

Citrus Genetics, Breeding and Biotechnology

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Citrus Genetics, Breeding and Biotechnology

Edited by

Iqrar Ahmad Khan



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Preface

Citrus fruits are regarded as major household items in more than 100 countries around the world. The world juice industry is also dominated by citrus juices. Citriculture is the foundation for the livelihood of millions of farm and industrial workers, entrepreneurs and businesses. There is a rich endowment of knowledge and technologies developed by dedicated researchers and academics around the world which addresses the issues faced by the citrus industry worldwide. Integration and application of knowledge from different disciplines has made its impact by guiding growers and investors to grow more and better citrus. Yet, we can see that the major citrus cultivars grown in different parts of the world are not the product of systematic breeding efforts. This book is intended to provide consolidated information on citrus breeding in the era of biotechnology which is likely to hasten the pace of variety development aimed at resolving the problems faced by grove owners growing currently available cultivars.

Citrus breeders and geneticists have pursued development of new cultivars for nearly a century. They do have clear goals to achieve. But, the traditional techniques have simply been insufficient to achieve the desired goals within reasonable time frames. The early researches focused on botanical and taxonomic subjects followed by interest in cytogenetics. The knowledge base has strengthened, but the outcome, in the form of commercially successful varietal releases, has remained slow, with a few success stories among rootstocks and fewer among scion cultivars. The recent advances in genetics, molecular biology and biotechnology have changed the pace of citrus breeding and genetics research. New approaches allow recombination within a broader and better understood germplasm pool, and more efficient selection methods have been developed for many traits. Progress has been also accelerated by the ease of communications via electronic media.

The scientific community is better organized than it was 50 years ago. The International Society of Citriculture has been holding its congresses at regular intervals since 1968. The amount of information presented on genetic improvement has grown manifold. There is hope that the flow of materials will follow the trends in the flow of information.

The idea of this book evolved from discussions held during citriculture congresses at Orlando, Florida (in 2000) and then at Agadir, Morocco (in 2004). Nearly all active citrus breeders of the world were willing to join hands to consolidate up-to-date information to accelerate citrus breeding. The subjects covered in the book are focused on citrus while

providing information equally useful to the breeders of other tree crops. This will also help students of genetics and breeding identify appropriate applications of biotechnology in citrus breeding. While providing information on future avenues, the authors have also reviewed the past progress and achievements ensuring continuity of the subject. Several chapters include protocols for novel techniques that should facilitate their broader application by citrus breeders.

Many colleagues at CREC-Lake Alfred, where most of the editorial work was undertaken, helped in vetting the drafts. While the ideas and refereeing help from many is acknowledged, the editor takes the responsibility for errors and omissions. Patience of CABI management and staff has been a source of encouragement. Many thanks to them for doing an excellent job of compilation. This task could not have been accomplished without support from the Sultan Qaboos University, Muscat. It is hoped that the book will prove beneficial to a wide range of readership.

Iqrar Ahmad Khan

1 Citrus Breeding: Introduction and Objectives

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As our early ancestors evolved from the process of nomadic food gathering to developing permanent food sources, crop breeding became an established practice. Over the centuries, humans selected, propagated and disseminated useful species that they chose to cultivate to feed the expanding populations. The hundreds of fruits, vegetables and grains that are now found on the supermarket shelves are each the result of the activities of plant breeders.

In 1865, the discovery of Gregor Mendel's laws of heredity became a turning point in the founding of the science of modern genetics, which transformed plant breeding into a modern scientific discipline. Mendel's critical and painstaking experiments allowed other plant breeders to conduct even more advanced studies on hybridization, which evolved into a remarkable success story. Consider the abundance of nutritious cultivars, the development of hybrid vigour in agronomic crops such as sorghum and maize, the development of short stature wheat and rice cultivars leading to the Green Revolution and elimination of extensive crop failures caused by southern corn leaf blight, or wheat rust or historic potato blight.

Until Mendel's paper on inheritance and its rediscovery at the turn of last century, plant breeding was based on traditional knowledge. Selection of desirable phenotypes was the primary means of perpetuating species. Plant breeding, now a scientific discipline, has resulted in major changes in how we feed the world's population through superior cultivars.

It can be said that the successful development of commercial crop industries around the world is directly related to the success of plant breeding programmes. The subject of citrus breeding and allied techniques has been reviewed previously (Cameron and Frost, 1968; Soost and Roose, 1996; Grosser and Gmitter, 2005). This book is a consolidation of the current status of science and technology relevant to citrus breeding.

Outlook

Tracking the ancestry of citrus is a complex process because of the great diversity and the distant centres of origin. Indeed, the evolution from wild citrus of Asia to modern day cultivars took hundreds of

thousands of years. Now grown in more than 100 countries in tropical, subtropical and Mediterranean climates, citrus (including oranges, grapefruit, tangerines and mandarins, and lemons and limes) is the leading fruit crop grown in the world. In 2004, world production of citrus was 108,535,000 Mt (www.fao.org) which is dominated by oranges (Fig. 1.1). Brazil and the USA (Florida and California) were leading producers of sweet oranges. The USA is the primary producer of grapefruit. China, Spain and Japan produce 65% of the tangerines grown in the world. Lemons are produced primarily in Argentina, Spain and the USA, while Mexico is the largest producer of small fruited limes. Lime is also a traditional crop in South Asia and the Middle East. While consumers are generally familiar with the edible citrus types, there is an equally large component of non-edible citrus used as rootstocks for successful production of edible citrus. Additionally, many citrus species have industrial significance as a raw material for cosmetic and pharmaceutical products.

In general, the citrus production areas are located within 35° north and south of the equator. The main citrus regions, how-

ever, are in the subtropics, which are more than 20° north or south of the equator. As with most agricultural crops, many factors are known to limit the production and processing of citrus. Most are dependent on problems related to scion and rootstock deficiencies. Major constraints to citrus production involve management inefficiencies, susceptibility to pests and diseases, and environmental challenges. These lead to increasing production costs, declining labour supply in many parts of the world and urban encroachment, especially on the most productive farm lands. Thus, new and improved scion and rootstock cultivars aimed at controlling these production and marketing constraints have been the primary aim of citrus breeding efforts.

The development of new and improved citrus cultivars by conventional methods is a slow and costly process. It may take as long as 20–35 years or longer to release a new cultivar from the time of making the cross. Kinnow mandarin was bred and released at the University of California-Citrus Research Center, Riverside (Frost, 1935). The parental cross was made in 1915 and official release took 20 years. However, it took another period of more than 30 years

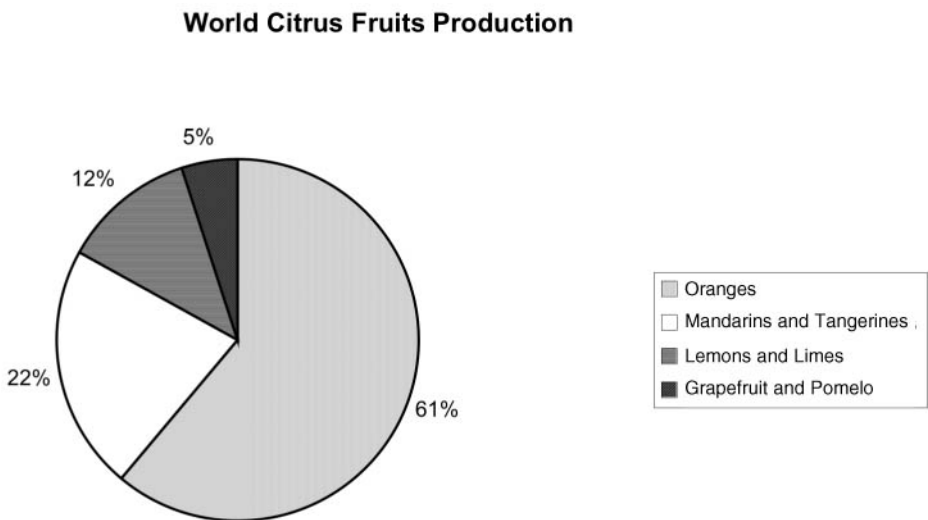


Fig. 1.1. Proportion of citrus fruits produced in the world.

before Kinnow became a successfully grown commercial cultivar in the Punjab region of Pakistan and India.

Several serious obstacles exist that hamper citrus hybridization. For example: (i) citrus is highly heterozygous; (ii) its unique reproductive biology such as apomixis and embryony; (iii) pollen and ovule sterility causing incompatibility; (iv) a long juvenile period taking as long as 5–10 years to express first flowering; and (v) adventitious embryos in the nucellar of developing ovules limit hybrid production. In developing seeds of polyembryonic cultivars, nucellar embryos compete with zygotic embryos. In addition to the long generation cycle, large seedling populations are needed (depending on species) that require extensive field space and labour.

The first formal citrus breeding programme was started by USDA in Florida in 1893 (Cooper *et al.*, 1962) which is still expanding. The University of California established the Citrus Research Center, Riverside in 1907. The University of Florida initiated its citrus breeding programme in 1924 which is now one of the largest breeding programmes, centred at the Citrus Research and Education Center, Lake Alfred (CREC). Today, there are numerous citrus breeding programmes spread in all major citrus-producing countries.

Most of the present day scion and rootstock cultivars of citrus are the progeny of chance seedlings or a mutant branch of a tree, called 'budspots'. The commercially successful cultivars now grown have resulted from the selection, propagation and advanced testing of thousands of such superior chance seedlings. This process of screening of potentially superior scion and rootstock candidates under commercial conditions involves a critical evaluation of tree performance and fruit quality traits. The above-mentioned breeding programmes played a major role in shaping today's citrus industry by providing information on the use of numerous scion and rootstock cultivars.

Breeders identify the most advanced candidates selected from initial screens and

usually propagate them on certain rootstocks, establish them in replicated blocks in different regions and environmental conditions, and compare them with known cultivars. After such rigorous evaluation of horticultural performance, economic analysis and test marketing for fresh market and processing use, superior survivors may finally result in the introduction of a named variety.

In some citrus breeding programmes, new selections are patented for economic and scientific protection. After receiving a patent, the new cultivar may be licensed to nurseries to multiply the trees for commercial distribution. Certification programmes may be established to ensure that disease- and pest-free budwood is provided to commercial nurseries.

Achievements in Cultivar Improvement

Although citrus breeding programmes have existed in citrus-producing areas around the world for many years, most commercially important scion and rootstock cultivars were developed by means of selection of mutations or by chance seedlings. Relatively few have arisen from conventional citrus breeding. Examples of cultivars from breeding programmes in the USA are the mandarin hybrids such as tangelos (mandarin \times grapefruit hybrids) and tangors (mandarin \times sweet orange hybrids) – specifically Minneola, Orlando, Nova, Page, Robinson, Sunburst, Fall Glo and Ambersweet. Swingle citrumelo (grapefruit \times trifoliolate orange hybrid) and sweet orange \times trifoliolate orange hybrids Carrizo and Troyer citranges are examples of citrus rootstocks originating from breeding efforts. Three mandarin varieties were released from the Citrus Research Center, Riverside (Frost, 1935). Only one of the three, Kinnow mandarin (King \times Willow Leaf), became a major success in the subcontinent of India and Pakistan. That reflects the value of international exchange of breeder's efforts. The process of release of new cultivars has now become more frequent, with expected

impact on the citrus industry in the years to come.

Breeding Objectives

Depending on the needs of a specific region of the world, the objectives of citrus breeding programmes may vary considerably. For example, some of the following objectives may have high priority in some regions and low or no priority in others.

Scion breeding

See Table 1.1 for the objectives of scion breeding.

Rootstock breeding

Nearly all commercial citrus in the world is grown as grafted trees, with the scion cultivar budded on a selected rootstock cultivar. A good scion and rootstock combination supports development of trees that bear large quantities of high quality fruit. Such a stoic combination can maintain health and productivity for 50 years or more with modest management. However, many available rootstocks are inadequate to meet the emerging needs and challenges. A large proportion of the problems faced by the citrus industry could be overcome by use of improved rootstocks (Wutscher and Hill, 1995; Bowman, 2000).

Benefits of the rootstock

EARLY BEARING. Budding a mature scion on to a rootstock plant takes advantage of the scion variety that has already passed juvenility which can start production within 1–2 years of planting in the grove, whereas citrus seedlings typically require many years to begin bearing fruit when grown from seed (3–15 years, depending on the species). In addition, citrus seedlings often maintain undesirable juvenile characteristics, such as excessively large thorns, for many years after fruiting begins.

TRUE TO TYPE. Propagation by budding/grafting on to a rootstock ensures that the trees will produce fruit that is identical to the source of budwood and thus allow plantings of a uniform type.

APOMIXIS. Most citrus rootstocks are apomictic, which can produce uniform plants from seed at a low cost. Seed propagation costs less and produces more vigorous and uniform nursery stock than by cuttings or tissue culture.

COMBINING TRAITS. Propagating citrus through budding on a rootstock is the benefit that probably has the most relevance in terms of rootstock breeding. It allows the graft creation of a tree that combines the best genetic fruit characteristics above ground with the strongest genetic root traits (adaptations to soil type, tolerance to salinity, resistance to diseases and nematodes, etc.) below ground as two separate units. Creating such a combination

Table 1.1. Objectives of scion breeding.

<i>Tree performance</i>	<i>Fruit characteristics</i>	<i>Postharvest of fruit</i>
Yield	Exterior appearance	Handling for fresh market
Cold hardiness	Size and shape	Economic and cultural importance
Adaptation to adverse climatic and soil conditions	Quality (ss/acid ratio)	Processing quality (processed into frozen concentrate or single strength juice products)
	Juice content, flavour/colour	
Adaptation to mechanical harvest	Ease of peeling	
Disease and pest resistance	Seedlessness	Storage life
	Season of ripening	Juice content and composition

of genetic traits in a single (self-rooted) genotype would be considerably more difficult.

Problems created by using a rootstock

EXPERTISE. Budding/grafting for propagation requires a significant expertise and adds cost when compared with seed propagation.

LACK OF APOMIXIS. A uniform nursery stand typically relies upon nucellar polyembryony (Frost and Soost, 1968) which results in the production of genetically uniform clonal seedlings. If a rootstock cannot be propagated uniformly by seed, clonal propagation must be accomplished by cuttings or tissue culture. The problem is further compounded when a rootstock strain produces a mixture of nucellar and zygotic seedlings which will require a rouging procedure (Khan and Roose, 1988).

GRAFT-TRANSMISSIBLE DISEASES. Vegetative propagation of the scion by budding can carry many virus and virus-like diseases and other pathogens. Hence, citrus has a long list of graft-transmissible pathogens that can debilitate trees and must be carefully excluded from propagation material (Roistacher, 1991).

GRAFT INCOMPATIBILITY. An important problem associated with using a rootstock is potential graft incompatibility between certain rootstocks and scions. Although generally not well understood, graft incompatibilities apparently arise when there is a conflict between the physiology of the rootstock and scion that causes the tree to grow

weakly or die. A similar reaction can be induced by some virus diseases, such as incompatibility which occurs when trees of sweet orange (*Citrus sinensis*) on sour orange (*C. aurantium*) are infected by isolates of citrus tristeza virus (Bowman and Garnsey, 2000).

ROOTSTOCK AFFECTS THE SCION. It must also be kept in mind that rootstock does affect many important traits of the scion grafted on to it, including tree size, productivity and fruit quality. Hence, a rootstock may degrade the tendency of a scion to be highly productive or to yield good quality fruit. One example of this is the rough lemon (*C. jambiri*) rootstock, which produces a vigorous and highly productive tree, but also induces the scion to yield low quality, low sugar fruit.

The Rootstock Development Process

Development of improved rootstocks is a long and many-faceted process. Typically, it takes 30–35 years from the time a new rootstock is created by cross-hybridization until it is released to the local citrus industry for commercial use. Commonly pursued objectives of rootstock breeding are listed in Table 1.2.

Molecular Genetics and Biotechnology

Citrus breeding and genetics has been fortunate to have an early and continuing history

Table 1.2. Commonly pursued objectives of rootstock breeding.

Apomixis
Improved productivity
Tree size reduction
Adaptation to soil conditions (salt, calcareous soils)
Improvement in poor nutrition
Resolution of bud union problems
Resistance to diseases (<i>Phytophthora</i> , citrus tristeza virus, citrus blight, etc.)
Resistance to soil borne pests (diaprepes, nematodes, etc.)
Improvement in fruit quality
High seed production

of meeting the needs of a changing industry through scientific research on genetic techniques. A new generation of scientific methodologies is now available for the study of genomics. It is possible to locate genes rapidly on chromosomes, to isolate genes from plants to study their function at the molecular level, to modify genes and to reintroduce them into living organisms. This is, indeed, a far cry from Gregor Mendel's 19th century tenant that plant characters are controlled by discrete hereditary units – which are now called genes.

Modern genetic engineering evolved from the discovery of DNA as the genetic material by Avery and co-workers followed by Watson and Crick's discovery in 1953 of the double helix structure of deoxyribonucleic acid (DNA). Since then, modern day geneticists have been able to use more precise and sophisticated technology to overcome the barriers and greatly to facilitate genetic procedures.

It is essential that future programmes for citrus cultivar improvement emphasize understanding the inheritance of fundamental qualitative and quantitative traits and also be comprehensive. For example, the study of genomics involves a molecular-based knowledge of the structure and function of genes. Such information will afford a precise use of such traits in the tree and fruit. Numerous examples can be cited where genomic technology has benefited plant improvement efforts. For example, in citrus, the long duration of field evaluation is a major obstacle for developing specific characters such as tree size, yield, disease and insect resistance. The use of marker-assisted selections linking molecular markers to genes, which control important traits, will significantly reduce the time required to screen mature tree characters. Other tools of biotechnology have been employed with equal ease to facilitate the progress of work in citrus breeding programmes. Shoot-tip grafting techniques have been used for sanitation of virus-infected germplasm, which has allowed wide dissemination and conservation of parental stocks.

Cytogenetics

Cytogenetic investigations in citrus have been restricted due to very small chromosome size and little implication for ploidy manipulations except for an interest in triploid breeding for seedlessness. In recent years, *Citrus* has been accepted as a model for the application of somatic hybridization and cybridization for crop improvement (Khan and Grosser, 2004; Grosser and Gmitter, 2005). Despite a huge variability in citrus, evolution has been restricted at the diploid level largely because of apomictic seed propagation. Somatic hybridization has offered a unique situation for citrus breeders to pursue citrus evolution at higher ploidy levels, particularly that based on allotetraploid combinations (Grosser *et al.*, 1996).

Research Diversity

To understand better the scope and depth of the citrus breeding/genetics research being conducted in the world today, the reader is encouraged to read the 2000 Proceedings of the International Society of Citriculture (ISC) held in Orlando, Florida (published in 2003) and ISC 2004 proceedings. Citrus breeders and geneticists presented a total of 114 papers in 2000 (71 oral and 43 posters). Of the oral presentations, topics included scion cultivar evolutions (seven), rootstock cultivar evolutions (eight), breeding (34), genetics and genes (eight) and biotechnology (14). These papers represented work done in over 20 different citrus-producing countries, including the USA, Brazil, Spain, France, China, Italy, Japan, Argentina, Cuba, South Africa, Australia, Oman, Israel, Morocco, Turkey, India, Pakistan, Egypt, Lebanon, Korea and New Zealand. In 2004, the number of genetics and breeding papers went up to 126 where a major shift was seen towards molecular genetics, genomics and transgenic citrus research.

The diversity and scope of global citrus genetic research projects are impressive.

Preservation of Citrus Germplasm

Citrus geneticists are concerned over the reported loss of citrus germplasm especially in the areas of origin. Such potential losses must be addressed on a global and cooperative basis before more valuable and irretrievable citrus materials are lost forever. Breeding of citrus requires a large diversity of citrus cultivars in a reliable and protected source. Since such citrus populations are rare, worldwide, habitat destruction threatens existing materials. The FAO sponsored Global Citrus Germplasm Network recommended in 1996 that (i) activities be initiated to identify the locations of gene banks of citrus and its relatives; (ii) it should be determined what needs to be preserved in collections and data banks; (iii) citrus germplasm should be characterized; (iv) procedures to conserve available and unique citrus germplasm should be developed; and (v) citrus germplasm should be evaluated and catalogued using molecular markers. Such activities, if properly conducted, would help ensure the availability of important germplasm for use by future citrus breeders and geneticists.

About this Book

Basic knowledge in plant genetics and biotechnology has opened the door to new concepts and methodologies, which have greatly advanced the scope and abilities of the citrus breeder. To define the progress in the field of citrus genetics, key scientists working on citrus cultivar improvement around the citrus-producing world have combined their talents to write chapters in areas of their expertise. Such information at this time of the genetic revolution will be of great value to the 'students of citrus'. These chapters characterizing the remarkable advances made in recent times will point out the success of past and current citrus genome research and the need for continued financial support for such research in the future.

The major topics covered in this book are:

- Origin and taxonomy
- Germplasm resources and shoot-tip grafting
- Cytogenetics
- Somoclonal variation
- Somatic hybridization and single chromosome transfer technology
- Mutation breeding
- Triploid hybrids (hybridization between diploids and tetraploids)
- Marker-assisted selection by linkage maps
- Gene cloning
- Genetic transformation.

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