Chapter

Introductory Chapter: Citrus for a Healthy Life

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1. An overview

Citrus is an extensively produced fruit crop and is cultivated predominantly in tropical and subtropical regions of the world. The genus Citrus and related genera (Fortunella, Poncirus, Eremocitrus, and Microcitrus) belong to the family Rutaceae. Of these genera, Citrus is the widely grown genus and is well known for fruits like oranges, mandarins, lemons, limes, and grapefruits [1]. The classification of citrus is complexed however, the genus Citrus consists of more than 100 species. The number of species is variable and this species variation in a single genus is due to the admixture of wide morphological diversity, intra- and interspecific sexual compatibility, apomixis, and spontaneous mutations. However, intergenic hybrids such as citranges, citrumelos, and citrandarins, citremons, citradias, and citrumquats are also reported and are getting increasing importance. Several indigenous varieties are developed and consumed locally in specific regions. The citrus fruits are tangy with pleasant flavor and taste, a combination of sweet and sour flavors. Oranges and mandarins are predominant species of genus Citrus, marketed as fresh or processed juice [2].

The citrus plants in the orchards are confronted worldwide with increasing biotic and abiotic factors due to the changing climate. Amongst abiotic factors, fluctuating temperature and unexpected frosts are the main limiting factors whereas, bacteria, viruses, viroids, nematodes, fungi, and phytoplasmas are major biotic factors. Some factors result in a massive reduction in production and quality while others may destroy altogether the citrus industry. Citrus improvement through conventional approaches is discouraged due to the genetic and reproductive characteristics of the plant. The omics and biotechnology-based interdisciplinary interventions may allow combating such external factors and improving the health, nutritional quality of the fruit. The book, Citrus: Research and Development describe the citrus plant, the biotic as well as abiotic challenges, nutrients, and nutritional value, and nutraceutical applications to improve human health. Citrus production, management, detection, and documentation of citrus pathogens and their management, fruit nutritional quality, and potential use as nutraceutical is an interdisciplinary endeavor; therefore, it is difficult to cover all aspects of this subject in a single book. The editor of the book is conscious of the fact that there is considerable scope for improving citrus production and controlling the diseases and benefitting from the availability of chemicals of nutritional and nutraceutical importance, novel approaches for detection of such chemicals, enriching the genetic information through next-generation sequencing and improving the genome by incorporating new genes through genetic engineering and knocking out genes using CRISPR/Cas technology, and hence the information relevant to the topics is covered in the book.

2. Citrus genealogy

Citrus domesticated in Southeast Asia started several thousand years ago and was distributed to different regions of the world through ancient land and sea routes. The genealogy of the modern cultivated citrus is controversial because these are either selections from or hybrids of wild progenitors (**Figure 1**). The biological features and cultivation of Citrus have further complicated the lineage of modern species. Citrus is being cultivated either through clonal grafting or by asexual means of propagation to maintain the identified superior traits however, diversity within such populations is due to somatic mutations. Further, spontaneous mutants have been reported and are occasionally selected from limb sports or nucellar seedlings. The genetic diversity in citrus is the major impediment in the classification of the ever-increasing number of varieties. Several methods have been used to classify the citrus varieties however the methods proposed by Swingle [3] and Tanaka [4] are commonly adopted. Swingle's classification is based on native varieties rather than cultivated, and he placed two subgenera Papeda with six species and Citrus with ten species in the genus Citrus [3], and the rest as natural

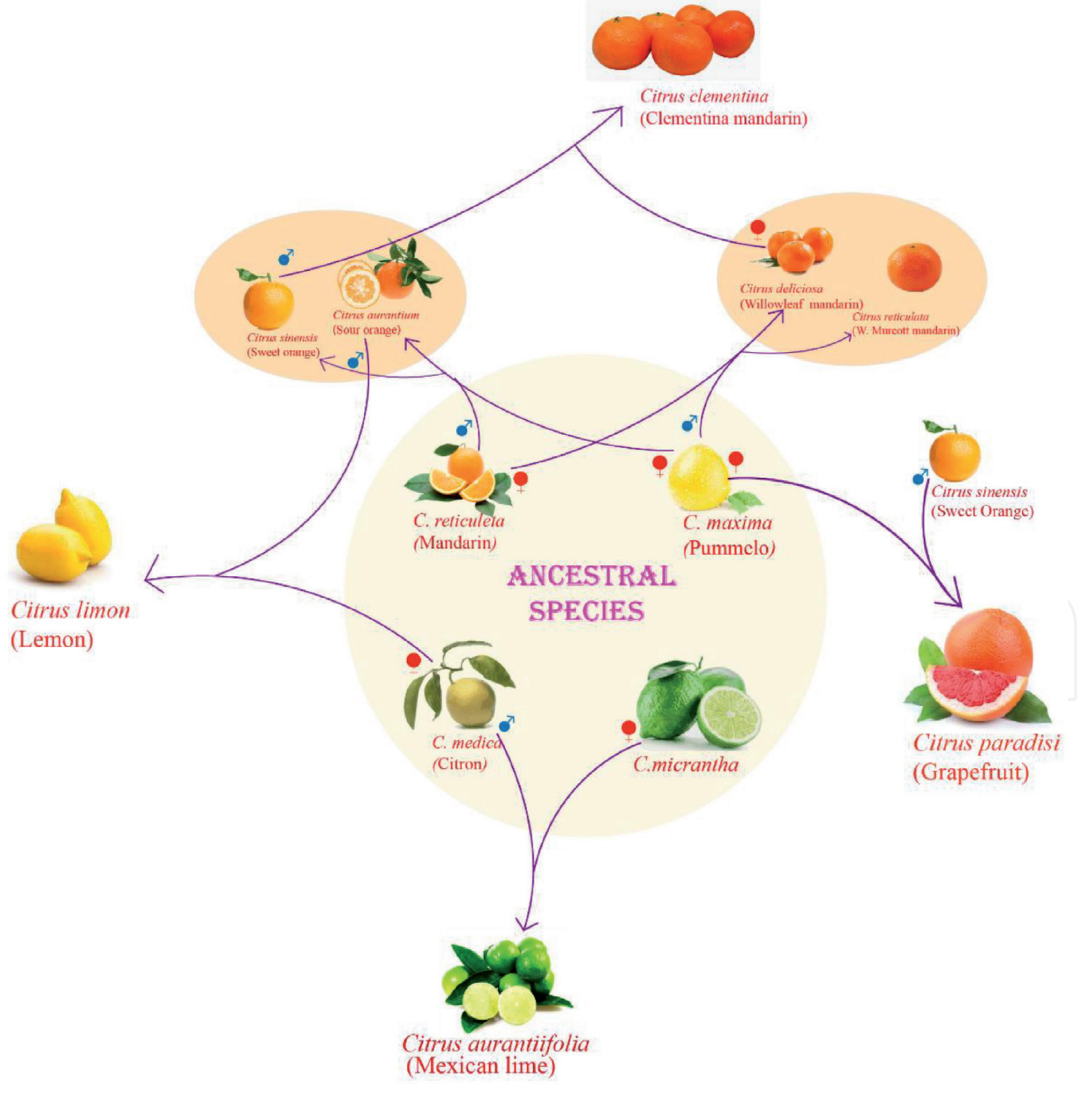

Figure 1.
The genealogy and evolution of citrus fruits. Four ancestral species namely; Citrus reticulata, Citrus maxima, Citrus medica, *and Citrus micrantha, depicted in the central circle, have contributed to the evolution of cultivated species. The figure is derived from Velasco and Licciardello, 2014 [8] and other published articles.*

hybrids of native species. Whereas Tanaka [4] classified both indigenous and cultivated varieties as species and placed two subgenera namely; Archicitrus with 111 species and Metacitrus with 48 species, the list of species was swelled to 159 [5]. The intervention of biotechnology approaches like RAPD, RFLP, AFLP, SSR, SRAP, and more recently the next-generation sequencing and pan-genomics has made it easier to determine the genealogy of citrus, extensively reviewed elsewhere [6]. The genomes of clementines, mandarins, pummelos, sweet oranges, and sour oranges have been sequenced using Sanger whole-genome sequencing method [7]. The sequence data together with earlier similar work was a step forward in elucidating the phylogenetic history of citrus domestication and highlights the genetic basis of the diversity in the colors, flavors, sizes, and aromas of citrus fruits, which could be introduced in novel varieties [8]. Another exciting and challenging work has been published, again by Wu et al. [9], where they worked out that distant genera i.e., Fortunella, Eremocitrus, and Microcitrus are a part of the citrus monophyletic group, whereas a related genus Poncirus, originally believed to be a part of Citrus group, is declared as a distinct clade based on whole-genome phylogeny. These findings have challenged the earlier taxonomic and phylogenetic developments and have warranted reformulation of the genus Citrus.

3. Citrus production and management

Worldwide, citrus is cultivated for consumption as fresh or processed fruit. Major citrus-producing countries are; China, Brazil, USA, Mexico, India, Spain, Iran, Italy, Nigeria, and Turkey. Production and consumption trends are diverse in different regions and countries however, variably 147 million tones citrus is produced, annually [10]. As citrus is grown from subtropical to tropical and the Mediterranean regions of the world, hence, its production is dependent on soil and climate conditions. The global orange production is projected to grow 3.6 million metric tons from the previous year due to favorable weather in Brazil and Mexico. Only a slight increase in production, as well as consumption, is expected for mandarins/tangerines. Unexpectedly, global grapefruit consumption and exports will rise to their highest levels in three years due to favorable weather conditions and expanded areas in China and Mexico. More than 80% of the fruit is being processed for juice production in developed countries, and the juice demand is increasing day by day. The market and consumption trends of major types of citrus fruit as well as of juice are not affected even by the COVID-19 pandemic, this is perhaps due to the perception of the consumers that citrus fruits are immunity boosters being rich in vitamin C.

In addition to climate, plant–soil interaction affects citrus production by affecting the availability of nutrients to the plants [11]. For efficient nutrient availability, the practices of controlled release of fertilizers are preferred [12]. The published data confirm that the nitrogen uptake is greater by using controlled means, compared to conventional approaches, resultantly, the plant growth is improved [13]. Hence, technology-based precision management of orchards including the application of balanced fertilizers, herbicides, and pesticides is required for sustainable citrus production. However, there are several biotic and abiotic factors including diseases that affect the citrus industry. Amongst diseases, bacterial, fungal, and viral are constant threats and cause substantial economic impact in all growing areas around the world. Citrus greening is an extremely dangerous disease, caused by different species of the bacterium Candidatus Liberibacter including Candidatus Liberibacter asiaticus, Candidatus Liberibacter americanus, and Candidatus Liberibacter africanus. Of these three species, the Candidatus Liberibacter asiaticus

(CLas) has become a serious threat to the citrus industry. The disease was reported from Brazil in 2004, from Florida, the USA in 2005, in 2007 from Cuba, and molecularl detection of the disease has been reported in 2007 from Pakistan, in 2008 from the Dominican Republic; in 2010 from Mexico [14–18]. However, the disease has been reported and described in China since 2019. The disease is transmitted by the psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), commonly called Asian citrus psyllid. As CLas is difficult to culture on artificial media hence its detection is possible through polymerase chain reaction (PCR) and particularly by quantitative real-time polymerase chain reaction (qPCR) targeting the 16S rDNA gene [14]. Recently, chloroplasts provide opportunities for pathogens to directly or indirectly target 'chloroplast immunity' as these organelles are the main sites for the synthesis of precursors of phytohormones, hence are coordinating plant defense responses. As hormonal crosstalk between host and pathogens is well established. The identification of chloroplast genes targeted by the CLas effectors will open the window to control this disease. The pathogen genome has been sequenced from *Diaphorina citri* from America [19] and the genome is composed of 1231639 bp with GC 36.5% contents. The pathogen genome has been sequenced from two strains from Pakistan. The CLas genomes of two strains (PA19 and PA20) were sequenced that is comprised of 1224156 bp and 1226225 bp, respectively with an average GC content of 36.4% [20]. The genome sequence of CLas from Thailand has also been reported with total GC contents of 36.4% and 1230623 bp genome. Several genes from the Candidatus spp. have been identified that interact with the genes of the plant defense system and scientists are working to identify the plant genes regulated by these pathogen effectors. The chloroplast being the main site of phytohormone precursor synthesis provides opportunities for pathogens to target, directly or indirectly, the 'chloroplast immunity'. As hormonal crosstalk between host and pathogens is now well established hence identification and editing of chloroplast genes using CRISPR/Cas technologies hold promise to control the disease. Similarly, the canker susceptibility gene, CsLOB1, of Duncan grapefruit has been knockout. The infection by Xanthomonas citri was significantly reduced with no disease development on plants [21].

4. Citrus nutritional and nutraceutical importance

Citrus fruits, diverse in color and size, are highly nutritious. They are beneficial for health, due to the presence of bioactive compounds such as carotenoids, flavonoids, and ascorbic acid [22]. These compounds have antioxidant, antitumor, anti-inflammatory and blood clotting inhibiting characteristics [23, 24]. These fruits are also a rich source of vitamins and minerals like vitamin C, A, and B-complex [25–27], vitamin A benefits skin and vision whereas vitamin B-complex like thiamin, folates, and pyridoxine are required as external sources to replenish. Of minerals, potassium, magnesium, calcium, and sodium are present in citrus from very high to low levels in citrus fruits [28]. However, zinc, iron, and manganese are present in trace amounts (**Figure 2**).

Nutraceutical is a combination of two words; 'nutrition' and 'pharmaceutical', hence the word infers that the nutraceuticals could be regulated as dietary supplements, medicine, and food ingredients. The word, 'nutraceutical' was first coined by Stephen L. DeFelice in 1989 who explained nutraceuticals as; "Food, or parts of food, that provide medical or health benefits, including the prevention and treatment of disease" [29, 30]. However, the government of Japan started approving foods with proven benefits for the general public in the 1980s, reviewed elsewhere [31]. Nutraceuticals protect against diseases developed due to nutrient deficiencies

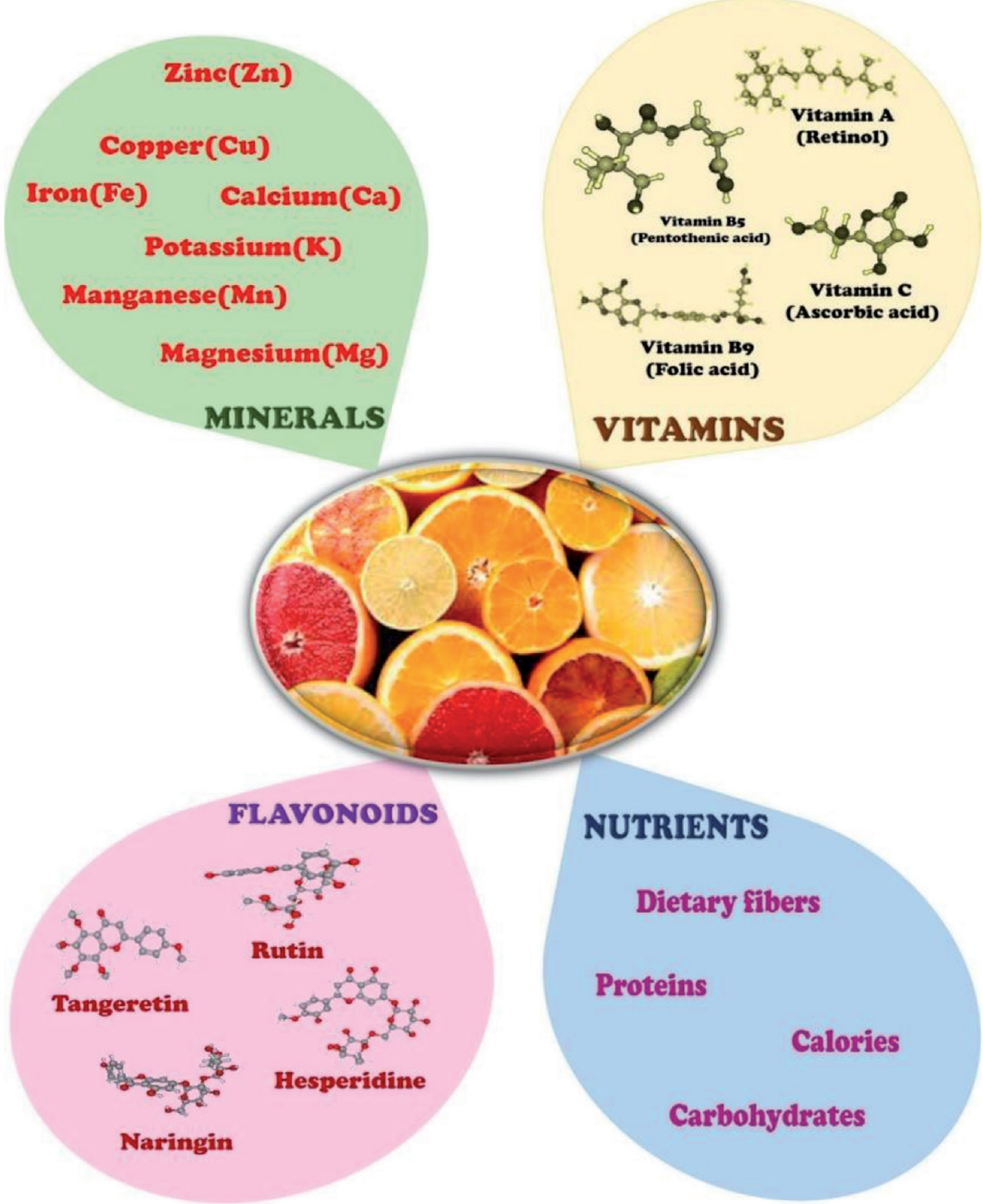

Figure 2.
The citrus, a source of nutrients and nutraceuticals. Citrus species are a rich source of carotenoids, flavonoids, minerals, and vitamins that function as antioxidants, antitumor, and anti-inflammatory compounds.

and also have physiological benefits. These are used as dietary supplements and food ingredients. Being dietary supplements, these may contain vitamins, minerals, botanical extracts, essential amino acids, Poly Unsaturated Fatty Acids (PUFA), and enzymes that are probably deficient in most of our diets. Another category of nutraceuticals is nutrient-fortified food. For example, the iron-fortified wheat flour protects the wheat-dependent population from diseases that develop due to iron deficiency. A salient example is iron deficiency anemias. Other examples include purple cauliflower and purple potatoes, having additional anthocyanin content. Golden rice and Golden potatoes as well as Provitamin-A-fortified maize are the rich sources of Provitamin-A and carotenoids. These crops could reduce the disease development in humans caused by Vitamin A deficiency diseases e.g. night blindness, xerophthalmia, prevalent in Africa and Asia. Similarly, Quinoa is of great nutritional value. Laden with fiber, vitamins, and minerals, this plant also is rich in lysine, hence its proteins are nutritionally more complete than many vegetables [32]. Thus, quinoa holds great potential as a Nutraceutical, to curb the malnutrition rampant in many third-world countries, the likes of Pakistan, India, Nepal, Bangladesh, and several African nations.

Several plants, including food crops, have been reported that contain compounds to prevent diseases. Even in this era of rapid medicine evolution, cancer remains a major threat to the population and a leading cause of mortality in developed nations. Introduction of plants into lifestyle at an early age could reduce cancer risk up to 33%. For instance, blue maize is useful in preventing different types of cancers, such as colon cancer [33]. Several chemotherapeutic agents such as Taxol, Vincristine, and Vinblastine are derived from plants such as *Taxus brevifolia* and alkaloids of Vinca species. Nutraceuticals have also been shown to reduce the toxic effects of chemotherapeutic agents and radiation therapies [34].

Bacterial infections and the growing resistance to synthetic antibiotics is a serious concern. It has been proven experimentally that medicinal plants are effective against bacterial infections. In this era of technology development, the introduction of medicinal and nutritional traits transgenically into food crops is on the top priority of biotechnologists, hence engineering the citrus genome will be a better choice.

5. Citrus improvement through biotech approaches

Citrus fruits are highly nutritious and are beneficial for health, due to the presence of bioactive compounds such as carotenoids, flavonoids, and ascorbic acid. However, citrus production and quality are challenged with several biotic and abiotic problems. Biotech interdisciplinary interventions including transgenesis, genome editing, and OMICS could offer solutions to the issues of this fruit crop. Genetic transformation has been established in many citrus species thereby transgenic plants have been developed against bacterial, viral, and fungal pathogens. Equally, OMICS approaches; genomics, transcriptomics, proteomics, metabolomics, interactomics, and phenomics are exploited to improve the citrus fruits. Since, first attempt to manipulate the citrus genome remained unsuccessful hence, the protocols for efficient regeneration from explants like seeds, embryogenic cells, epicotyls, callus, nodal stem segments, and protoplasts, followed by transformation and selection have been optimized for different citrus species. Maximum regeneration potential has been observed in explant 'epicotyl' hence, the epicotyl has been used as an explant for the genetic transformation of citrus plants. Transgenically stable plants were recovered from Agrobacterium treated Duncan grapefruit epicotyls. The recovered plants were confirmed for transgene presence using PCR and Southern blotting techniques. Similarly, transgenic plants using epicotyl tissues as explants were developed from sweet orange, and citrange. The transformation efficiency remained as high as 93%. Hence, transgenic technology is proven as one of the most reliable interventions to genetically improve tolerance/resistance to abiotic/biotic factors in citrus [35, 36].

Using the technology, the nutrition and medicine-related traits have been successfully tailored in citrus fruits. For example, the expression of genes that encode enzymes like phytoene synthase, lycopene-β-cyclase, and phytoene desaturase of the carotenoid biosynthesis pathway have been modulated to supplement human nutrition with vitamin A and antioxidants. The Valencia orange is majorly grown for its juice but the quality of the juice is deteriorated due to the degradation of an enzyme, named thermostable pectin methylesterase (TSPME). Hence, the gene (CsPME4) that encodes TSPME was downregulated to improve the juice quality [37]. Further, an environmentally friendly technology named, 'chloroplast transformation' is available to develop transgenic plants [38–46]. This technology offers several superior advantages like overexpression of transgenes up to 70% due to polyploidy at organelle and genome (plastome) levels, accumulation of functional proteins, and natural containment of transgenes since plastids are transmitted to the next generation through ovary, rather than pollens that cause horizontal gene transfer, in most of the cultivated plant species. The chloroplast genome of several citrus species has been sequenced [47–51], thus necessary genetic information of the subcellular organelle is available to develop chloroplast transformation vectors and achieve chloroplast transformation, successfully. Therefore, the development of transgenic plants through chloroplast genome engineering is a promising way forward for cost-effective production of nutraceuticals.

6. Conclusions

Conventional research has played a pivotal role in the improvement of citrus. Enhanced heterozygosity has helped in the development of genetically diverse germplasm in most of the citrus species and numerous varieties have been released for commercial cultivation. However, with the advent of modern biotechnological tools, the period involved in crop improvement through indirect mutagenesis and polyploidization could be further reduced and enhance cost-effectiveness. Transgenic technology and OMICS have great potential to improve this fruit crop.

Acknowledgements

The author would like to thank Miss Rimsha Riaz, a Ph.D. scholar for her contribution to the graphical presentations in the chapter.

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