out for [1] breeding of early cultivars for subtropical regions and [2] breeding of late ripening cultivars for cold regions, during 1995–1999. In the breeding experiments, carried out in Adana for early peach cultivars, Springtime, Suncrest, Flavorcrest and Redcap peach cultivars were used. Embryo rescue method was used with the combinations, where Springtime was used as a mother parent. As a result, embryo size was found to be important for the success of embryo rescue, and early cultivars should be used as pollinators. In the breeding studies to obtain late ripening peach cultivars, 'Monroe', 'Rio-Oso-Gem' and 'Ustun' were crossed reciprocally, while 'J.H. Hale' (pollen sterile) was used only as a mother plant at Pozantı Agricultural Research Center of Cukurova University. Seeds from crosses were grown in the orchards, of the Horticultural Department in Adana, under subtropical conditions. Morphological, pomological and isozyme analysis were also carried out in Adana [14].

Among the peach hybrids, especially Rio-Oso-Gem × Ustun combination yielded very good results, and six hybrids of this combination were placed among 20 promising candidate cultivars. As a result of this study, several high-quality, very late ripening hybrids were identified [15]. The best results for late ripening, yield and fruit quality characteristics were obtained from the hybrids of Rio-Oso-Gem × Ustun combination, Nos. 24 and 19. These were followed by Ustun × Monroe 14, J.H. Hale × Rio-Oso-Gem 14, Rio-Oso-Gem × Ustun 21, and Independence open pollinated hybrid No. 8. The promising genotypes were planted at the orchards of Pozanti Agricultural Research and Application Center to see the real performances of these high chilling requiring peach and nectarine genotypes. According to the observations among the hybrids, some of them were found to be resistant to *Taphrina deformans*. The band profiles of 12 enzyme systems of parent cultivars were investigated and polymorphism obtained in 7 enzyme systems (MDH, PRX, ADH, AMY, IDH, PEP and ACP). In 31 hybrids, polymorphism in enzyme systems was found to be suitable to Mendel Segregation rates. As a result of this experiment, two very late ripening peach cultivars from Rio Oso Gem × Ustun combination and two peach cultivars from J.H. Hale open pollination were registered and patented. All these new cultivars are of good quality and high chilling requiring cultivars.

b. The aim of the second peach breeding program was to improve some quality characteristics of Ustun peach to obtain high chilling, late ripening, good quality and good yielding peach, as well as nectarine cultivars. In this study, the pollen of Ustun cultivar was crossed with Venus and Stark Red Gold nectarines [16]. By crossing Venus and Stark Red Gold nectarines, with Ustun peach cultivar, 61 genotypes from VxU combination and 115 genotypes from SRGxU combination were obtained. A total of 176 genotypes were investigated for their morphological as well as phenological characteristics and were analyzed pomologically. Also, some pomological characters were compared by BPPCT009, MA014, MA040, and STS-OPAG8 SSR primer pairs to investigate the effectiveness of the marker-assisted selection in F_1 genotypes.

In the weighted ranking method, VxU-55, VxU-41, VxU-34, VxU-14, VxU-1, VxU-13, VxU-24, VxU-26 peach genotypes and VxU-31, VxU-42, VxU-53, VxU-15 nectarine genotypes in VxU combination gave the highest points. In SRGxU combination, SRGxU-101, SRGxU-28, SRGxU-88, SRGxU-84, SRGxU-36, SRGxU-57, SRGxU-23, SRGxU-92, SRGxU-93 peach, and SRGxU-5 nectarine genotypes gave the highest points for the same scaling method.

No gene amplification was obtained from the PCR reactions among VxU and SRGxU populations analyzed by MA040 and STS-OPAG8 SSR primer pairs. BPPCT009 and MA014SSR primer pairs were also insufficient to determine fruit shape and free stone characteristics for VxU and SRGxU populations. These selected genotypes will eventually be taken under registration very soon.

Detailed information on this research is given below.

c. Another peach and apricot breeding program resistant to Sharka has been completed with a cooperative research between BETA Private Research Center, Malatya Fruit Research Institute and Cukurova University. This study aimed to obtain peach, nectarine, and apricot genotypes resistant to "Sharka" with crossbreeding method. Plum pox virus (PPV) causing Sharka disease is the most important viral agent for stone fruits. This disease is most harmful to apricot, plum, and peach trees. In Valencian Institute of Agricultural Research (IVIA), peach breeding program on apricot and on peach was started in 1993 and 1997, respectively. The aim of the study on apricot was to adapt to Southern Europe and to obtain high-quality cultivars resistant to the Sharka disease. Sharka or PPV is the most important limiting factor in the production of apricot. It was first described in Spain in 1984, causing serious loss of fruit and destruction of more than 1.5 million trees. The disease resistance breeding program is based on the transfer of resistance from local cultivars of Sharka disease to other cultivars by crossbreeding experiments. With 15 genotypes selected in accordance with the program's objectives, they were worked on apricot production areas in Spain. The aim of the peach breeding program was to obtain new cultivars of peach and nectarine that ripen early and provide good quality cultivars found in the market. The main market in Spain is the European countries, just as the big world producers are in the countries. In some parts of Valencia, Murcia, and Andulacia, climatic conditions allow the production of early cultivars that mature so as not to conflict with other European countries. In this study, 15 apricot genotypes and 12 peach genotypes selected for the purposes of the peach breeding program have been defined as resistant to the Sharka disease [17].

The Sharka disease is a race such as PPV-D, PPV-M, and PPV-Rec, and new breeds can be taken out from a combination of these races. PPV-T is a combination of PPV-M and PPV-D races, and it was found to be a race belonging to Turkey. In this study, local apricot cultivars Hacıhaliloglu and Kabaası were crossed with foreign apricot cultivars such as Stark Early Orange, Rojo Pasion, Murciana, and P 1908 (peach clone from *Prunus davidiana*), which are known to be resistant to PPV. For peaches, commercial peach cultivars such as Flored and Carolina were crossed with PPV resistant Stark Early Orange (apricot) and P 1908 peach clone. In the hybridization studies, embryo rescue was performed with the combinations in which Flored peach variety was used as a mother parent, and in other combinations, the seeds were folded. Murashige & Skoog (MS) and Woody Plant Medium (WPM) nutrient media were used for embryo rescue combinations. Molecular studies were used to determine early resistance to Sharka disease in the hybridized individuals. Studies of the PGS1.21, PGS1.24, and ZP002 markers in hybrid subjects revealed the presence of resistance, tolerance, and susceptibility alleles. A total of 365 genotypes from crossing among 12 combinations of apricot and peach were tested with SSR markers (P GS1.21, PGS1.24, and ZP002). Approximately, 138 genotypes were found to be candidates for PPV resistance in future studies [18]. Individuals with endurance allele at the next stage of the study will be protected for other tests and observations to be made by grafting on the clone rootstock.

d. Current peach breeding work is focused on breeding of low chilling, good quality genotypes, and to obtain flat and nonacid peaches (*Prunus persica* var. platycarpa). For this aim, Venus, Maycrest, Early Silver, and Gransun peach and nectarine cultivars and four flat and nonacid local peach genotypes, which were obtained by a selection study (Flat peach genotype 1, Flat peach genotype 2, Flat peach genotype 4, and Flat peach genotype 5), were used as parents. Phenological observations (blistering, green tip, pink tip, balloon, full bloom, and fall of petals) and pomological analyses (fruit weight, fruit height, fruit length, fruit width, total acid, pH, firmness, fruit top color, ground color, fruit pulp color, freestone state, fruit shape, and pubesence) were also studied. Wang et al. [19] stated that the nonacid peaches are preferred in the market, and this trait is usually selected in the commercial breeding programs. A major gene (D/d) located on chromosome 5 of peach has been described for this character, where the nonacid character is determined by the dominant D allele. Flavor analysis in fruit juice samples taken from genotypes will be carried out using the HS-GCMS technique, while sugar and organic acids will be carried out by the HPLC technique. Thus, the aroma, sugar, and organic acid levels of each individual will be determined.

In this chapter, brief information on the results of the second (b) peach breeding experiment at Cukurova University is provided.

2. Material and method

In this breeding study, Ustun peach cultivar was used as a father parent, and Venus and Stark Red Gold nectarine cultivars were used as mother parent. In the trial, 61 F_1 hybrids obtained

from Venus × Ustun crossbreeding and 115 F_1 hybrids obtained from Stark Red Gold × Ustun crossbreeding, and a total of 176 F_1 hybrids were used as plant material.

Ustun peach cultivar has been emerged as a result of bud mutation from peach cultivar of J.H.H. It matures between the first week and the third week of October (1400–1500 altitude). Fruit peel is fairly hairy, red cheek on yellow ground, fruit stalk is short, flesh is hard and yellow color and it has a very good aroma (**Figure 1**).

Venus and Stark Red Gold are kinds of nectarine with a yellow flesh. They have quite good properties in terms of fruit weight, color, and taste. Venus matures 10 days later than the Stark Red Gold.

2.1. Phenological, morphological, and pomological analyses

A total of 176 F_1 hybrids, that is, 61 from Venus × Ustun crosses and 115 from Stark Red Gold × Ustun crosses were compared for their correlative, phenological (pink tip, balloon, first bloom, full bloom, harvest date), morphological (plant length and trunk diameter, tree form, flower property, flower color), and pomological (fruit weight, fruit height, fruit length, fruit width, seed weight, Brix, % acidity, pH, pulp/seed rate, fruit shape, fruit tip, rupture state of the fruit, pubesence, fruit top color, fruit ground color, red blush in flesh, fruit attractiveness, firmness, taste, freestone, and fruit flesh color) characteristics in this experiment [20, 21]. Also, the marker-assisted selection effectiveness of F_1 peach hybrids was determined by two SSR markers.

2.2. Molecular analysis (SSR)

High molecular weight genomic DNA was extracted from the leaf samples of each F_1 hybrids for SSR analysis. SSR analysis was performed in accordance with Aka-Kacar et al. [22]. Two primer pairs (BPPCT009/b, MA014a) were used to generate the SSR genotyping. DNA profiles of parents and F1 hybrids were recorded, and their allelic profiles were compared. Results obtained from the SSR analysis were evaluated with the pomological features of genotypes.

Figure 1. Ustun local peach cultivar.

3. Results and discussion

3.1. The morphological, phenological, and pomological analyses

If we consider the trunk diameter measurements on F_1 hybrids, following results were obtained; In VxU combination, the highest value (87.65 mm) was obtained from VxU-18 genotype, whereas the lowest one (33.54 mm) was obtained from VxU-56 genotype. In SRGxU combination, the highest trunk diameter value (99.44 mm) was determined in SRGxU-9 genotype, while the lowest one (21.92 mm) was determined in SRGxU-100 genotype. The genotypes reached full bloom phase on March 11, the earliest and on April 6, the latest. The hybrids showed the characteristics of rosaceae and campanula flower [16].

To get faster growth and earlier fruit set, F_1 seeds of the genotypes were sown and grown under the subtropical conditions of Adana at the Cukurova University (50 m elevation). The genotypes could not show their best performances in Adana, but they gave us early selection opportunities. Although to the low chilling and sea-level conditions in Adana, some genotypes showed very late ripening habit such as, September 3–18. If we consider Ustun late peach cultivar is ripening at the end of September in Pozanti, some of these genotypes could be ripen later than the parents under Pozanti conditions.

The highest mean fruit weights in VxU combination were obtained from VxU-58 (137.30 g) and VxU-55 (136.95 g) genotypes. In SRGxU combination, the highest fruit weights were obtained from SRGxU-32 (154.30 g) and SRGxU-82 (150.90 g) genotypes.

Brix values among all hybrids (**Table 1**) were the highest in VxU-4 (14.40), SRGxU-110 (13.0), and SRGxU-111 (13.0) genotypes. These results are in accordance with the results of Tanriver and Kuden [13] and Monet [7, 20, 23] who stated that the hybrids showed their best performances (fruit weight, color, Brix value, and especially for their harvesting dates) in the areas convenient for their chilling requirements. Harvesting dates and fruit characteristics of the selected genotypes are given in **Tables 2** and **3**.

The data obtained from the observations and analyzes were weighed out in the individuals in both combinations. According to results, VxU-55, VxU-41, VxU-34, VxU-14, VxU-1, Vx-13, VxU-24, and VxU-26 genotypes gave the best results among the peach genotypes in VxU combination. In the same combination, VxU-31, VxU-42, VxU-53, and VxU-15 were found to be the best nectarine genotypes. In the other combination (SRGxU), the performances were obtained from SRGxU-101, SRGxU-28, SRGxU-88, SRGxU-84, SRGxU-36, SRGxU-57, SRGxU-23, SRGxU-92, and SRGxU-93 peach genotypes and SRGxU-5 nectarine genotype (**Table 1**).

Harvest date	20	Fruit shape	7
Fruit weight	20	Fruit ground color	7
Brix	13	Fruit tip state	5
Attractiveness	12	Red color under skin	3
Freestone state	10	Red color around seed	3

Table 1. Weighted grading types and scores.

Genotypes	Harvest date	Fruit weight (g)	TSS (%)	Acidity
VxU-55	08/14–27	136.95	12	0.73
VxU-41	08/27	173.20	12	0.68
VxU-34	08/26	163.10	15	0.64
VxU-14	08/26	171.28	12	0.68
VxU-1	07/12–21	108.60	12.9	0.75
VxU-13	08/20–27	120.12	13.15	0.82
VxU-24	08/1–13	142.25	11	0.54
VxU-26	08/14–09/18	129.15	12.9	0.66
VxU-31	09/03	79.8	13	0.77
VxU-42	08/02–09/03	108.23	11.5	0.51
VxU-53	06/28	131.74	10	1.03
VxU-15	06/10–29	87.42	10	0.60
SRGxU-101	08/14	161.75	12	0.53
SRGxU-28	07/24	109.8	12	0.50
SRGxU-88	08/14–27	136.95	12	0.48
SRGxU-84	07/04	164.21	12	0.67
SRGxU-36	08/12	152.89	10	0.38
SRGxU-57	07/27	106.6	10.5	0.66
SRGxU-23	07/23–08/27	118.37	11	0.69
SRGxU-92	07/12	110.64	10	0.58
SRGxU-93	07/08	209.17	12	0.46
SRGxU-5	08/24	108.09	11.5	0.49

Table 2. Harvesting dates and the fruit characteristics of the selected genotypes.

3.2. The molecular analysis

A total of 176 F_1 hybrids were examined by using two different SSR primer pairs for early marker-assisted selection. The fruit characteristics of some F_1 hybrids are found to be different as compared to their parents, while some of them were almost found to be the same.

Among the molecular data determined from MA014a primer pair, with pomological analysis of hybrids, more correct results were obtained from MA014a primer pair in the genotypes of SRGxU combination (42.02%) and in the genotypes of VxU combination (85.29%). Also, BPPCT009/b primer pair was used to determine freestone characteristics of the hybrids. SRGxU combination gave 38.35% of correct results, and VxU combination gave 30.50% of

Genotypes	Firmness (kg/cm²)	Freestone	Fruit shape	Pulp color	Pubescence
VxU-55	2.92	Semi freestone	Oval	Yellow	Medium
VxU-41	2.67	Semi freestone	Round	Yellow	Medium
VxU-34	2.19	Cling stone	Round	Yellow	Medium
VxU-14	2.06	Cling stone	Round	Yellow	Medium
VxU-1	3.12	Freestone	Oval	Yellow	Medium
VxU-13	1.88	Cling stone	Oval	Yellow	Medium
VxU-24	2.68	Freestone	Oval	Yellow	Medium
VxU-26	2.9	Semi freestone	Oval	Yellow	Medium
VxU-31	1.5	Freestone	Oval	Yellow	Nectarine
VxU-42	3.10	Cling stone	Oval	Yellow	Nectarine
VxU-53	3.06	Cling stone	Oval	White-Red	Nectarine
VxU-15	2.28	Semi freestone	Round	White-Red	Nectarine
SRGxU-101	2.07	Freestone	Oval	Yellow	Medium
SRGxU-28	3.26	Freestone	Round	Yellow	Medium
SRGxU-88	2.92	Semi freestone	Oval	Yellow	Medium
SRGxU-84	2.65	Cling stone	Oval	Yellow	Medium
SRGxU-36	1.86	Freestone	Round	Yellow	Medium
SRGxU-57	2.75	Freestone	Round	Yellow	Medium
SRGxU-23	2.45	Freestone	Round	Yellow	Medium
SRGxU-92	2.34	Semi freestone	Oval	Yellow	Medium
SRGxU-93	3.47	Cling stone	Round	Yellow	Medium
SRGxU-5	2.75	Freestone	Round	Yellow	Nectarine

Table 3. The fruit characteristics of the selected genotypes.

correct results. As a conclusion, for early selection criteria, SSR primer pairs are good molecular markers to be used for this aim. However, in this research, we could not obtain a very good relationship among our hybrids.

Dirlewanger et al. [24] stated that MA014a SSR primer pair is associated with fruit shape and flat fruit. In our experiments, we found that MA014a primer pair was more suitable to VxU population, but not for SRGxU population. For freestone character, BPPCT009/b SSR primer pair was used (**Figure 2**) to determine the freestone character. This primer was not compatible for SRGxU and VxU F_1 populations. Freestone characters of hybrids and allelic profiles did not match each other properly. The photos of some of the selected genotypes are shown in **Figures 3–16**.

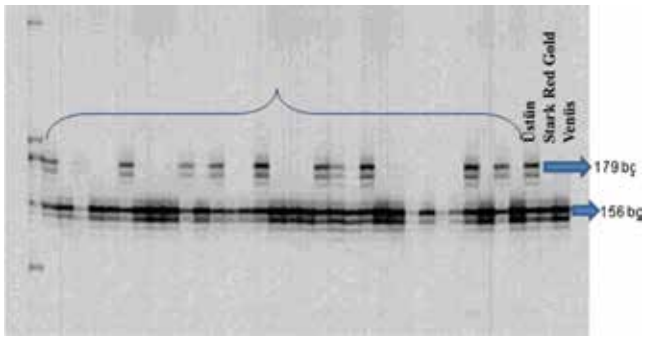

Figure 2. Polyacrylamide gel image of SSR bands of BPPCT009 primer pair with SRGxU population.

Figure 3. Fruits of VxU-55 genotype.

Figure 4. Fruits of VxU-41genotype.

Figure 5. Fruits of VxU-14 genotype.

http://dx.doi.org/10.5772/intechopen.73440

Figure 6. Fruits of VxU-1 genotype.

Figure 7. Fruits of VxU-13 genotype.

Figure 8. Fruits of VxU-24 genotype.

Figure 9. Fruits of VxU-26 genotype.

Figure 10. Fruits of SRGxU-101 genotype.

Figure 11. Fruits of SRGxU-28 genotype.

Figure 12. Fruits of SRGxU-88 genotype.

Figure 13. Fruits of SRGxU-84 genotype.

http://dx.doi.org/10.5772/intechopen.73440

Figure 14. Fruits of VxU-53 genotype.

Figure 15. Fruits of VxU-15 genotype.

Figure 16. Fruits of SRGxU-5 genotype.

4. Conclusion

This peach breeding study was carried out to obtain late ripening, high chilling, good quality peaches, and nectarines suitable for more continental climates. As a result of the experiment, some genotypes were found to be later fruit ripening producers than their parents (September 3–18) under Adana subtropical climatic conditions (23 m elevation). The results showed that these late genotypes could ripen later under the continental climatic conditions. This means that these genotypes could have better performances at Taurus Mountains in Pozanti under higher elevation conditions (1100 m).

One of the parents of these genotypes was Ustun, late peach cultivar, which ripened at the end of September in Pozanti. This observation lead us to think that some of these genotypes ripen on 3rd-18th September. Under Adana subtropical climatic conditions, they could ripen later than their parents in Pozantı (may be at the end of September or at the beginning of October). Thus, this will be a very good opportunity to get very high market prices with these very late season peaches and nectarines.

Considering the fruit quality characteristics of the genotypes, among F1 hybrids, VxU-18 and SRGxU-9 genotypes gave better trunk development than the others. For the fruit weight characteristics, SRGxU-32, SRGxU-82, VxU-58, and VxU-55 gave the biggest fruits among all the genotypes. The highest brix values were obtained from VxU-4, SRGxU-110, and SRGxU-111 genotypes.

In conclusion, the genotypes of VxU-55, VxU-41, VxU-34, VxU-14, VxU-1, Vx-13, VxU-24, VxU-26, SRGxU-101, SRGxU-28, SRGxU-88, SRGxU-84, SRGxU-36, SRGxU-57, SRGxU-23, SRGxU-92 and SRGxU-93 peaches and VxU-31, VxU-42, VxU-53, VxU-15 and SRGxU-5 nectarines were found to be promising ones.

The selected genotypes which were grafted onto GF-677 rootstock were taken to Pozanti Agricultural Research and Application Center at the Taurus Mountains in Pozanti to compare their performances at high chilling area under second selection. Also in the future studies, more locus specific molecular marker systems such as SSRs or SNPs could be used to find out better characterization of the hybrid populations.

Author details

Ayzin Baykam Kuden[1]*, Songul Comlekcioglu[1], Kadir Sarier[2], Burhanettin Imrak[2] and Ali Kuden[1]

*Address all correspondence to: abkuden@gmail.com

1 Department of Horticulture, University of Cukurova, Faculty of Agriculture, Saricam, Adana, Turkey

2 Pozanti Agricultural Research and Application Center, University of Cukurova, Saricam, Adana, Turkey

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Chapter 4

Genetic Apricot Resources and their Utilisation in Breeding

Boris Krška

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77125

Abstract

This chapter outlines the evolution of apricot which took place not only in its original gene centers but also after its domestication in new, secondary areas. During this process, Ice Age, migration of nations as well as the influence of mountains played a significant role in the diversity of this fruit species where many clones of genetically similar cultivars and ecological groups of apricots were formed. The chapter presents the list of donors of main biological and economic properties which are important in breeding to increase the adaptability of the species. The chapter summarizes some of the breeding results and inheritance of characters related to frost hardiness of blossom buds, fruits and plum pox virus (PPV).

Keywords: apricot, *Prunus armeniaca* L., germplasm, inheritance, breeding

1. Introduction

Man has participated in the evolution of cultivated plants by selection as well as by controlled evolution, that is, crossing. In the past, most or all wild fruit trees possessed certain properties that were beneficial and tempting for humans. Plants were also know for their changeability due to the influence of external conditions but also to breeding with related varieties. As a result, even in their wild form, hybrids with complex genetic bases were created. Their seeds gave rise to many distinct types which were preserved and in fruit trees this initiated either accidental or intentionally developed bud mutations which also led to a greater diversification of species. This method of propagation is being used in some areas of Central Asia and China to this day, and, as a result, even within the European group of apricots, we have been

able to select some interesting varieties with higher adaptability to the environment (Sucre de Bohutice, Rosa Early, Holubova, Pourtáleská, Keczkemete Rozsa, Kamenickyi and so on).

In all countries where apricots can be grown, apricots enjoy great attention. With the change of economic factors and growing influence of globalization, further development of apricot-growing activities is determined mainly by the three following factors:

1. Costs are on the increase, mainly the cost of labor which is not offset with corresponding yield per hectare and price, particularly where traditional varieties of apricots are grown.
2. Due to the spread of plum pox virus (PPV)—Sharka and European stone fruit yellows (ESFY) phytoplasma into previously unaffected areas—the yields here are lower and so is the quality of fruits.
3. An unresolved problem in apricot growing persists which causes early death and therefore results in significant economic losses.

The overall trend of world apricot production is rising, currently at approximately 2.2 million tons of apricots a year. From 1950 to 2000 the worldwide production of apricots increased four times. Some major European countries growing apricots include Spain, Italy, France, Greece, Ukraine, Moldova and others. In addition to these main producers, there are also other world producers such as Turkey, Iran, Uzbekistan, Algeria, Pakistan, and Morocco, as shown in **Figure 1** [1].

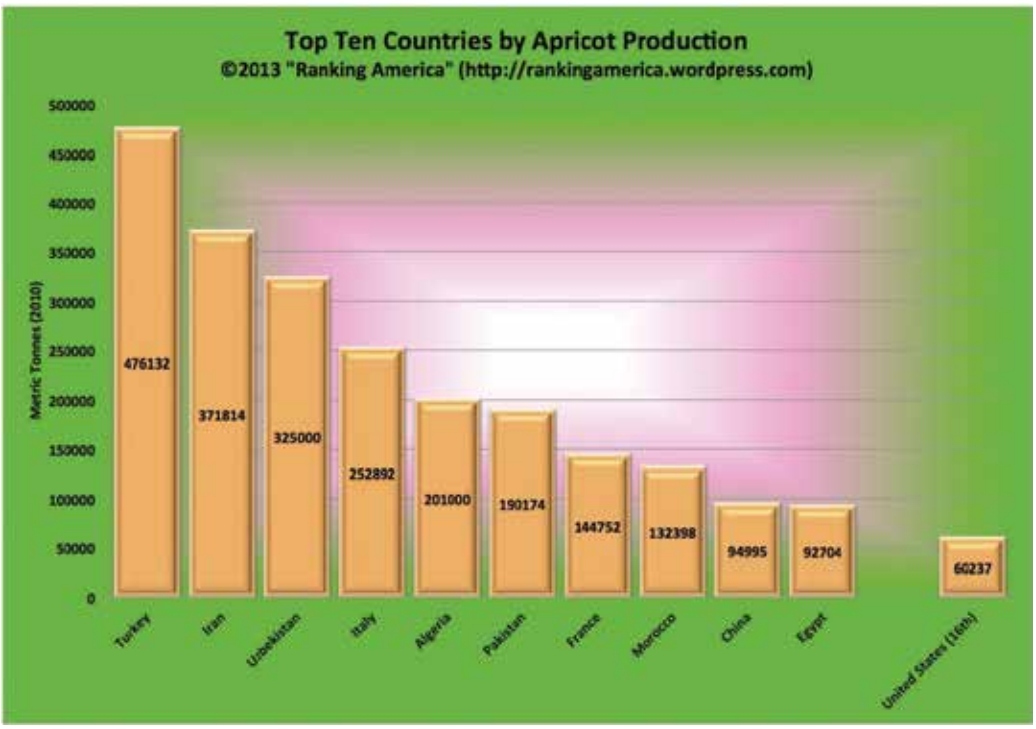

Figure 1. Top ten counties by apricot production.

http://dx.doi.org/10.5772/intechopen.77125

2. Domestication and selection of apricots

2.1. Domestication and genetic diversity of apricots

The origin of all eco-geographic groups of apricot dates to the beginning of the Tertiary Period and is associated with the Northern and Middle China centers, which gave origin to more than 100 species of stone fruit, particularly to cherry, peach and apricot. In the Tertiary Period, apricot trees were found abundantly in extensive mountainous parts of Northern and Middle China, where, because of changing conditions, a process of forming and creation of new ecotypes was developed through natural selection best adapted to the changeability of the environment.

Ice Age, as stated by Kostina [2], played a significant part in the formation of individual apricot varieties mainly on the edges of apricot-growing areas. In the North, frost-resistant varieties were formed with shortest vegetation period, that is, the xerophytic dwarf variety of *Armeniaca sibirica* and its related variety of *A. Davidiana* and a forest tree, *A. mandshurica.* Kostina further suggests that the greatest diversity of varieties and forms can be found from the middle to northern area of the overall species region of China which is represented by *A. vulgaris, A. Davidiana, A. mandshurica* as well as *A. ansu* and *A.mume* varieties. The connection of different areas of several varieties with easy breeding potential facilitates great diversity among both domesticated and wild apricot trees.

In relation to the presence of the only variety *A. vulgaris* in the western part of China (Tian Shan), and, with regard to comparable unity of these apricot forms, we can deduce that this area is of secondary and later origin as a result of migration of Common Apricot from the primary Chinese area westward. At the time, when the climate in these mountains was not so arid and the mountains were covered in woody vegetation, they could provide a sort of bridge between woody flora of China and the eastern part of Tian Shan.

The formation and movement of apricot was probably a lengthy one and commenced with the beginning of human agricultural activities. It was then when humans started to plant first orchards in the mountains by cutting the trees around their settlements, leaving only those who provided edible fruits. This activity had a considerable influence on the diversity of wild apricot trees with juicy and sweet flesh. The earlier natural biodiversity of more abundant varieties represents even today's abundance of apricot varieties in the mountainous parts of Tian Shan and Northeastern China. Subsequent introduction of apricots as a domesticated species means a new era of apricot tree evolution influenced by artificial selection.

Man has influenced the growth of the apricot for a significant period of time. Historical records prove that as early as 4000 B.C., apricot trees accounted for widespread cultivated fruit trees in China. Later, via Central Asia, the apricot spread to Western Asia. It was only at the beginning of our century that the apricot made its way from Armenia to ancient Rome and was called the Armenian apple at the time. Simultaneously, the apricot made its way from China to Central Asia where it was possible for it to be formed autonomously by domestication of local wild apricot trees from Tian Shan. This process of transmittal into areas of wild growing apricots can be observed in many regions of Eastern Kazakhstan and Kyrgyzstan where "mountain" apricots are regarded as the most frost-resistant types and less sensitive than cultivated species [2].

Cultivated types of the basic *A.vulgaris* variety went through significant changes and took on various characters when moving west and south from China to Central Asia. This happened because of many evolutionary factors (external conditions, environment, changeability of inheritance and natural or artificial selection). As the role of artificial selection was of most important significance, these changes were reflected mainly in the quality of fruits (size and taste) but also in some economic traits such as frost resistance, immunity to main fungal diseases and biology of propagation.

The simplicity of generative propagation and a commonly known method of vegetative apricot propagation have played a key role in the intensity of selection and vegetative propagation of most economically valuable differences. The comparison of the current cultivated range of apricot with wild varieties in Western and Central Tian Shan provides evidence of gradually acquired biodiversity of traits in apricots. Primary differences between these two groups are based on the size of the fruit. Wild grown apricots in Tian Shan weigh from 3.0 to 35 g (an average of 8–12 g), domesticated varieties of Central Asia from 5.5 to 55 g (an average of 15–30 g) and Irano-Caucasian and European domesticated varieties weigh from 10 to 165 g (an average of 30–55 g). Other traits include sugar and acidity contents, taste, kernel taste, skin pubescence and stone size [2].

The evolution of cultivated apricots in Europe took on a slightly different direction because of its shorter history in this region. The apricot first arrived from Iran to Ancient Greece and Rome and to southern Europe. A more or less substantial spread of the apricot in Europe was not achieved until the seventeenth century. At the same time, a brief period of growing and limited original material domination of vegetative propagation and a very low degree of seed propagation in the apricot have all led to a lot more limited diversity in European apricots. Direct consumption of apricot fruits initiated the selection and vegetative propagation of those varieties which had been formed as random seedlings in orchards and nurseries. Basic characteristics that presented value in introducing apricots as fruits used in their fresh form were the size of fruits, a comparable low stone-to-flesh ratio, excellent taste, harmonious constitution of sugars and acids, aroma and flesh firmness.

Vavilov highlights the importance of mountains when clarifying the variety of cultivated plants [3, 4]. Vavilov states that apricots are grown in their three centers of origin. Key areas of these centers are Central China (Gansu Province, mountain areas of Northeastern and Central China and Northeastern Tibet) and Central Asia (mountain areas stretching from Northwestern Iran, the Caucasus Region to Central Turkey). Vavilov found that the center of Near East is a secondary center for apricots in terms of originally grown varieties. As these findings on the origin and evolution of growing apricots outline, the importance of mountains when clarifying biodiversity of apricot species and varieties is unquestionable. The connection of wild species and forms, old and formerly grown varieties with mountains is also confirmed when studying the terrain of regions in Central Asia [3] (**Picture 1**).

The apricot spread to Europe from Central Asia, through Iran and into the trans-Caucasus Region and then further West. This movement was conditional to war campaigns and economic and cultural exchange during the arrival of Alexander the Great in the area from Turkistan to the Fergana Valley in the fourth century BC. Another shift of the apricot westward

http://dx.doi.org/10.5772/intechopen.77125

Picture 1. The mountains and valleys of Western Pamir (photo by Saodatkadamova T.M.).

took place in two stages. Apricots were known in Italy and Greece as a result of the Roman-Persian wars in the first century BC. The species of *Armeniaca* suggests that apricots were first brought to Italy and Greece by Armenian traders. This happened much later when the apricot was already grown in other parts of Southern Europe [5].

Because of lack of biological material, the specification of local varieties in North Africa and Spain is not highlighted. Here, apricots were brought mainly by the Arabs from Syria and they kept their Syrian names (mesh mesh and mush mush). For the character of its fruits and biological properties, this group can be assigned to the Irano-Caucasian ecological geographic group. However, these were classified as a North African group adapted to warmer climate [6].

The process of domestication in Mediterranean species results in a loss of diversity which is far greater in fruit species introduced into Mediterranean areas compared to the species native to this region (olive and grape). By comparing genetic diversity among regional apricot gene pools in several Mediterranean areas, the loss of genetic diversity associated with apricot selection and diffusion into the Mediterranean Basin was investigated [7]. Microsatellite markers were able to detect a marked domestication bottleneck in the Mediterranean apricot material. This led to the depiction of a global image of two diffusion routes from the "Irano-Caucasian" gene pool: North Mediterranean and Southwest Mediterranean. It also assessed a significant loss of genetic diversity from the "Irano-Caucasian" gene pool, considered as a secondary center of diversification, to the Northern and Southwestern Mediterranean Basin. A substantial proportion of shared alleles were specifically detected when comparing gene pools from the "North Mediterranean Basin" and "South Mediterranean Basin" to the secondary center of diversification. Based on the three main identified gene pools, we observed a significant and substantial loss of apricot genetic diversity, ranging from about 37 to 49%

from the secondary apricot diversification zone ("Irano-Caucasian") to the Southwestern Mediterranean Basin, depicting a genetic signature of apricot domestication and diffusion into the Mediterranean Basin. Unlike Kostina's assumptions, we proposed an evolutionary scenario in favor of two diffusion routes in Southern Europe and North Africa as revealed by a substantial proportion of shared alleles that were specifically detected along each of the two diffusion routes [8]. This study generated genetic insight that will be useful for management and conservation of Mediterranean apricot germ-plasm as well as genetic selection and breeding programs related to adaptive traits [7].

2.2. Selection of donors of characters related to breeding aims

Breeding programs of all countries with economically significant apricot production are based on the effort of obtaining varieties with wide or varied ecological adaptability regarding their production areas. The efforts are to create varieties that are adaptable to low temperatures, mainly to sudden temperature changes during the post-dormancy period, but also to meet requirements for a small number of chilling units in winter. Since the apricot requires specified eco-climatic conditions as outlined by its phylogenesis, it lacks the ability for wide environmental adaptability as opposed to, for example, the apple variety Golden Delicious or the peach variety Redhaven. The change in the traditional range of apricots is also caused by globalization, methods of sale, requirements of vendors and the fact that in the European market apricots are of increasingly higher importance as a fresh commodity.

Successful introduction of new apricot varieties should focus on main targets which are similar in most growing periods. Generally, it is possible to summarize breeding aims into several objectives:

- Adaptability to both cold and warm areas: To achieve an appropriate level of adaptability, it is important to assure a good degree of frost resistance. Furthermore, it is a requirement for a small or large number of chilling units, long or short dormancy periods, slow development of pollen microsporogenesis during the post-dormancy period, late blooming period, frost hardiness and autogamy.
- Ideotype of fruits: For their utilization, it is important fruits comply with certain criteria, that is, the size and firmness of fruits, attractive appearance, taste, aroma, sugar and acid contents.
- Resistance to diseases and early death: In Europe, for example, the main objective is breeding apricots resistant to Sharka—PPV, European stone fruit yellows (ESFY), brown rot, *Cytospora* and bacteria such as *Pseudonamas* spp. and *Xantonomas* spp. and early decline as a result of pathological factors and environment.

It is possible to say that all the aforementioned breeding aims are of fundamental importance and are pursued by a majority of breeders worldwide. Complimentary breeding objectives include growth control, so called dwarfism or semi-dwarfism, the compatibility of varieties with diverse rootstock, crown habitat and its suitability for different modern breeding shapes and resistance to drought.

Michurin used the seedlings of Mongolian, Siberian and Manchurian apricots as donors for frost hardiness and increased adaptability. Abrikos No. 84, No. 86, No 241, No 246, Mongol, Sacer were utilized as Mongolian apricot seedlings and Lučšij Mičurinskiy and Tovarišč as the seedlings of *P. sibirica*. To increase frost hardiness, it is recommended to breed apricot varieties with varieties that are native to countries further away from the center of cultivated varieties, such as those of Asia Minor, Palestine and Persia [9].

To increase the frost hardiness of flower buds, breeding apricots with *P. salicina* L. while creating several fertile types with transitional traits called "Plumcot" have been suggested. It has been highlighted that the Royal variety can be grown in many regions where other apricot varieties do not bear fruit and may prove to be a valuable material for further selection [10].

Hummel created a list of *Prunus* varieties which are known as frost hardy or grown with minimal damage in zones classified according to frost hardiness from 4b (from −30 to −20°F, which is −34.4 to −28.9°C) to zone 1 (below −50°F, below −45.6°C). For zone 4a varieties such as Anda, Harbin and Labin—all seedlings of *P. mandshurica* L.— and then Manchu and Moongold, Morden 604, Morden 601 and Scout and others have been chosen [11].

The basic requirement for breeding, selection and further introduction of new varieties is knowledge of a wide collection of genotypes, varieties, clones and landraces of all ecological groups of *P. armeniaca* L. This enables breeders to select donors of individual properties which they expect to appear in the progeny's genotype. At Horticulture Faculty in Lednice, studies have been carried out since 1985 on several apricot collections of genotypes, and several years of observations have helped in choosing the most important characters connected with adaptability to environment [12]. The analysis of these characters enables breeders to choose parents—donors of characters and conservation of genetic characters. Genetic improvement of apricots as a species is possible in all characters relating to increase in adaptability. However, it usually takes a longer period of time. The following donors of characters have been chosen.

2.2.1. Frost hardiness of flower buds

In different years and in different collections, the frequency of genotypes having a high level of frost hardiness of flower buds varied from 8.6% to almost 18% of all observed apricot progeny. Genotypes having fruits presenting a good market value and a high frost hardiness had a frequency of occurrence varying from 2 to 48% (**Table 1**). Some of the frost-hardy cultivars are Harlayne, Harval, Leala, Lejuna, Leronda, Leskora, Lenova, Lejara, LE-498, LE-806, NJA 1, Vivagold, Volschebnyi, Vynoslivyi, Yulskyi, Strepet, Harrow Star, NJA 35, NJA 62, NJA 77, NJA 44, Veharda, Vegama, Harcot, Saldcot, Vyndrop, Alfred, Reliable, apricot seedlings of the serious M-LE-1, M-VA-3, M-VA-2, M-VA-1, Dzhankojskiy rannyi, Arzami,Henderson, Morden,Senetate, VS 023/187, Riland, Veecot, Vestar, Lunnik, Harglow, Stark Early Orange, Orange Red, Scout and Lel.

2.2.2. Late termination of dormancy

A higher frequency of phenotypes (16%) was observed with late ending of dormancy character, globally. From the breeding and practical points of view, genotypes presenting a combination

Characters	Categories	No. of individuals	Frequency
Frost hardiness of flower buds		**346**	**1**
	High hardiness	182	0.135
	Hardiness + market value of fruits	56	0.042
Termination of bud dormancy		**82**	**1**
	Late	13	0.159
	Late + market value of fruits	6	0.073
Frost tolerance of juvenile fruits more tolerant		**209**	**1**
	Than control	37	0.177
	More tolerant + market value of fruits	14	0.066
Blooming time		**489**	**1**
	Late	29	0.059
	Late + market-value of fruits	13	0.030
Self-fertility		**124**	**1**
	Self-fertile	44	0.354
	Self-fertile + market value of fruits	31	0.250

Table 1. Frequency of the most important characters of apricots connected to their adaptability [12].

of both characteristics (late dormancy time and fruit quality) are very interesting, but this was the case for only 7% of analyzed progeny (Vestar × Stark Early Orange). Henderson, ChuanZhi Hong, Lebela, LE-498, Oranzevo-krasnyi, Stark Early Orange, Vegama, Veharda, Zard, Vynosliviy, Reliable, NJA2 and Curtis are varieties exhibiting late termination of dormancy.

2.2.3. Frost tolerance of juvenile fruits

Almost 18% of observed genotypes showed a higher frost hardiness in young fruits than the control clone Velkopavlovická LE 12/2 /type Hungarian Best. Other genotypes having pomological characters answering market requirements were altogether 6.7% in the whole collection. For example, cultivars such as Leala, Lemira, Ledana, Leskora, Lejuna, Lefrosta, Neptun, Re Umberto, Henderson, Early Gold, Zard, Oranzevo-krasnyi, Marculešti 17/2, Horákova raná, Fara, Rakovskyi, Bergeron, Reumberto,Marlen, Detskyi, M 146, Neptun, Triumf severa, Patriarca tempráno, M 90B, Baneasa 16/3, Kamenickýi, Selena, Mari de Cenad, NJA 33, Morden 604, Sephora, Bergeval, Big Red, Orange Rubis, Anegat, Primaya and Orangered had juvenile fruits with frost tolerance.

2.2.4. Late blooming

On the contrary, the character of late blooming was observed in the lowest number of phenotypes (0.061). The frequency of individuals presenting both a late blooming and a good market value of fruit appeared to be very rare, only about 0.8% of all evaluated genotypes of apricots (Early Gold, Machova, Marculesti, *P. brigantiaca* L. xOlymp, Re Umberto, Sulmona, Stella, Pozdněkvetoucí, Frostina, Farclo, Fardao, Dolgocvetna, Polyus yuznyi, Zard, Oranzevo-krasnyi, Venus, Tilton, Selena, Badami, Kamenický, Rosa Late, Yulskyi, Ambrosia, Farbaly and Harglow).

2.2.5. High level of self-fertility

High frequency of the requested values of characters was achieved on self-fertility. Of the overall number of self-fertile genotypes, 25% showed significant pomological traits. Examples of such cultivars are Bergeron, Minaret, Vestar, Kostinskyi,Leala, Marlen, Pisana, Kioto and Gergana.

2.2.6. Climatic adaptation

Bergeron, Goldrich, Marculesti, Tilton, Kecskemete Rose, Leala, Lejuna, Leskora, Re Umberto, Rose Early, Vynosliviy, Bergarouge, Harrow Star, Harlayne, Tomcot, Bronzoviy and Kostinskyi show climatic adaptation. Harglow, NS-4 and Strepet also had a higher level of adaptability in the European eco-geographical groups of apricots. It includes some types bearing small fruits which come from a European subgroup as well as species from the groups Rosa Early, Holubova, Pourtal abricose, Sucre de Bohutice, Keczkemete Rozsa, Bergeron, Kamenickiy and other types of natural population.

2.2.7. Field tolerance to European stone fruit yellows (ESFY)

Vestar and Royer were the only two cultivars which were tolerant to European stone fruit yellows.

2.2.8. Tolerance or resistance to plum pox virus (PPV)

Stark Early Orange, Harlayne, Henderson, Orangered, Betinka, Adriana, Candela, Sophia, Veecot, Leronda,Harval, Sundrop and Harcot are some of the varieties resistant to PPV.

2.2.9. Attractiveness of fruits

Harrow Joy, Bergarouge, Betinka, Orangered, Rubista, Pincot, Chuan Zhi Hong, Laycot, Bobcot, Roxana, Neptun, Gergana, Veselka, Gama, Robada, Big Red, Tsunami, Carmen Top, Harrow Star, Flavor Cot, Kioto, Harrow Blush, Harogem, Magic Cot, Cegledi Piroska, Sophia, Bergeval, Sephora,Orange Rubis,Pricia,Bergeval, Swired, Gilgat, Rougemont and Montier are some of the attractive varieties.

2.2.10. Big fruit size

Gergana, Roxana, China, Hargrand, Senetate, Goldrich, Olymp, Gama, Velikiy, Exnerova, Agat, Jumbo Cot, Goldstrike are some of the varieties that have big fruit sizes.

2.2.11. High sugar content

Shalach, Sekerpare, Forum, Lakaniy, Strepet, Nadezda, Olymp, Kabaasi, Hacihaliloglu, Bronzoviy, Abutalibi, Vynosliviy, Hasanbey, Suphani and Isfarah are some of the varieties that have high sugar content.

2.2.12. Excellent taste and apricot aroma

Velkopavlovická, Sucre de Bohutice, Sabinovská, Hungarian Best, Klosterneuburg, Krasnoscokiy, Bronzoviy, Vynosliviy, Bobcot, Harrow Joy, Bergarouge, Hargrand, Skopljanska krupnoplodna, Royer, Paviot, Sucre de Holub, Nancy apricot, Luizet, Polonais, Betinka, Bergeval, Breda and Paviot are some of the varieties that have an excellent taste and aroma.

2.2.13. Extension of the ripening time

In relation to late ripening, many potentially interesting parents are also available. Pisana Bergeron, Tardif de Bordaneil, Tardicot, Anegat, Farbaly, Farclo, Fardao, Kechpsar,Vynoslivyi, Helena de Roussilon, Rosa Late, Keczkemetr rozsa and Borsi-félekései rózsa are some of them.

In relation to early ripening, many potentially interesting parents are also available such as Tomcot, Magic Cot, Wonder Cot, Pricia, Spring Blush, Big Red, Bukuriya, Early Samarkand, Tsunami and Banzai [11].

All the evaluated traits exhibited a different value of variability and frequency enabling the breeders and geneticists to make more informed choices on the selection of donors for a particular character. This variability allows the integration and combination of each single character in the breeding process which will lead to the production of hybrids with a higher level of adaptability and fruit market value [12].

Increased adaptability to environment is also essential for the selection of genotypes with frost-tolerant fruits. Late spring frost which damages small developing fruits after the blooming period is more common in the Mediterranean, but from time to time it can also occur in the Central European climate. As the climate changes (milder winters, early onset of spring), spring frost after the blooming period is more frequent.

A degree of hardiness in fruits of individual varieties is of the greatest difference as conditioned by the development phase and the length and time of the critical period.

Different authors suggest that the temperatures from −0.5 to −1.6°C are dangerous for apricots after the blooming period. Djurič found no relation between the development (size) of fruits and their frost hardiness. He found that varieties with the smallest percentage of frozen fruits (Hindukush, Overnskyi, Zimostojki and Novosadski clone BC-1) had fruits of lower weight than 1 gram; however, varieties Blenril, Nugget, Royal, SEO, Kostjuženskij and NJ 27, NJ 26

and hybrid 252 had heavier fruits than 1 gram and the percentage of frozen fruits were from 69 to 100%. This means that the size of fruits is of lesser significance to sensitivity or hardiness than genetic foundation of a variety character.

Among hardy varieties, Djurič further included Sacharijstyi. Melitopol early, Keč-pšar, RuchiDžuvanon and Zemljaničniy (frost damage to fruits varied from 40.1 to 60%) were categorized as semi-sensitive varieties [13].

In Italy, Bassi et al. [14] evaluated a collection of apricots at a temperature of −5.8°C when in full bloom and for the next four consecutive nights. In 1993, when apricots were losing petals, frost occurred 6 times (from 0.0 to −4.8°C). What the team in Italy found in 1990 was that some most productive varieties had more than 80% of brown pistils and the relation between the detected percentage of brown pistils and crop yield (r = 0.04) was not therefore confirmed.

Varieties that had fruits mostly in the upper part of the crown were Alfred, Bella di Casale, Farmingdale and Harlayne. Other varieties such as Bergeron, Precoce di Cesena, Bulida, Canino, Goldcot and Ivonne Liverani a Mandorlou showed a relatively uniform location of fruits. Very late blooming varieties from Hungary and Romania showed a very low count of buds during all the years as well as a high percentage of brown pistils even though they had bloomed after a period of frost. This defect is caused by bad adaptability of varieties from Central to Eastern Europe which demands cooler conditions [14].

Concerning the damage to the apricot fruits during spring frost at Mendel University in Brno, Horticulture Faculty in Lednice, from 1989 to 1996, the following was observed:

i. The genotypes Leala, Lemira, Ledana, Leskora, Lejuna, Lefrosta, Henderson, Early Gold, Zard, Oranžovokrasniy, Marculešti 17/2, Horákova raná, Fara, Rakovského, Reumberto, Lednická, Detskij, Neptun, Triumf severa, Patriarca tempráno, Baneasa 16/3, Kamenický, Selena, Mari de Cenad and NJA 33 conclusively or highly conclusively increased tolerance of fruits to frost than the control variety (Velkopavlovická LE-19/2,) in 2, possibly 3 years.

ii. The genotypes Orange Red, Legolda, Shalah, Nugget, Kecskemetr rozsa, Leronda, Skaha and Lerosa showed lower resistance of fruits to frost.

It was possible to detect a highly conclusive impact of genotype, year and blooming period during these years of observation when the conditions differed each time either because of vegetation development or because of critical temperatures. The impact of blooming period was recorded as highly conclusive in relation to the percentage of frozen fruits (r = 0.41++). In all years, the fruits of late blooming varieties sustained less damage (Reumberto, Venus, Zard, Marculesti 17/2, Neptun and Leala). However, there were certain genotypes which, despite their early or mid-early blooming period, were not significantly damaged (Bukurija, Junskij, Senetate and Henderson) [15].

Breeders also face another challenge, which is to single out the most harmful diseases and pests relevant to a particular area, recognize their biology and inheritance and explore the mechanism of resistance. They would then be able to, based on such selection, use disease-resistance donors in breeding programs with quality donors. When growing plants, resistance breeding is the most efficient, natural and widespread method of protection against pathogens [16].

Greek colleagues have worked very hard studying apricot varieties mainly in a stage after a strong natural infection. Syrgiannidis was first to describe the Stark Early Orange and Stella cultivars as resistant against *plum pox virus* based on his fieldwork between the years of 1967–1970. He then sorted the varieties into very sensitive (ProimoTyrinthos, Rouge de Sernhac), quite sensitive (Blenril, Canino, Docteur Mascle, Bergeron, Moorpark, Nugget, Rouge de Fournes, Sungold and Tilton a Stavropoulos) and less sensitive (Blenheim Royal, Ricordo di Amic and Grossa del Giardino) [17].

Murg et al. [18] recorded the results of observing 64 apricot species infected with PPV in the region of Oradea and found that Stark Early Orange, Manitoba, Farmingdale, Bolšoj Rozovoj, Blenril, King, Doty, Arzami, Pize, Smyrna, Sophie, Tarzii de Bucuresti, Selena, Favorit, Timpurii de Chisinau, Rozova Scora, Rosii de Banesa, Magyar Kayszi and Borozi Rozsa varieties were not infected. Varieties which had no symptoms in fruits and weak symptoms on leaves were Venus, Tuzla, Saturn, Calatis, Dalia, Neptun, Sulina, Excelsior, Čačak, Raske Carpo, Joubert, Toulon and Precoce de Italia [18].

2.3. Achieved findings in apricot breeding

Clone selection can be regarded as an evolution of varieties when clones with higher adaptability are selected during a long-term growing period in a relatively vast area, whereas clones with lower adaptability to the environment cease to exist or are only relevant to a smaller section of areas. This is called the plasticity of a given cultivar which, influenced by its environment and length of growing, provides an array of clones with various adaptabilities. This plasticity is conditioned by the age of varieties. Hence, clone selection provides one of the solutions for increased adaptability for varieties such as Velkopavlovická, Hungarian best and Sabinovská. Clone selection can increase environmental adaptability as can the selection of additional properties other than just fertility. In the scope of several cycles of clone selection of apricots which Professor Vachůn initiated in the 60s, variability of the blooming period was also detected (a 1–2-day delay of full bloom can in some years positively influence the harvest), as was the number of blossoms with two pistils making the clones with higher number of pistils more efficient. Of other variable properties, clone selection can be used to improve the health of trees (early death or economically significant viral diseases) when even in these traits conclusive differences were found [19].

In Hungary, Nyujtó chose large fruits of local cultivars and saw the European group of apricots as being suitable enough to achieve a faster and significant improvement in cultivar adaptability using clone selection as opposed to the creation of new cultivars through crossing. His program saw the creation of varieties such as Cegledi Biborkajzsi C.244, Kéczkei rozsa barack C.171, Cegledi oriás and Mandula kajzsi C.712. When selecting 532 items of apricot, it was discovered that the size of fruits and excellent taste are rarely related to high productivity and even less to frost hardiness and drought tolerance [20]. Later, Szabó showed that Keczkei rozsa and Mandula kajzsi varieties introduced frost hardiness into its progeny dominantly and irrespective of them being used as female or male parents [21].

The degree of apricot adaptability is, to a certain extent, conditioned by the level of frost hardiness in buds, blossoms and juvenile fruits. Therefore, the period of vegetative dormancy

(its intensity and length) ensures less dependence on external environmental conditions and is a crucial factor for frost hardiness. Based on its origin, the apricot is a mountainous plant and as such is typical for its adaptability to cold winter without fluctuating temperatures. Results of many authors have shown that buds of stone fruit experience important stages of micro- and macrosporogenesis at the time of autumn, winter and spring [22].

Development of blossom buds at the time of dormancy is assured by individual changes and intensity of metabolism. Jablonskij and Elmanova found a relation between accumulated hydrocarbons and the pace of morphogenesis as well as a relation between morphological and anatomical changes and the dynamics of phenol contents in buds (r = 0,83). This occurrence relates to the fact that most phenols inhibit the growing process. They found that the highest contents of phenols in blossom buds were present (as detected in the beginning of April) in the following varieties: Narjadniy, Vynosliviy and Orfyand Amur [23].

Kostina et al. compared the inheritance of dormancy length in reciprocal crossing of varieties Zard and Shalah, which belong to two different eco-geographical groups with different dormancy lengths. In this cross, 34.8% of progeny inherited the character of slow development of Zard and 21.9% of progeny inherited the character of its father variety Zard [24].

The study on two progenies, where the late terminating dormancy variety Stark Early Orange (SEO) was the male variety for both of them and female varieties from early to mid-early-breaking dormancy of Vestar and Velkopavlovická LE-19/2 were utilized, observed that in both the combinations 82.1% of hybrids terminated their deep vegetative dormancy after their parents and intermediates. New traits appeared in 17.9% of progeny, of which being positive (late dormancy termination) appeared in 9.4% [25].

In her breeding work, Kostina established a dominant inheritance character in apricots from Central Asia regarding several biological traits including late blooming and increased frost hardiness if apricots in this group had been crossed with apricots in the Irano-Caucasian group or European group [8]. Crossa-Raynaud (when crossing Canino and Amerleuch showed the intermediate inheritance character of development rhythm in flower buds of blooming period [26]. Zagorodnaya also found that more than a half of observed progeny possessed frost-hardiness properties of blossom buds among parents and quite a considerable number of progeny were drawing nearer to their frost-hardy parent, particularly if this parent was used as a mother cultivar [27].

Oranževokrasniy is also recommended as a frost-hardy donor for crossing by Kostina together with Churmai variety (long dormancy period and late blooming) [24]. Hough also recommended (written statement, 1989) the crossing of Oranževokrasnyi × Orange Red with the aim of obtaining frost-hardy individuals with firm fruits and early ripening [28].

Plum pox virus (PPV) resistance presents one of the most discussed topics in European breeding programs. This topic has been reviewed by Zhebentyayeva et al. [29]. All apricot cultivars of European origin are susceptible to PPV. Currently, most apricot breeding programs in Europe use the PPV-resistant North American cultivars to introduce this trait into European germplasm. Resistance among apricots has been found only in some North American cultivars such as “Goldrich,” “Harlayne,” “Stark Early Orange,” (SEO) “Stella” and “Harcot” [30, 31].

Therefore, most conventional breeding programs very often use one of these as a source of resistance in the development of new varieties. Badenes et al. were the first to suggest the role of Eastern Asiatic species, particularly *P. mandshurica,* as a potential source of PPV resistance into North American germ-plasm [32]. The results from Karayiannis et al. [31, 33] gave more support to this idea, even if not all the progenies of *P. mandshurica* were PPV resistant [34]. North American selections derived from *P. mandshurica* were introduced for their cold hardiness in midwinter and spring, late blooming and the ability to set fruit under adverse conditions for pollination [35]. Besides *P. mandshurica,* other East Asian species such as *P. sibirica* var. *davidiana* and *P. mume* may also have been involved in the pedigree of PPV-resistant North American apricots. A likely scenario for introgression of resistance into North American germplasm might include hybridization of European apricots with Northern Chinese varieties cultivated in overlapping areas of *P. armeniaca* and East Asian apricot species [28]. The histories of apricot domestication and of its resistance to Sharka are however still poorly understood. In another piece of work, Decroocq et al. used 18 microsatellite markers to genotype a collection of 230 wild trees from Central Asia and 142 cultivated apricots as representatives of the worldwide cultivated apricot germ-plasm. The genetic markers confirmed highest levels of diversity in both wild and cultivated apricots in their original areas (Central Asia, China). Furthermore, high frequency of resistance to Sharka was detected in apricots native to Central Asia [36].

The PPV resistance trait in apricots was first mentioned by Dosba et al. [37]. In the population of Stark Early Orange × Screara, 64 inoculated (by both methods, chip budding and aphids) hybrids were tested for PPV and a polygynous character of heredity for PPV was found. Dosba et al. mentioned 30% resistant hybrids in the same hybrid combination [37]. Moustafa et al. agreed with Dosba [37]. A ratio of 3:1 sensitive/resistant genotypes in hybrid populations of North American PPV resistant varieties and local Spanish sensitive cultivars corresponds with the hypothesis of heredity to PPV resistance by two independent dominant genes. Resistance donors who used incrossing could be heterozygous for both loci. Only those heterozygous seedlings in both loci as the parental donors could be resistant [38].

On the contrary, Dicenta et al. established the ratio of 1:1 in 291of seedlings of 20 different crossing activities between resistant and sensitive parents. Based on these results, the authors conclude that PPV resistance in apricots is controlled by one dominant gene and that resistant parents could be heterozygous even in this trait [39].

Faculty of Horticulture in Lednice has got a long tradition of apricot breeding which started in 1990 and focuses on resistant breeding against Sharka (PPV). One of the parents used in the breeding program in Lednice (Harlayne) was assessed as resistant by Martínez-Gómez et al. [40], while both Dosba et al. [37] and Fuchs et al. [41] classified it as immune.

When crossing the parents of resistant to susceptible, using Harlayne species, a hypothesis was reached on heredity of three dominant genes which are responsible for PPV resistance [42]. In the progeny derived from the four apricot crosses (resistant to susceptible), the segregation ratios were compatible with the hypothesis of three dominant genes being responsible for PPV resistance, with "Harlayne" being heterozygous for all three genes [43]. Four quantitative trait loci (QTLs) were identified of which three mapped on linkage group 1 (LG1) which explained between 5 and 39% of the phenotypic variance. This happened when analyzing quantitative

resistance of Harlayne cultivar in a large F1 population [44]. Dondini et al. established that a major QTL for resistance to both PPV-M and PPV-D strains was found in the top half of "Lito" LG1 (just like in "Stark Early Orange") and when the resistant, tolerant and recovered seedlings were pooled together, the ratio of these to susceptible plants was fixed as 1:1, explaining why such a large part of the phenotypic variability is accounted for by a single QTL in the LG1 [45].

A first determinant was mapped on linkage group 1 (LG1) by using an F1 progeny of Goldrich 9 Valenciano [46]. These studies were also verified by a quantitative trait locus (QTL). Goldrich is known to be tolerant to the pathogen while Valenciano was highlighted as susceptible [39, 47]. This preliminary result was recently confirmed by a quantitative trait locus(QTL) analysis carried out on another F1 progeny, Goldrich 9 Currot [47]. A major QTL was also found in LG1 by the analysis of F1 and F2 progenies of Stark Early Orange (SEO) [48] and its progeny Lito [46, 49]. Minor QTL were discovered in the Polonais 9 SEO progeny in LG3 and LG5 of both SEO and Polonais [50]. The main QTL on LG1 was upheld by Sicard et al. [51] establishing new microsatellite (SSR) markers flanking the QTL and by Lalli et al. [52] in a backcross population of SEO 9 Vestar and was again confirmed by Soriano et al. [46] in the extended F2 Lito selfed progeny. Most of the resistance determinants shown above were characterized using the PPV-D strain as a source of inoculum and, at present, the only resistances specific to the PPV-M strain are those endorsed in the BC1 SEO xVestar [52] and in *P. davidiana* [44]. Dodini et al. [45] introduced a potentially different QTL which either explained a very small part of the variance or was below the LOD threshold. The markers closer to the QTL peaks would already be suitable for marker-assisted breeding. Those plants that recovered should be subjected to another observation to evaluate their tolerance or resistance.

Krška et al. studied the inheritance of resistance to PPV in F1 progeny of cross "Harlayne" × "Betinka" obtained within gene pyramiding. The observed segregation ratio (153,30,16) for the progeny of "Harlayne" × "Betinka" was not significantly different from the predicted 12:3:1 segregation ratio ($\chi^2 = 2.551$, P = 27.9%). These findings showed that PPV resistance in apricot is controlled by two independent dominant genes with epistatic interaction, where resistance would be a dominant trait and the resistant parents ("Harlayne" and "Betinka") would be heterozygous for both loci. Tolerant plants would be heterozygous for the hypostatic (masked) locus, and susceptible plants would be homozygous recessive for both loci. They established that it is possible to pyramidize genes for resistance to PPV in apricot and gain a cultivar with durable resistance to PPV. This principle was upheld in the apricot breeding program at the Mendel University in Brno, Czech Republic [53].

3. Conclusion

1. The apricot is a species with lower adaptability to the environment, which has, in the scope of its species, many genotypes—donors that can be used in breeding against biotic factors.
2. At present, there are substantial gene resources for apricot breeding and scientific activity relating to this species. These are used intensively to achieve mainly higher quality of fruits and resistance to Sharka.

3. In chosen and traditionally most significant apricot cultivars, it is essential to carry out clone selection and it is important to maintain this process so that the biological quality of clones is maintained. The use of local genotypes and clones of genetically similar cultivars should not just be the nostalgia of "good old days" but the reality of economically justified growing and agri-tourist utilization.
4. The study of apricot germ-plasm so far has enabled not only the selection of suitable genotypes to be tested in growing practice but also the selection of suitable genotypes for targeted breeding while aiming to use gene variability. Results obtained using the analysis of polymorphic markers have enabled breeders to carry out their work. It was possible to use them in the context of evaluated collection of apricot genotypes to identify the difference to assess the genetic similarity and more detailed characteristics of varieties, genetic resources and breeding material as well as for methods of early selection of progenies with chosen traits. Developed molecular markers for selection in breeding programs (MAS) represent almost a routine method today, particularly in the area of resistant breeding.
5. New methods of selection (MAS) have so far aided in early selection of traits of PPV resistance and determination of self-compatibility.
6. Currently, newly created cultivars present the opportunity of genotype selection with appropriately combined traits of resistance to abiotic or biotic pathogens but also in terms of requirement for an ideotype of fruits with increased adaptability, so that the apricot could again, as stated by Hough and Bailey, descend from the mountains.

Author details

Boris Krška

Address all correspondence to: borokrska@gmail.com

Independent Researcher, Lednice, Czech Republic

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Section 2

Health Benefits

Chapter 5

Myrciaria dubia "Camu Camu" Fruit: Health-Promoting Phytochemicals and Functional Genomic Characteristics

Juan C. Castro, J. Dylan Maddox,
Marianela Cobos and Sixto A. Imán

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73213

Abstract

Camu camu is a typical Amazon native fruit shrub that possesses a diploid genome, moderate genetic diversity, and population structure. The fruits accumulate several essential nutrients and synthesize L-ascorbic acid (vitamin C) in great quantities and an array of diverse secondary metabolites with corroborated *in vitro* and *in vivo* health-promoting activities. These beneficial effects include antioxidative and antiinflammatory activities, antiobesity, hypolipidemic, antihypertensive and antidiabetic effects, DNA damage and cancer protection effects, and other bioactivities. Many health-promoting phytochemicals are biosynthesized in several metabolic pathways of camu camu. Their reconstruction from the fruit transcriptome database was accomplished by our research group. These include basic metabolic pathways such as glycolysis and pentose phosphate pathway, vitamin C biosynthesis pathways, and pathways involved in secondary metabolites production. Due to their agronomic potential and fruits growing demand, recently, based on an ideotype, programs were initiated for their domestication and genetic improvement, but so far with very negligible achievements. Consequently, we propose new strategies to accelerate the processes of domestication and genetic improvement based on state of the art technologies for multiomic data analysis and innovative molecular tools.

Keywords: genetic diversity, health-promoting phytochemicals, phenolic compounds, transcriptome, vitamin C

1. Introduction

Myrciaria dubia Kunth (McVaugh) "camu camu" is a typical native Amazonian fruit shrub that thrives in areas exposed to periodical flooding on the banks of rivers, streams, lakes, and swamps

of several Amazonian countries [1, 2]. This plant species possesses a diploid genome, and their genome size (~230 Mb) is in the range of other Myrtaceae species [3–6]. Populations exhibit moderate genetic diversity and genetic structuring [7–11]. Camu camu produces several essential nutrients such as amino acids, polyunsaturated fatty acids, B-complex vitamins, and high quantities of vitamin C [2, 12–15]. Additionally, the fruits (including peel, pulp, and seeds) and several other tissues/organs (leaves, roots, etc.) accumulate numerous health-promoting phytochemicals with powerful antioxidant, antiinflammatory activities, antiobesity, hypolipidemic, antidiabetic effects, DNA damage and cancer protection effects, hepatoprotective properties, and other beneficial effects [16–24]. These bioactive phytochemicals, in addition to vitamin C, are secondary metabolites that primarily include various phenolic compounds, carotenoids, terpenoids, and several other bioactive metabolites. These associated beneficial effects of phytochemicals were corroborated by numerous *in vitro* and *in vivo* studies with several animal models (i.e., flies, mice, rats, etc.) and human volunteers [16, 17, 22, 24–28]. In the health-promoting phytochemicals section of the book chapter, we will illustrate the chemical structures of several of these phytochemicals that were isolated from various tissues of camu camu by bioassay-guided approaches. These secondary metabolites were biosynthesized in several metabolic pathways of camu camu. In the functional genomic characteristics section, we have presented the reconstruction of some metabolic pathways from the fruit transcriptome database that was accomplished by our research group. These include, for example, basic metabolic pathways (i.e., glycolysis, pentose phosphate pathway), vitamin C biosynthesis pathways, shikimate pathway, and pathways directly involved in secondary metabolites production (i.e., anthocyanins, carotenoids, flavonoids, phenylpropanoids, and terpenoids biosynthesis pathways). Finally, we have included a section about domestication strategy and genetic improvement efforts, where we examine the strategies implemented by Peruvian Institutions to achieve these goals. We have also proposed new strategies to significantly accelerate the domestication and genetic improvement of this species based on the state of the art technologies for multiomic data analysis and innovative molecular tools.

2. General description

2.1. Geographical distribution

Camu camu is a typical native shrub from the tropical rainforest of the Amazon. Wild populations of this species grow in dense areas exposed to periodical flooding (complete submergence for 4–5 months) on the banks of rivers, streams, lakes, and swamps of Guyana, Venezuela (Casiquiare Oreda, Pargueni, Caura, and Orinoco), Colombia (Putumayo and Inirida), Ecuador, Brazil (Trombetas, Cachorro, Mapuera, Maçangana, Urupa, Javari, Solimões, Madeira, and Negro), Peru (Amazonas, Curaray, Itaya, Nanay, Napo, Putumato, Ucayali, Marañon, and Tigre), and Bolivia (**Figure 1**) [1, 2]. In Peru, wild populations only exist in the Loreto Region, consisting of approximately 1345 ha [29], whereas artificial plantations have been established in the Regions Loreto (~6475 ha), San Martin (~110 ha), and Ucayali that consist of approximately ~5930 ha [29–32].

http://dx.doi.org/10.5772/intechopen.73213

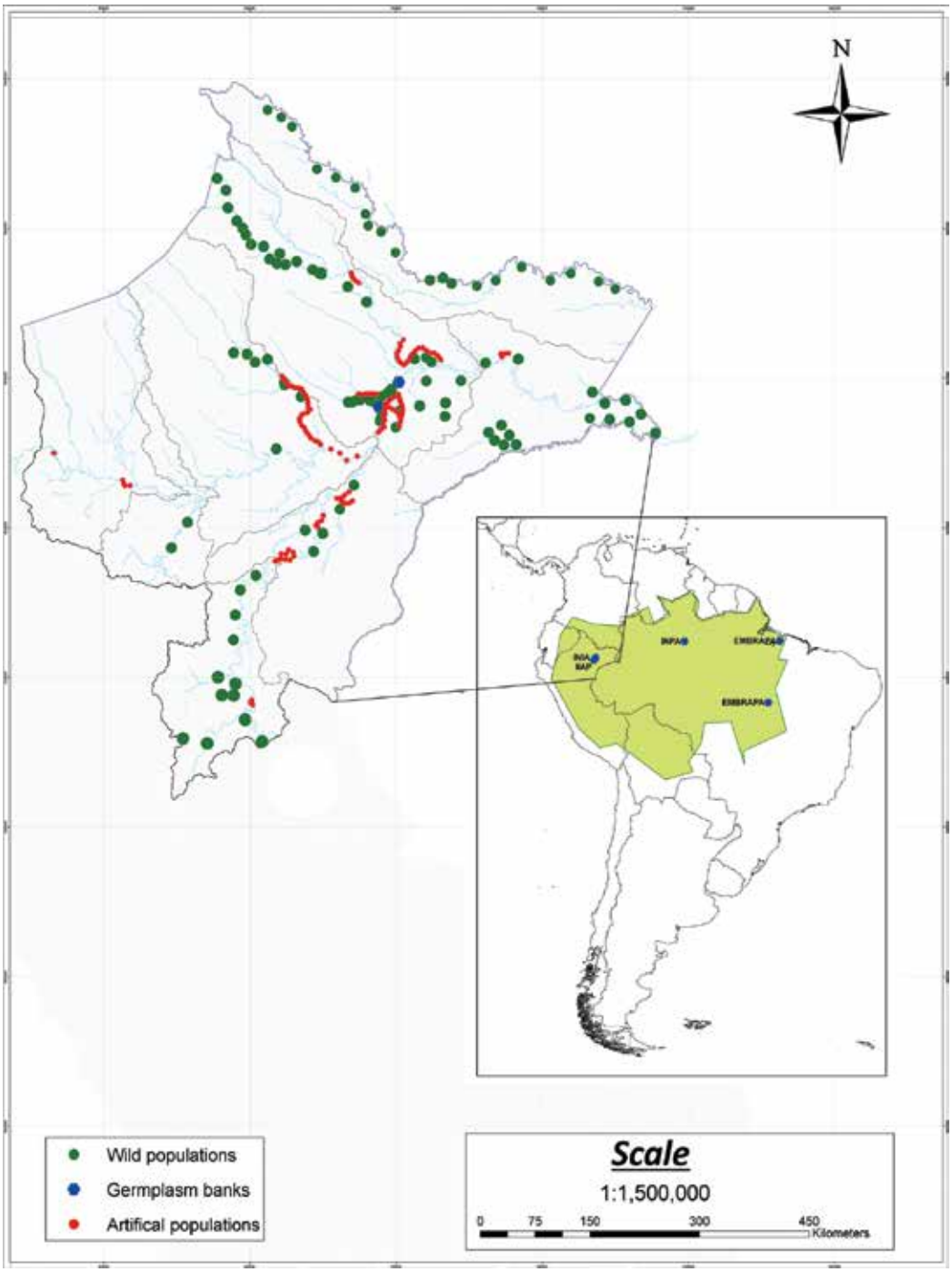

Figure 1. Geographical distribution of camu camu in South American and the Peruvian Loreto region.

2.2. Botanical characteristics

Typically, the camu camu shrub achieves a height of 4–8 m, branching from the base to form several secondary stems, which in turn branch out as an open vessel. The trunk and branches are glabrous, cylindrical, and smooth, and the bark is light or reddish brown, which peels off naturally in periods of drought [14, 33]. The deep-rooted shrub contains numerous absorbing roots. The leaves are opposed, single, petiolar, elliptic-lanceolate (ca. 3–12 × 1.5–4.5 cm), with acuminated apex and oval base, with primary and secondary veins (18–20 pairs). Petioles that are cylindrical have a length of 3–9 × 1–2 mm [34]. The inflorescences are axillary with 1–12 (generally four) subsessile and hermaphrodite flowers arranged in two pairs on the axis. The rounded ciliated bracts and bracteoles are persistent. The calyx is approximately 2 mm long and 2 mm wide and includes four sepals with broad apex and the hypanthium is prolonged and circumscissile at the summit of the ovary and falls with the calyx as a unit after anthesis [35]. The corolla has four white ovate petals which are 3–4 mm long with a ciliated margin. The ovary is inferior with a simple style that is 10–11 mm long, and the androecium has 125 stamens of 6–10 mm in length and anthers of 0.5–0.7 mm length. Although camu camu flowers are hermaphrodite, inbreeding is largely prevented by the lack of synchrony between the development of the gynoecium and androecium, leading to facultative allogamy [14, 33, 36]. The fruits are globular and measure 1.0–5.0 cm in diameter, and their weight averages 11.7 ± 1.4 g [34]. Based on the fresh weight, the fruits are comprised, on an average, of 65.2% pulp, 19.5% seeds, and 15.3% peel [34, 37]. The shiny peel can be pink to deep red or even black when completely ripe, with slightly pinkish pulp [2, 14, 33]. The seeds are kidney-shaped to ellipsoid, flattened bilaterally and are exalbuminous. The fruit contains one to four seeds with an average length of 13.5 ± 1.6 and width of 4.8 ± 0.6 mm. The average fresh seed weight is 440 ± 170 mg. The elongated seed coats are brown and thin and are covered with spiny-celled villi (**Figure 2**) [38, 39].

2.3. Nutritional composition

Pioneering work on the high L-ascorbic acid (vitamin C) content of camu camu fruits was published in 1964 [15]. In this report, the authors indicate that camu camu fruits are among the highest natural sources of vitamin C. Approximately 30 years later, these observations were corroborated by several investigations on the chemical and nutritional composition of camu camu [2, 12, 13, 34, 37, 40–44]. These findings from approximately 60 years of camu camu research are consolidated in **Table 1**. These fruits are composed of nutrients such as protein, carbohydrate, lipids, ash, and crude fiber. Additionally, they have essential amino acids (valine, leucine, phenylalanine, etc.), essential fatty acids of the families omega 3 and 6, vitamin C, and vitamins of the B-complex and several essential minerals for human nutrition, such as potassium, phosphorous, sulfate, calcium, magnesium, cobalt, iron, and several others.

2.4. Chromosome number and genome size

Some standardized karyotype analyses conducted on meristematic cells from root apices have demonstrated that camu camu is a diploid plant with 2n = 22 chromosomes [3, 4], which is consistent with several other Myrtaceae species (i.e., *Acca sellowiana*, *Callistemon citrinus*, *Eucalyptus*

Figure 2. Botanical characteristics of camu camu and harvest strategies. Wild populations in flooding soils (A) and nonflooding soils (B), culture population in nonflooding soils (C), blooming flowers (D), unripe, semiripe, and ripe fruits (E), variations in seeds size (F), typical plant height and architecture (G), manual harvest during flooding period using canoes (H), and manual harvest in non-flooding soils.

grandis, *Gomidesia affinis*, etc.) [5]. Using flow cytometry, our research group recently determined that the genome size of camu camu is ~ 230 Mb (unpublished data), which is similar to *Myrciaria glazioviana* (234 Mb) [6] but is on the lower end of the range (234–1110 Mb) reported for Myrtaceae species [5].

2.5. Genetic diversity

Pioneering research analyzing the genetic diversity of camu camu was carried out with biochemical markers (esterases) by Brazilian researchers [7]. At that time, the presence of genetic structure was demonstrated among populations (two genetic groups) with an average heterozygosity of 0.08–0.14. Subsequent studies have analyzed the genetic diversity in germplasm collections and cultured populations using DNA markers, such as rapid amplification of polymorphic DNA (RAPD) [45], inter simple sequence repeat (ISSR) [8], expressed sequences tag-simple sequence repeats (EST-SSR) [9, 46, 47], and SSR [10, 11], also known as microsatellite markers. Overall, these investigations found that the average expected heterozygosity (He = 0.67 ± 0.19, range of 0.45–0.88) was greater than the average observed heterozygosity

Component per 100 g	Contents
Bromatological analysis	
Energy (kcal)	19.48 ± 3.68
Water	93.83 ± 0.51
Protein	0.51 ± 0.07
Carbohydrate	4.84 ± 0.80
Lipids	0.17 ± 0.10
Ash	0.22 ± 0.03
Crude fiber	0.56 ± 0.40
Total soluble solids (°Brix)	6.18 ± 0.99
pH	2.84 ± 0.31
Essential amino acids (mg/100 g)	
Valine	242.00 ± 104.65
Leucine	210.50 ± 111.02
Phenylalanine	32.50 ± 14.85
Threonine	32.00 ± 5.66
Essential fatty acids (% of total lipids)	
C18:3ω3 (α-Linolenic)	16.00 ± 0.70
C18:2ω6 (Linoleic)	9.70 ± 0.40
C18:3ω6 (γ-Linolenic)	9.30 ± 0.20
C20:5ω3 (EPA)	7.00 ± 0.10
Vitamins (mg/100 g)	
Vitamin C	2210.00 ± 650.00
Niacin	0.48 ± 0.28
Riboflavin	0.03 ± 0.02
Thiamine	0.01 ± 0.00
Minerals (mg/100 g)	
K	87.020 ± 29.382
PO_4	18.183 ± 8.122
SO_4	14.750 ± 2.192
Ca	14.510 ± 9.346
Cl	9.100 ± 3.536
Mg	7.393 ± 4.323
Co	1.173 ± 0.807
Na	0.934 ± 1.546
Mn	0.820 ± 1.118
Fe	0.424 ± 0.152

Component per 100 g	Contents
Al	0.255 ± 0.064
Zn	0.230 ± 0.138
Cu	0.117 ± 0.072
B	0.050 ± 0.000
Br	0.021 ± 0.005
Cr	0.015 ± 0.004
Mo	0.004 ± 0.002
Se (μg)	0.429 ± 0.089

Table 1. Nutritional composition of camu camu fruit pulp.

(*Ho* = 0.41 ± 0.06, range of 0.33–0.49). Also, the populations exhibited high inbreeding coefficients (f = 0.31 ± 0.13, range of 0.20–0.49) and high genetic differentiation values (F_{ST} = 0.26 ± 0.08, range of 0.21–0.32). Likewise, the average intrapopulation genetic diversity (average 74.89%, range of 65–79%) is ∼3 times greater than average interpopulation genetic diversity (average 25.10%, range of 20–35%). Also, these molecular markers studies demonstrated the presence of genetic structure among populations (from 2 to 3 genetic groups). However, two of these reports have shown that genetic and geographic distances are uncorrelated (r = 0.31, range of 0.23–0.39). These peculiar genetic population characteristics of camu camu can be partially explained as the result of their undomesticated condition and isolation of the populations by natural barriers, which limit gene flow and favor inbreeding. In conclusion, the genetic diversity characterization of camu camu in wild and artificial populations, and germplasm banks are still too fragmented and deficient to make any strong conclusions. Consequently, an in-depth knowledge of the genetic diversity of this species will be essential to implement programs for genome-wide genetic marker discovery and genotyping using next-generation sequencing technologies, which could then be used to quantify, with more precision and accuracy, the genetic diversity of camu camu across the entire Amazon region. Following this line of thought, our research group explored the assembled transcriptome of this species for molecular marker discovery. We identified more than 3200 SSR motifs that would be appropriate for developing a comprehensive set of genic-SSR markers. Also, the transcriptome contained a large number (>23,000) of high-quality single-nucleotide polymorphisms (SNPs) and marks the highest number of SNP markers discovered to date for camu camu using transcriptome sequencing [48]. Both types of potential molecular markers, however, will require validation.

3. Health-promoting phytochemicals

An ethnopharmacological survey of medicinal plants in the northeastern Amazon region of Peru showed that several botanical parts of camu camu such as immature and mature fruits, stems, leaves, roots, seeds, and barks are used to prepare remedies in folk medicine to treat numerous diseases such as arthritis, diabetes, hypercholesterolemia, bronchitis, inflammation,

asthma, atherosclerosis, cataracts, depression, flu, gingivitis, glaucoma, hepatitis, infertility, migraine, osteoporosis, Parkinson's disease, and malaria [49, 50]. Additionally, Steele [51] showed that camu camu is used traditionally for the treatment of malaria by indigenous people of South America. All these traditional uses are in concordance with multiple scientific researches showing that several botanical parts of camu camu are a rich source of various health-promoting phytochemicals with proved health beneficial properties. Among these bioactive phytochemicals, in addition to vitamin C, several secondary metabolites exist such as polyphenols, carotenoids, and other chemicals, which are presented in **Figures 3** and **4** and detailed below.

3.1. Antioxidative and antiinflammatory activities

A large amount of scientific information currently exists regarding the antioxidant properties of camu camu fruits that were collected by diverse methods, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH assay), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric reducing ability of plasma (FRAP assay), oxygen radical absorbance capacity assay (ORAC assay), total radical-trapping antioxidant parameter (TRAP assay), β-carotene bleaching method, cupric ion reducing antioxidant capacity (CUPRAC assay), total oxidant scavenging capacity (TOSC assay), Trolox equivalent antioxidant capacity (TEAC assay), peroxyl radical scavenging capacity (PSC assay), and pulse voltammetry measurements (voltammetric electronic tongues) [16, 17, 21, 52–54]. Pioneering work on antioxidant properties of camu camu was realized by Reynertson et al. [25], who obtained a IC_{50} value of 57.2 μg/mL on the dried, powdered fruit with the DPPH assay. This low value indicates large antiradical activity, and compared with other Myrtaceae fruits, it was considered very active. These properties were attributed to the high content of vitamin C and total phenolic phytochemicals (101 ± 0.25 mg gallic acid equivalent/g dry weight). Furthermore, Sotero et al. [55] reported that a methanolic extract of fruit pulp, fruit peel, and seeds have antioxidant activities with IC_{50} values of 167.7, 146.9, and 399.8 μg/mL, respectively, with the DPPH assay. Also, De Souza Schmidt Gonçalves et al. [56] demonstrated that lyophilized pulp presented the highest antioxidant capacity with the DPPH assay (∼1450 μmol trolox equivalent/g dry weight) and ORAC assay (∼800 μmol trolox equivalent/g dry weight), which was ≥10 times higher than 21 other native Brazilian fruits analyzed. Positive correlations were high and significant ($r = 0.989$ [DPPH vs. total phenolics content], and $r = 0.795$ [ORAC vs. total phenolics content]) between the antioxidant capacity and total phenolics content (∼285 mg catechin equivalent/g dry weight). Additionally, in the fresh fruit pulp, Sánchez [57] found antioxidant capacity values of 219.7 μmol trolox equivalent/g fresh weight and 214.1 μmol trolox equivalent/g fresh weight with the DPPH and ABTS assays, respectively. Another study conducted by Villanueva-Tiburcio et al. [52] compared the antioxidant activity in fruit peel of three maturation stages (unripe, semiripe, and ripe). Semiripe fruits showed the highest antiradical power with the DPPH (IC_{50} = 46.20 μg/mL), ABTS (IC_{50} = 20.25 μg/mL), and PSC (IC_{50} = 8.30 μg/mL) assays. Again, the authors corroborated a highly positive correlation of vitamin C and polyphenol content with the ability to inhibit the free radical DPPH ($r = 0.999$ with both compounds). Several other investigations have corroborated the highly positive correlations between polyphenol content and antioxidant activity with different assays. Likewise, the superior antioxidant capacity of camu camu fruits was established by the comparison

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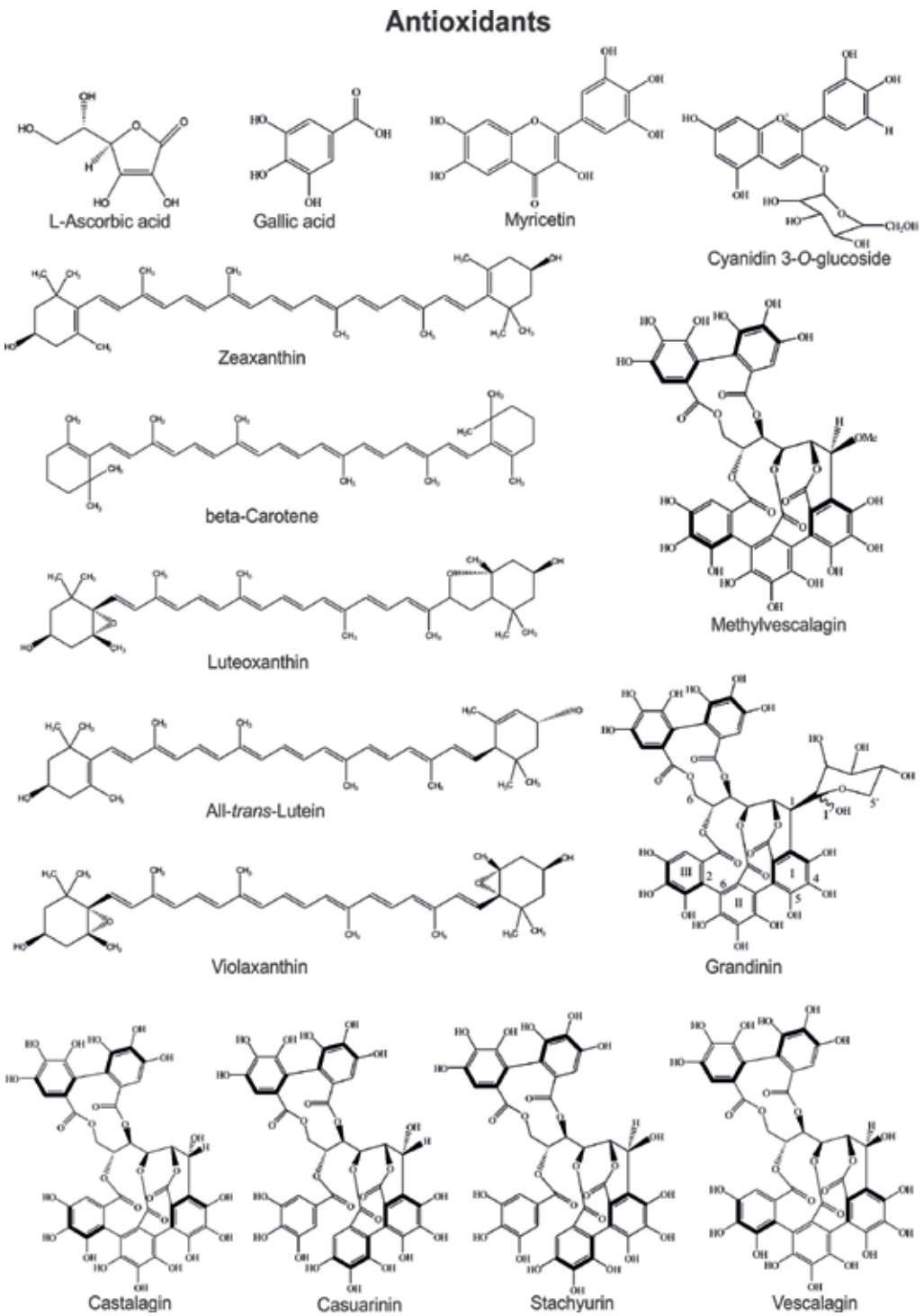

Figure 3. Some phytochemical compounds with antioxidant activities identified in fruits of camu camu.

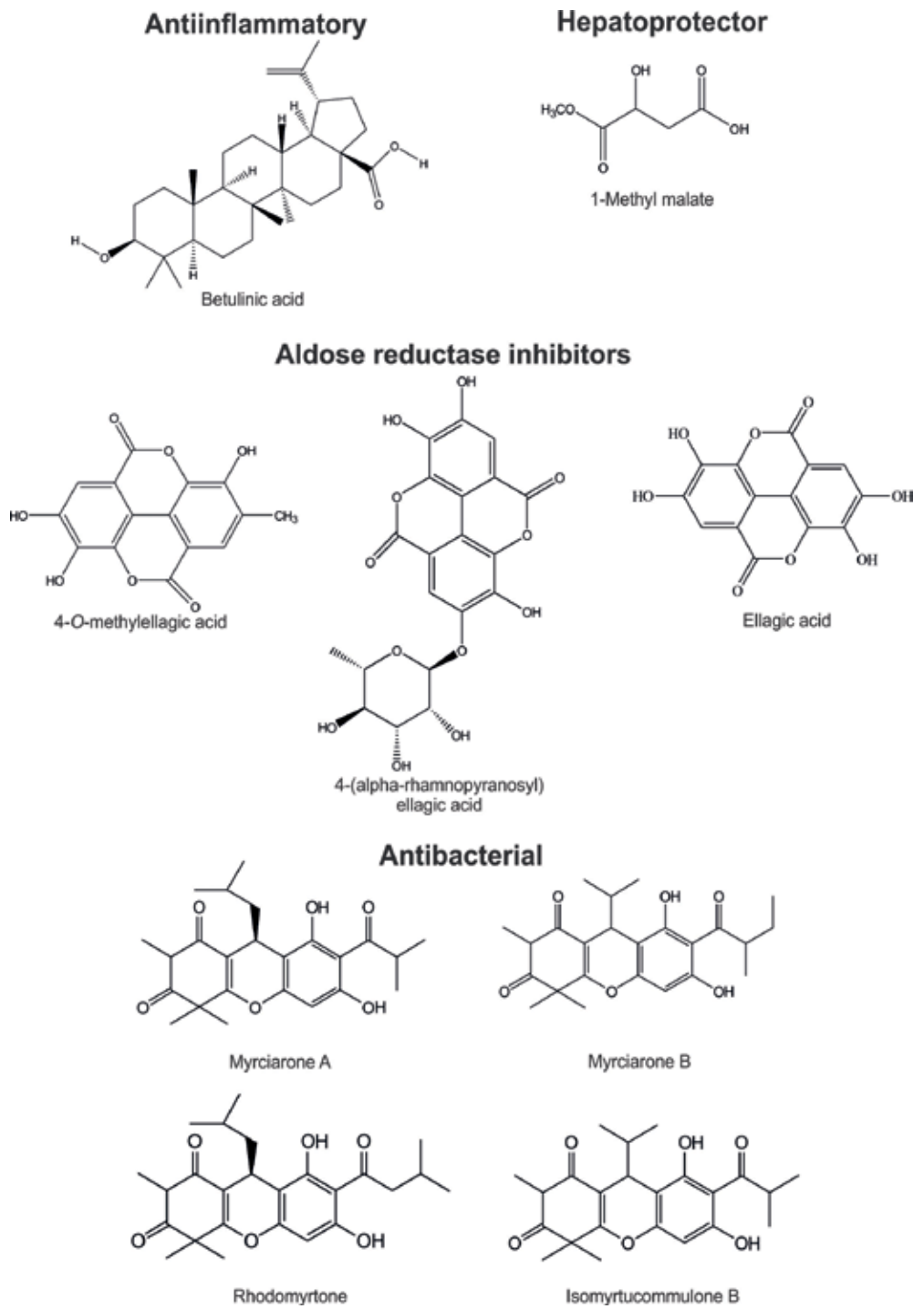

Figure 4. Phytochemical compounds with corroborated health-promoting phytochemicals isolated by bioassay-guided approaches from camu camu tissues.

of other plant species [16, 17, 19, 58–60]. An in-depth analysis of phenolics compounds was realized by Fracassetti et al. [17]. These researchers identified 53 different phenolics (flavonols [6], anthocyanins [1], ellagic acid and derivatives [10], ellagitannins [10], gallic acid and derivatives [2], and proanthocyanidins [24]) and found differential quantities of these compounds in fruit pulp, peel, seeds, and two powder products from camu camu fruits. Interestingly, the flour produced from the remaining peel, seeds, and adhered pulp after pulp extraction showed a superior vitamin C and phenolics content than the pulp powder (2.6 and 13.9 times, respectively). In addition, the flour displayed greater antioxidant capacity than the pulp power (from 2.0 to 4.5 fold).

In vivo studies using *Rattus norvegicus* (Wistar strain) as an experimental model that were treated orally with fruit pulp and tropical juice containing 5% of camu camu fruit pulp demonstrated a significant increase in plasma antioxidant capacity, liver glutathione peroxidase, superoxide dismutase, and catalase activities and thiobarbituric acid-reactive substances were reduced when compared with control [61, 62]. Finally, Inoue et al. [22] analyzed the antioxidant and antiinflamatory activities of camu camu juice in 10 healthy habitual smokers. The participants ingested 70 mL of juice (100% fruit pulp) daily for 7 days. After the experimental period, the oxidative stress markers such as levels of urinary 8-hydroxy-deoxyguanosine and total reactive oxygen species decreased significantly ($p < 0.01$) in the camu camu group. Similar trends were evident with inflammatory markers such as serum levels of high-sensitivity C reactive protein, interleukin 6, and interleukin 8, while the group that ingested vitamin C tablets remained unchanged. The researchers concluded that the fruit juice of camu camu may have powerful antioxidant and antiinflammatory properties compared to vitamin C tablets, due to the existence of unknown antioxidant substances modulating *in vivo* vitamin C in camu camu. These health-promoting activities can be partially explained based on current evidence that the ingestion of the fruit pulp and other derived products significantly increase the postprandial antioxidant capacity of the plasma [63] due to the high vitamin C and polyphenolics content.

Additionally, in previous animal experiments using *Mus musculus* as carrageenan-induced paw edema model, a Japanese research group demonstrated that the methanolic extract of seeds significantly reduced edema formation with regard to size and volume in a dose-dependent manner. These antiinflammatory effects were associated with inhibition of the localized nitric oxide production by macrophages. Further, by bioassay-guided fractionation, the researchers identified the active compound to be 3β-hydroxy-lup-20(29)-en-28-oic acid (betulinic acid) [23].

3.2. Antiobesity, hypolipidemic, and antihypertensive activities

Several studies in animal models and humans have corroborated the beneficial effects of camu camu fruits on the improvement of biochemical lipid profiles. For example, in animal experiments conducted by Schwertz et al. [24], Wistar rats (*R. norvegicus*) were induced to a dyslipidemic condition by a high-fat diet and then were subjected to various treatments with camu camu fruit juice (0.4–10 mL/kg) for 2 weeks. All dosages showed an improvement in the biochemical lipid profile in a dose-dependent manner, which was evident by a significant reduction in triacylglycerols, total cholesterol, and hepatic cholesterol. Further, fecal cholesterol

excretion was increased. In addition, assays performed by Nascimento et al. [44] in a rat model of diet-induced obesity that ingested daily 25 mL of camu camu fruit pulp by 12 weeks resulted in a significant weights reduction of the fat in white adipose tissues. Triacylglycerols (↓40.6%), total cholesterol (↓39.6%), LDL cholesterol (↓2.14%), and VLDL cholesterol (↓36.4%) levels were also decreased, and HDL cholesterol (↑12.3%) increased in experimental groups. Similar experiments conducted by De Souza Schmidt Gonçalves et al. [61] in a type 1 diabetic rat model (streptozotocin induced) receiving oral administration of 1 or 3 g/kg by body weight of aqueous fruit pulp extract by 30 days, significantly reduced triacylglycerols and total cholesterol levels, and an increase in HDL cholesterol. Additionally, some controlled clinical trials with healthy young adults (20–35 years old) who received oral administration of pulp nectar [64] or encapsulated freeze-dried pulp for approximately 2 weeks [65, 66] displayed a significant improvement in their biochemical lipid profiles, due to hypolipidemic effects. The improvement in the biochemical profile of obesity is associated with an increase in lipid elimination in feces (50%) and by the liver (140%) due to the existence of dietary fiber in the fruit pulp [2, 41]. Dietary fibers have shown multiple beneficial health properties by their role in energy intake regulation and obesity development, which are related to its peculiar physicochemical characteristics. Some mechanisms suggested how dietary fiber aids in weight management include promoting satiation, extended signals of satiety, decreasing transport of macronutrients, and altering secretion of gastrointestinal hormones [67–71].

An *in vitro* study based in the angiotensin converting enzyme-1 (ACE) inhibition displayed that the camu camu fruit pulp lacks antihypertension properties in the 10-mg/mL aqueous extract [26]. However, when spray-dried powders of fruit pulp were added (0.5–1.0%) to samples of lactic acid bacterial fermented soymilk, there was a higher ACE inhibitory effect, suggesting a synergic interaction between camu camu and soymilk active compounds.

3.3. Antidiabetic activity

Some studies have shown that camu camu has antidiabetic activities, which may indicate its potential to treat this disease. For example, studies conducted by De Souza Schmidt Gonçalves et al. [56], De Azevêdo et al. [59], and Fujita et al. [26, 72] with fruit methanolic and polyamide-purified extracts, fruit depulping residue, pulp extract powders (spray drying and freeze-drying), and a probiotic beverage from dried powder of fruit pulp combined with soymilk, respectively, have shown *in vitro* antidiabetic properties due to the combination of moderate alpha-amylase and potent alpha-glucosidase inhibitory activities. This antienzymatic association is considered appropriate as means of modulating carbohydrate digestion and retarding postprandial glycemia, which is an efficient strategy to manage early stages of diabetes type 2 [73]. Also, an *in vivo* assay with obese male *R. norvegicus* conducted by Nascimento et al. [44] treated with 25 mL of pulp for a duration of 4 weeks showed a significant reduction in both blood glucose (23%) and insulin activities (44.5%). The authors attribute the hypoglycemic activity to the large quantity of soluble fibers (**Table 1**) that form complexes with monosaccharides. Furthermore, studies with healthy young adults (20–35 years old) who received oral administration of pulp nectar [64] or encapsulated freeze-dried pulp displayed a significant hypoglycemic effects in 15 days [65, 66]. Finally, Balisteiro et al. [63] showed that healthy subjects that have ingested a polyphenols-rich juice of camu camu significantly reduced the blood glucose levels

(31%) after a carbohydrate-rich meal ingestion. The hypoglycemic activity of these preparations can be attributed to polyphenols of the camu camu fruits. Polyphenols have two corroborated hypoglycemic mechanisms. The first is related to inhibitory action of the carbohydrate digestive enzymes alpha-amylase and alpha-glucosidase [26, 44, 56, 63, 72] because these inhibitory activities have high-positive correlations (Pearson's correlation coefficient ≥ 0.50) with the concentration of different phenolic compounds (casuarictin, ellagic acid, quercetin, syringic acid, myricetin, etc.) [26, 56]. Second, intestinal monosaccharides transporters such as sodium-dependent glucose transporter 1 (SGLT1) and glucose transporter 2 (GLUT2) were inhibited, which was demonstrated in several studies [74–79].

Additionally, Ueda et al. [80] isolated three aldose reductase (AR) inhibitors from an 80% methanolic leaf extract: ellagic acid, 4-*O*-methylellagic acid, and 4-(α-rhamnopyranosyl)ellagic acid. The last phenolic compound showed the strongest uncompetitive inhibition against human recombinant AR and rat lens AR. Also, the inhibitory activity of 4-(α-rhamnopyranosyl)ellagic acid was 60 times more powerful than quercetin. Consequently, these AR inhibitors are able to prevent the biochemical conversion of glucose to sorbitol in the polyol pathway and then reduce diabetic complications [81–83].

3.4. DNA damage and cancer protection effects

Mus musculus (CF-1™ strain) were used by Da Silva et al. [27] to test the genotoxic and antigenotoxic potential of camu camu fruit juice after acute (single dose for one day), subacute (for 28 consecutive days), and chronic (for 56 consecutive days) oral administration. None of the fruit juice concentrations (25, 50, and 100%) tested exerted any genotoxic effect on blood cells in male and female mice. In the *ex vivo* test, with the alkaline comet assay, the fruit juice demonstrated antigenotoxic effect after acute, subacute, and chronic treatments. However, the acute administration of the fruit juice produced the lowest values in both damage index and damage frequency. The researchers associated the protective effect, against DNA damage caused by hydrogen peroxide, to the elevated levels of vitamin C, as well as to the flavonoids and phenolic compounds present in the fruit juice of camu camu; together these phytochemicals are very able to eliminate free radicals.

Furthermore, several studies using microbial and animal models have demonstrated the antimutagenic properties of the camu camu fruits. In Peru, pioneering investigations were conducted by Gutiérrez [28], who tested the antimutagenic properties of an aqueous extract of fruit using *in vitro* and *in vivo* models. In the *in vitro* model, cultures of the CHO-K1 cell line from hamster (*Cricetulus griseus*) ovary were exposed to hydrogen peroxide and co-treated with fruit extract (concentrations at 1, 5, and 10%). The camu camu fruit extract had a significant capacity to protect against chromosomal aberrations induced by reactive oxygen species in a dose-dependent manner. Also, in the *in vivo* model using fruit fly (*Drosophila melanogaster*) specimens, the antimutagenic activity of the fruit extract against the mutagenic effect induced by N-ethyl-N-nitrosourea (concentrations at 0.01 and 0.1 mmol) was demonstrated. This DNA damage protection effect was tested by the somatic mutation and recombination test that displayed a significant reduction in the frequency of wing spots (55.0–74.4%) in flies co-treated with 25% of fruit aqueous extract. In addition, Sánchez [57] demonstrated with the Ames test that a phenolic compound-rich fraction from the fruit pulp displayed antimutagenic activity (36.7–91.5%) in a

dose-dependent manner against the mutagenic compound 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole. Further, in the two investigations on the frequency of micronucleated polychromated erythrocytes of bone marrow cells of albine mice (*M. musculus*) induced with cyclophosphamide and co-treated with fruit pulp by orogastric gavage for 10–15 consecutive days, there was a significant and drastic (from 16 to 90%) reduction of micronucleous frequency in a dose-dependent manner [21, 84]. Additionally, the cytoprotective activity of the camu camu fruit against the mutagenic damage caused by potassium bromate ($KBrO_3$) was also demonstrated. Animals were treated by oral administration of a fruit aqueous extract (50 mg/kg) for 35 consecutive days. In the tenth day of treatment, albine mice were intraperitoneally injected with a solution of $KBrO_3$ (dosage of 68.5 mg/kg) to induce mutagenic injury. After a meticulous comparative analysis of the DNA fragmentation degree, with the alkaline comet assay, a great inhibitory capability of the fruit pulp against the DNA-damaging effects of $KBrO_3$ in blood, kidney, and liver cells was noticeable. This strong protective action was potentially attributed to the high content of antioxidants such as vitamin C and flavonoids present in the fruits of this fabulous Amazonian plant [85].

Recent reports also demonstrated the anticancer properties of camu camu fruit juice. In the first research conducted by Carvalho-Silva et al. [21], the authors used an *in vitro* cytotoxicity and antiproliferative assays. In these tests, the HepG2, a liver hepatocellular carcinoma cell line, was grown for 72 hours in media containing increasing concentrations (0–25 mg/mL) of a tropical fruit juice mix that contained fruit pulp of camu camu at 5%. The results indicate that the tropical fruit juice mix provided anticancer activity against HepG2 cell line because the proliferation ratio was inhibited in a dose-dependent manner (from 10 to 80%, approximately). Also, there was no cytotoxic effect up to 150 mg/mL, suggesting that the anticancer effect was independent of cytotoxicity. These health-promoting actions were associated with the high levels of vitamin C and anthocyanins (i.e., cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, cyanidin-3-*O*-rhamnoside, etc.) present in the tropical plant beverages that together contribute to the great antioxidant capability, consequently decreasing the free radicals to the lowest level and then the risk of cancer. The second study conducted by Asmat and Benites [86] used an *in vivo* experimental model. In this study, one group of *R. norvegicus* (Albinus strain) was injected subcutaneously with 21 mg/kg of 1,2-dimethylhydrazine from week 2 to 22 to induce colorectal cancer and co-treated from week 1 to 32 with fruit extract (containing fruit pulp and peel) at a dosage of 4.37 g/kg. The control group also received treatment to develop colorectal cancer but received a standard diet and water *ad libitum* for 32 weeks. The fruit extract of camu camu interfered with the progression to histological alterations such as metaplasia. In contrast, the control group showed major structural damage, pseudostratification, necrosis, and high mitotic index. Once again, this demonstrated the multiple beneficial properties of camu camu fruits.

3.5. Hepatoprotective activity

Akachi et al. [18] conducted experiments with *R. norvegicus* (Wistar strain), which were fed for 7 days with lyophilized fruit juice of camu camu or one of 11 other fruit juices (*Averrhoa carambola*, *Citrus depressa*, *Hippophae rhamnoides*, *Hylocereus costaricensis*, *Litchi chinensis*,

Malpighia glabra, *Passiflora edulis*, *Punica granatum*, *Ribes nigrum*, *Vaccinium corymbosum*, and *V. oxycoccus*). The liver of the rats was then injured by the injection of D-galactosamine (GalN). Only the juice of camu camu significantly suppressed the GalN-induced liver injury. This hepatoprotective activity was due to the active compound 1-methylmalate, which was isolated by bioassay-guided solvent fractionation and silica gel column chromatography of the camu camu fruit juice. To date, however, the molecular mechanism by which this active compound suppresses GalN-induced liver injury remains undiscovered. Similarly, the specific metabolic pathway and enzymes involved in the biosynthesis of 1-methymalate in camu camu are unknown. It is probable that the Krebs cycle provides malate for the biosynthesis of this active compound, and specific S-adenosyl-L-methionine-dependent methylation of carboxyl groups methyl transferase adds the methyl moiety in carbon 1 of malate, which remains undiscovered.

3.6. Neuroprotective and immunological effects

The effect of hot air-dried residue of camu camu fruits (seeds, peel, and residual pulp) on the neuroprotective effects in experimentally induced neurodegeneration was evaluated in *Caenorhabditis elegans* transgenic models for Alzheimer's disease and Parkinson's disease [87]. Treatments with low molecular weight fraction of hot air-dried residue significantly extended the life span in *C. elegans* by 20% and delayed $A\beta_{1-42}$ aggregation induced paralysis by 21% in the Alzheimer's disease model. Additionally, in the 1-methyl-4-phenylpyridinium-induced oxidative dopaminergic neurotoxicity model for Parkinson's disease treatment with the same fraction of the fruit extract resulted in significant abrogation in neurotoxicity by 15–21%. These health-relevant effects were inferred mostly due to polar acidic low-molecular-weight bioactive fractions.

Recently, a Brazilian research group [88] evaluated the effect of the oral administration of the fruit powdered pulp extract in immunological parameters in *Oreochromis niloticus* (Nile tilapia). Fishes were fed for 5 weeks using various dosages (0–500 mg of camu camu extract/kg of feed). At the end of the trial period, fishes were inoculated in the swim bladder with the pathogenic bacteria *Aeromonas hydrophila* to induce an acute aerocystitis. The immunological parameters were then analyzed after 6, 24, and 48 h of the infection. Results revealed that fish supplemented with camu camu fruit extracts had significantly increased immunological responses by increasing the white blood cells counts and exudate (lymphocytes, monocytes, neutrophils, and thrombocytes), leukocyte respiratory burst, serum lysozyme activity, serum bactericidal activity, direct agglutination, and melanomacrophage centers count. Notably, an increase in fish growth after 5 weeks, especially, at a dose of 500 mg/kg was detected.

Also in pre-clinical studies using *R. norvegicus* (Holtzmann strain) with oral administration of an aqueous extract of fruit pulp (5 and 10%) by 5 days, the authors displayed a greater immunostimulatory activity in a dose-dependent manner, after the treatments with the vegetable beverage [89]. This immune stimulant action was in two ways, first was by increasing the number of circulating mature lymphocytes (~2 times) and the second mechanism was by stimulating the phagocytic activity of the reticulum endothelial system (in average 75.71%).

3.7. Antibacterial and antiparasitic activities

Recently, several research groups have investigated the antibacterial and antiparasitic activities of botanical extracts from camu camu. With respect to antibacterial activity, the first report was from a Japanese research group (Myoda et al. [19]) who showed that the methanolic extracts (100% methanol) from fruit peel and seeds have strong antimicrobial activity against *Staphylococcus aureus* at 5 mg/mL, suggesting that lipophylic chemicals are responsible for the selective antistaphylococcal activity. Also, a Peruvian research group reported the antistaphylococcal activity of hydroalcoholic extracts (70% ethanol) derived from leaves and bark [90]. Again, Brazilian researchers recorded antistaphylococcal activity of the lyophilized pulp, spouted bed dried pulp, and spray-dried pulp with minimum inhibitory concentration (MIC) of extracts of 0.08 mg/mL [26, 91]. Similarly, another Brazilian research group showed the antistaphylococcal activity of the fruit industrial residue (seeds and peel). Both fresh, freeze dried, and hot air dried residues showed higher inhibition zones (>10 mm) and lower MIC (0.31–2.50 mg/mL) against *S. aureus*. In addition, the polyphenolic-rich fractions provided these antibacterial activities with inhibition zones from 13.1 to 16.1 mm and MIC value of 2.5 mg/mL. Furthermore, another Peruvian research group demonstrated that methanolic extracts from pulp and seeds possess higher antibacterial effects against the cariogenic bacteria *Streptococcus mutans* and *S. sanguinis*. The MIC of the pulp extract showed a range of 50–75 μg/mL; however, the seed antibacterial activity was detected at very low levels [92]. Finally, recently another Japanese research group isolated four polyphenolic antimicrobial constituents (acylphloroglucinols) from the n-hexane extracts of peel and seeds, these compounds were Isomyrtucommulone B, Myrciarone A, Myrciarone B, and Rhodomyrtone. The second and third compounds were confirmed to be new acylphloroglucinols [20]. The four compounds showed antimicrobial activities against Gram-positive bacteria such as *Bacillus subtilis*, *B. cereus*, *Micrococcus luteus*, *S. aureus*, *S. epidermidis*, and *S. mutans* but were inactive against Gram-negative bacteria and fungi. Several investigations at transcriptomic, proteomic, and metabolomic levels have revealed the molecular mechanism involved in the antimicrobial activity of Rhodomyrtone against Gram-positive bacteria [93–96].With respect to antiparasitic activities, researchers from England [51, 97] reported that a mixture of 10 myricetin and quercetin glycosides isolated from the aqueous acetic acid–soluble fraction of methanolic extracts of camu camu were potent inhibitors of the GSH-haemin reaction. Also, the aqueous ethanolic extract (70%) of fruit peel, leaves, and seeds exhibited antiplasmodial activity with the ferriprotoporphyrin inhibition test with IC_{50} < 5.0 μg/mL [50]. Additionally, the aqueous and the ethanolic (70%) extracts from the bark displayed inhibitory activity against the *Plasmodium falciparum* strain FCR3 (chloroquine resistant) with IC_{50} values of 3 and 6 μg/mL, respectively [98]. In addition, the dichloromethanolic extract of camu camu leaves exhibited a significant in vitro growth inhibition of *P. falciparum* and *Leishmania amazonensis* with IC_{50} values of 1.05 and 6.41 μg/mL, respectively [99].

3.8. Other bioactivities

Animal experiments using *R. norvegicus* to test the effect of a fruit extract of camu camu alone and in combination with a powdered tuber extract of black maca (*Lepidium meyenii*) showed

that camu camu fruits extract significantly increased the daily sperm production, stages IX and XI of mitosis and stage XII of meiosis. In combination with black maca, spermiation stages, mitosis, and meiosis were increased. The authors concluded that camu camu fruits potentially improve spermatogenesis and mixing with black maca tubers increased the stages of mitosis, meiosis, and spermiation of the spermatogenic cycle as assayed by the transillumination technique [100].

Additionally, two *in vitro* investigations demonstrated that the hydroalcoholic and aqueous fruit extracts (containing peel, pulp, and seeds) of camu camu could be added in cosmetic formulations, since fruit extracts empower the sun protection factor against UVB radiation. This peculiar characteristic is attributable to the high levels of vitamin C and phenolic compounds in the fruit [101, 102].

Finally, a research of Yuyama et al. [103] demonstrated the beneficial impacts of mixing fruits pulp of camu camu and *Euterpe oleracea* to treat preschool children (2–6 years old) with anemia and chronic malnutrition.

4. Functional genomic characteristics

4.1. Transcriptome *de novo* asembly and annotation

Recently, our research group used a total of 24,551,882 high-quality reads to assemble the fruit (unripe, semiripe, and ripe) transcriptome of camu camu. In total 70,048 unigenes were obtained in the meta-assembly (mean length of 1150 bp and N_{50} = 1775 bp). These unigenes were annotated by searching homologous sequences in multiple databases (i.e., NCBI nonredundant (nr), UniProtKB, TAIR, GR_protein, FB, MGI, etc.). The top three plant species that contributed the greatest number of gene annotations were *Vitis vinifera*, *Theobroma cacao*, and *Populus trichocarpa*. Of the three core GO annotation categories, biological processes comprised 53.6% of the total assigned annotations, whereas cellular components and molecular functions comprised 23.3 and 23.1%, respectively. In total, 160 metabolic pathways were reconstructed [48].

4.2. Metabolic pathways for vitamin C biosynthesis

Based on the fruit transcriptome analysis, five metabolic pathways for vitamin C biosynthesis [48] were reconstructed: animal-like pathway, myo-inositol pathway, L-gulose pathway, D-mannose/L-galactose pathway, and uronic acid pathway. Gene coding enzymes involved in the ascorbate-glutathione cycle were also identified (**Figure 5**). From these pathways, the D-mannose/L-galactose pathway is the best characterized in several plant species [104–106]. This pathway involves the sequential enzymatic conversion of D-mannose-1-phosphate in to Vitamin C. These enzymatic reactions are as follows: GDP-D-mannose synthesis from D-mannose-1-phosphate and GTP is catalyzed by GDP-D-mannose pyrophosphorylase (E.C. 2.7.7.13), and then, GDP-D-mannose is converted to GDP-L-galactose by a reversible double epimerization, catalyzed by GDP-mannose-3′,5′-epimerase (E.C. 5.1.3.18); further, GDP-L-galactose is

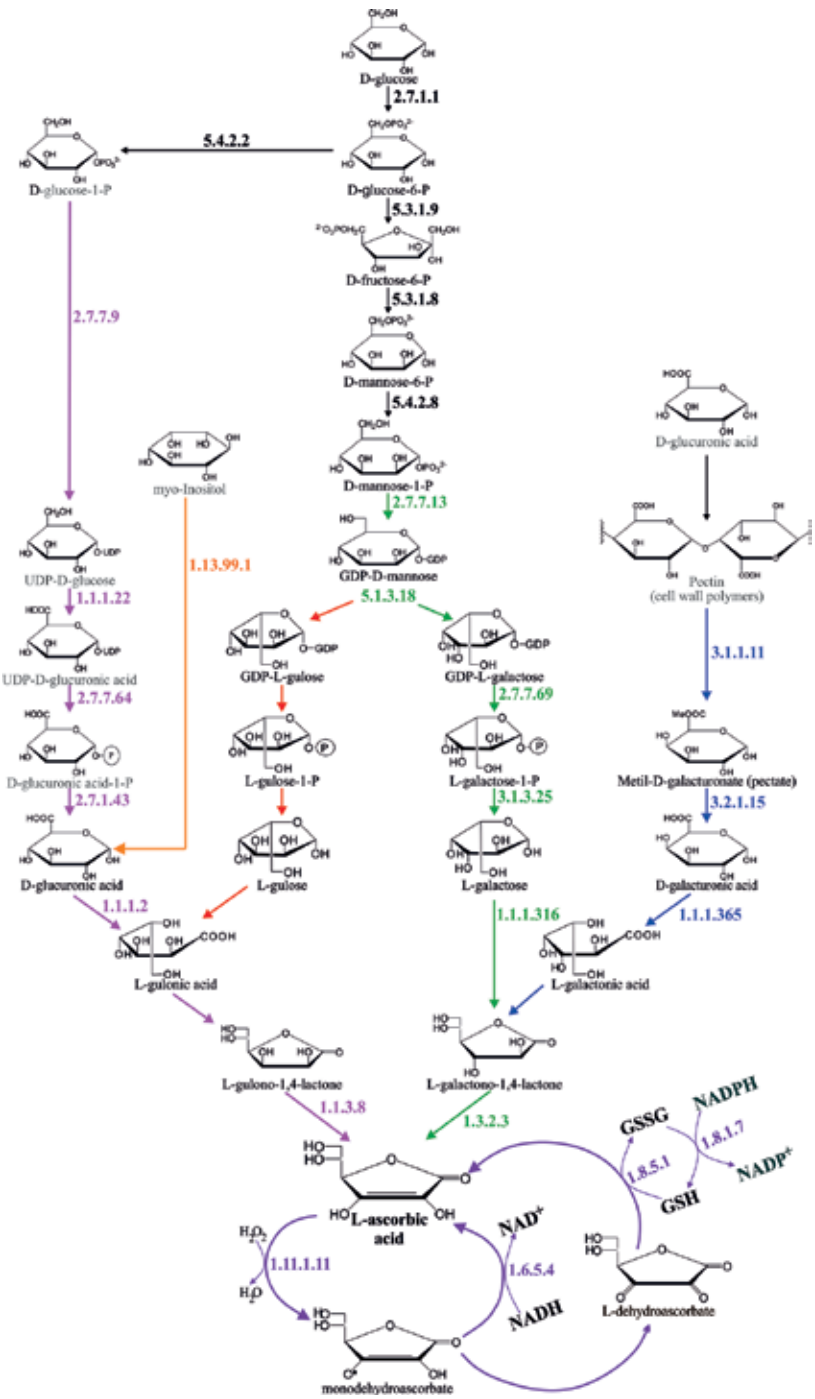

Figure 5. Vitamin C biosynthesis and recycling pathways reconstructed from the fruit transcriptome database of camu camu. Source: Castro et al. [48].

transformed by GDP-L-galactose:hexose-1-phosphate guanyltransferase (E.C. 2.7.7.69) to L-galactose-1-phosphate, which is subsequently hydrolyzed to L-galactose and inorganic phosphate by L-galactose-1-phosphate phosphatase (E.C. 3.1.3.25). L-galactose is next oxidized to L-galactono-1,4-lactone by the NAD-dependent L-galactose dehydrogenase (E.C. 1.1.1.316), and finally, L-galactono-1,4-lactone is oxidized to vitamin C by L-galactono-1,4-lactone dehydrogenase (E.C. 1.3.2.3).

4.3. Metabolic pathways involved in health-promoting phytochemicals biosynthesis

As previously mentioned, most of the health-promoting phytochemical compounds identified in camu camu are specialized metabolites, commonly known as secondary metabolites. Biosynthesis of these structural diverse molecules starts from key basic pathways, for instance the Embden-Meyerhof-Parnas pathway (also known as the glycolysis), pentose phosphate pathway, and the Shikimate pathway. The latter pathway produces chorismate, a common precursor for the tryptophan pathway, the phenylalanine/tyrosine pathways, and the metabolic pathways for the biosynthesis of folate, salicylate, and phylloquinone [107]. Subsequently, these three aromatic amino acids are used as biosynthetic precursors in several metabolic pathways to produce a diverse array of secondary metabolites (i.e., terpenoids, phenolic compounds such as flavonols, anthocyanins, ellagic acid and derivatives, ellagitannins, gallic acid and derivatives, etc.), depending on several biological and environmental factors [108]. From the annotated fruit transcriptome of camu camu, we were able to reconstruct more than 160 metabolic pathways [48]. These include several pathways involved directly in secondary metabolite biosynthesis, for example, the anthocyanins, carotenoids, flavonoids, phenylpropanoids, and terpenoids biosynthesis pathways. The universal biosynthetic precursor (chorismate) for all these pathways is synthesized in the Shikimate pathway (**Figure 6**). In this pathway, seven enzymatic reactions biochemically transform phosphoenolpyruvate (PEP) and D-erythrose 4-phosphate (metabolic intermediates in glycolysis and the pentose phosphate pathways, respectively) to chorismate. The first committed step of the shikimate pathway is an aldol condensation of phosphoenolpyruvate (PEP) and D-erythrose 4-phosphate to produce 3-Deoxy-D-arabino-heptulosonate-7-phosphate (DAHP), this reaction is catalyzed by DAHP synthase (E.C. 2.5.1.54). Further, 3-Dehydroquinate synthase (E.C. 4.2.3.4) converts DAHP to 3-dehydroquinate using a divalent cation (i.e., Co^{2+}) and NAD+ cofactors via five consecutive chemical reactions: alcohol oxidation, β-elimination of inorganic phosphate, carbonyl reduction, ring opening, and intramolecular aldol condensation. The third enzymatic reaction catalyzed by 3-dehydroquinate dehydratase (E.C. 4.2.1.10) includes the dehydration of 3-dehydroquinate to 3-dehydroshikimate to introduce the first double bond in the ring, and the fourth reaction catalyzed by shikimate:$NADP^+$ oxidoreductase (E.C. 1.1.1.25) is a reversible reduction of 3-dehydroshikimate into shikimate using NADPH. The fifth enzyme (shikimate kinase [2.7.1.71]) catalyzes the phosphorylation of the C3 hydroxyl group of shikimate using ATP as inorganic phosphate donor to yield shikimate-3-phosphate. Then, 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase (E.C. 2.5.1.19) catalyzes the formation of EPSP, by transferring the enolpyruvyl moiety of PEP to the 5-hydroxyl position of shikimate-3 phosphate. Finally, chorismate synthase (E.C. E.C. 4.2.3.5), the last enzyme of the shikimate

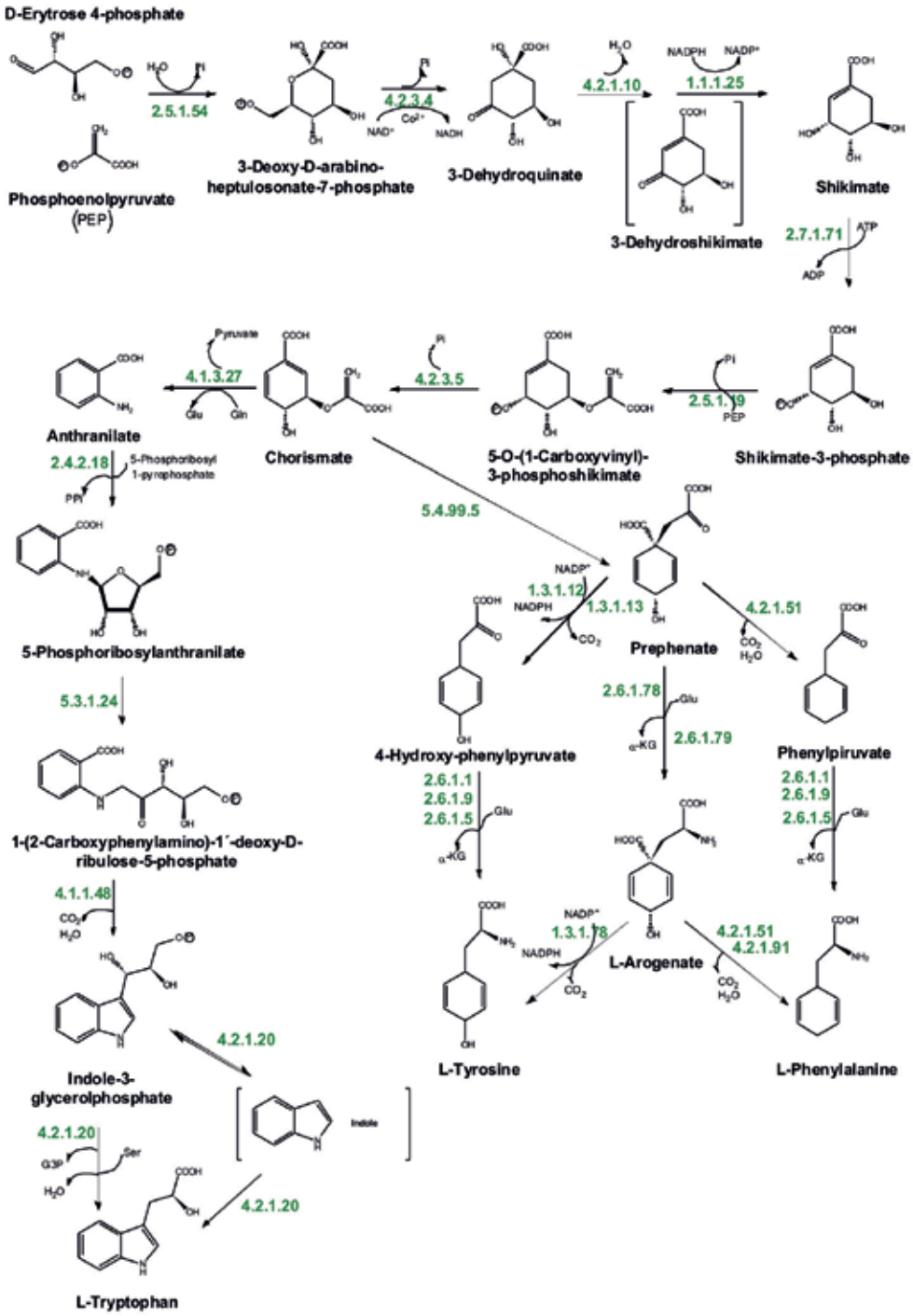

Figure 6. The Shikimate pathway reconstructed from the fruit transcriptome database of camu camu.

pathway, is itself biochemically unique in nature and catalyzes a 1,4-antielimination of the 3-phosphate group and C6-pro-R hydrogen from EPSP, introduces the second double bond in the ring to produce chorismate [107].

5. Domestication strategy and efforts for genetic improvement

Some Peruvian research Institutions such as the National Institute of Agricultural Innovation (INIA), the Research Institute from the Peruvian Amazon (IIAP), and the Veterinary Institute for Tropical and High Altitude Research (IVITA), as well as Brazilian research Institutions such as the National Institute of Amazonian Research (INPA) and the Brazilian Agricultural Research Corporation (EMBRAPA), have implemented programs for *ex situ* conservation of camu camu. These genetic conservation programs involve the establishment of germplasm banks composed by accessions of botanical material collected from wild populations. In Peru, germplasm banks were established about 37 years ago [109] from seeds (due to the lack of vegetative propagation techniques) obtained from only 40% of the wild populations in the Loreto region (Imán S., personal communication, September 15, 2017). Consequently, further prospecting and collecting of botanical samples need to be carried at regional and distribution wide levels to maximize greatest genetic diversity. The efficiency of increasing banked accessions could be improved by incorporating vegetative propagation techniques. The two most plausible alternatives are improved grafting and root cuttings techniques, developed and refined by Peruvian and Brazilian researchers [110–112].

The domestication process of camu camu was promoted by INIA and IVITA at the beginning of the 1990s with the installation of seven demonstration parcels in the community of Santa Ana, which are located in the Amazon River ~30 km from Iquitos [109]. Further, since the beginning of the twenty-first century, INIA in association with IIAP implemented a genetic improvement program using an active community participation strategy and conventional plant breeding methods, based on Mendelian principles of inheritance [109]. This improvement program was focused on an ideotype characterized by precocity of fructification (beginning with the third year after germination, but with ≥0.5 kg of fruits per plant), high vitamin C content in fruit pulp (≥ 2.0 g per 100 g of fruit pulp), and larger fruits (fresh weight ≥ 10 g). The promoters of these programs touted that the first generation of genetically superior plants would be ready by 2010 and superior homozygous lines by 2016. Thus far, none of these goals have been achieved.

To overcome these drawbacks, a radical redefinition of ideotypes is necessary. Our current knowledge affords us the opportunity to create comprehensive ideotypes that is built upon detailed knowledge of plant genetics, biochemistry, physiology, anatomy, morphology, phenology, and ecology [113]. Additionally, including the state of the art technologies for multiomic data analysis (i.e., genomic, epigenomic, transcriptomic, proteomic, metabolomic, phenomic, etc.) will enable the rational design and application of innovative strategies for the domestication

and the genetic improvement program for camu camu. For example, using genome editing tools such as clustered regularly interspaced short palindromic repeats/associated protein-9 nuclease [CRISPR/Cas9 system], transcription activator-like effector nucleases [TALENs], and zinc finger nucleases [ZFNs] could be the molecular tools of choice to achieve the desired ideotypes [114–118], after obtaining the complete genome sequence of camu camu.

To accelerate the domestication and genetic improvement program to obtain *de novo* elite commercial varieties, the five-step strategy of genomics-based plant germplasm research recommended by Jia et al. [119] should be implemented: (1) the detection of genomic diversity in germplasm banks, (2) the conservation and protection of germplasm based on the knowledge of genomic diversity, (3) the use of diversity information to design a representative core collection, (4) the enhancement of germplasm banks using core collections, and (5) the discovery of new alleles and/or genes in the core collections.

To date, our research team has generated fruit transcriptome data and identified several of the genes involved in vitamin C biosynthesis that have proved to be polymorphic. For example, the D-mannose/L-galactose pathway mannose-1-phosphate guanylyltransferase (E.C. 2.7.7.13) contained >20 SNPs, GDP-mannose-3′,5′-epimerase (E.C. 5.1.3.18) had 13 SNPs, whereas L-galactono-1,4-lactone dehydrogenase (E.C. 1.3.2.3) only had 5 SNPs. The animal-like pathway UTP:glucose-1-phosphate uridylyltransferase (E.C. 2.7.7.9) contained 7 SNPs. In the uronic acid pathway pectin esterase (E.C. 3.1.1.11) and galacturan-1,4-alpha-galacturonidase (E.C. 3.2.1.15) showed more than 20 and 14 SNPs, respectively. Finally, in the ascorbate-glutathione pathway, the unigenes monodehydroascorbate reductase (E.C. 1.6.5.4) and glutathione reductase (E.C. 1.8.1.7) contained 2 and 3 SNPs, respectively [48]. It is likely that these mutations are associated with the high variation of vitamin C production reported between both individuals and populations of camu camu [13], as well as the differential gene expresssion and enzyme activities of the D-mannose/L-galactose pathway [120]. Our research group is currently finishing the transcriptome analysis of plantlets after germination and initial growth process and a draft genome sequence (using PacBio and Illumina technology) and annotation of camu camu. These forthcoming as well as previous functional and structural genomic resources will greatly accelerate the domestication process and the genetic improvement program of camu camu.

Acknowledgements

We thank Dr. Jorge L. Marapara for his help with the infrastructure and equipment of Unidad Especializada de Biotecnología and Instituto Nacional de Innovación Agraria (INIA) - San Roque-Iquitos for access to the germplasm collection of camu camu. Special thanks to our students (Jhoao Flores, Jhon Vargas, Stalin Tirado, and Andry Mavila) for their great support in the design of maps, chemical structures, and metabolic pathways.

Author details

Juan C. Castro[1]*, J. Dylan Maddox[2,3], Marianela Cobos[4] and Sixto A. Imán[5]

*Address all correspondence to: juan.castro@unapiquitos.edu.pe

1 Specialized Unit of Biotechnology, Research Center of Natural Resources of the Amazon (CIRNA), National University of the Peruvian Amazon (UNAP), Iquitos, Perú

2 Pritzker Laboratory for Molecular Systematics and Evolution, The Field Museum of Natural History, Chicago, IL, USA

3 Environmental Sciences, American Public University System, Charles Town, WV, USA

4 Laboratory of Biotechnology and Bioenergetics, Scientific University of Peru (UCP), Iquitos, Perú

5 Conservation Area of Plant Genetic Resources, National Institute of Agricultural Innovation (INIA), Iquitos, Perú

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Chapter 6

Qualitative and Quantitative Assessment of Fatty Acids of Hazelnut by GC-TOF/MS

Jian Ding, Chengjiang Ruan, Ying Guan and Susan Mopper

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73016

Abstract

On the basis of gas chromatography coupled with time-of-flight mass spectrometry, we assessed the constituents and relative quantities of fatty acids extracted by supercritical carbon dioxide in seeds of hazelnut. Hazelnut seeds contain four fatty acids (palmitic, stearic, oleic, and linoleic acids). The content of unsaturated fatty acids is more than 92.9% in hazelnut seed oil. Oleic acid, which constitutes 76.1%, has a high boiling point and low volatility. Hazelnut oil has good storage stability and is recommended as senior edible oil for health and the food industry. Our study reveals the important contribution of hazelnut in the production of bioactive oils and compounds that prevent obesity, cancer, coronary disease, and many other human health as well as pharmaceutical challenges.

Keywords: hazelnut, fatty acid, gas chromatography coupled with time-of-flight mass spectrometry, supercritical carbon dioxide extraction

1. Introduction

The expanding population and improved living standards have increased the demand for edible vegetable oils. In 2016/2017, the global consumption of vegetable oils amounted to 168.53 million metric tons, compared to just 71.7 million metric tons in 1995/1996 [1]. The fastest increase has occurred in China, which in 2014 was 31.67 million metric tons, about 3.2 times greater than the consumption in 1996 [2]. Over the past decade, obesity rates, cerebrovascular, coronary disease, and cancers have increased dramatically [3–5]. This is because the changes in diets and lifestyles resulting from industrialization and market globalization have increased rapidly. However, a general improvement in the standard of living often has been

accompanied by unhealthy dietary patterns and insufficient physical activity to maintain an optimal energy balance and a healthy weight. The net result has been increased prevalence of diet-related chronic diseases.

Fatty acids form the building blocks of lipid molecules, contribute to the structure of cell membranes and hormones, and provide cells with energy [6]. Palmitic acid (C16:0) and stearic acid (C18:0) are common saturated fatty acids (SFA) in edible oils and are thought to raise blood cholesterol and low-density lipoprotein (LDL) levels, leading to many diseases [7]. Monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are considered a healthy source of dietary fat for humans. Originally, the American Heart Association (AHA) recommended a fatty acid balance of approximately 1:1.5:1 ratio of SFA:MUFA:PUFA [8]. Oleic acid (an omega-9 MUFA) is defined as "conditionally essential," because it can be synthesized *in vivo*. Any amount of omega-9 is beneficial [9]. Linoleic acid (an omega-6 PUFA) is a desirable and an essential fatty acid, as humans cannot synthesize double bonds in the n-6 positions of their hydrocarbon chains.

Hazelnut (*Corylus avellana*) is the second most popular nut worldwide, and it is distributed in several areas of Europe and Asia [10–14]. Hazelnut seeds are high energy food rich in fats as well as proteins. Seed oil contents range from 41.96 to 63.73% of hazelnut kernel dry weight [11], and fatty acids of hazelnut are similar in composition to those of olive oil. The nutrition and health benefits of UFA in hazelnut oils can reduce or prevent cancer, cardiovascular, and autoimmune diseases, and have anti-ulcerogenic, regenerating, and anti-inflammatory properties [3, 10–13]. Hazelnut seeds contain high concentrations of bioactive compounds (such as tocopherols, polyphenolics, neolignans, and 1,1-diphenyl-2-picrylhydrazyl radical) [10–14]. These are valuable sources of phytonutrients, fiber, and antioxidants [15].

Few studies have compared the fatty acid composition of seed oils extracted by supercritical carbon dioxide extracting method (SCDE) and commercially prepared product oils. In this chapter, we (1) extracted seed oils using SCDE, (2) identified the constituents and relative contents of fatty acids in the extracted oils and in products purchased from Dihao company in China, using gas chromatography coupled with time-of-flight mass spectrometry (GC-TOF/MS), and (3) compared the differences in fatty acids between extracted oils and commercially prepared product oils. Finally, we also discuss the beneficial functions of these oils and provide useful information for producing this bioactive oil that reduces or prevents obesity, cancer, coronary disease, and many other human health as well as pharmaceutical challenges.

2. Materials and methods

2.1. Chemicals and reagents

Carbon dioxide gas (99.999%) was purchased from Airichen (Dalian, China). Hexane, methanol, and methylene chloride (≥97%, GC grade) were obtained from Honeywell (Ulsan, Korea). The boron trifluoride and fatty acid methyl ester mix was obtained from Sigma-Aldrich (Steinheim, Germany).

http://dx.doi.org/10.5772/intechopen.73016

2.2. Materials

We collected seeds of hazelnut hybrids (*Corylus heterophylla* × *C. avellana*) from Liaoning Provinces of China (**Figure 1A**). Oils from seeds were extracted by SCDE (**Figure 1C**). Seeds were stored in closed plastic bags in dark at 4°C. Commercially prepared seed oils were provided by Dihao (Liaoyang, China) company (**Figure 1C**).

2.3. Oil extraction by supercritical carbon dioxide

Seed samples were powdered using laboratory plant grinder and air-dried [16]. Ground sample (100 g) was then placed into the extraction kettle of SFT-110 supercritical carbon dioxide extracting device from Supercritical Fluid Technologies, Inc. (Newark, USA). The pressure parameter and temperature of kettle heating were set at 5500 PSI and 60°C, respectively. The carbon dioxide flow and extraction time followed was 18 mL·min^{-1} [17].

2.4. Determination of fatty acids

Fatty acid profile was determined as fatty acid methyl esters (FAME) by gas chromatography. The methyl esters were prepared according to Wang et al. [18] and Sanchez-Salcedo et al. [6] with some modifications. Twenty milligram of oil was added to a test tube, followed by the addition of 2 mL of n-hexane and 5 mL of methanol-potassium hydroxide solution (1 M). The mixture was placed in a blender shock with water bath at 60°C for 30 min. After the reaction, 10 mL of boron trifluoride (BF_3) in methanol was added to the mixture, and the samples were left at 60°C for 30 min. FAME were then extracted using saturated sodium chloride solution (2 mL) and n-hexane (2 mL) through vigorous shaking for 1 min. Top layer was transferred into a vial and stored at −20°C.

The fatty acid compositions were analyzed using a Clarus 680 GC coupled with AxION iQT TOF/MS system (PerkinElmer, Shelton, USA). The system was equipped with Agilent J&W DB-23 capillary column (60 m × 0.25 mm × 0.25 μm). The flow rate of carrier gas (Helium) was 1 mL·min^{-1} with a split mode (1:20). The temperature program was started at 50°C, raised

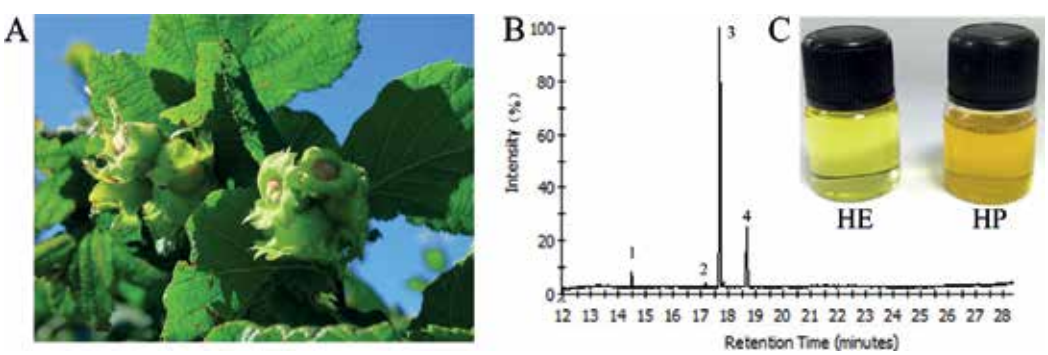

Figure 1. Fruits (A), fatty acid composition (B), and oils (C) of hazelnut. HE, oils extracted from seeds of hazelnut; HP, commercially prepared product oil of hazelnut seed.

to 200°C at 15°C·min^{-1} up, and finally to 230°C for 10 min. The temperature of EI ion source was 230°C, and the injection volume was 1 μL. Fatty acids were identified based on the mass spectra of 37 FAME standards. Fatty acid composition was expressed using peak area normalization method. All the analyses were conducted in three replicates.

2.5. Statistical analysis

The results were expressed as mean ± standard deviation (n = 3). The p-value ≤0.05 was used to denote significant differences between mean values determined by one-way analysis of variance (ANOVA). All statistical analyses were performed using SPSS Statistics 20.0 software (IBM SPSS Statistics 20.0, Armonk, NY, USA) [19].

3. Results and discussion

3.1. Hazelnut species and distribution in China

C. America, *C. avellana*, *C. colurna*, and *C. mandshurica* are widely distributed species in the world. The world's hazelnut production is mainly covered by two main market players (Turkey and Italy). Turkey is the major hazelnut (*C. avellana*) producing country, supplying 65% of the world's total production. However, USA, Azerbaijan, Georgia, China, Iran, Spain, France, Kirgizstan, Poland, and Croatia are smaller but significant producers [15, 20]. *C. mandshurica*, also known as pilose hazelnut, is an economically and ecologically important species in China [21]. There is currently more than 4 million acres of natural hazel groves in northeastern China alone. Zong et al. [21] applied 10 polymorphic simple sequence repeat (SSR) markers to evaluate the genetic diversity and population structure of 348 *C. mandshurica* individuals among 12 populations in China and found that there was obvious genetic differentiation among populations from Northeast China to North China. The hybrid varieties of *C. heterophylla* and *C. avellana* have been widely planted in North China because of the cold resistance and high yield, which are superior to *C. avellana* in terms of unsaturated fatty acid content and antioxidant activity [22, 23].

3.2. Fatty acid composition in hazelnut seed oils

Hazelnuts are a high energy food with functional fats and proteins, which are the main components of the hazelnut kernel. The lipid portion represents a major determinant of kernel flavor, particularly following roasting [11]. We determined four fatty acids in hazelnut seed oils by GC-TOF/MS (**Figure 1B**). Oleic acid was the main fatty acid followed by linoleic acid, and palmitic and stearic acids were also measured in hazelnut oil. Hazelnut oil has low concentrations of SFA, and palmitic and stearic acids (4.7 and 2.4%, respectively) were quantified in hazelnut oil. Palmitic acid and SFA concentrations of oils extracted by the method of SCDE were significantly higher than in the commercially prepared product (**Table 1**).

Oleic acid (C18:1) concentrations were 76.1 and 74.5% in oils extracted from seeds of hazelnut (HE) and commercially prepared product oil of hazelnut seed (HP, **Table 1**), respectively.

Koksal et al. [20] investigated the oleic acid contents (74.2–82.8%) among 17 different hazelnut varieties grown in the Black Sea Region of Turkey. Because of its high percentage of oleic acid, hazelnut oil is stable edible oil and considered beneficial for a healthy diet. Oleic acid naturally exists in many plant and animal products and is considered one of the healthiest sources of dietary fat. It can reduce ratios of LDL/HDL and triglycerides in the blood, prevent coronary heart disease, hypertension, cerebrovascular disease, arteriosclerosis, stomach ache, and burn injuries; a high MUFA diet is recommended in diabetes mellitus patients [24].

PUFA are precursors of potent lipid mediators, important structural components of cell membranes, and play an important role in inflammation regulation and cell function [25]. Omega-6 fatty acids are necessary for healthy brain function, skin and hair growth, bone density, energy production, and reproductive health. Meat, eggs, and nut-based oils are the main dietary sources of omega-6 fatty acids [26]. The linoleic acid (one of omega-6 fatty acids) concentrations in HE and HP oils was 16.8 and 19.7%, respectively (**Table 1**). The linoleic acid concentration of HP oil was significantly higher than HE oil. Bacchetta et al. [11] found the percentage of linoleic acid ranged from 5.91 to 19.01% among 75 European hazelnut germplasm oil samples and detected its content was inversely correlated with oleic acid, because oleic acid is the precursor of linoleic and linolenic acids [11]. Because of the high level of oleic and linoleic acids, the composition of TUFA was more than 92%

Fatty acids	HE	HP	Dif.	Std.	Student *t*
Palmitic acid (C16:0)	4.73 ± 0.03	3.33 ± 0.22	1.40	0.16	0.71**
Stearic acid (C18:0)	2.40 ± 0.08	2.39 ± 0.14	0.01	0.11	0.07
Oleic acid (C18:1)	76.07 ± 0.79	74.54 ± 0.62	1.53	0.71	2.66
Linoleic acid (C18:2)	16.80 ± 0.84	19.74 ± 0.98	−2.94	0.91	3.96*
SFA	7.13 ± 0.05	5.72 ± 0.36	1.41	0.26	6.72*
MUFA	76.07 ± 0.79	74.54 ± 0.62	1.53	0.71	2.66
PUFA	16.80 ± 0.84	19.74 ± 0.98	−2.94	0.91	3.96*
TUFA	92.87 ± 0.05	94.28 ± 0.36	−1.41	0.26	6.72*
MUFA/SFA	10.67 ± 0.04	13.03 ± 0.72	−2.39	0.51	5.79*
PUFA/SFA	2.35 ± 0.14	3.45 ± 0.39	−1.11	0.29	4.72**

Data expressed as mean ± standard error (n = 3); nd, not detected; HE, oils extracted from seeds of hazelnut; HP, commercially prepared product oil of hazelnut seed; SFA, saturated fatty acids, are the sum of palmitic and stearic acid; MUFA, monounsaturated fatty acids, are the sum of stearic, eicosenoic, erucic, and nervonic acids; PUFA, polyunsaturated fatty acids, are the sum of linoleic and linolenic acids; MUFA/SFA, monounsaturated/saturated fatty acids ratio; PUFA/SFA, polyunsaturated/saturated fatty acids ratio; Dif., difference between oils extracted from seeds and commercially prepared product oils; Std, standard deviation (n = 3)

*$P < 0.05$.

**$P < 0.01$.

Table 1. Fatty acid composition (weight % of total fatty acids) and comparison of the mean values (%) of fatty acids between the oils extracted from seeds and the commercially prepared product oils.

(**Table 1**). Ciemniewska-Zytkiewicz et al. [15] quantified the concentrations of TUFA and oleic acid as 94.01 and 80.25%, respectively, in hazelnut seed oil, which were higher than the previous reports.

3.3. Fatty acid ratios in hazelnut seed oils

Fatty acids and their ratios affect lipid oxidation and physiological functions of oils and fats [7, 8]. Hazelnut oils have low concentrations of SFA and high concentrations of MUFA. The main sources of SFA in food are animal products (such as milk, meat, salmon, and egg yolks) and some plant products (such as chocolate and cocoa butter, coconut, and palm kernel oils). In modern time, people can easily consume sufficient SFA. SFA are thought to raise total cholesterol (TC) and low-density lipoprotein (LDL), which are undesirable to human health. But certain SFA (as consumed in our daily diet) have beneficial effects on the ratio of LDL to high-density lipoprotein (HDL) [7]. Recently, Souza et al. [27] and Mancini et al. [28] showed that saturated fat intake was not associated with mortality, cardiovascular disease, coronary heart disease (CHD), ischemic stroke, or type 2 diabetes, whereas trans fats were associated with mortality, total CHD, and CHD mortality, probably because of higher levels of intake of industrial trans fats than ruminant trans fats. No trans fatty acid occurred in hazelnut oil, and it had high MUFA/SFA (10.67) and low PUFA/SFA ratio (2.35) in HE oil (**Table 1**). So hazelnut oil has a high boiling point and low volatility and can improve the nutritional quality and shelf-life of processed foods [11]. Hazelnut oil has good storage stability and is recommended as senior edible oil for health and the food industry.

In addition, the abundant tocopherol, sterol, phenolic compounds, fiber, and antioxidants such as Vitamin E were also determined [29]. Hazelnut oil is suitable for cooking, salad oils, and for the manufacture of margarine. Meanwhile, a high level of MUFA and a low quantity of SFA in hazelnut oil enhance its usefulness in food as well as oleochemical applications [29].

4. Conclusions

Hazelnut seed oil contains four fatty acids, and the content of unsaturated fatty acid is more than 92.9% in hazelnut seed oil. The oleic acid concentration is 76.1%, which has a high boiling point and low volatility. Hazelnut oil is recommended as senior edible oil for health and the food industry. Our study reveals the important contribution of hazelnut for producing bioactive oils and compounds that reduce or prevent obesity, cancer, coronary disease, and many other human health as well as pharmaceutical challenges.

Acknowledgements

This research was funded by the National Natural Science Foundation of China (Project No. 31570681) and a Marie Curie International Incoming Fellowship from the 7th European Community Framework Program (Project Nos. PIIF-GA-2010-272048 and PIIFR-GA-2010-910048).

Author details

Jian Ding[1], Chengjiang Ruan[1]*, Ying Guan[2] and Susan Mopper[3]

*Address all correspondence to: ruan@dlnu.edu.cn

1 Institute of Plant Resources, Key Laboratory of Biotechnology and Bioresources Utilization, Ministry of Education, Dalian Minzu University, Dalian, P.R. China

2 Institute of Berries, Heilongjiang Academy of Agricultural Sciences, Suiling, P.R. China

3 Department of Biology, University of Louisiana, Lafayette, USA

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Chapter 7

Clinical Applications of Pomegranate

Sally Elnawasany

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75962

Abstract

Pomegranate, *Punica granatum* L., is an ancient, unique fruit borne on a small, long-living tree in the Mediterranean region, Southeast Asia, and tropical Africa. Pomegranate was mentioned in ancient times in the Old Bible, the Jewish Torah, and mentioned three times in the holy Quran where it was described as one of the paradise fruits. In ayurvedic medicine, pomegranate is used in the treatment of parasitic infection, diarrhea, and ulcers. Recently, pomegranate has been studied in several systems of medicine for its pharmacological actions: anti-inflammatory, antioxidant, and anticarcinogenic. The aim of the chapter is to summarize pomegranate efficacy in many preclinical and clinical studies.

Keywords: pomegranate, ayurvedic medicine, pharmacological activities, preclinical, clinical studies

1. Introduction

Pomegranate, (*Punica granatum* L.), a paradise fruit, has a great value throughout history. It had been mentioned in Judaism, Christianity, and Islamic religions [1]. From ancient times, pomegranate was used in treatment of diarrhea [2], parasitic infections [3], and diabetes mellitus [4]. Greco-Arab and Islamic medicine prescribed pomegranate for sore throat, inflammation, and rheumatism [5]. Various pomegranate activities (anti-inflammatory, antioxidant, and anticancer) encouraged growing number of studies to apply it in solving multiple medical problems [6]. Pomegranate plant is a small tree (**Figure 1**) that is cultivated in the Middle East, Mediterranean region, China, India, California, and Mexico. The fruit (**Figure 2**) is composed of many parts such as seeds, peels (pericarp), pulp, and juice [6].

Figure 1. Pomegranate tree.

Figure 2. Pomegranate fruit.

2. Clinical applications

For its multiple pharmacological potential, pomegranate has been investigated by variable preclinical and clinical studies in a wide variety of health disorders:

2.1. Inflammation

Pomegranate exhibits a potent anti-inflammatory effect through inhibition of cyclooxygenase (COX) and lipoxygenase (important inflammatory mediators) [7].

2.1.1. Gastrointestinal inflammation

2.1.1.1. Gastric inflammation

2.1.1.1.1. Preclinical studies

Helicobacter pylori (*H. pylori)* are major etiological agents in peptic ulcer. Pomegranate methanol extract produced a remarkable anti-*H. pylori* activity with mean diameter of inhibition zone 39 at 100 μg $disc^{-1}$ [8]. This activity is explained by altering bacterial cell surface hydrophobicity and prevention of bacterial adhesion to gastric mucosa [9]. Moreover, pomegranate revealed gastroprotective potential via antioxidant mechanism in aspirin- and ethanol-induced gastric ulceration in animal models [10]. Gastroprotective property of pomegranate is attributed to its constituents (saponin, tannins, and flavonoids) as demonstrated in another study on wistar rats where oral administration (490 and 980 mg/kg body weight) of pomegranate aqueous methanolic extract significantly reduced gastric ulcer index in alcohol-, indomethacin-, and aspirin-induced ulcers [11]. Tannins are high molecular weight phenolic compounds present in many plants, including pomegranate fruit pericarp (peels). These compounds have the capacity to form complexes mainly with proteins [12, 13]. Pomegranate tannins form a protective layer (tannin-protein/tannin-polysaccharide complex), upon damaged epithelial tissues, thus allowing the healing process below to occur naturally through prevention of bleeding and acceleration of ulcer healing [14, 15]. All parts of the pomegranate tree have been used as a source for tannin in the leather industry, changing animal hide into leather. About 10–25% of tannin are present in the trunk bark and were important in leather production in Morocco. In this process, collagen chains in the hide are cross-linked by tannin to give leather. The formation of various complex bonds helps the tannin-protein polymer combination [16, 17]. These facts take our attention to the Islamic advice "To *eat pomegranate with its pericarp as it is tanning for the stomach*".

2.1.1.2. Intestinal inflammation

2.1.1.2.1. Preclinical studies

Inflammatory response is induced by transduction cascades initiated by many inflammatory mediators, that is, tumor necrosis factor α (TNF-α) and nuclear factor κB (NF-κB). Pomegranate inhibited TNF-α-induced NF-κB activation and COX-2 expression in colon cell line. This effect was highly presented by pomegranate juice compared to single constituents, that is, tannin and punicalagin. This highlights the synergism between all bioactive pomegranate compounds [18]. Prebiotics are food agents that stimulate the growth or activity of beneficial microorganisms. Pomegranate peel extract (6 mg/d for 4 weeks) increased the cecal pool of beneficial bifidobacteria when given to high-fat diet mice. Additionally, it counteracted the high-fat-induced expression of inflammatory markers both in the colon and in the visceral adipose tissue [19]. Through its antioxidative action, pomegranate elagic acid (EA) (10 mg/kg) in colonic-delivering microsphere significantly ameliorated the severity of colonic lesions and reduced myeloperoxidase (MPO) activity and lipid peroxidation. This effect was obtained by orally administrating it to rat model of dextran sulfate sodium (DSS)-induced ulcerative colitis [20]. Mast cells are

important inflammatory cells that release histamine. Mast cell stabilizing is an additional anti-inflammatory mechanism of pomegranate where its hydroalcoholic extract significantly lowered DSS-induced elevated histamine level in mice colon tissue [21].

2.1.1.2.2. Clinical studies

The only human trial is the ongoing phase I study on the role of pomegranate juice ellagitannins in the modulation of inflammation in inflammatory bowel diseases. This has been registered since December 2016. Available online: http://www.clinicaltrials.gov

2.1.2. Joint inflammation

2.1.2.1. Preclinical studies

A pomegranate compound, delphinidin, attenuated the inflammatory signaling that results in rheumatoid arthritis. This mechanism was mediated by inhibition of the histone acetyl transferase and NF-κB activation in human rheumatoid arthritis synovial cell line [22]. Pomegranate alleviated features of arthritis in collagen-induced arthritic mice (CIA). This effect was associated with histopathological evidence of reduced inflammatory cells and joint tissue damage. Moreover, pomegranate decreased the interleukin 6 (IL-6) level and suppressed inflammatory signal transduction pathways in mouse macrophages [23].

2.1.2.2. Clinical studies

Pomegranate (2 capsules of 250 mg pomegranate extract/day for 8 weeks) improved disease activity, some inflammatory blood biomarkers and oxidative stress (increased glutathione peroxidise) in 30 rheumatoid arthritis patients in a double-blind, placebo-controlled, randomized study [24].

2.1.3. Respiratory inflammation

2.1.3.1. Preclinical studies

Pomegranate peel aqueous extract attenuated lipopolysaccharide (LPS)-induced lung inflammation in mice. Furthermore, it inhibited the production of human neutrophil reactive oxygen species (ROS) and myeloperoxidase [25]. Synergistic anti-inflammatory effect of pomegranate extract (encapsulated into microparticles) with dexamethasone was demonstrated in asthma model mice. The microparticles attenuated leukocytes' recruitment to bronchoalveolar fluid, particularly eosinophils, reduced cytokines (IL-1β and IL-5), and reduced protein levels in the lungs. These findings supported the alternative/complementary use of pomegranate in treatment of lung inflammation [26]. Pomegranate (80 μmol/kg/day) significantly attenuated the expression of inflammatory mediators, apoptosis, and oxidative stress that were induced by acute mice exposure to cigarette smoke (for 3 days). Additionally, on chronic cigarette smoke exposure (1–3 months) pomegranate reduced expression of TNF-α and normalized lung cell architectures. Moreover, pomegranate juice attenuated the damaging effects of cigarette smoke extract on cultured human alveolar cells [27]. Pomegranate juice diminished

inflammatory changes in pulmonary tissue via its antioxidative capacity in a study that was carried out on 27 streptozotocin-induced diabetic rats, which were given either pomegranate or saline for 10 weeks [28].

2.2. Cancer

2.2.1. Prostate cancer

2.2.1.1. Preclinical studies

Prostate cancer suppression was exerted by different pomegranate fruit parts (juice, peel, and seed oil) on LNCaP, PC-3, and DU 145 human cancer cell lines. This effect was manifested by inhibition of proliferation, invasion, phosholipase A2 (PLA2) expression, and apoptosis induction [29, 30]. Pomegranate fruit extract inhibited cell growth and induced apoptosis via remodeling of apoptosis regulating proteins in prostate cancer PC-3 cell line. In addition, oral administration of pomegranate fruit extract to mice implanted with CWR22Rnu1 cells significantly suppressed tumor growth and decreased prostate-specific antigen (PSA) in the serum [31, 32]. Oral pomegranate fruit extract (100 mg/kg) for 4 weeks inhibited testosterone-induced prostatic hyperplasia, prostate weight, prostatic acid phosphatase activity, and total glutathione in rats [33].

2.2.1.2. Clinical studies

A two-stage phase-II clinical trial on 46 subjects with recurrent prostate cancer and rising serum prostate-specific antigen (PSA) after surgery or radiotherapy was carried out. The participants consumed daily eight ounces of pomegranate juice (570 mg of total polyphenol gallic acid equivalents) until meeting the disease progression endpoints. About 35% of patients achieved a significant decrease in serum (PSA). There was a significant increase in mean PSA doubling time from baseline of 15–54 months post-treatment. In a parallel in vitro study of patients' serum on LNCaP cell growth, there was a significant reduction in cell proliferation and induction of apoptosis after treatment with pomegranate juice [34].

2.2.2. Breast cancer

Pomegranate constituents have been proved to be antiproliferative, noninvasive [35], apoptotic [36] angiogenesis [37], and tumor growth inhibitors [38]. Pomegranate seed oil and fermented juice polyphenols exhibited antiangiogenesis potential by suppression of vascular endothelial growth factor in MCF-10A and MCF-7 and upregulated migration inhibitory factor (MIF) in MDA-MB-231 breast cancer cell lines [38].

2.2.3. Colon cancer

Pomegranate juice derived ellagitannins and their intestinal bacterial metabolites, urolithins, exhibited dose- and time-dependent decreases in cell proliferation, and clonogenic efficiency of HT-29 cells. The half maximal inhibitory concentration, IC50 values, ranged from 56.7 μM for urolithin A to 74.8 μM for urolithin C [39].

2.2.4. Hepatocellular carcinoma

Oxidative stress is a precipitating factor of hepatocellular carcinoma (HCC), one of the most lethal cancers. Pomegranate emulsion (1 or 10 g/kg) was given 4 weeks before dietary carcinogen diethylnitrosamine (DENA)-induced rat hepatocarcinogenesis and 18 weeks thereafter. Pomegranate revealed chemopreventive activity manifested by reduced incidence, number, multiplicity, size, and volume of hepatic nodules. This effect was mediated by pomegranate antioxidant activity and inhibition of nuclear factor-kappaB (NF-κB) (a potent stimulant of Wnt/β catenin signaling which is involved in cell proliferation, cell survival, and apoptosis) [40, 41].

2.2.5. Bladder cancer

Transitional cell carcinoma results in most of the bladder tumors [42]. The tumor suppressor gene p53 which is essential for cell cycle arrest and apoptosis [43] was believed to be inactivated in more than 50% of carcinogenesis of bladder cancers [44]. Polyphenols in pomegranate rind extract was shown to inhibit bladder cancer cell EJ proliferation via p53/miR-34a axis [45].

2.3. Cardio vascular disorders

2.3.1. Preclinical studies

Pomegranate protected against cardiovascular injury initiated by cigarette smoking in rats through its antioxidative property [46]. Moreover, antioxidative and anti-inflammatory effects of pomegranate extract reduced the size of atherosclerotic plaques in the aortic sinus and reduced the proportion of coronary arteries with occlusive atherosclerotic plaques when it was given orally in a dose of 307.5 μl/L of drinking water/day for 2 weeks to mice model of coronary heart disease [47]. Furthermore, pomegranate extract supplementation (625 mg/day) for 10 days to pigs prevented hyperlipemia-induced coronary endothelial dysfunction via a stimulation of the Akt/endothelial nitric oxide-synthase pathway [48].

2.3.2. Clinical studies

Natural pomegranate juice (150 ml/day) succeeded to significantly lower systolic and diastolic blood pressure 4–6 h post-consumption in 13 hypertensive patients [49]. Furthermore, a 1 year consumption of pomegranate juice by 10 atherosclerotic patients with carotid artery stenosis significantly reduced common carotid intima-media thickness (IMT), systolic blood pressure, and serum lipid peroxidation. Whereas after 3 years of pomegranate consumption, no additional beneficial effects occurred except for further reduction of serum lipid peroxidation by up to 16% [50].

2.4. Metabolic disorders

2.4.1. Preclinical studies

High level of low-density lipoprotein (LDL) is a risk factor for cardiovascular disease. The esterase paraoxonase1 (PON1) prevents oxidation of LDL. Decreased levels of PON1 increase the incidence of cardiovascular disease. Pomegranate juice (12.5 mL/L of juice in 1 l of water/day for 4 months) significantly induced PON1 gene expression and activity when given daily

to streptozotocin-induced diabetic mice fed with a high-fat diet. Furthermore, pomegranate reduced blood glucose level and body weight [51]. Metabolic syndrome includes common clinical disorders such as obesity, hypertension, dislipidemia, and diabetes. Pomegranate juice and fruit extract induced a significant decrease in vascular inflammation markers; thrombospondin (TSP), and cytokine TGFβ1 and increase in plasma nitrate, nitrite levels, and nitric oxide-synthase expression (important factors for arterial function enhancement) in a metabolic syndrome rat model [52]. Pomegranate extract (300 mg/kg/day for 8 weeks) reduced the levels of high-fat diet-induced elevated serum interleukin 6 (IL6) and corticosterone in rats [53]. Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver diseases in the world [54]. The pathogenesis of NAFLD includes the increased accumulation of triglyceride in hepatocytes, which progresses to nonalcoholic steatohepatitis (NASH) due to oxidative stress. In high-fat, high-sugar-diet-fed rats, pomegranate juice (60 ± 5 ml /day for 7 weeks) exhibited a significant modulation in hepatic steatosis, ballooning, lobular and portal inflammation, as well as significant attenuation of hepatic pro-inflammatory and pro-fibrotic gene expression. It significantly decreased plasma levels of alanine, aspartate aminotransferase, insulin, triglycerides, and glucose with respect to control [55]. A study comparing the antidiabetogenic effect of glibenclamide (5 mg/kg) and pomegranate juice (1 ml/day) was carried out on 40 streptozotocin (STZ)-nicotinamide (NAD)-induced type 2 diabetes mellitus rats for 21 days. Pomegranate juice (1 mL/day) showed significant repair and restoration signs in islets of Langerhans. Additionally, it significantly lowered the level of plasma total cholesterol, triglyceride, and inflammatory biomarkers, which were actively raised in diabetic rats [56].

2.4.2. Clinical studies

Concentrated pomegranate juice (50 g daily for 4 weeks) exerted a significant increase in total and high-density lipoprotein cholesterol from baseline levels in 40 type 2 diabetic patients. Only serum interleukin-6 (IL-6) was significantly reduced among other tested inflammatory markers. There was about 75% increase in mean value of serum total antioxidant capacity (TAC) [57]. In a double-blinded, randomized crossover controlled study, daily 500 mL of pomegranate juice was introduced to 30 individuals with a metabolic syndrome for a week. Systolic and diastolic blood pressure as well as high sensitivity C-reactive protein was significantly reduced. However, pomegranate consumption significantly increased the level of triglyceride and low-density lipoprotein cholesterol which is attributed by the authors to the more lipogenic effect of fructose than glucose after hepatic metabolism into triglycerides [58]. On the other hand, administration of 400 mg of pomegranate seed oil capsules twice daily for 4 weeks to 25 dyslipidemic patients insignificantly reduced serum of TNF-α level [59].

2.5. Infections

2.5.1. Bacterial and fungal infection

2.5.1.1. Preclinical studies

Antimicrobial activity of pomegranate has been widely investigated in many studies. *Escherichia coli* (*E. coli* O157:H7) is associated with many disorders: diarrhea, hemorrhagic colitis, thrombocytopenic purpura, and hemolytic uremic syndrome. Pomegranate ethanolic

extract was shown to be bacteriostatic and bacteriocidal against *E. coli* with minimal inhibitory concentration (MICs) from 0.49 to 1.95 mg/ml and minimal bactericidal concentration (MBCs) from 1.95 to 3.91 mg/ml [60]. Tuberculosis is an infectious disease with a time long emergence of drug resistance. Pomegranate *juice*, and *peel extracts* prepared with methanol/water, and its polyphenolic byproducts namely caffeic acid, ellagic acid, epigallocatechin-3-gallate (EGCG) and quercetin, were examined against drug-resistant clinical isolates of *Mycobacterium tuberculosis* and β-lactamase producing *Klebsiella pneumoniae*. The peel extracts exerted higher antimycobacterial activity (MIC 64–1024 μg/mL) than the juice (MIC 256 - > 1024 μg/mL). EGCG and quercetin showed more antitubercular and antibacterial activity than caffeic acid and ellagic acid [61]. Biofilm is a protective layer made of extracellular polymeric substances where the pathogen hides with subsequent modulation of its virulence and pathogenicity. Pomegranate methanolic extract was believed to counteract the formation of biofilms by *Staphylococcus aureus*, methicillin-resistant *S. aureus, E. coli*, and *Candida albicans*. Moreover, pomegranate extract disrupted the preformed biofilms with inhibition of germ tube formation, a virulence trait, in *C. albicans*. Further studies revealed the ability of ellagic acid to inhibit the growth of all species in suspension at higher concentrations (>75 μg ml^{-1}) and biofilm formation at lower concentrations (<40 μg ml^{-1}) [62]. Besides single antifungal activity, pomegranate extract showed a synergistic effect with other antimicrobial agents. Punicalagin synergism with fluconazole against *C. albicans* and *C. parapsilosis* was demonstrated in an in vitro study with a twofold decrease of MIC for fluconazole [63]. Pomegranate methanolic extract showed synergistic effect with five antibiotics: chloramphenicol, gentamicin, ampicillin, tetracycline, and oxacillin against methicillin-resistant *S. aureus* strains (MRSA) and methicillin-sensitive *S. aureus* (MSSA). Most potent synergism was noticed when pomegranate was combined with ampicillin. This combination increased the post-antibiotic effect (PAE) of ampicillin from 3 to 7 h. as well as it reduced cell viability by 99.9 and 72.5% in MSSA and MRSA populations, respectively [64]. Methanol extract of pomegranate showed a synergistic action with ciprofloxacin against extended-spectrum β-lactamase (ESBL) producing *E. coli* and metallo-β-lactamase (MBL) producing *Pseudomonas aeruginosa;* that effect was attributed to bacterial efflux pump inhibitor (EPI) activity of the pomegranate polyphenolic constituents [65]. In an ongoing study of our work where we are testing the antifungal activity of some medicinal herbs against clinical isolates of *C. albicans* strain, pomegranate methanolic extract showed an inhibitory zone of 12 mm and synergistically augmented the action of fluconazole by increasing the inhibition zone from 25 to 35 mm after combination. The potent antifungal action of pomegranate led some researchers [66] to design a new antifungal peptide, pomegranin, with an N-terminal sequence from fresh pomegranate peels by ion exchange chromatography. Pomegranin suppressed mycelial growth in the fungi *Botrytis cinerea* and *Fusarium oxysporum* with half maximal inhibitory concentration (IC50) of 2 and 6.1 μM, respectively.

2.5.2. Virus infection

2.5.2.1. Preclinical studies

Pomegranate showed antiviral action against many viruses: influenza, human immunodeficiency virus (HIV), herpes simplex virus (HSV), and adenoviruses in multiple studies. Of pomegranate polyphenol extract (PPE) constituents (ellagic acid, caffeic acid, luteolin, and

punicalagin), punicalagin had the highest affect against influenza A virus through suppression of viral RNA replication and agglutination of chicken RBCs. In addition, pomegranate polyphenol extract augmented the anti-influenza effect of oseltamivir when given together [67]. Pomegranate juice prevented HIV-1 binding to CD4 and blocked viral entry [68]. Moreover, agents present in pomegranate juice (polyphenols, beta-sitosterol, sugars, and ellagic acid) and fulvic acid were demonstrated as envelope virus neutralizing compounds that neutralize the viral infectivity by binding to the envelope lipid or sugar moieties [69]. Adenoviruses are a group of non-enveloped viruses that give rise to in a wide range of illnesses. Pomegranate peel ethanol extract exhibited anti-adenovirus activity on HeLa cell line where the half maximal inhibitory concentration (IC50) and 50% cytotoxicity concentration (CC50) of the extract were 165 ± 10.1 and 18.6 ± 6.7 μg/ml, respectively. The selectivity index (SI), the ratio of CC50 and IC50, was 8.89 [70]. Moreover, pomegranate tannins were shown to have anti-HSV-1, HSV-2 effect via blocking of virus adsorption to African green monkey kidney and human adenocarcinoma cells [71]. Hepatitis C virus (HCV) is the leading cause of end-stage liver disease. Ellagitannins from pomegranate peel crude extract, punicalagin, punicalin, and ellagic acid, specifically blocked the HCV NS3/4A protease activity in an in vitro study. Furthermore, punicalagin and punicalin significantly suppressed HCV replication in cell culture system. Moreover, these compounds arewere well tolerated ex vivo and "no-observed adverse effect level" (NOAEL) was established up to an acute dose of 5000 mg/kg in BALB/c mice. Additionally, these components were bio-available by pharmacokinetics study [72].

2.5.3. Parasitic infection

2.5.3.1. Preclinical studies

From ancient times, pomegranate was described as an antihelminthic agent. Malarial infection represents a public health and economic burden in tropical and subtropical regions of the world [73]. Pomegranate gallagic acid and punicalagin exerted an antiplasmodial activity against *Plasmodium falciparum* D6 and W2 clones with IC50 values of 10.9, 10.6, 7.5, and 8.8 μM, respectively [74].

Schistosomiasis is a morbid widely distributed tropical disease [75]. Blood flukes of the genus *Schistosoma* pass a complex life cycle including multiple morphologically distinct phenotypes in definitive human and intermediate snail hosts [76]. In vitro and in vivo studies were designed to evaluate pomegranate impact on *Schistosoma mansoni* (*S. mansoni),* one of the three major species infecting humans. Pomegranate peels and leaves extracts significantly affected both adult *S. mansoni* worms and schistosomules with 100% death rate, after 24 h of exposure to plant extracts. Oral administration of the pomegranate extract to mice at a dose of 800 mg/kg, 45 days post-infection and on three consecutive days yielded a high percentage of dead adult worms (77.30 and 72.2) with either leaves or peels extract, respectively. In addition, reduction in tissue egg load, liver, and intestinal ova counts was observed. This antiparasitic effect was confirmed by electron microscopic examination that revealed ultrastructural alterations in the tegument and the male genital systems of the worms. Bone marrow examination of pomegranate-treated *S. mansoni*-infected mice showed eosinophilic degranulation that indicates reduced *S. mansoni* activity [77].

2.6. Central nervous system disorders

2.6.1. Cognitive disorders

2.6.1.1. Preclinical studies

Cognitive disorders affect learning, memory, perception, and problem-solving. These disorders include amnesia, dementia, and delirium. Pomegranate ellagic acid (30 and 100 mg/kg) ameliorated scopolamine- (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.)-induced amnesia in mice. Furthermore, chronic administration of ellagic acid (30 mg/kg) improved the memory deficit induced by diazepam (1 mg/kg) in rats [78]. Memory impairment, a feature of Alzheimer's disease (AD), is initiated by neuroinflammation and impairments in synaptic plasticity. These disorders are induced by the effect of extracellular amyloid-beta (Aβ) deposits called senile plaques. The generation of Aβ is dependent on the proteolytic processing of amyloid precursor protein (APP) [79]. Pomegranate is believed to slow the rate of neurodegeneration in Alzheimer's disease. At a cellular level, pomegranate compound, punicalagin, was examined for its memory protective anti-inflammatory effect on lipopolysaccharide (LPS)-induced neuroinflammation in astrocytes and microglial BV-2 cells. In a dose of 1.5 mg/kg punicalagin attenuated LPS (250 μg/kg daily 7 times) induced memory impairment and blocked the LPS-induced expression of inflammatory proteins via suppression of NF-κB activation [80]. In addition, freeze-dried pomegranate (25–200 μg/ml) in a dose-dependent manner reduced COX-2-dependent prostaglandin E2 (PGE2) production in SK-N-SH cells stimulated with IL-1β [81]. The neuroprotective action of pomegranate was obscured in an animal study in which dietary supplementation of 4% pomegranate extract to APPsw/Tg2576 mice for 15 months ameliorated the loss of synaptic structure proteins, inhibited neuroinflammatory activity, and enhanced autophagy (degradation and recycling of cellular components). Moreover, it reduced β-site cleavage of APP [82]. Along with figs and dates, pomegranate dietary intake attenuated the levels of inflammatory cytokines in APPsw/Tg2576 mice a model of Alzheimer disease, as well as delayed the formation of senile plaques [83].

2.6.2. Ischemic stroke

2.6.2.1. Preclinical studies

Ischemic stroke is one of the neurodegenerative diseases. An in vitro study utilized serum glucose deprivation (SGD) as a model for ischemia-induced brain injury in PC12 cells. Pretreatment with different pomegranate extracts, namely, pulp hydroalcoholic extract (PHE), pulp aqueous extract (PAE), and pomegranate for 2 h significantly and concentration-dependently, increased cell viability and decreased DNA damage initiated by SGD insult [84].

2.6.3. Multiple sclerosis

2.6.3.1. Preclinical studies

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system and is associated with demyelination, neurodegeneration, and sensitivity to oxidative stress.

Pomegranate seed oil (PSO) in nanodroplet formulation induced more significant beneficial effects the in mice model of multiple sclerosis (MS) than natural pomegranate seed oil. This effect was evident by dramatic alleviation of lipid demyelination and oxidation in mice brains [85].

2.6.4. Neonatal hypoxic-ischemic brain injury

2.6.4.1. Preclinical studies

Neonatal hypoxic-ischemic (HI) brain injury is a fatal condition that affects preterm very low birth-weight infants. After administration to pregnant mice, pomegranate juice revealed anti-oxidant-driven neuroprotective effect in experimentally induced HI brain injured neonatal offsprings [86–87].

2.7. Miscellaneous disorders

2.7.1. Skin disorders

2.7.1.1. Preclinical studies

Prolonged human exposure to sun's ultraviolet (UV) radiation, especially its UV-B, causes many adverse effects. Pomegranate fruit extract was proved to be *a* photo-chemo preventive agent on human epidermal keratinocytes. It alleviated ultraviolet A and B radiation-induced cell damage in a dose- and time-dependent manner [88, 89].

2.7.1.2. Clinical studies

Oral elagic acid-rich pomegranate extract either in high (200 mg/d ellagic acid) or low doses (100 mg/d ellagic acid) improved ultraviolet-induced skin pigmentation of 26 subjects in 4 weeks double-blind placebo-controlled trial [90].

2.7.2. Male infertility and erectile dysfunction

2.7.2.1. Preclinical studies

Pomegranate juice improved epididymal sperm concentration, spermatogenic cell density, diameter of seminiferous tubules, and sperm motility. It decreased the number of abnormal sperms compared to control rat animals. Moreover, pomegranate juice resulted in improvement of antioxidant enzyme activity in both rat plasma and sperm [91]. Pomegranate juice significantly increased intracavernous blood flow and smooth muscle relaxation in a rabbit model of arteriogenic erectile dysfunction [92].

2.7.2.2. Clinical studies

In a randomized, double-blind, placebo-controlled, 10-week crossover trial, pomegranate juice (1.5 mmol polyphenols daily) showed insignificant improvement when introduced to 53 men with mild-to-moderate erectile dysfunction [93].

2.7.3. Dental disorders

2.7.3.1. Preclinical studies

Bacterial and fungal co-infection initiates oral diseases. Pomegranate phytotherapeutic gel was shown to be superior to miconazole in attenuation of microbial adherence with three and four associated organisms: *Streptococci* strains (*mutans* ATCC 25175, *sanguis* ATCC 10577 and *mitis* ATCC 9811) and *C. albicans* [94]. Elagic acid exerted a moderate inhibitory effect at 12.5 mg/mL with inhibition to adherence <50% against different strains of Streptococcus mutans bacteria that induced dental caries [95].

2.7.3.2. Clinical studies

In a human study, pomegranate hydroalcoholic extract was superior to chlorhexidine (standard and positive control) in decreasing the colony forming unit (CFU)/ml by 84 and 79%, respectively, of dental plaque microorganisms [96]. Pomegranate along with *Centella asiatica* extracts significantly improved clinical signs of chronic periodontitis and IL-1beta level when it was applied with biodegradable chips on periodontal disease in 20 patients with remaining probing pocket depths after conventional periodontal therapy [97]. Pomegranate gel was compared to miconazol gel (3 times daily for 15 days) in 60 patients suffering from denture stomatitis. The patients were randomly distributed into two groups of 30 patients each. Clinical response was statistically better in miconazol group ($P < 0.01$) with similar fungal negativity in both groups. Clinical response and fungal negativity was achieved in 21 and 23 patients of pomegranate group as compared to 27 and 25 subjects who received miconazol, respectively. Side effects were only reported from all miconazol-treated patients. The authors explained the better miconazol clinical response by the bigger number of subjects with good oral hygiene score in the miconazol group and the longer duration of miconazol (sticky formulation) in the mouth than pomegranate gel that was washed away on mixing with saliva [98].

3. Pharmacokinetic studies

Pomegranate ellagitannins release ellagic acid in the gut, and this compound is poorly absorbed in the small intestine, while it is largely metabolized by human gut microflora into urolithins, such as urolithins A and B and urolithin-8-methyl ether in the large intestine [99]. Pomegranate anthocyanins (the 3-glucosides and 3, 5-diglucosides of delphinidin, cyanidin, and pelargonidinare) are stable in the stomach. While in the neutral pH of the small and large intestines, anthocyanins become less stable and are converted into a variety of metabolites [100–102].

The maximum plasma concentration (Cmax) of ellagic acid was 33 ng/mL and time of maximum concentration (Tmax) was 1 h [103]. A pharmacokinetic study on 18 healthy volunteers proved the rapid absorption and plasma clearance of ellagitannins as well as long persistence

(48 h) of urinary excreted urolithin metabolites after 180 ml of pomegranate juice consumption. Prolonged stay of urolithins in the human body is responsible for the health benefits of chronic pomegranate consumption [104]. A 1 liter pomegranate juice containing 4.37 g/L punicalagins and 0.49 g/L anthocyanins was introduced to six healthy individuals for 5 days; urolithin A, urolithin B, and a third unidentified minor metabolite were detected in plasma as well as in urine analysis at 24 h besides an aglycone metabolite corresponding to each of three plasma metabolites. Maximum excretion rates occurred 3–4 days after juice ingestion. The concentrations of urinary metabolites varied significantly in the subjects which may be attributed to colonic microflora variability and the site of ellagitannins metabolism [105]. A crossover pharmacokinetic study reported that higher free ellagic acid EA intake does not enhance its bioavailability in healthy volunteers who consumed two pomegranate extracts of 130 mg punicalagin+524 mg ellagic acid or 279 mg punicalagin+25 mg ellagic acid. The study showed high inter-individual variability; Cmax ranged from 12 to 360 nM that may be attributed to the ellagitannin pH and protein environment [106].

4. Safety

Pomegranate is safe when it is used in normal doses [107]. The median lethal dose, LD 50 of the whole fruit extract, was 731 mg/kg after intra-peritoneal administration to OF-1 mice [108]. Standardized pomegranate extract of 30% punicalagins showed acute oral LD50 in wistar rats and in Swiss albino mice it was more than 5000 mg/kg. Subchronic no-observed adverse effect level (NOAEL) was 600 mg/kg body weight/day [109]. Pomegranate ellagitannin-enriched polyphenol extract in a daily dose of 1420 mg (870 mg of gallic acid equivalents,) for 28 days showed no adverse effects in 64 overweight subjects [110].

5. Conclusion

Pomegranate's uncountable beneficial pharmacological properties encourage more and more studies to discover other secrets for solving mankind health problems.

Acknowledgements

First and foremost, my deepest gratitude to GOD, for his uncountable gifts including pomegranate. Second, I would like to thank my parents and family for continuous encouragement. I would also like to express my thanks to Dr. Farid Badria, Professor of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Egypt, and Dr. Khalil Mahfouz, Assistant Professor of Botany, Faculty of Science, Tanta University, Egypt, for their generous advice and help. My great appreciation to Professor Dr. Said Shalaby, Vice President, Academy of Scientific Research and Technology, Cairo, Egypt, for his unforgettable support.

Author details

Sally Elnawasany

Address all correspondence to: elnawasany_s@hotmail.com

Faculty of Medicine, Tanta University, Tanta, Egypt

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Edited by Jaya R. Soneji and Madhugiri Nageswara-Rao

The fruit and nut crops are laden with health benefits. As people are becoming more conscious about their health and nutritional uptake, the worldwide demand and consumption of fruit and nut crops are steadily increasing. This has made it hard to keep pace between the rate of fruit and nut production and its consumption. To meet this increasing demand, there is a need to produce improved, better yielding, and high-quality fruit and nut crops. This book intends to provide the reader with a comprehensive overview of the current status and future prospects of fruit and nut crops. Such information covered in this book will directly enhance both basic and applied research in fruit and nut crops and will particularly be useful for students, scientists, researchers, teachers, breeders, policy-makers, and growers.

Published in London, UK

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ISBN 978-1-83881-439-7