

Compendium of Plant Genomes  
*Series Editor: Chittaranjan Kole*

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Alessandra Gentile  
Stefano La Malfa  
Ziniu Deng *Editors*

# The Citrus Genome

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# **Compendium of Plant Genomes**

## **Series Editor**

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

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# The Citrus Genome

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*This book series is dedicated to my wife Phullara, and our  
children Sourav and Devleena*  
Chittaranjan Kole

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## Preface to the Series

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

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

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

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

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

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

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

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

Plant genome science has emerged as an important subject in academia, and the present compendium of plant genomes will be highly useful both to students and teaching faculties. Most importantly, research scientists involved in genomics research will have access to systematic deliberations on the plant genomes of their interest. Elucidation of plant genomes is of interest



not only for the geneticists and breeders but also for practitioners of an array of plant science disciplines, such as taxonomy, evolution, cytology, physiology, pathology, entomology, nematology, crop production, biochemistry, and obviously bioinformatics. It must be mentioned that information regarding each plant genome is ever growing. The contents of the volumes of this compendium are, therefore, focusing on the basic aspects of the genomes and their utility. They include information on the academic and/or economic importance of the plants, description of their genomes from a molecular genetic and cytogenetic point of view, and the genomic resources developed. Detailed deliberations focus on the background history of the national and international genome initiatives, public and private partners involved, strategies and genomic resources and tools utilized, enumeration on the sequences and their assembly, repetitive sequences, gene annotation, and genome duplication. In addition, synteny with other sequences, comparison of gene families, and, most importantly, the potential of the genome sequence information for gene pool characterization through genotyping by sequencing (GBS) and genetic improvement of crop plants have been described. As expected, there is a lot of variation of these topics in the volumes based on the information available on the crop, model, or reference plants.

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

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

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

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## Preface

*Citrus* (L. 1753) is one of the most important genera of the *Rutaceae* family. Genus *Citrus* encompasses most of the widespread fruit crops such as oranges, mandarins, clementines, lemons, limes, pummelos, and grapefruits in the Mediterranean and subtropical environments. The wide diversity among such species is accompanied by an overall positive appreciation by consumers, thanks to the outstanding organoleptic characteristics of citrus fruits and, not secondarily, to their health and nutritional value. Citrus was originated in Southeast Asia and diversified during the late Miocene epoch. The genealogy of the different citrus species indicated that they were originated through successive events of hybridization that occurred among the three “true” or “biological” ancestral species: citron (*Citrus medica*), mandarin (*Citrus reticulata*), pummelo (*Citrus maxima*), and/or their hybrids.

Despite the efforts on genetic improvement, citrus species cultivation undergoes several limitations especially for the adaptability to different environmental conditions and for the risks derived from pests and diseases spreading at the global level. In citrus, traditional breeding enabled the setup of several varieties and rootstocks showing improved agronomical traits; however, in these species, conventional breeding techniques are hampered by several aspects such as the long juvenile phase, the high level of heterozygosity, the male and female sterility, and the occurrence of apomixis.

In such a picture, the recent advances of molecular biology and molecular-based breeding techniques greatly helped researchers to speed up the entire breeding process. In particular, the release of the genome sequence of most of the economically important *Citrus* species is facilitating the critical investigation of several aspects spanning from *Citrus* phylogeny, till the study of their complex biological features and the understanding of the genetic basis underlying traits of agronomic interest.

This book is aimed at reviewing with a multidisciplinary approach, the state-of-the-art of the researches in Citrus, highlighting both novel discoveries and open questions for future works. The book provided an exhaustive overview of the Citrus phylogeny with particular interest in the description of the genetic resources and approaches at the base of conventional breeding of varieties and rootstocks. The rapid increase in efficiency and reliability of the high-throughput sequencing platforms enabled the use of both molecular markers and sequencing data to get novel insights both on the genetic architecture of traits of interests and on the genetic diversity among Citrus species. To this extent, the recent findings on the genetic basis

underlying traits of agronomical interests (either related to fruit quality, tree production, or resistance to biotic/abiotic stress) were presented and discussed. Citrus fruit quality was also addressed from a metabolic point of view highlighting two of the most peculiar aspects of fruits: the color and the presence of essential oils. The book will be a guide for those who are interested in a comprehensive overview of the progress in the scientific research related to Citrus with attention also to the transferability of such findings in the context of breeding. For such reason, these pages will be particularly useful for the scientists, breeders, and students of the universities, public sector institutes that are involved in research for the development of citrus industry for updating the amount of knowledge generated in recent years.

We are grateful to all our colleagues for their contribution. We wish to record our thanks and appreciation to Prof. Chittaranjan Kole, the Series Editor, for his assistance and guidance right from the inception till the publication of this book.

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# The Citrus Genome: Past, Present and Future

1

Eliezer E. Goldschmidt

## Abstract

Within this introductory chapter, I would like to briefly discuss the contribution of the genomic revolution to the science of Citrus. Genomics undoubtedly plays a central role in the ongoing reconstruction of evolutionary history of the Citrus clade, but elucidation of the role played by man in the more recent wandering and distribution of citrus relies on other disciplines, such as Archaeobotany and Historic documentation. As to the current challenges faced by the Citrus Industry, the most serious threat of the 21st century is the pandemic, devastating HLB disease. In this case implementation of the genomic tools has not yet lead to a real breakthrough. Can anything meaningful be said about the future? Two major frontiers are evident; breeding and physiology. Modern plant breeding includes by now techniques for precise editing of the genome, which hold a bright promise for targeted crop improvement. The term ‘physiology’ means, in this context, an advanced understanding of all the developmental and biochemical traits responsible for the citrus phenotype. This is a tremendous challenge, since the citrus phenome is still rather

mysterious, even the genetic basis of the ‘Hesperidium’ fruit unit is as yet poorly understood.

As already indicated in the title, the genomic era has brought new insights for studies of the past of the citrus tribe, as well as new vistas for the future of citrus as a horticultural crop and industry.

Within this preface, I would like to address the following issue: What did we, as citrologists, or, what did the science of Citrus gain from the recent genomic revolution? I am asking this question as an old-fashioned horticulturist–physiologist who was not personally involved in genomic research, but attempts nevertheless to evaluate the contribution of genomics to our broader understanding of Citrus’ universal survival.

Thinking about the general evolution and domestication of fruit tree genera (Janick 2005), *Citrus* appears to be one of the most, if not the most complicated case. Genomics is evidently the principal tool through which the history of citrus can be reconstructed although one must not forget the seminal work of Barrett and Rhodes (1976) who came up with the ‘Three Ancestors’ theory long before the molecular biotechnological approach took the lead. Within the efforts to reconstruct the history of citrus, one may distinguish between two major research areas: (1) The extensive, ancient botanical evolution scenery which goes back to the previous geological era and must take into account presumed

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continental movements and climate changes. (2) The role played by man in the prehistoric and historic expansion of citrus species from their centers of origin, leading to the current worldwide distribution of citrus and the shaping of the present, extremely diverse population of citrus phenotypes. Several research teams have joined forces during the last decade or so in an attempt to resolve the mysteries of the 1st research area, based on sequencing and comparative analysis of an increasing number of citrus genomes. Although a multidimensional, unifying theory has recently been proposed (Wu et al. 2018), solid evidence with regard to the emergence sites of some of the citrus clades is still fragmentary.

As to the 2nd area mentioned above, this aspect has not received sufficient attention in recent years. Here genomics alone is not enough. The contribution of humans to the spread and diversification of citrus genotypes must be studied by a combination of historical, archaeological and biological methods. A noteworthy example of this kind of research is the archaeobotanical study by Langgut et al. (2013), that provided unequivocal evidence for the presence of the citron (*Citrus medica* L.) in the fourth century B. C., Palestine by fossil pollen found in the plaster of the Persian palace excavated in Ramat Rahel, just south of Jerusalem.

Moving now from the past to the present and future, it seems appropriate to begin by considering the current challenges faced by the citrus industry. It is rather obvious that the most serious threat of the twenty-first century is the pandemic HLB disease, which has destroyed by now most of Florida's citrus and might spread within the foreseeable future to other, as yet unaffected areas. But, this is not the first time that an erupting disease endangers the citrus crop worldwide. In the twentieth century, it was the devastating tristeza viral disease, which appeared in South America and gradually spread to the Mediterranean. Prior to that, in the nineteenth century, it was the phytophthora citrus foot rot, which was discovered in Madeira (1854) and enforced the introduction of grafting on the sour

orange rootstock. The assertion that pests are the most troublesome threats for agricultural crops is also valid with other crops, e.g. the nineteenth-century phyloxera French grapevine catastrophe. Turning back to the HLB malady, we may wonder whether the progress in genomics has brought us closer to the solution of the HLB distress; and the answer is, I am afraid, rather disappointing.

The reason is, in my opinion, that deciphering the genomic sequence of the DNA does not in itself tell us the whole story, just some kind of a frame—background. Only a very detailed enquiry of the genetic regulation of every biological trait and biochemical activity (as partially achieved with *Arabidopsis*) will lead to the desired understanding of the observed phenomena; and pathological phenomena are even more complex since they generally involve interaction of several organisms.

That sequencing the genome does not solve all mysteries can be easily demonstrated by the case of the 'fingering citron' (Fig. 1.1). The 'fingering citron', also named 'Buddha's hand' is widespread in China, where it apparently originated (Karp and Hu 2018). In the 'fingering citron' the locules, which are normally united in the *hesperidium*, develop as separate 'fingers'. This malformation has probably arisen by some kind of mutation (Karp and Hu 2018). This mutation apparently interferes with a very basic step in the formation of the hesperidium fruit unit but, in spite of rather detailed enquiry of the citron genome (Ramadugu et al. 2015) there is as yet no clue as to the genomic site responsible for this mutation. Furthermore, we cannot even be sure that the 'fingering' phenotype is brought about by a change at the DNA level or through other molecular regulatory mechanisms.

How about the future? Although it is dangerous to engage in prophecy, two major frontiers are evident: breeding and physiology. Modern plant breeding includes by now, techniques for precise editing of the genome, such as CRISPR, which hold a bright promise for targeted crop improvement. However, the long time

**Fig. 1.1 Fingered citron.**  
Zhaocai fingered citron,  
Jinhua, Zhejiang, China.  
(Photo credit: Xulan Hu)



required for the establishment of new, successful citrus cultivars is still a major barrier. The term ‘physiology’, in my opinion, means an advanced understanding of all the developmental and biochemical processes responsible for the citrus phenotype. However, as indicated in the previous sections, the progress in both breeding and physiology areas rests heavily on detailed mapping of the genes and resolution of their regulatory loops. Although the molecular tools are available, will there be sufficient funds and research power to confront these challenges? This leaves us certainly with a great room for concern.

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# Citrus Origin, Diffusion, and Economic Importance

# 2

Guangyan Zhong and Elisabetta Nicolosi

## Abstract

Citrus are widely cultivated in more than 140 countries in the world, in tropical, subtropical and Mediterranean climates, in the “citrus belt” between approximately 40° N and 40° S latitude, but their natural distribution areas before domestication must be much smaller. The chapter briefly outlines the issues related to the exact identification of the geographical origin and spread of citrus fruits: these simple questions have bothered citrus breeders and taxonomists for centuries. Nevertheless, we have witnessed a tremendous progress in the past two decades since the introduction of molecular tools in citrus researches, and the release of the first citrus genome has made an unprecedented breakthrough in our understanding of citrus genetics, taxonomy and evolution. Currently, on the basis of genomic, phylogenetic, and biogeographic analyses, scientists from different part of the world agree and haven’t doubt that the main centers of origin for the citrus species are the tropical and subtropical regions of south-east Asia, the northeastern India in the Himalayan foothills,

Yunnan province of south-west China, northern Myanmar, the Indochinese peninsula, and the Malaysian archipelago, from which Citrus began the spread into the other continents. The genus *Citrus* is the result of a long and complex domestication process and this, together with sexual compatibility between *Citrus* and related genera and the frequency of bud mutations, makes citrus taxonomy and phylogeny very complicated. The chapter focuses on the genetic origin of the main cultivated species such as sweet oranges, clementine, citron, pummelo, grapefruit and mandarins. Finally, the major citrus production areas and the main commercial citrus groups and producing countries are reported.

## 2.1 On the Origin Center of Citrus

The citrus is certainly one of a few most delicious fruits that are generous gifts of Mother Nature. Most of us will intuitively fall in love with the attractive, fragrant, and delicious fruits at first sight, and wonder where and how they have come into existence. These seemingly simple questions, however, have bothered citrus breeders and taxonomists for centuries. Two peculiar reproductive behaviors of citrus should be heavily blamed, first, the citrus is notorious for their sexual promiscuity, and hybrids between species and even genera are not only very easy to produce but also quite normal in fecundity, making it difficult to delineate a clear genealogy

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of them; second, many citrus including mandarins, kumquats, oranges, and *Poncirus* are polyembryonic and propagated apomictically through nucellar embryos of maternal origin that are dominant over sexual embryos, freezing the normal evolutionary clock of the affected species and causing problems for scientists, especially the early taxonomists, who relied heavily on morphological characters to identify hybrids from true species. Additionally, long-distance dispersal should have occurred frequently in the history of evolution and spread of citrus, for their fruits are not only favorite foods of migrating birds and other herbivorous animals including humans but also, with sponge tissues and water-repellant wax skins, floatable for a considerably long time that certainly facilitate their dispersal through rivers that flow across their vast habituating areas, which adds a lot of difficulties to trace their origins. Moreover, fossil evidence is very limited since only one of the three fossil specimen reports described definitely a citrus species. Not to mention that not many wild citrus have been discovered and recognized, and the population sizes of the extant wild species, without exception, are too small to do population genetics studies.

Without enough corroborating evidence from geographical, genetic diversity, wild population, fossil, paleoclimate, and domestication history studies, the questions about the origin, dispersal, divergence, and evolution of citrus are difficult to answer. Nevertheless, we have witnessed tremendous progress in the past two decades since the introduction of molecular tools in citrus researches, and the release of the first citrus genome has made an unprecedented breakthrough in our understanding of citrus genetics, taxonomy, and evolution (Xu et al. 2013).

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## 2.2 Inference from Citrus Domestication History

Though citrus is widely cultivated in more than 140 countries in the world, its natural distribution areas before domestication must be much smaller.

Clearly, the citrus are not native to America since it had not been known by native Americans before the Italian explorer and navigator Christopher Columbus brought the first citrus to North America in 1493, even though the American countries, USA and Brazil, have been the two of the three most important and biggest citrus producers in the past half centuries in the world. Evidently, the birthplace of citrus must be within the borders of the Eurasian continent.

Citron (*Citrus medica*) was probably the first citrus plant known to Europeans. In Africa, citron must be cultivated by ancient Egyptians since it was depicted on the walls of the botanical garden at the Karnak Temple that dates back to 3000 years ago. The Jews might be familiar with citrus as early as the sixth century BC when they were about to depart from Sinai since the “fruit of the beautiful (‘hadar’) tree” was mentioned in Leviticus, although there was no direct mention of citrus in the Bible. The spread of citron to European countries is believed to be accredited to Alexander the Great and his armies who brought it from the east, probably India, in the late fourth century BC, since Theophrastus who lived in Greece in the same era recorded that citron was already grown in the east (Median and Persia). According to Langgut (2017), citron was brought from Persia to the Western Mediterranean during the early Roman period, and lemon was the second introduced citrus, while Sour orange (*C. aurantium*), lime (*C. aurantifolia*), and pummelo (*C. maxima*) were introduced in the tenth century AD. Sweet orange (*C. sinensis*) and mandarin (*C. reticulata*) were introduced in the late fifteenth century, and the early nineteenth century, respectively. Introduction of them to the west must be via southern trade routes that spread from South China to the west, and in the process, Northern Africa and the Iberian Peninsula served apparently as the relay stations (Ramón-Laca 2003). Considering that the time needed for a citrus species to arrive at a certain location must be correlated to the geographical distance between the location and the origin place of the species and that the reachable distances of the ancient traders were limited by their travel capabilities, it is reasonable to

conclude that Citron must be geographically closer than other citruses to the west. In other words, the later arrivals must come from more distant regions.

Evolution theories tell us that the genetic diversities of a given species are gradually reduced as disperses away from its origin center. In fact, the unique morphological attributes of the citrons also suggest that they must be derived from a geographically marginal population that was distant or isolated from the core citrus population. We know that wild citrons are still grown in valleys at the foothills of the Eastern Himalayas, a region stretched from Northeast India to Myanmar. To the east of this area are China, Japan, Australia, and the Southeastern Asian countries where numerous different native citruses have been reported. The geographical center of the vast area is located in the middle of South China sea if we exclude the northern areas where the winter temperatures can drop to a lower than normal survival level for citrus.

Citrus plants were rarely mentioned in ancient literature in most countries of the aforementioned regions, but abundantly documented in Chinese literature in the past 4000 years. A very ancient book, “Yu Kung 禹贡”, mainly documenting the geography of China in Xia dynasty, mentioned that mandarins and pummelos were listed as tributes to the emperor Yu (ca 2205–2197). “Lu Shi Chun Qiu 吕氏春秋”, a cyclopedia edited by the prime minister of Qin state in 241 BC, described that the most beautiful and delicious fruits were the mandarins produced in “Jiangpu” and the pummelos produced in “Yunmeng”. Qu Yuan (340–278 BC), one of the most famous poets in the history of China, wrote a long piece of poem to eulogize mandarin trees. “Han Shu 汉书”, edited between 54 and 74 AD, stated that a person who grew 1000 mandarin trees in Jiangling area (between nowadays East Sichuan and West Hubei provinces) was as rich as a marquis who possessed 1000 households, which indicated that there was large-scale citrus production in the region at that time. In the following centuries, the Chinese Citrus industry had been developed to such a level that permitted Han Yenchu to edit the world first citrus variety atlas, “Chu Lu”, in 1127

AD, in which a total of 27 citrus (mostly mandarins and kumquats) cultivars were meticulously documented.

The earlier human activities indicate that the citrus were known, domesticated, and exploited earlier in Southern China than in other regions. The suggestion is that the center of origin of citrus must be somewhere in Southern China or somewhere in the surrounding areas.

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### 2.3 Inference from the Distribution of Extant Wild Citrus

The pattern of a species’ natural distribution should provide a valuable clue to where its origin center is. *Citrus* and its close relatives, *Poncirus* and *Fortunella*, are naturally distributed in a vast area bordered by the Yellow River to the north, Japan and Taiwan to the east, Northeastern Australia to the south, and from India down to Indonesia to the west. The geographic center of the areas is approximately in South China. Generally, the center of a species distribution area is the center of origin of the species if it is overlapped with the genetic diversity center. This is because as individuals migrate further away from their distribution center, the chances for them to exchange genetic materials with others with diverse genetic backgrounds are exponentially decreased. Now, where is the center of the genetic diversity of citrus?

There exist Australia limes in Australia. Australia limes, as their names suggest, are indigenous to Australia and nearby Papua New Guinea. These special types of citrus have very distinctive characters such as much smaller and thicker leaves and higher tolerance to drought stress, probably resulted from long time adaptive evolution in Australian desert environments. Though many different species were recognized in Australia limes, they are in fact closely related to each other and most probably only one or two true species since modern molecular genetics studies have repeatedly shown that there are limited genetic diversities among them. Considering that the Australian continent has always been separated from Asian continent by ocean

waters even during the greatest glacial epoch, and that the vast, almost insurmountable waters are definitely natural hindrances preventing against cross-water spread of most land animals and plants, it is conceivable that only a few seeds of citrus happened to arrive at the continent and have since thrived there. In other words, their limited DNA polymorphisms can be best explained by the theory of “founder effect”. There might be only one or a few lucky founders (seeds or fruits) brought there by cross-ocean migrating birds or ocean currents flowing between the continents. The cross-ocean dispersal event(s) should have most probably occurred during the greatest glacial epoch(s) when the sea level was very low, and many nowadays sea floors were then drylands, and the waters between the continents were much narrower.

Papedas are mostly uncultivated for their inedible fruits and hence less disturbed by humans and animals. Though named after their characteristic large leaf wings, different papedas do not in fact belong to the same clade when classified by modern molecular taxonomic tools. Ichang papeda is widely distributed in the area that stretches from Northeast Yunnan to Southeast Shanxi, approximately along the east margin of the Yunnan–Guizhou plateau where the winter is very harsh and capable of killing other papedas and other citrus species. In contrast, other papedas spread mostly southward and westward from southern Yunnan to Indonesia, Philippines, and Malaysia although Khasi papeda can also be found in Northeastern India.

Citrons are native to Yunnan, Tibet, Guizhou, Guangxi, Assam, and Myanmar. As mentioned above, many slightly different types of citrons grow wild in Yunnan and surrounding areas including Northeast India and Myanmar. The citrons are mono-embryonic yet they keep a surprisingly high degree of genetic homozygosity. The high homozygosity can be attributed to their reproductive behavior that their flowers tend to be self-fertilized before opening. There might be other possibilities, anyway. First, the citron’s founder population might be separated by high mountains from the main population, which generated a bottleneck effect and as a result, a

considerable number of ancient variations of the main population was lost in citrons. Second, the speciation of citrons might have occurred relatively later and there might not be enough time for as many genetic variations as other citrus to accumulate.

Wild kumquats have been naturally growing in southeastern provinces in China, including Guangdong, Guangxi, Hunan, Hainan, Jiangxi, and Zhejiang. The most primitive kumquat is *Fortunella hindsii* (Shan Jin Gan) which is also the bushiest and the smallest fruited citrus fruit. Shan Jin Gan, with inedible fruits, can still be found in the wild although some good-looking trees are occasionally removed for use as pot materials. Other kumquats with larger and fleshier fruits have been domesticated and cultivated for at least more than 1000 years in the southeast of China. Though hybrids can be obtained without any difficulty when kumquats are artificially fertilized with other citrus, some degrees of natural reproductive isolation do exist among them. The barrier comes from the difference in their flowering seasons. Kumquats sprout and blossom later than any other citrus, leaving no chance for natural pollination to occur between them. The late blossom and high tolerance to low temperatures, compared to other *Citrus* species, suggest that they have evolved from an area with a longer and harsher winter season. That is, they may have originated from either a higher latitude, a higher altitude or the colder side (e.g., the north side) of a high mountain.

Mandarins (*C. reticulata*) vary greatly in fruit size, shape, color, and other morphological characters but their fruits are usually smaller and easy to peel. Several wild mandarins have been recognized as true species by different taxonomists. One of them, *C. indica*, that was considered as a primitive mandarin by Tanaka, is in fact a type of citron or a hybrid of citron. The remaining wild mandarins are mostly native to South China except that the *C. tachibana* is a native species of Taiwan and Japan. Various wild mandarins have been discovered in Hunan, Jiangxi, and Guangxi provinces in the past four decades.

A unique citrus, longmengxiangcheng, was discovered on the peak of the Luofu mountain of

Longmeng county, Guangdong province. Its leaves resemble those of mandarins but its fruit looks more like a small pummelo. Its juice pulps are sour and bitter. Studies showed that it should be a true citrus species (Zeng et al. 2014).

Trifoliolate oranges (*P. trifoliata*), named after their characteristic trifoliolate compound leaves, are widely distributed in a vast area boarded by the yellow river, the east coastal line, the south coastal line and the east Yunnan, and the Yunnan–Guizhou plateau in China. Although only one species and one subspecies have been recognized in *Poncirus*, there are in fact many different types of trifoliolate oranges that vary greatly in sizes of fruit and leaves according to our surveys. Most trifoliolate oranges, except for fomingzhi, shed their leaves completely in winter, and are thus the most hardy citrus that can tolerate temperatures lower than  $-10^{\circ}\text{C}$ , suggesting strongly their northern origin. Trifoliolate oranges open flowers before they expand leaves, and are in fact the earliest in flowering season compared with *Citrus* and *Fortunella*. Early flowering of *Poncirus* isolates them reproductively from *Citrus* and *Fortunella*. But hybridization does occur when their pollens are fallen onto each other's stigmas, and surprisingly, these intergeneric progenies have shown no apparent abnormalities in fertility and many of them are as normal as intraspecific hybrids. Though artificial hybrids such as citranges and citramellos are well known, thanks to the great work of the worldwide renowned breeder W. T. Swingle, natural hybrids between *Poncirus* and *Citrus* were reported in China. Yongshunzhi is, for example, a natural citrange that was found growing in the border area between Hunan and Guizhou. Fomingzhi (*P. polyandra*) which was discovered in Yunnan province, is the only evergreen trifoliolate orange. It is not clear if fumingzhi is a natural hybrid between *Poncirus* and *Citrus*.

The aforementioned natural distributions of the species of the three close relative genera, *Citrus*, *Fortunella*, and *Poncirus*, which are still inter-fertilizable, suggest in a broad sense that their common origin is somewhere in the Southern China.

## 2.4 Inference from Genomic Data

Genome sequence is a faithful record of almost all genetic changes that are inheritable. The changes are mostly substitutions of nucleotides which mainly result from errors in the incorporation of nucleotide during DNA replication, deletions/insertions from slipped strand mispairing, and far less frequently large structural rearrangements. Both the single nucleotide substitutions and the small deletions/insertions are regarded as single nucleotide changes (variants). When a single nucleotide change is benign or neutral to the survival of the affected individual, it may spread in the population. When the changed single nucleotide reaches a certain frequency (e.g.  $>1\%$ ), allowing coexistence of two to four alleles (A, T, G, C) at the affected locus in the population, the so-called single nucleotide polymorphism (SNP) can be defined. SNPs can be identified by comparison of genome sequences of the investigated samples. SNPs are more informative for tracing ancestry of any species, hybrid, or individual due to their incomparable abundance. Apparently, the number of individuals carrying an ancient SNP is greater than that carrying a novel SNP. In other words, the number of specific SNPs accumulated in a species is positively correlated to how long the species has diverged from the most recent common ancestor. An evolutionary tree based on SNPs or haplotypes (closely linked SNPs) can thus be established and the chronological order of many key evolutionary events can be determined.

Wu and his colleagues compared the genomes of 58 citrus accessions representing 10 taxonomic groups as well as two related genera, *Poncirus* (*Poncirus trifoliata*) and Chinese box orange (*Severinia*), including some pure accessions of important progenitor species (Wu et al. 2018). By using 362,748 genome-wide ancestry-informative SNPs from nongenic and non-pericentromeric genomic regions, they established so far the most correct evolutionary tree for citrus. Their conclusion was that the center of origin of citrus was the southeast foothills of the Himalayas, a region that includes the eastern area

of Assam, Northern Myanmar, and western Yunnan. The proposed origin center is rich in geographical and climate diversities and should therefore be an ideal cradle for citrus to evolve. However, this area is in the strict sense not the only biodiversity center. As mentioned previously, there may be even more diversified citrus species in Southern China. More importantly, one of the area's indigenous species, *C. manshanensis*, is more primitive than citron, papeda, and pummelo as revealed by genomic studies (Wu et al. 2014a, b). Recently, we found that the Guangdong wild citrus, longmengxiangcheng, should also be a species that diverged as early as citron and pepeda (unpublished data). Moreover, it is well known that the South Central China inhabitants, trifoliolate oranges, kumquats, and citrus, have not yet developed a true reproductive isolation mechanism among them although they have been traditionally treated as three separate genera.

More probably, the Central Southern China is the primary origin center of all citrus including trifoliolate oranges. As the ancient citrus spread to a much wider area, geographic isolation of them has occurred although not long enough to allow them to develop reproductive isolation. Anyway, two secondary biodiversity centers, i.e., the southeast foothills of the Himalayas and the Southern China areas, have been naturally formed.

## 2.5 On the Origins of Some Important Citrus Species

### 2.5.1 Sweet Orange

Sweet orange is economically the most important species and has been planted more than any other citrus species in the world. Why it is superior to other citrus species in fruit quality is a fascinating question that both the breeders and the physiologists have been trying to answer. Undoubtedly some clues could be obtained by deciphering its exact genetic origin.

It was suggested that sweet orange might be the first generation hybrid between pummelo (P) and mandarin (M) (Barrett and Rhodes 1976;

Nicolosi et al. 2000; Nicolosi 2007). Analysis of SSRs suggested that the sweet orange should have originated from one or more backcrosses of a pummelo–mandarin ( $P \times M$ ) hybrid to mandarin since there were more mandarin alleles than pummelo alleles in its genome (Barkley et al. 2006). Analysis of more (172) SSR markers assigned 25% of the sweet orange alleles to pummelo and 46% to mandarin with many regions having two alleles derived from mandarin, which strongly suggested that sweet orange was a backcross of a mandarin–pummelo hybrid to mandarin ( $P \times M$ )  $\times$  M (Roose et al. 2010). Xu and his colleagues assembled the first citrus genome using sweet orange as material and concluded that sweet orange should be derived from ( $P \times M$ )  $\times$  M (Xu et al. 2013).

Wu and Zhong proposed that sweet orange was most probably from [ $(P \times M) \times P$  or  $P \times (P \times M)$ ]  $\times$  M after analyzing 121 randomly selected loci/unigene segments that should have a statistically guaranteed genome-wide representativeness (Zhong 2013; Wu 2016). The involvement of an immediate mandarin parent in the generation of sweet orange was clearly shown by the fact that at least one allele at every analyzed sweet orange locus was from mandarin. In addition, the ratio of P/M loci to M/M loci was 95:26, which suggested that the other non-mandarin parent was most probably from a backcross of a pummelo–mandarin hybrid to a pummelo, i.e., ( $M \times P$  or  $P \times M$ )  $\times$  P or  $P \times (M \times P$  or  $P \times M)$ . Since the sweet orange carries pummelo maternal materials (Luro et al. 2000), the proposal that its maternal parent was a ( $M \times P$ )  $\times$  P was invalid. The origin of sweet orange could therefore be expressed as [ $(P \times M) \times P$  or  $P \times (P \times M$  or  $M \times P)$ ]  $\times$  M. When this model was tested by Markov chain method, the chance to produce a sweet orange like genome was 30%. The ( $P \times M$ )  $\times$  M hypothesis was also tested under the extreme condition that no crossover event occurred during gamete formation in the ( $P \times M$ ) parent, but the chance to produce a sweet orange like genome was about 7% which was very slim. The  $P \times M$  hypothesis is invalid since there is an unignorable large proportion (26/121) of M/M regions in sweet orange, which clearly excluded the direct

involvement of a pure pummelo. A search for the paternal parent of sweet orange was also tried but none of the 69 investigated mandarins was found to be a perfect match when genotyped for the paternal-parent specific SNPs (our unpublished data). The  $[(P \times M) \times P \text{ or } P \times (P \times M)] \times M$  scheme of sweet orange origin was confirmed by PM haplotype ratio whereas the  $(P \times M) \times M$  model was rejected by the existence of P/P loci, as shown by a more detailed assembly of the sweet orange genome (Wu et al. 2014a).

### 2.5.2 Clementine

Clementine has been treated as a mandarin. It is one of the two most popular mandarin cultivars (the other one is Satsuma mandarin). Though its leaves are mandarin-like, its fruits are more compact and relatively more difficult to peel than other mandarins. Clementine was thought to be a mysterious Algeria hybrid and was assigned taxonomically to *C. reticulata* by Swingle (1967). Clementine was also suggested to be a hybrid between a mandarin and sweet orange (Bayer et al. 2009), and indeed. Clementine and sweet orange share at least one common allele at all analyzed loci, clearly showing that sweet orange is a parent of clementine. The other parent which is Mediterranean mandarin was easily identified by genotyping related mandarins (Ollitrault et al. 2012; Wu 2016). Thus, it is undoubted that clementine derived from the cross between Mediterranean mandarin and sweet orange in which Mediterranean mandarin acted as the female parent since clementine possesses a different-than sweet orange's chloroplast (Penjor et al. 2010). Genome sequencing result showed more clearly that the genome of clementine mandarin came from a hybridization between Willow leaf mandarin and sweet orange (Wu et al. 2014a).

### 2.5.3 Citron

Citrons have two morphologically distinct types of fruits, non-fingered and fingered. The non-fingered fruit is usually ovate or oblong with a

protruding stylar end. Pulp are often not well developed, mostly taste sour. Some varieties are even pulpless. Rinds are thick and very fragrant. Leaves are more lignified and petioles are often not articulate. Citron is monoembryonic but has maintained a surprisingly high intraspecific homozygosity, probably due to the cleistogamy of its flowers that facilitates self-fertilization in unopened flower buds (Wu et al. 2018). All the morphological characters could be explained by their isolated habitats that should have efficiently prevented them from exchanging genes with other citrus.

Investigations on the genetic diversity of citron have mostly shown that it is a pure species with relatively low intraspecific diversity, which is very rare in *Citrus*. Genome-wide SNP analysis showed indeed that only 0.1% of intraspecific diversity existed in four representative citrons which is significantly lower than those in other citrus species (0.3–0.6%) (Wu et al. 2018). Such a low intraspecific diversity can also be explained by either geographic isolation during their early evolution and/or their cleistogamy of flowers. Nevertheless, two gene pools were still recognizable in 36 citron accessions collected from China by analyzing nSSR marker and SNPs/Indels on six chloroplast genes; the first one included fingered citrons and the wild citrons from Tibet while the other consisted of non-fingered citrons mostly from Yunnan (Yang et al. 2015). Ramadugu et al. (2015) also surveyed the intraspecific variations of 47 citron accessions collected not only from China but also from Mediterranean region by using SSR markers, and SNPs from one nuclear and one chloroplast genes, and confirmed the existence of two groups of citrons in Yunan province of China. They also found a third group that consisted of the citrons cultivated in Mediterranean region and pointed out that the group should have originated from India. Interestingly, both studies observed considerably higher intraspecific variations at some investigated loci although the overall intraspecific variations are low. This is a good example that shows that the high local variation signals can be easily inundated by a way larger number of genome-wide variations.



### 2.5.4 Pummelo

Pummelos (*C. maxima*) are widely cultivated in China, Thailand, Vietnam, Malaysia, and other Southeast Asian countries while its hybrid, grapefruit (*C. paradisi*), is mostly cultivated in western countries. Mature pummelo fruits are greenish to light orange and very large. Pummelo rinds are very thick, with white or rarely pink sponge-like albedo. Fleshes are mostly white or less frequently pink; taste varies from very sweet to moderately sour. Compared to other citrus, the leaves of pummelo are usually larger and thicker, and with a more prominently winged petiole. Similar to Citron, pummelo is also mono-embryonic. Yet true to type progenies can be normally obtained from seed propagation even though some types of pummelos are self-incompatible and thus prone to cross-pollination, although modern molecular evidence have constantly shown that introgression of pummelo genes into other citrus especially mandarins are quite common.

Pummelo is thought to be native to South-eastern Asia but no true wild pummelo population has been convincingly identified so far anywhere in the world. *Citrus lingcangensis*, the only well-recognized citrus fossil specimen, resembling modern pummelos in that it had a very typical heart-shaped leaf wing, was excavated from Lingcang county of Yunnan province. The fossil leaves were believed to be buried in the late Miocene (Xie et al. 2013).

Taxonomic studies showed that pummelo was related to papeda (Federici et al. 1998). More specifically, it was closely related to Honghe papeda that is still growing in the Honghe river valleys in southern Yunnan province (Zhong et al. 1993; Pang et al. 2003). RAPD, SCAR, and cpDNA data grouped pummelo into the same clade with Khasi papeda, micrantha papeda, and lime (Nicolosi et al. 2000; Nicolosi 2007).

Genome sequencing of two pummelos showed that the overall genome-wide nucleotide heterozygosity was 5.7 heterozygous sites/kb which was not a high number, indicating their ancestors experienced a strong bottleneck ~100,000–300,000 years ago. Analysis of

12 gene segments of more than 20 true pummelos also showed that the nucleotide diversity varied from 0.4/kb to 6.1/kb, and the haplotype diversity varied from 2/locus to 9/locus but there might still be four founder populations that might have contributed to today's pummelo genomes (Wu et al. 2014b). Though different studies told different stories, they were most probably originated from Yunnan (277). The reasons are two fold, first the fossil pummelo, *Citrus lingcangensis*, was excavated from Yunan, second Yunan is renown not only for its rich pummelo resources but also for its rich river systems that could facilitate the downstream dispersions of the thick-skinned pummelos that can float on water for a considerably long time.

### 2.5.5 Grapefruit

Grapefruit was thought to have originated from Barbados island from a chance cross between a pummelo and a mandarin. Evidence from cpDNA and AFLP further confirmed its pummelo–sweet orange hybrid origin (Nicolosi et al. 2000; Li et al. 2010). Moreover, genome sequencing provided unquestionable evidence that it is a half pummelo half mandarin hybrid (Wu et al. 2018).

### 2.5.6 Mandarins

Mandarins are worldwide cultivated and ranked the second, next only to sweet orange in acreages among the cultivated citrus species. Collectively, mandarins are a group of smaller citrus fruits, mostly easy-peelers with darker juice and peel colors in comparison with Citron and Pummelo. Seeds of mandarins are mostly polyembryonic but also occasionally mono-embryonic. Morphological variations are larger in mandarins than in any other citrus species, which has caused a lot of confusions and disagreements in studies of their taxonomy. More than 60 mandarin species were recognized by Tanaka whereas only one species and two sub-species were recognized by Swingle. With the



accumulation of molecular data, it is now generally agreed that mandarins are only one species which can be represented by ponkan (*C. reticulata*).

Several wild mandarin populations have been discovered in Nanling mountain and surrounding areas in the past half century (Liu et al. 1990; Wang et al. 2018). A comparative analysis on the genomes of 40 mandarins by Xu's lab showed that there were three distinct wild citrus genomes in the area (Wang et al. 2018). Two of them including Mangshanyejun wild mandarin are true mandarins while the third one, Mangshanyegan (*C. mangshanensis*) is quite different. Their analysis showed that two independent domestication events might have respectively occurred in the north and the south sides of the Nanling mountain. As a result, two different groups of cultivated mandarins have come into existence, the northern group with sourer, deeper colored fruits and the southern group with lighter colored, sweeter fruits (Wang et al. 2018). A comprehensive comparison between the genomes of 28 mandarin accessions, except for Tachibana, showed that there was extensive haplotype sharing among their ancestors, and three types of extant mandarins could be identified (Wu et al. 2018). According to Wu et al., tachibana mandarin, which is indigenous to Taiwan and Southern Japan, was dispersed from the mainland China to its nowadays island areas during the early Pleistocene (around 2 Ma) via the land bridges between the mainland and the surround islands that were exposed by sea level dropping, and has since separated from other mandarins. But a different story that it was a close relative of mangshanyejun and daoxianyejun was told by Wang et al. (2018). Interestingly, both researches noticed the introgression of pummelos into mandarins, which were thought to have helped reduce the acid content, and thus facilitated the domestication, of mandarins.

Mangshanyegan (*C. mangshanensis*) that was discovered in Mangshan of Hunan province was initially considered as a mandarin species for its resemblance to mandarins (Liu et al. 1990). Its habitat is not far from that of the wild

Daoxianyejun mandarin. Its fruit is pubescent, round, and with thicker peel and taste bitter for containing oil drops in juice pulps, which contrasts it clearly from other mandarins. Its unique and homozygous  $\beta$ -carotene hydroxylase gene sequence argues that it should be treated as a new citrus species (Zhong 2013). Whole genome analysis by Wu et al. (2014a) also concluded that the *C. mangshanensis* is a distinct species.

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## 2.6 Citrus Economic Importance

### 2.6.1 Major Citrus Production Areas

The cultivation of citrus fruits involves a large number of countries with great geographical dynamism, so the role of the various states changes rapidly (an important development is currently in Brasil and in China). The expansive trend is constantly growing and we can affirm with certainty that Citrus is the most extensively produced tree fruit crop in the world, with total global production reported to be 124.246 million tons in 2016 ([www.fao.org](http://www.fao.org)). Nowadays, citrus fruits are grown in more than 140 countries, in tropical, subtropical, and Mediterranean climates, in the "citrus belt" between approximately 40° N and 40° S latitude, where the winter temperatures are suitable for tree survival and escaping freeze destruction, and where there are sufficient water and appropriate soils to sustain tree growth and fruit production. In the most extreme areas it is possible to grow citrus fruits, thanks to the temperatures moderated by ocean winds. The world production is localized both in the northern and in the southern hemisphere; particularly, in the northern hemisphere in the South and East Asian regions such as China (with a total production of more than 32.7 million tons), India (9.7 million tons), Pakistan (1.9 million tons), Indonesia, Japan, and Thailand, in the United States (7.8 million tons), and in Mexico (6.6 million tons), in the Mediterranean regions (totally production around 25.2 million tons) such as Spain (6.8 million tons), Egypt (4.9 million tons), Turkey (3.6 million tons), Italy (3.1 million tons),

Morocco (2.0 million tons), and some other regions. In the southern hemisphere, Brazil leads in citrus production, with more than 16.55 million tons followed by Argentina with 2.8 million tons, South Africa (2.4 million tons), Peru (1.1 million tons) and then Australia (0.58 million tons), and Paraguay (0.43 million tons) (Table 2.1).

Commercially, several species, are considered as a group under the term citrus, including lemons, limes, mandarins, satsumas, clementines, common mandarins and tangerines, oranges, grapefruit, and pummelos. In addition to these species, other citrus fruits such as kumquats, Calamondins, citrons, and many other hybrids are also commercially important. The global citrus industry contribution is immense and it provides employment to millions of people around the world considering all industry chains, starting from production to harvesting, handling, transportation, storage, processing, and market operations. As well known, although many citrus fruits can be eaten fresh, about a third of them worldwide is utilized after processing. In fact there are two types of distinct markets, one linked to fresh fruit and the other one to the processed citrus product, consisting mainly of citrus juice. Sweet orange is the predominant species for both of these markets. As indicated by FAO, in the industrialized countries, the consumption of fresh oranges is reducing while growing in the emergent developing countries like India, Brazil, Argentina, Mexico, and China. Moreover, over the last 20 years, two important market changes are to be recorded, one is that related to the increase of mandarin production, that consist also of tangerines, clementines, and satsumas, to the detriment of oranges; the second important change is related to the increase in juice consumption mainly due to the improvements in quality associated with technological progress and the price reduction.

According to FAO 2016, in order, China, Brazil, India, USA, Spain, Mexico, Egypt, Iran, Turkey, Italy, Argentina, and South Africa are the world's leading citrus fruit-producing

countries, representing around 80% of global production (Table 2.1). Spain is the principal producer among the Mediterranean region regarding the citrus fruits produced for the fresh fruit market, which represents the prevailing production, with more than 6 million tons. Production trend indicates that the leading species is orange that contributes significantly to the bulk of world's citrus fruit production accounting for more than 54% of the global citrus output, followed by the group of tangerines, which includes mandarins, clementines, and satsumas, with 26%, the group of lemons and limes (13%), and grapefruit and pummelos comprise roughly 7%.

Although many citrus fruits can be eaten fresh, about 20% of citrus fruit worldwide is utilized after processing and orange juice production accounts for nearly 79% of total processed consumption. Overall, in the last few years, however, the production of juices has decreased compared to fresh citrus fruit (FAO 2016). In particular, Brazil is the leader with 50% of global transformation, followed by USA (around 16%), Mexico and Argentina (7% each), China (5%), and among the Mediterranean region, only Spain has a certain importance contributing with 4% of citrus processing (Table 2.2).

Major fresh fruit exporting and importing countries are shown in Tables 2.3 and 2.4. Fresh citrus fruit exports (about 16 million tons) account for more than 12% of the world production. The Mediterranean area is the major exporter of fresh citrus with 55% of the volume and among the Mediterranean region Spain has become the major exporting country with more than 4 million tons of fresh fruit export, around 26% of the total world export. Follow Turkey and Egypt which contribute greatly to exporting with respectively 1.5 and 1.4 million tons (around 9% each). The export main destination from Mediterranean region is toward the European countries, principal to Germany, Netherlands, France, and the Russian Federation. The main exports from United States to other states,

**Table 2.1** World citrus production and major producing countries by species. All values are expressed in 1000 tons

	Total	Oranges	Tangerins	Lemons/Lime	Grapefruits
<i>World</i>	124246.0	66974.1	32968.5	15981.8	8321.6
<i>Northern hemisphere</i>	97848.9	42242.3	30609.4	12365.6	7631.6
Cina	32705.9	7000.0	19000.0	2405.9	4300.0
Mediterranean region	25216.0	14654.8	6901.9	3034.1	625.1
Spain	6882.0	3642.4	2222.6	950.0	68.0
Egypt	4930.4	3610.4	948.5	369.6	1.9
Turkey	3652.1	1700.0	1040.0	670.0	242.1
Italy	3150.2	1854.9	855.1	434.4	5.8
Morocco	2018.9	925.0	1065.0	28.4	0.5
Algeria	1372.4	1025.5	251.3	93.2	2.4
Greece	1041.5	808.1	165.8	62.6	5.0
Syria	882.4	715.5	–	166.9	–
Israel	476.0	76.0	164.0	67.0	169.0
Tunisia	331.4	127.9	46.0	56.2	101.3
India	9755.8	6850.2	–	2613.8	291.8
United States	7829.0	5371.0	876.0	847.0	735.0
Mexico	6634.0	3535.0	499.0	2270.0	330.0
Iran	4067.6	2717.3	742.4	509.4	98.6
Pakistan	1907.4	1320.1	504.5	82.8	–
Indonesia	1574.8	1574.8	–	–	–
Japan	1143.3	20.3	1115.0	8.0	–
Thailand	1102.1	368.9	137.8	135.1	460.3
Viet Nam	998.7	520.0	–	–	478.7
<i>Southern hemisphere</i>	26397.1	19731.9	2359.1	3616.2	690.0
Brazil	16555.1	14350.0	910.8	1214.5	79.8
Argentina	2800.7	800.0	350.0	1500.0	150.7
South Africa	2409.2	1560.0	174.2	345.0	330.0
Perù	1112.1	446.9	393.7	265.4	6.1
Australia	584.6	455.0	95.0	27.2	7.4
Paraguay	431.4	323.3	48.7	9.3	50.0
Tanzania	426.8	426.8	–	–	–
Bolivia	371.4	183.5	153.5	25.9	–

Source FAO (2016)

5% of world export, are those toward Canada and the countries of Southeast Asia. In the countries of the Southern hemisphere, only South Africa has a considerable export volume, about 1.7 million tons (10% of global world export).

Anyway, other countries of the Southern hemisphere, such as Argentina and Australia, are increasing their export volumes, thanks to the interest of the Northern hemisphere regions to the “out of season” productions.

**Table 2.2** Citrus utilized for processing. All values are expressed in 1000 tons

	Total	Oranges	Tangerins	Lemons/Lime	Grapefruits
<i>World</i>	23538.9	18460.9	1821.5	2469.0	787.5
<i>Northern hemisphere</i>	9215.9	6360.3	1374.6	941.2	539.8
China	1200.0	600.0	600.0	–	–
Mediterranean region	1925.0	1072.0	356.0	358.2	138.8
Spain	951.9	516.4	204.8	222.6	8.1
Turkey	202.4	115.0	34.9	48.1	4.4
Italy	372.9	237.6	61.9	73.4	–
Morocco	73.1	58.6	14.5	350.0	–
Greece	120.5	120.1	–	–	0.4
Israel	168.9	20.0	27.0	2.0	119.9
United States	3760.0	3080.0	135.0	230.0	315.0
Mexico	1703.7	1225.0	42.7	350.0	86.0
Japan	93.0	–	90.0	3.0	–
Thailand	38.2	38.2	–	–	–
Republic of Korea	150.9	–	150.9	–	–
<i>Southern hemisphere</i>	14323.1	12100.7	446.9	1527.8	247.7
Brazil	11791.5	11180.0	244.0	311.0	56.5
Argentina	1670.0	350.0	150.0	1150.0	20.0
South Africa	684.6	436.0	22.8	54.6	171.2
Australia	105.4	100.0	5.4	–	–
Uruguay	71.6	34.7	24.7	12.2	–

Source FAO (2016)

## 2.7 Main Commercial Citrus Groups and Producing Countries

As well known, on a worldwide basis, citrus fruits are divided into five groups of significant economic importance: sweet oranges (*C. sinensis* L. Osbeck), mandarins (*C. reticulata* Blanco and *C. unshiu* Marc.), grapefruit (*C. paradisi* Macfadyen), lemons (*C. limon* L. Burmann f.), and limes (*C. aurantifolia* Christm. Swingle). Other species such as pummelo, citron, *Fortunella* sp., and others have lesser commercial importance although, obviously, they have an economic interest in the places of origin and production.

### 2.7.1 Sweet Oranges

The principal sweet oranges world producer is Brazil with a production of about 14 million tons (21%), the orange production is concentrated mainly in the state of Sao Paulo. China and India are almost on the same level of production, about 7 million tons each, the second-largest orange producer in the world accounting for more than 10% of the world's production. In the United States, with an orange fruit production of 5 million tons, representing 8% of orange world production, Florida is the major orange-producing state and most of that produce is used for juice processing. Orange fruits from

**Table 2.3** Fresh citrus export by principal countries. All values are expressed in 1000 tons

	Total	Oranges	Tangerins	Lemons/Lime	Grapefruits
<i>World</i>	15912.8	7361.6	4404.8	3055.8	1090.6
<i>Northern hemisphere</i>	13030.93	5857.2	3961.7	2331.6	879.8
United States	825.1	550.0	40.1	100.0	135.0
Mediterranean region	8842.5	4479.7	2778.3	1226.0	358.5
Spain	4114.1	1870.5	1553.7	624.3	65.6
Egypt	1386.5	1286.4	45.7	34.1	20.4
Turkey	1495.1	403.4	460.0	449.3	182.4
Italy	198.6	123.4	33.2	39.9	2.1
Morocco	524.2	135.0	380.0	8.7	–
Greece	611.3	459.9	127.4	23.0	–
Israel	155.2	5.0	87.0	2.2	61.0
Mexico	693.6	45.0	–	627.4	18.0
Cina	683.1	59.2	445.9	40.4	137.5
<i>Southern hemisphere</i>	2882.6	1504.4	443.1	724.2	210.9
Argentina	394.3	64.8	49.7	279.3	–
Brazil	126.8	31.1	–	95.7	–
Chile	250.2	75.2	96.6	77.3	1.1
Perù	37.6	10.3	21.0	4.7	1.6
Australia	220.0	165.9	48.9	4.1	1.1
South Africa	1701.3	1064.1	189.7	245.0	202.5

Source FAO (2016)

California, Arizona, and Texas are sold for fresh consumption due to their appreciative quality. The Mediterranean region, Spain and Egypt, with around 3.6 million tons of orange production (5% each) are placed before Mexico and Iran (4% each) and Italy (3%).

Major cultivars in the sweet orange group are classified as “Blonde oranges”, “Navel oranges”, and “Blood oranges”. The main cultivars, with commercial importance in the world, belonging to “Blonde oranges” are as follows: Hamlin, marked fresh in Florida, Brazil, South Africa, and in many other countries; Valencia Late (and its selection), the most widely grown in the world both in the Northern and in the Southern hemisphere, but most of the produce is processed; Shamouti, cultivar from Israel utilized as fresh fruit; Pineapple, grown mainly in USA, Mexico, South Africa, Brazil, and India, appreciate for the

high juice content; Salustiana, commercial important seedless cultivar in Spain, similar to Cadenera also grown in Spain; Pera, in Brazil; Belladonna, and Comune widely grown in Italy.

“Navel oranges” fruit is characterized by the presence of a distinctive secondary fruit (navel) at the stylar end of the fruit. Usually are seedless due to complete pollen and partial ovule sterility; generally the fruits are larger than the other sweet orange cultivars. Navel is grown for fresh market. The highest quality fruit is produced in Mediterranean country such as Spain and also in the California coastal region. Washington Navel is the most widely grown important navel cultivar in the world. Its commercial importance is mainly in California, Florida, South Africa, Spain, Australia. Navel in a cultivar is very well established in Spain and Italy; Navelate, is a late maturing bud sport of Washington navel selected

**Table 2.4** Fresh citrus import by principal countries. All values are expressed in 1000 tons

	Total	Oranges	Tangerins	Lemons/Lime	Grapefruits
<i>World</i>	15037.6	7011.6	4210.4	2828.0	987.5
<i>Northern hemisphere</i>	14858.2	6885.5	4190.1	2803.5	979.2
Canada	450.0	180.0	140.0	90.0	40.0
United States	965.0	160.0	215.0	580.0	10.0
Mediterranean Region	2065.3	981.2	544.2	417.2	122.8
France	1099.2	502.4	365.2	155.9	75.8
Spain	267.4	163.4	10.0	86.1	7.9
Italy	301.3	119.1	60.0	96.7	25.5
Germany	1118.1	477.5	403.6	176.0	61.1
Netherlands	1132.6	539.4	193.5	237.9	161.7
Poland	447.3	153.3	149.7	94.3	49.9
Russian Federation	1455.0	440.0	700.0	210.0	105.0
China	287.0	215.3	–	–	32.4
Hong Kong	341.7	260.0	–	37.6	16.1
Saudi Arabia	688.2	550.0	4.7	100.0	–
UK	796.6	292.4	324.9	147.6	31.6
United Arab Emirates	412.0	215.0	107.0	90.0	–
<i>Southern hemisphere</i>	179.3	126.1	20.3	24.6	8.3
Brazil	25.4	14.6	9.1	1.4	0.3
Australia	29.8	18.9	3.4	6.5	1.0
New Zeland	17.6	10.7	4.7	1.9	0.2
Paraguay	14.5	12.3	–	1.7	–
Kenya	44.4	44.4	–	–	–
South Africa	10.9	3.5	1.3	2.1	4.0

Source FAO (2016)

in Spain that produces a very vigorous tree; Cara Cara, Powell, Chislett, Rhode Navel, and others are navel oranges grown in several citrus regions.

The third group is that of “Blood or pigmented oranges” so called because of light to deep blood red colored anthocyanin pigments in rind and flesh of the fruit. Red color develops in the Mediterranean climate characterized by warm days and cool nights. In particular are of commercial importance in several Mediterranean countries including Italy, Spain, Morocco, Algeria, and Tunisia. In Italy are widely spread Moro, Sanguinello, and Tarocco, all with several clones. Moro cultivar has fruit subglobose to round with deep red flesh, particularly juicy; Moro is considered the most pigmented variety,

at peak maturity, both the pulp and the peel, have an intense red-violet color. Sanguinello fruits are round to slightly oblong with slightly loose rind, deep red-fleshed. Tarocco: it cannot be considered a single cultivar because there are several clones with very different characteristics regarding the ripening period, the shape of the fruits, the pigmentation levels.

## 2.7.2 Mandarins

Commercially citrus fruit included in this group belong to several separate species, natural hybrids, man-made hybrids, selection, and mutants. The term mandarins is often replaced by