A GLOBAL STRATEGY FOR THE CONSERVATION AND USE OF CITRUS GENETIC RESOURCES





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A global strategy for the conservation and use of citrus genetic resources

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COVER

Citrus diversity. Photo: T Siebert-Wooldridge and K Trunnelle, UC Riverside



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EXECUTIVE SUMMARY

Citrus, including oranges (*Citrus × sinensis* (L.) Osbeck), lemons (*C. × limon* (L.) Osbeck), limes (*C. × aurantiifolia* (Christman.) Swingle), pummelos (*C. maxima* (Burm.) Merr.), grapefruits (*C. × paradisi* Macfad.), and mandarins (*C. reticulata* Blanco) as well as other fruits, are among the most widely grown fruit crops globally. Citrus is sold and consumed fresh and processed into juice, juice concentrate, oils, and other products. Citrus crop wild relatives have local economic importance. Citrus fruit are healthy; they are rich in vitamins, secondary metabolites, antioxidants, and other bioactive compounds. Sugar to acid ratios in the fruit, as well as some recently identified aromatic compounds, determine the degree to which fruit and juice taste sour or sweet.

Citrus is produced in subtropical, semi-tropical, and tropical regions around the world, with most commercial production between 20° and 40° latitude in the northern and southern hemispheres. It requires sufficient heat during the growing season and moderate winters to survive. Oranges represent the largest global harvested area, global production (in tons), and value, followed by mandarins, lemons/limes, and pummelos/grapefruits. Citrus species, as well as other genera in the sub-tribe Aurantioideae of the Rutaceae family, are found in the wild throughout Asia, with some also present in Africa and Australia. Market classes of citrus fruit resulted from introgressive hybridization (admixture) among wild ancestral taxa: C. medica (citron), C. reticulata (mandarin), C. maxima (pummelo), C. hystrix (makrut lime) and C. micrantha (papeda), among others. These primary progenitor species are native to Southern China, India, and other regions of Southeast Asia. Sexual hybridization resulted in oranges, lemons, limes, and others that were transported from Asia to locations around the world over thousands of years. Grapefruit is the most recent hybridization event leading to a category of citrus fruit in the marketplace—occurring between sweet orange and pummelo most probably in Barbados no more than 400 years ago.

Conservation of citrus trees and their crop wild relatives is critical. This diversity is necessary for use in breeding programs that seek to improve overall plant performance against biotic and abiotic challenges, as well as to improve product quality to benefit the wellbeing and health of consumers. Citrus collections are Goal for global *ex situ* conservation of citrus: The diversity of citrus and its wild relatives is conserved and available in a disease and pathogen-free state in perpetuity in a secure, distributed network of genebank collections that provide collection data (passport, phenotypic, genotypic) in a standardized common information system.

maintained primarily as field plantings or as potted trees in greenhouses or screenhouses. Fungal, bacterial, viral, viroid, phytoplasma, and arthropod pathogens and pests are significant concerns for both genebank collections and commercial production. Plant quarantine and sanitation programs are employed both nationally and regionally to limit the spread of pathogens and pests. This also limits the movement of citrus genetic resources on an international level, and sometimes even within countries. Citrus trees are usually clonally propagated by grafting, although most rootstocks are propagated from seeds containing nucellar embryos identical to the seed parent tree.

Genebanks seek to acquire, maintain, perform trait evaluations on, carry out molecular characterization on, and distribute citrus genetic resources. Collection back-ups are important because materials may be lost due to abiotic and biotic threats, limited financial support, and changing institutional priorities. Genebank collection maintenance is labor intensive, ideally requiring specific expertise in propagation and physiology, taxonomy, plant pathology, molecular genetics, database management, and breeding.

The Global Strategy for the Conservation and Use of Citrus Genetic Resources results from a background study of citrus genetic resources conservation and use, responses to a widely distributed survey of major citrus collections, and online consultations with genebank curators. The findings from the survey provide insights into the current status of citrus collections on a global scale. Based on this information, a global system by which citrus conservation and use efforts could become more coordinated, systematic, and unified is described.

The 2021 Global Citrus Survey collected information about worldwide citrus collections. Results revealed a total of 15,555 genebank accessions maintained in 33 collections around the world, more than 4-fold the quantity recorded in the Genesys and FAO-WIEWS databases. Survey respondents reported collections that ranged in size from fewer than 100 accessions to 1735 accessions at the Instituto Agronômico de Campinas/Centro de Citricultura Sylvio Moreira in Brazil. Other large collections were: Citrus Research Institute, Chinese Academy of Agricultural Sciences and Southwest University in China (1700 accessions); USDA-ARS National Clonal Germplasm Repository for Citrus and Dates in the United States (1632 accessions); Institute of Fruit Tree and Tea Science (NARO, NIFTS) in Japan (1261 accessions); National Research Institute for Agriculture, Food and Environment (INRAE)-Corsica in France (1100 accessions); and Queensland Department of Agriculture and Fisheries in Australia (1000 accessions). Smaller collections had significant numbers of local cultivars and wild species representatives, making their conservation efforts critical to the global conservation of citrus. Collections are rarely duplicated or backed-up at secondary locations, although a substantial portion of the USDA-ARS collection is being cryopreserved as shoot tips in liquid nitrogen. Materials from collections are distributed for propagation for re-sale, certification programs, breeding, plant and/or pathogen research, phenotypic evaluation, and molecular characterization.

Priority Actions were identified that seek to unify the citrus genebanking community with respect to sharing maintenance, inventory, and associated data through compatible online resources. With <u>funding</u>, Priority Actions will result in shared online resources, training opportunities, and standardized collection data as well as healthy, secure plant collections.

Priority Actions:

- Increase citrus genebank community cooperation by establishing an international working group and developing/using a Citrus Community Information System (CCIS) for citrus and related genera.
- 2. Support data collection and documentation efforts for citrus collections.
- 3. Identify taxonomic gaps (cultivars and related genera) in citrus collections and fill gaps through collections and exchange.
- Increase citrus collection health and security (backup), particularly collections that have vulnerable unique plant genetic resources.
- Provide training opportunities for the citrus genebanking community on a wide range of topics through a combination of affordable in-person and online options.
- Develop, maintain, and distribute materials from a clean, secure international citrus collection at one or more locations that captures taxonomic and genetic diversity of citrus.



BACKGROUND

Citrus is a perennial crop that was domesticated in ancient times and is now cultivated wherever a suitable climate is available. It is one of the most widely cultivated fruit crops in the world. Billions of people savor its spritely flavor and benefit from its nutritional properties.

"Citrus" in the context of this document refers to economic species in the genus Citrus and its closely related genera Fortunella and Microcitrus (see commentary in taxonomy section below), but also to crop wild relatives in the subfamily Aurantioideae of the family Rutaceae. Citrus fruits are classified by FAO (2022) as: oranges, lemons and limes; pummelos and grapefruits; tangerines, mandarins, and Clementines; and other citrus fruits (Figure 1). These commodities are consumed fresh, processed into juice or juice concentrate, or have oils, citric acid, pectin, and other compounds extracted from them (Di Giacomo, 2002). Some crop wild relatives have limited or local economic exploitation (Krueger and Navarro, 2007). For instance, bael (Aegle marmelos) is used as a food source and in religious ceremonies in South Asia, Clausena spp. are used as food sources in China and neighboring areas, and Microcitrus is used as a food source in Oceania.



Figure 1. Diverse citrus fruit (P. Greb, USDA).

1.1 Health benefits

Although fruit composition varies with the type of citrus, region of cultivation, and cultural practices, citrus fruit is low in protein and extremely low in fat, the chief source of calories being sugars (Erickson, 1968). The "sweetness" of citrus is determined by the sugar/acid content of the fruit, and to some extent by recently identified aromatic compounds that influence the perception of "sweetness". Some types of citrus, for instance lemons, are considered "acid" fruit because this ratio is lower than in "sweet" fruit, such as sweet oranges. Most of the sugars are in the juice portion of the fruit. Citrus fruit juice therefore may have similar calorie content to fresh fruit, but the latter contains many other compounds that contribute to the overall taste sensation of eating fresh fruit and has significantly more fiber than the juice consumed by itself. Citrus fruit have high levels of soluble fiber compared to insoluble fiber and thus may be beneficial in preventing diabetes and lowering cholesterol levels.

The potential benefits of citrus consumption on human health were recognized centuries ago as demonstrated by its use in Traditional Chinese Medicine and Ayurvedic Medicine. More recently, studies have demonstrated that citrus consumption may reduce lifestyle-related diseases such as cancer, cardiovascular disease, and type 2 diabetes. The health benefits of citrus, primarily recognized by the presence of secondary metabolites, has been reviewed (Patil et al., 2006; Ma et al., 2020). Citrus fruits have high concentrations of bioactive compounds, the best known of which is ascorbic acid (vitamin C). The health preserving effects of citrus consumption against scurvy was noted as early as the 17th century, although the role of ascorbic acid in preventing scurvy was not demonstrated until the 20th century. More recent studies suggest that ascorbic acid has strong anti-oxidant properties that may improve collagen formation, iron absorption, and immune function; prevent the occurrences of cardiovascular diseases and age-related macular degeneration; and reduce the risk of certain types of cancers.

Citrus also has high levels of other bioactive compounds, particularly antioxidants including carotenoids, flavonoids, and limonoids. Carotenoids have been shown to reduce the risks of eye diseases, certain cancers, and inflammation. Flavonoids potentially reduce the risk of developing cardiovascular disorders and have other pharmacological properties. Limonoids have potential anti-cancer properties. Most of these benefits have been demonstrated in *in vitro* and *in vivo* studies; additional therapeutic studies are needed to validate their value in this area (Ma et al., 2020).

1.2 Production

Currently, citrus is produced in most areas with suitable tropical, semitropical, or subtropical climates, resulting in a "citrus belt" between approximately 40° N and 40° S and comprising 140 countries, according to FAO (2022)(Figure 2, 3). The main constraints for most citrus production are temperatures during the growing season sufficiently warm for quality fruit development and winter temperatures mild enough for tree survival. Although citrus grows well in the tropics, subtropical climates are better for production and fruit quality (Burke, 1967; Spiegel-Roy and Goldschmidt, 1996).

For the last 60 years, global citrus production has trended upward in cultivated area, total production, and value (FAO, 2023) (Figures 4, 5, 6). However, the increases for sweet oranges and mandarins have been greater than for other types of citrus, with the increase in production area for mandarins being higher than for sweet orange, particularly in the last two decades. The total production and value of mandarins has not increased at the same rate as the area of production, possibly due to more recent plantings and lower per tree and per area yields. Production of specific types of citrus varies with geography (Figure 7). For instance, China produces large amounts of mandarins, sweet oranges, and pummelos, while Brazil produces mainly sweet oranges (Figure 8). Although Brazil is the largest producer of sweet oranges, most of its production is exported for use in the global orange juice industry. Spain is also a major producer of sweet oranges, but its fruit are mostly exported for fresh consumption (Figure 9). Mexico is a major producer of lemons and limes, with a large proportion of its production being used industrially in beverage or aroma production. The largest citrus producing countries are shown in Figure 8.

1.3 Taxonomy and classification

The taxonomy of citrus has always been complicated and is currently particularly fluid due to advances in molecular systematics and comparative genomics. W.T. Swingle, of the U.S. Department of Agriculture, spent over 40 years studying the taxonomy and botany of *Citrus* and its related genera. His many publications in this area are summarized in Swingle (1943) and its slight revision as Swingle and Reece (1967)¹. Swingle (1943) placed *Citrus* into the subfamily Aurantioideae of the family Rutaceae, comprising genera further divided into tribes and subtribes (Table 1) and recognized 16 species of *Citrus* (Table 2). The basic system

¹Since the revision of Swingle and Reece (1967) only slightly altered the original of Swingle (1943), reference will be made henceforth to Swingle (1943) with the understanding that the information is available in both sources



Figure 2. Citrus production A) Family Satsuma farm in Jeju Island, South Korea (F. Gmitter), B) Hillside orchard in South Hunan Province, China (F. Gmitter), and C) Washington navel orange grown in Florida, USA (USDA).



Figure 3. A) Citrus production in the San Joaquin Valley, California, USA. B) Citrus trees ready to ship at a commercial nursery in the San Joaquin Valley, California, USA (R. Krueger).

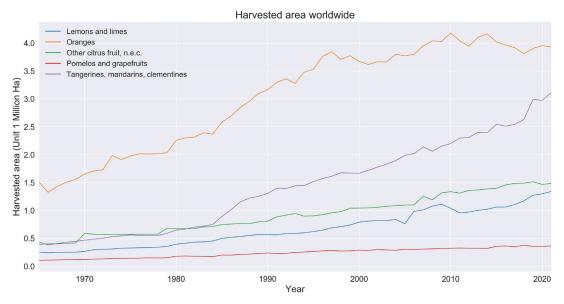


Figure 4. Global harvested area of citrus types, 1961–2020 (FAO, 2023).

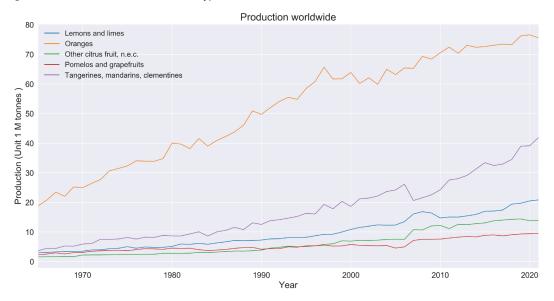


Figure 5. Global production of citrus types, 1961–2020 (FAO, 2023).

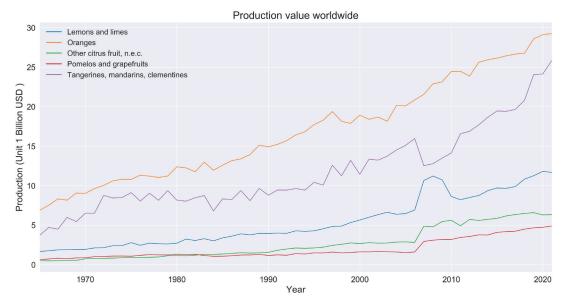


Figure 6. Global value of citrus production (constant USD), unit on the vertical axis is 10°9 (1 billion), 1961–2020 (FAO, 2023). NOTE: n.e.c. is "other citrus fruit not elsewhere classified." This subclass includes: – bergamots, *Citrus aurantium* subsp. *bergamia* – chinottos, fruit of the myrtle-leaved orange, *Citrus aurantium* var. *myrtfolia* – citrons, *Citrus medica* – kumquats, species of Fortunella



Figure 7. Citrus in markets in A) Cairo, Egypt, B) Quito, Ecuador, and C, D) Munich, Germany (G.Volk).

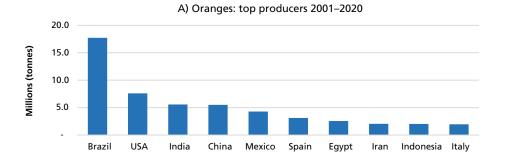
of Swingle has been modified to recognize 17 species (Bhattacharya and Dutta, 1956; Stone, 1994a), 31 species (Singh and Nath, 1970), or 36 species (Hodgson, 1961). In contrast with the Swingle (1943) classification, the Tanaka system of classification recognizes up to 162 species (Tanaka, 1954; 1977). This reflects disagreements as to what degree of difference justifies species status and whether supposed naturally occurring hybrids should be assigned species status. The Tanaka system has been used widely in most countries outside the USA and is useful in recognizing horticulturally important cultivars and characteristics.

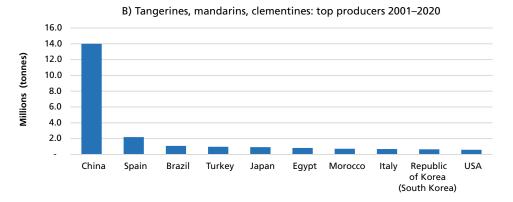
The taxonomic treatments of Mabberley (1997, 1998, 2004, 2022) and Zhang et al. (2008) recently modified Swingle (1943) by pulling Poncirus, Fortunella, Microcitrus, and Eremocitrus back into the genus Citrus from whence they came. This system and its modifications are becoming more widely used as molecular and genomic data have become increasingly available. Genome sequence data are yielding new insights into citrus classification and genetic relationships (Wu et al., 2014; 2018). The evolution of Citrus classification has been reviewed recently by Luro et al. (2017) and Ollitrault et al. (2020); the latter group also proposes a new taxonomic treatment recognizing 42 "phylogenomic classifications." Another recent taxonomic system is that of Schori (2022), which is based upon more classical taxonomic principles.

family are used much less frequently and therefore exist most often as "wild" unselected types (Table 1). Overall, there has been less attention focused upon them except by local populations. The fact that these wild relatives are not commercially useful, however, does not imply that they do not have useful genes that may be used in citrus improvement. For instance, fruit and juice of C. trifoliata (also known as Poncirus trifoliata) are unpalatable, and although much effort has been expended over more than 100 years to use this germplasm for scion improvement, to date there are no commercially successful new scion types incorporating C. trifoliata genetics. However, it has several genes that have been extremely useful in developing rootstock varieties, and its various disease resistance characteristics, such as resistance to citrus tristeza virus (CTV) and a high level of tolerance of Huanglongbing (HLB), likewise are being explored for use in scion breeding.

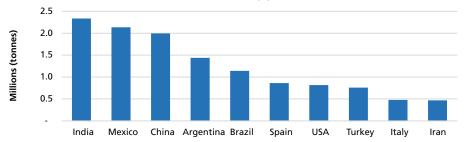
Therefore, maintenance of a wide range of genetic material in genebanks, whether of immediate commercial use or not, is essential to the long-term survival of citrus as a crop. Review of the taxonomy of Swingle (1943) indicates that in many cases, new species were named based upon a single collection or herbarium specimen; at least some are probably best treated as synonyms. Research into the related Aurantioideae genera has been limited in recent years, as summarized in Krueger and Navarro (2007) and Krueger (2010). The classic taxonomic treatments

The 32 non-Citrus genera in the Aurantioideae sub-





C) Lemons and limes: top producers 2001–2020



D) Pomelos and grapefruits: top producers 2001–2020 3.0 2.5 **Millions (tonnes)** 2.0 1.5 1.0 0.5 USA China Thailand Vietnam Mexico South Sudan Israel Turkey India Africa

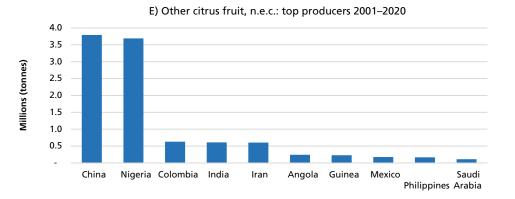
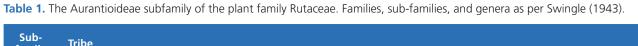


Figure 8. Top 10 international producers of citrus, 2001–2020. A) Sweet oranges, B) Tangerines, mandarins, and clementines, C) Lemons and limes, D) Pomelos and grapefruit, and E) Other citrus (FAO, 2023).



Figure 9. Commercial nursery association production facility in A) Alcalá de Xivert, Castellón de la Plana, Spain (R. Krueger), and B) Chongqing, China (F. Gmitter)



Sub- family	Tribe	Subtribe	Genus	Origin
		Micromelinae	Micromelum	SE Asia, Oceania
	eae		Glycosmis	SE Asia, Oceania
	Clauseneae	Clauseninae	Clausena	S Asia, Oceania
	Clau		Murraya	S & SE Asia, Oceania
		Merrilliinae	Merrillia	SE Asia
			Wenzelia	Oceania
			Monanthocitrus	Oceania
			Oxanthera	Oceania
		Triphasiinae	Merope	SE Asia, Oceania
			Triphasia	SE Asia, Oceania
			Pamburus	S & SE Asia, Oceania
			Luvugna	S & SE Asia, Oceania
			Paramignya	S & SE Asia
		Citrinae	Severinia	S China, SE Asia
υ	Citreae		Pleiospermium	S Asia, Oceania
idea			Burkillanthus	SE Asia, Oceania
Aurantioideae			Limnocitrus	SE Asia
Aura			Hesperethusa	S & SE Asia
			Citropsis	Central Africa
			Atalantia	S & SE Asia
			Fortunella	S China
			Eremocitrus	Australia
			Poncirus	Central & N China
			Clymenia	Oceania
			Microcitrus	Australia
			Citrus	S & SE Asia, S China
		Balsamocitrinae	Swinglea	Phillipines
			Aegle	India
			Afraegle	West Africa
			Aeglopsis	W Africa
			Balsamocitrus	Uganda
			Limonia	S & SE Asia
			Feroniella	SE Asia

have been updated for *Clausena* (Stone, 1978b; Molino, 1994; Lu et al., 2016; Mou et al., 2018, 2021a), *Clymenia* (Stone, 1985a), *Glycosmis* (Brizicky, 1962; Huang, 1987; Stone, 1978a, 1985b, 1994b; Mou and Zhang, 2009; Mou et al., 2012; Toyama et al., 2016), *Luvunga* (Stone, 1985c; Ling et al., 2009; Tagane et al., 2020), *Monanthocitrus* (Stone, 1985c; Stone and Jones, 1988), *Murraya* (Huang, 1978; Stone, 1985c; Jones, 1995; Kinoshita, 2014; Astuti and Rugayah, 2016; Mou et al., 2019, 2021b; Nguyen et al., 2019), *Oxanthera* (Stone, 1985b), *Paramignya* (Phi et al., 2020), and *Wenzelia* (Stone, 1985b). However, more work is undoubtedly needed in this area, particularly with genera and species that are rare or difficult to acquire.

1.4 Geographic distribution of wild species

Citrus and closely related genera in the sub-family Aurantioideae of the family Rutaceae are mostly native to the monsoon regions of southeastern Asia (northeastern India, southern China, the Indo-Chinese Peninsula), eastern Australasia, and Polynesia (Talon et al., 2020; Table 1). Recent research places the center of origin of citrus in the southeastern Himalayan area, including eastern Assam (India), western Yunnan (China), and Myanmar (Wu et al., 2018; Talon et al., 2020).

It is now generally accepted that *C. medica* (India, Bhutan, Bangladesh, Myanmar and China), *C. reticulata* (China), *C. maxima* (Malaysia, Indochina, China), *C. hystrix* (Southeast Asia), and *C. micrantha* (Philippines), and possibly a few more species are true wild species of *Citrus* sensu Swingle (Figure 10; Wu et al., 2018, Talon et al., 2020). These "primordial" species later hybridized naturally to create most of the commonly cultivated *Citrus* species (Figure 11; Scora, 1975; Barrett and Rhodes, 1976; Wu et al., 2014, 2018, 2021; Luro et al., 2017; Talon et al., 2020; Ollitrault et al., 2020). Interestingly, the earliest workers also believed that there were only three or four true species of wild *Citrus* (Linnaeus, 1753; Hooker, 1875).

From southeastern Asia, wild proto-citrus spread northeastward through China and into Japan, and southeastward into the Indo-Chinese peninsula, during which time it speciated into the ancestral citrus species (Figure 12). Citrus crop wild relatives currently are found in China, west to India, east to Japan, and as far south as Australia.

There are additional wild *Citrus* species that are not believed to be the progenitors of commercial cultivars, including *C. ichangensis* (China, India, Myanmar), *C. mangshanensis* (China), *C. japonica* (kumquat, Japan), *C. margarita* (kumquat, China), *C. ryukyuensis* (Okinawa), *C. latipes* (India), *C. glauca* (Australian desert lime, Australia), *C. australis* (Australia), and *C. australasica* (Australia) (Gmitter et al., 2020; Talon et al., 2020; Wu et al., 2021). *C. trifoliata* (trifoliate orange, China) is not a progenitor of scion types of citrus, but it has been utilized extensively by humans to produce rootstocks for citrus propagation. Australian citrus migrated from the Asian landmass no earlier than the late Oligocene (Pfeil and Crisp, 2008).

1.5 Domestication

Before domestication, citrus had apparently spread

Citrus species	Common name	Probable origin	Probable native habitat	Seed reproduction
C. medica	Citron	true species	India	sexual
C. aurantium	Sour orange	Hybrid	China	nucellar
C. sinensis	Sweet orange	Hybrid	China	nucellar
C. maxima	Pummelo	true species	China	sexual
C. limon	Lemon	Hybrid	India	partly sexual
C. reticulata	Mandarin	true species	China	variable
C. aurantifolia	Lime	Hybrid	Malaya	partly sexual
C. paradisi	Grapefruit	Hybrid	Barbados	Nucellar
C. tachibana	Tachibana	Unknown	Japan	Sexual
C. indica	Indian wild origin	Unknown	India	Sexual
C. hystrix	Mauritius papeda	Unknown	SE Asia	Sexual
C. macroptera	Malesian papeda	Unknown	SE Asia	Sexual
C. celebica	Celebes papeda	Unknown	Celebes	Sexual
C. ichangensis	Ichang papeda	Unknown	China	Sexual
C. micrantha	Papeda	Unknown	Philippines	Sexual
C. latipes	Khasi papeda	Unknown	Assam	Sexual

Table 2. Species within the genus Citrus as per Swingle (1943).

from its center of origin to be more widely dispersed in eastern and southeastern Asia (Figure 12; Wu et al., 2018, Talon et al., 2020). This includes the origin and diffusion of Tachibana (native to Taiwan and Japan) and Shekwasha (Shiikuwasha, in Okinawan dialect, native of Okinawa), derived from *C. reticulata* and the recently described *C. ryukyuensis* (Wu et al., 2018, 2021). Domestication of citrus likely began in areas in which it had become endemic, particularly in China and India, where citrus was probably being cultivated 5–6,000 years ago (Tolkowsky, 1938, Deng et al., 2020). The ancestral taxa *Citrus medica* (citron), *C. reticulata* (mandarin), and *C. maxima* (pummelo), *C. hystrix* (makrut lime), and *C. micrantha* reproduce sexually and when different genotypes within the species are crossed, the progeny are similar to their parents (Figure 13). These citrus types produce seeds with primarily zygotic (sexually derived) embryos (Table 2). The other important edible types of citrus (orange, grapefruit, lemon, and lime) are believed to have originated from one or more generations of hybridization between the ancestral species (Figure 11). Many of the embryos produced by these hybrid species are nucellar

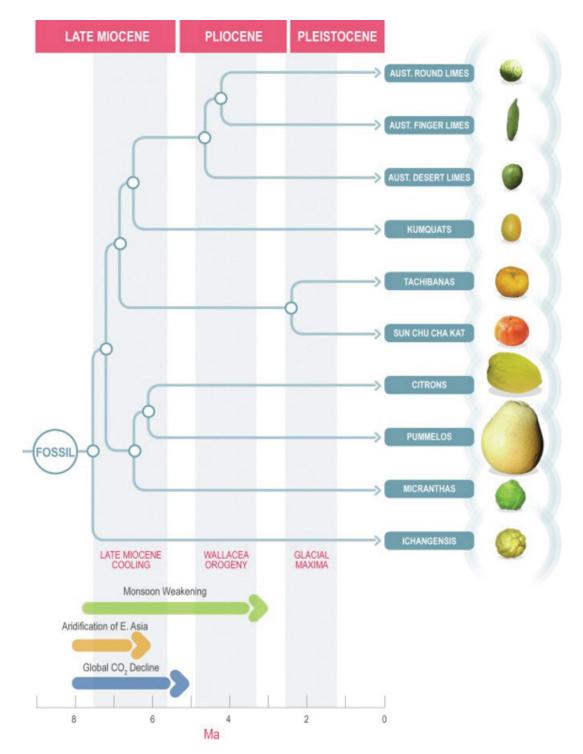


Figure 10. Diagram of Citrus speciation (Talon et al. 2020).

or apomictic types (Table 2). Wu et al. (2021) recently traced the origin and diffusion of the gene responsible for apomixis in citrus scion cultivars to a subpopulation of *C. reticulata* in southeastern China and highlighted the central role this mutation has played in the domestication processes that have led to all the major cultivar groups of commercial significance. Most of the cultivars of orange, grapefruit, and lemon originated as apomictic seedlings or bud sports and were subsequently cultivated vegetatively by grafting. Consequently, the amount of genetic diversity within most commercially significant citrus cultivar groups is relatively low, despite the many named cultivars. Conversely, mandarins, pummelos, and citrons have higher levels of genetic diversity because many of the cultivars arose through sexual hybridization prior to their vegetative propagation.

Vegetative propagation of citrus is traditionally performed by growing seedling rootstocks and then grafting the scion variety onto the rootstock. The number of rootstocks currently being used is limited but increasing. Most citrus accessions used as rootstocks are not useful as scions due to their poor fruit

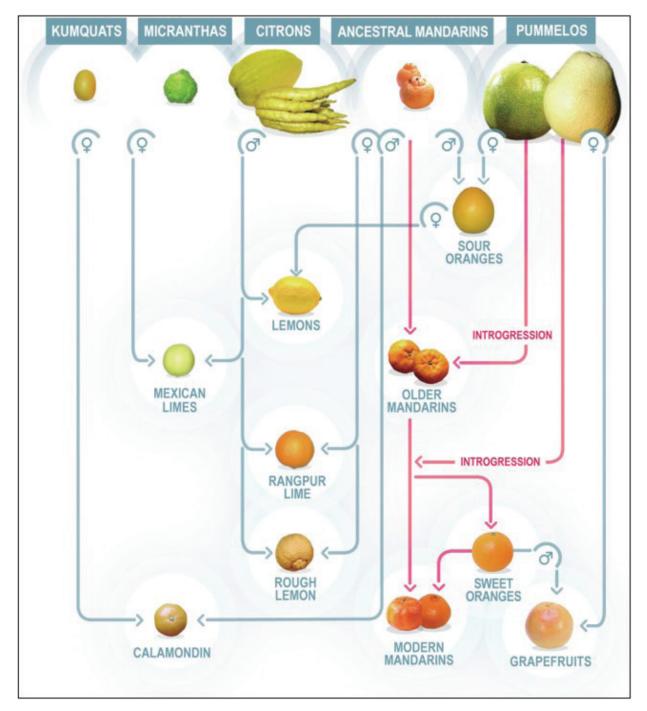


Figure 11. Admixture of primordial citrus species into modern citrus types (Talon et al. 2020).

quality (high acidity and bitterness). *C. trifoliata*, as mentioned, has been used extensively as a pollen parent in developing rootstocks for many types of commercial citrus. Amongst its other qualities, *C. trifoliata* is resistant or tolerant to citrus tristeza virus, *Phytophthora* spp., Huanglongbing, cold temperatures, and use of it or its hybrids as rootstocks generally produce high-quality fruit. These properties can be passed down to its progeny. Most *C. trifoliata* accessions and their hybrids are also highly apomictic, making it possible to produce genetically identical rootstocks from seeds.

The genomic relationships between wild and hybrid citrus species continue to be resolved, and the phylogenetic relationships among progenitor species and their hybridized offspring have been significantly simplified in Figure 11. Some natural hybrid and admixed species have been identified in places where progenitor species are sympatric. For example, C. × limonia (Rangpur lime = C. reticulata × C. medica) were found in China, Myanmar, Thailand, Laos, and Vietnam. Citrus × jambhiri (rough lemon, a different C. reticu*lata* × *C. medica* hybrid) was found in the Himalayan region. Sour oranges (C. × aurantium = C. maxima × C. reticulata) were found in India and China, and sweet oranges (C. × sinensis = C. maxima × C. reticulata) were found in Mynamar, Vietnam, Thailand, and China. Even lemons (C. × limon = (C. aurantium ×

C. reticulata) × *C. medica*) have been found in the wild in the Eastern Himalaya region (Talon et al., 2020).

Tanaka (1954) proposed a theoretical dividing line (the Tanaka line), which runs southeastward from the northeast border of India, above Myanmar, through Yunnan Province of China, to south of the island of Hainan (Figure 14). Citron, lemon, lime, sweet and sour oranges, and pummelo originated south of this line, while mandarins, kumquats, and trifoliate oranges originated north of the line, according to Tanaka's proposition. Gmitter and Hu (1990) proposed that Yunnan, China, through which the Tanaka line runs, is itself a major center of origin for citrus.

Citrus was spread by humans from its early centers of cultivation to regions worldwide that have a suitable tropical, semitropical, or subtropical climate (Figure 15) (Tolkowsky, 1938; Webber, 1967; Calabrese, 2002). The first type of citrus to diffuse westward was apparently the citron, which arrived in Persia in the first millennium BCE and was taken further west by the Romans (first century BCE) and the Arabs (700 CE). Citron, lemon, and sour orange arrived in Europe around the 9th century CE, via Spain during the Arab occupation. Sweet oranges were a later introduction around the 15th century, with mandarins not arriving until the 1700s or 1800s.

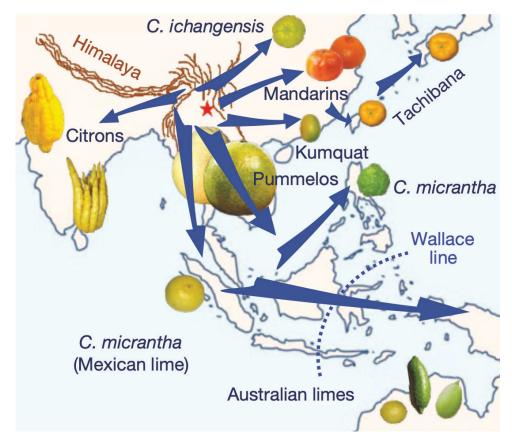


Figure 12. The spread of wild Citrus species from their center of origin (Wu et al., 2018).

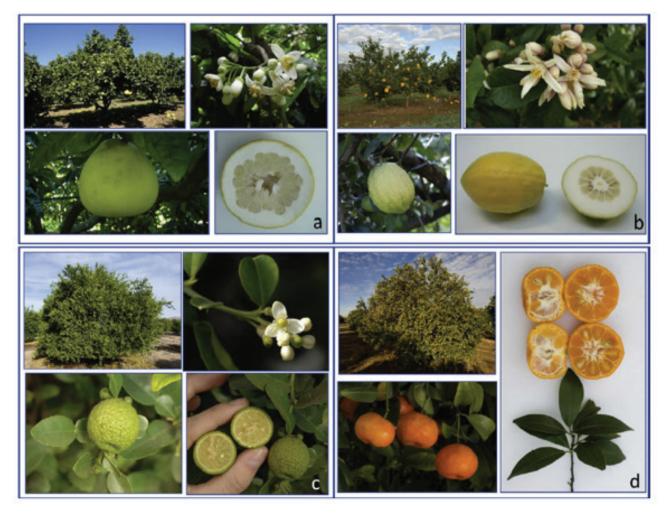


Figure 13. Morphological traits of 4 ancestral taxa of Asian edible citrus. A) *C. maxima* (F. Curk-INRA); B) *C. medica* (F. Curk-INRA); C) *C. micrantha* var *macrocarpa*; D) *C. reticulata* var *austera* 'Sun Chu Sha Kat' (C, D University of California, Riverside Citrus Variety Collection).



Figure 14. Geographical distribution of the origin areas of the Asian Citrus species divided by Tanaka's line (Luro et al. 2017).

Citrus was taken to the Western Hemisphere around the fifteenth century by the Spanish and Portuguese during the initial colonial era because their colonies had suitable climates for citrus production. Initially introduced into the Caribbean basin, citrus later spread into other areas. Citrus was introduced into Mexico early in the 16th century CE and spread thence into California (approximately 1800 CE) and Texas (approximately 1880 CE). Sweet orange (*C. sinensis*) and pummelo (*C. maxima*) hybridized most likely in Barbados to create grapefruit (*C. paradisi*) around 1750 CE and then spread into present-day Florida in the early 19th century CE.

1.6 Conservation

Access to a broad range of diversity, both at a cultivar and species level, is critical for the long-term sustainability of agricultural production, and agricultural production is essential for the survival of the human species. Agricultural production may be threatened by various biotic and abiotic factors (discussed below). Adapting to these threats involves using genetic resources to develop new varieties that are adapted to or tolerant of these threats. This means that diverse plant genetic resources must be available to the plant breeding community.

Genetic resources conservation is sometimes classified as *in situ* and *ex situ*. *In situ* conservation takes place at a local level and has the goal of preserving genetic resources in place, generally in their naturally occurring area and as naturally growing or minimally manipulated populations. An *in situ* gene sanctuary for wild citrus with 627 accessions was established in the Garo Hills in northeast India (Singh, 1981), and more recent surveys have shown wild citrus is currently only in the Tura range of the Garo Hills (Borah et al., 2018; Malik et al., 2006).

Ex situ preservation, in contrast, consists of formally "held" accessions that are managed to maximize their utility, health, and continued preservation. These collections may or may not be in areas where the species naturally exist, although they are generally in areas with climates conducive to their holdings. These collections could be maintained as formal genebank programs, local diversity collections, botanic gardens, or as breeding or industry resources.

1.7 Ex situ genebank collections

Plant collections can be maintained by national, regional, educational, non-profit, botanic gardens, or industry organizations. These each have different purposes and missions, but generally have a shared interest in acquiring and maintaining genetic diversity for public or private use. In some cases, materials are also distributed. The following sections focus on citrus conservation in *ex situ* genebank conditions.

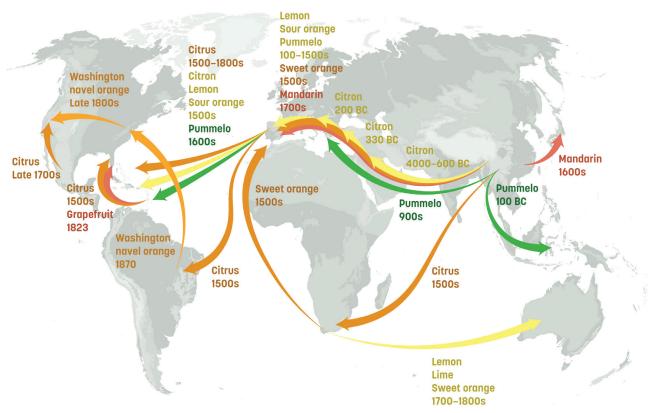


Figure 15. A simplified diagram of citrus movement by humans from 4000 BC to the 1800s (K. Chen).

1.7.1 Collection acquisition

Acquisitions for genebank programs generally have the goal of increasing the genetic diversity of the collection. Ideally, these acquisitions are targeted based upon existing knowledge of the collection. Identification of collection gaps and redundancies allows a more targeted approach. This is a curatorial responsibility that may or may not be feasible depending on available resources as it depends on thorough genotypic and phenotypic characterization of the collection and, ideally, of potential additions. In some cases, stakeholders can suggest useful additions to a genebank. Although genebanks do not always maintain commercial accessions, these can sometimes be the source of useful genes or unique combinations of genes and alleles, and so would be appropriate to acquire.

It is critical that new citrus accessions are incorporated into the collection following phytosanitary and regulatory requirements. This may or may not require assistance from an outside program such as a quarantine office or sanitation program. In these cases, the new acquisitions are processed outside the genebank and then incorporated into the holdings when processing is complete, and the materials are compliant with phytosanitary regulations. In some cases, this process may be carried out within the genebank program. Additional import regulations are provided in section 1.10 below.

1.7.2 Collection maintenance

Germplasm holdings can include cultivars (usually maintained clonally), seedling populations, seeds, or a combination of these. In the case of citrus, holding germplasm accessions as seeds is not a prudent strategy, due to several factors. First, unlike annual crops, citrus seeds cannot readily be used to regenerate mature clones due to the extended juvenility period of citrus; further, citrus seed viability is not retained for very long unless complex cryopreservation techniques are available and can be utilized. Although citrus seed storage methods have been proposed, they are not routinely implemented, and longterm viability assessments have not been published. Acquisition of seedling populations may or may not increase genetic diversity compared to acquisition of clonal (vegetative propagative) material. Some types of citrus (for instance, sweet oranges, lemons, limes, grapefruit, and some mandarins) have high levels of apomixis (asexual reproduction). If an accession is acquired as seed but only nucellar seedlings result, a clone has essentially been acquired, but one with the disadvantage of having juvenile characteristics for 7-10 years. From a genebank perspective, if diverse sexually hybrid seeds are acquired, the individual trees

grown from them are essentially managed like clonal acquisitions.

For a citrus genebank to be as useful as possible, the accessions must be maintained as living trees in an active (or working) collection. If resources permit, a citrus genebank should maintain trees both in the field and in protective structures (insect-proof screenhouses or greenhouses). Field orchards with mature trees can be used for evaluations and characterizations and to provide flowers and fruit from which pollen and seeds can be collected and distributed (Figure 16, 17). Field-grown trees are not protected from insects and diseases that they carry. Even fieldplant materials that are clean upon planting can be reinfected with diseases, and do not meet phytosanitary regulations that are required for budwood distribution and propagation. Plants in insect-proof protected structures such as screenhouses and greenhouses can be maintained in a pathogen- "free" state (Figure 18). Budwood from these pathogen-tested trees can generally be distributed. Due to the small stature and growth habits of trees grown in pots, it is not possible to collect accurate morphological and reproductive phenotypic data from these materials. They also may not produce flowers or fruits in protected conditions, making it impossible to distribute pollen or seeds.

Accessions maintained *in vitro* are expensive to maintain but can be useful as backups for actual trees and as a distribution form when propagation methods, skilled staff and specialized laboratories are available.

1.7.3 Phenotypic Evaluation

Genebanks often use the International Plant Genetic Resources Institute (IPGRI) crop descriptors for phenotypic evaluations, which are available for citrus (IPGRI, 1999). These are useful for a basic morphological description of the accessions and have the advantage of being standardized so evaluations made by different teams or in different climates can be compared. However, they are only a snapshot of the accession's characteristics, particularly fruit characteristics, which vary between seasons and locations. Ideally, these data would be taken from multiple locations in multiple years for better assessments. Evaluations of additional useful traits, including resistance or tolerance to biotic and abiotic stressors and temporal evaluations of fruit quality, are also needed.

1.7.4 Molecular Characterization

The past 40 years have seen the incredibly rapid and profound evolution of molecular technologies that can be applied to characterizations of any living organisms, including citrus. In the 1970s and 1980s,



Figure 16. Citrus in the University of California, Riverside collection A) Aerial view of entire collection (T. Kahn); B) Fruit on tree (G. Volk); C) Field collection (R. Krueger).





Figure 17. Citrus A, B) flowers and C) seeds (G. Volk).

protein-based techniques were employed to genotype citrus plants in a rather primitive way, with a limited number of genetic markers and alleles that could be used to characterize and distinguish different citrus accessions. As DNA-based systems developed, such as restriction fragment length polymorphisms (RFLPs) and amplified fragment length polymorphisms (AFLPs), it became possible to increase the numbers of genetic markers and gradually to decrease the costs per data point to be used to genotype accessions. As DNA and RNA sequencing technologies improved, gene expression data were coupled with genomic information, and newer and more powerful, cost-effective marker systems were developed, such as expressed sequence tags-simple sequence repeats (EST-SSRs) and more widely distributed single nucleotide polymorphisms (SNPs), enabling a much deeper molecular characterization and more thorough and discriminative genotyping of citrus germplasm. The ultimate technology is whole genome sequencing and assembly, and as sequencing technology continues its rapid development, this platform now provides the opportunity to characterize and distinguish in great detail even mutants from a single germplasm resource or cultivar.

Various approaches to genotyping citrus accessions have been employed in different programs over time, including RAPD and DaRT markers, simple sequence repeats (SSRs), genotyping by sequencing (GBS) and SNP arrays, among others. High-density SNP arrays, such as developed by Hiraoka (2020) (~58K SNP features) would be desirable to use as a system to compare genotypes among and within collections because of the power that so many polymorphic features can provide. However, it is worth noting that SNP arrays for citrus have been developed using a more limited range of genetic diversity than might be found in global germplasm collections, and therefore will be of limited value for broad application. Clearly, there is a need to develop new arrays that would include more of the taxonomic diversity found in the citrus germplasm pool to best genotype collections in the future. With the rapidly expanding number of citrus genome sequences being produced, the goal of developing a more robust array platform is within reach.

Genebanks seeking to use molecular characterization tools within their collection to scan the genotypic diversity and to understand the relatedness among accessions must consider the absolute need for such information balanced against the costs to employ these new platforms, and use limited financial resources most carefully for maximum management decision impact.

1.7.5 Documentation

Documentation of data associated with genebank materials is essential. Information may be stored in spreadsheets, in local databases, or in shared database structures. Passport data should be available to the user community and so is most appropriately maintained in a database that has some public accessibility. Critical data for managing a genebank include the location, quantity, propagation history, pathogen test results, etc. of each inventory item held. In some cases, a hybrid system may be in place wherein a central server houses publicly available information as well as information not shared with the public but accessible to the genebank team. In any case, it is critical that good data management practices be followed, guar-



Figure 18. Citrus under protective insect-proof screen at the USDA National Plant Germplasm System citrus collection in Riverside, California A) Screenhouse; B) Citrus trees in pots on the screenhouse bench with drip irrigation (G. Volk).

anteeing system integrity, security, and backup. One example of a genebank management tool is GRIN-Global (USDA, 2022). <u>Genesys</u> aggregates information for 10 citrus collections worldwide (Appendix 1).

Other relevant databases may play a role in documentation of germplasm collections. These include the National Center for Biotechnology Information (NCBI) of the U.S. National Institutes of Health, a globally recognized and used repository for genomic and transcriptomic information, which includes an abundance of information on citrus accessions. Citrus specific databases maintain such information, as well as information on metabolomics, breeding, and each has a suite of tools developed specifically for the research community to query the databases for information on specific genes, species, cultivars, etc. These include, among others: the Citrus Genomic Variation Database housed at the Southwest University/Chinese Academy of Agricultural Sciences in Beibei, Chongqing, China; the Citrus Pan-genome2breeding Database housed at Huazhong Agricultural University in Wuhan, Hubei, China; and the Citrus Genome Database housed at Washington State University, in Pullman, Washington, USA.

1.7.6 Security back-ups

Genebank collections are vulnerable to abiotic and biotic threats. Accessions can be lost due to natural disasters (floods, hurricanes, etc.), infrastructure disasters (long-term electrical or water outages), biotic stresses (pests, diseases), and operator error (not turning on irrigation, cooling system malfunction, etc.). Plant materials can be duplicated at the genebank site, either as replicated field or protected-structure plants, or as plants in both the field and in protected structures. In addition, genebank materials can be duplicated at multiple geographically distinct locations.

For example, recognizing the need to protect the USDA citrus collection from possible disease threats, a shoot tip cryopreservation program was developed and implemented to back-up a portion of the collection in liquid nitrogen at a secondary location (Volk et al., 2012, 2017). Briefly, actively growing shoots from the National Plant Germplasm System (NPGS) citrus collection are harvested from screenhouse-grown trees about 4 to 9 months after pruning (September to January). Shoots are surface sterilized, one millimeter shoot tips excised, and then treated with cryoprotectant solutions containing glycerol, sucrose, ethylene glycol, and dimethyl sulfoxide. They are then placed onto foil strips and plunged into liquid nitrogen for long-term storage (Figure 19). For regeneration, shoot tips on foil strips are removed from liquid nitrogen and immediately submerged into sucrose solution and

placed on medium overnight (Volk et al., 2012, 2017, 2020b). They are micrografted onto Carrizo seedling rootstocks and established in tissue culture (Volk et al., 2020a). After 2–3 months, recovered plantlets can be transferred to the screenhouse to establish trees. At this time, the methods have been used to cryopreserve 438 of the 540 pathogen-tested citrus accessions in the NPGS citrus collection (Figure 19; Volk et al., 2019, 2022).

Citrus seeds can also be cryopreserved. This is useful for capturing the diversity of wild species with zygotic embryos and some cultivars/rootstocks that produce nucellar embryos (Kaya et al., 2017; Lambardi et al., 2004). Citrus seeds, which are classified as having intermediate storage physiology, must be adjusted to an optimal equilibrium relative humidity prior to liquid nitrogen exposure. Graiver et al. (2011) reported optimum equilibrium relative humidities between 64 and 85% for C. sinensis, C. paradisi, and C. reticulata, with some variation across species. Citrus limon, C. aurantium, and C. aurantifolia are tolerant to desiccation, and C. sinensis, C. deliciosa, C. sinensis x P. trifoliata, and C. halimii require precise moisture adjustment (Cho et al., 2002). It is critical to remove unfrozen water prior to liquid nitrogen exposure (Graiver et al., 2011; Hamilton et al., 2009). In some cases, it may be necessary to remove seed coats or excise embryonic axes prior to dehydration (Cho et al., 2002).

No citrus genetic resources are backed-up at the Svalbard Global Seed Vault because liquid nitrogen storage is necessary for long-term seed and shoot tip preservation.

1.7.7 Distribution

There are several distribution forms possible for citrus germplasm. Citrus germplasm is often distributed as budwood. This ensures that a true-to-type plant can be propagated. Budwood should generally only be distributed from sanitized, pathogen-tested, protected trees, unless the requestor has the resources to accept unsanitized or untested budwood. Seeds are often distributed when large numbers of plants are needed for experimental purposes. They are sometimes distributed for propagative purposes when phytosanitary restrictions are in place for budwood. Leaves are distributed for extraction of nucleic acids (particularly for specialized extraction methods), although the latter can be sometimes extracted at the genebank and sent directly to the requestor. Fruit is sometimes distributed for specific phenotypic evaluation, such as for secondary metabolite analyses. Other distribution forms are uncommon but sometimes used for specific purposes (e.g., roots for rhizobiome characterization). In some instances, resources may limit what forms

can be maintained or distributed. Resources might not allow extraction of nucleic acids on site. In other instances, phytosanitary restrictions may be in place for certain distribution forms. Restrictions on vegetative material are much more common than on seeds or nucleic acids.

1.8 Abiotic threats

Citrus genetic diversity is severely threatened in situ by habitat losses caused by deforestation, population pressure, fire, hydroelectric development, clearance for agriculture or other development, tourism, etc. (WWF and IUCN, 1994-1995). These factors may be

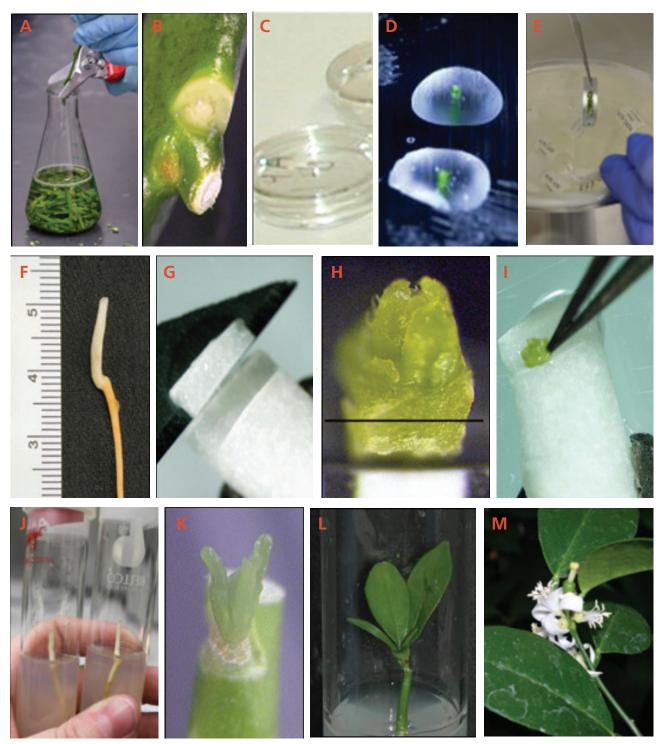


Figure 19. Citrus shoot tip cryopreservation: A) Surface sterilized nodal sections; B) 1 mm shoot tips are excised from nodal sections; C) Shoot tips treated with cryoprotectants; D) Shoot tips cryopreserved in droplets of vitrification solution; E) Cryovials placed and stored in liquid nitrogen; Micrografting: F) Carrizo seedling rootstocks grown *in vitro*; G) Ledge cut on rootstock; H) Base of shoot tips trimmed; I) Shoot tip placed on rootstock ledge; Regrowth after liquid nitrogen exposure: J) Plant immediately after micrografting; K) Micrografted plant after two weeks; L) Micrografted plant after eight weeks; M) *Citrus aurantifolia* flowering in greenhouse, after 13 months regrowth (G. Volk, USDA).

especially important in countries such as India and China, which have or have had rapidly expanding populations coupled with rapid economic/industrial development. Southern China is one of the centers of diversity for Citrus and related genera and a wide range of genetic diversity is apparently still present in situ. Some (not all) of these areas are threatened with habitat degradation or lack of proper management that could result in decreases in genetic diversity. In India, the northeast region is the center of origin/ diversity. Unfortunately, this region sometimes experiences civil unrest, making evaluation of genetic diversity and plant exploration difficult. Southeast Asia (including Malaysia) is rich in indigenous germplasm, with chance seedlings, semi-wild, and wild types. This genetic diversity is threatened by deforestation, development, and disease. China and India, have ex situ collections of citrus genetic resources to reinforce whatever in situ efforts may exist.

Climate change is modeled in various ways with differing assumptions and conclusions (CCSP, 2008; USGCRP, 2017). Most likely scenarios project increases in average and extreme temperatures, but the magnitude of these changes varies from slight to large, depending on the model and location. In contrast, the effect on precipitation is not as well understood, and varies depending on the region of the earth. As a crop adapted to relatively high temperatures and little or no chilling requirement (Krajewski and Rabe, 1995), citrus may be less threatened by modeled climate changes than some other crops; however, changing temperature conditions may shift the areas capable of citrus production to the north and south of traditional cultivation areas in the Northern and Southern Hemispheres, respectively.

There have been a few reports on observed and predicted effects of climate change on citrus growth and production. In growth chambers, Baker and Allen (1993) observed increases in growth and photosynthesis and decreases in water use by citrus when CO, concentrations increased from 330 µmol mol⁻¹ to 840 µmol mol⁻¹. Water use increased with increasing temperature. Martinez-Ferri et al. (2013) modeled increased irrigation requirements of 6-16 % for citrus in Spain under various climate projections. In contrast, Fares et al. (2017) modeled decreases in evapotranspiration and irrigation requirements of up to 12% and 37%, respectively, resulting from CO₂ increases under a number of temperature and precipitation models. Canopy light interception and subdrainage were modeled to increase under these models. In contrast to Martinez-Ferri et al. (2013), Fares et al. (2017) modeled at a global level and reported great variability from region to region and month to month. Downton and Miller (1993) described changes in cold temperatures in Florida in response to climatic oscillations

(CO₂ was not included in this report). Because many of these climate models predict that the increases in average temperature will be associated with increases in minimum temperature, it is possible that climate change may result in fewer losses due to cold in citrus production. However, warmer nights during fruit maturation may result in lower sugar levels and other negative changes in fruit quality parameters.

Rosenzweig et al. (1996) estimated citrus production at 22 simulated sites under nine different temperature/CO₂ scenarios. Results of the simulations without CO₂-induced yield improvement indicated that production may shift slightly northward in southern U.S. states, but yields may decline in southern Florida and Texas due to excessive heat during the winter. CO₂ effects tended to counteract the decline in simulated citrus yields. Tubiello et al. (2002) simulated five different climate change models in eight current citrus-producing areas and five areas that may become suitable for citrus production. Yields increased 20-50% with less water use and fewer freeze losses in areas currently suitable for citrus production. Yield increases were lower in areas currently marginal for citrus production and the northward expansion of production was minimal. Similarly, Du et al. (2010) and Duan et al. (2010) assessed the possibilities of climate change affecting citrus production in China. They predicted more threats to citrus production from high temperatures in certain regions of the country and increased "adaptability" towards the northeast.

1.9 Biotic threats

As is the case for all plant species, citrus is attacked by a spectrum of pests and diseases. Reviewing all pests and diseases is beyond the scope of this assessment. Citrus pests include vertebrates, arthropods, and nematodes. These are present in all citrus-growing areas and cause economic damage and adverse health effects and, if not managed, can result in tree death. With proper control and maintenance conditions, citrus pests do not threaten collections at this time.

Numerous viroid, viral, bacterial, and fungal pathogens can infect citrus and result in economic damage, tree health decline, and sometimes tree death (Figure 20). Most of these diseases are managed in part by the development of certification and registration programs mandating the use of clean propagative stock (Navarro, 1993; Vidalakis et al., 2010 a,b). Phytophthora-caused root rot and citrus tristeza virus are currently mostly managed using a combination of cultural (fungicide treatment) and genetic (tolerant/resistant rootstocks) practices. Huanglongbing (HLB) does not currently have effective management options. Observations have shown that after it is introduced to a citrus-producing area, HLB becomes established

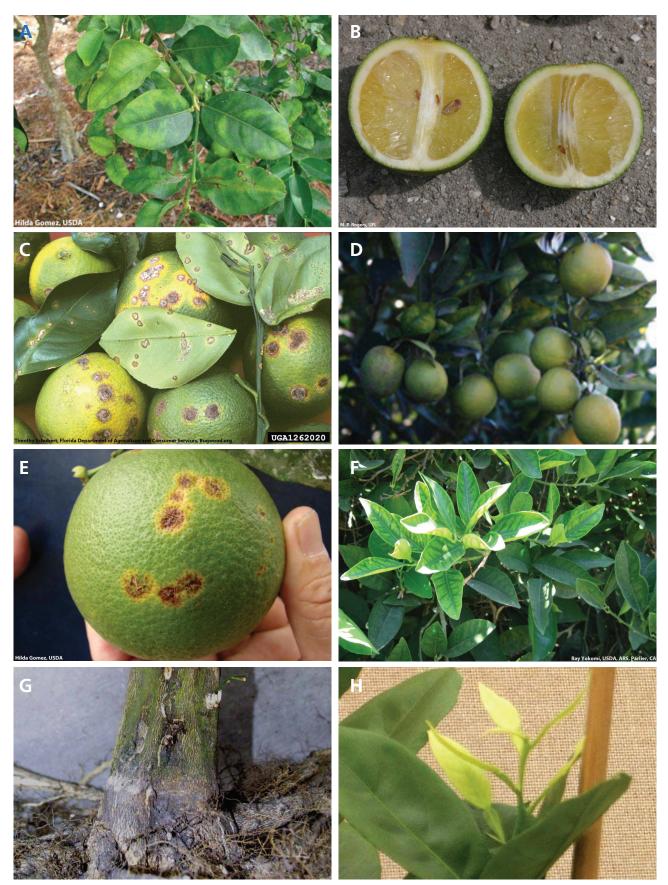


Figure 20. Symptoms of citrus pathogens A, B) Huanglongbing (HLB) caused by bacteria *Candidatus* Liberibacter spp.; C) Citrus canker caused by bacteria *Xanthomonas axonopodis* pv. *citri* (Hasse) Vauterin et al.; D) Citrus variegated chlorosis caused by bacteria *Xylella fastidiosa* Wells et al.; E) Citrus Leprosis virus (CiLV); F) Citrus Stubborn Disease caused by bacteria *Spiroplasma citri*; G) Oomycete *Phytophthora* spp.; H) Citrus tristeza virus (CTV) (Serrano et al., 2010).

and widespread (Gottwald, 2010). Given its level of potential destructiveness, HLB must be considered the greatest current threat to citrus production, and likewise to the conservation of citrus germplasm resources globally. Consequently, most citrus research is currently aimed at HLB-related topics. At this time, even countries severely affected by HLB, such as Brazil, continue to have a viable citrus industry. This situation could change, however, as potentially more destructive or infectious strains emerge. Therefore, continued research into the biology and management of HLB and its vector is necessary, along with vigilance on the part of citrus producers and researchers.

There may be effects on pest pressure from climate change. Narouei-Khandan et al. (2016) modeled the global distribution of HLB and its vector, the Asian Citrus Psyllid (ACP), finding a good correlation between the models and existing presence data. The model identified areas having suitable climates for the expansion of HLB and ACP ranges, which were not always the same. Jesus Junior et al. (2009) predicted increases in vector populations and disease severity for citrus variegated chlorosis, Huanglongbing, and citrus leprosis, as well as increased severity of Citrus black spot and Citrus floral rot, under conditions predicted by climatic change models in Sao Paulo State, Brazil. Conversely, Aurambout et al. (2009) modeled decreased ACP activity in Australia with climate change due to decreases in the flushing period in the spring; however, they predicted an increase in the area suitable for ACP presence. Although HLB has received the most attention as a citrus disease that may be affected by climate change, additional pathogens, insect pests, and other biotic stressors may increase pressure on citrus and other crops due to climate change (Juroszek et al., 2020).

1.10 Import regulations and phytosanitary restrictions

The main constraint to acquiring new and interesting germplasm is the inability to identify unique, desirable accessions and to find sources that are willing and able to provide them. Although individual scientists may be willing to exchange germplasm, in some cases this is strictly regulated and controlled by national governments. China and India are countries that are home to potentially valuable wild germplasm diversity, but many desirable wild types are forbidden to be sent abroad. There are political challenges with other countries where citrus is native, as well, in gaining access to the diversity. It is hoped that continuing engagement between governments regarding mutually beneficial germplasm exchange can open up these resources in the future, for the benefit of all humanity. In addition, certain international treaties and agreements regulate the

exchange of plant genetic resources. Notable are the Conventional on Biological Diversity (CBD) (accessed 2022-10-09) and the FAO International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) accessed 2022-10-09). The CBD mandates benefit sharing and other restraints on the sharing of plant genetic resources. The ITPGRFA is less restrictive. Citrus is an "Annex 1" crop in the ITPGRFA, so material can be transferred using the Standard Material Transfer Agreement. In some cases, acquisition of new accessions is limited by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (accessed 2022-10-09).

Movement of vegetative propagative materials (such as budwood) of citrus and its wild relatives between countries is generally prohibited or restricted due to the possibility of introducing diseases exotic to the importing country. In citrus, virus, viroid, and some bacterial pathogens are vascular-limited (usually phloem-limited) and thus might invisibly enter a new area within innocent appearing budwood. Consequently, a strict program of pathogen-testing and therapeutics is necessary to ensure that new pathogens are not introduced along with new germplasm (Frison and Taher, 1991). Many or most citrus growing countries require an import permit to introduce new accessions of citrus, and these permits specify the conditions under which the new material may enter. In some cases, countries may allow the importation of new accessions from trusted sources without an introduction protocol. This is particularly true of low resource countries and so the availability of "clean source" materials maintained by more resource-rich countries is vital. Compared to budwood, seeds are not a major source of potential pathogen introductions, but they are also often restricted at the country level.

1.11 Breeding

1.11.1 Importance

The collection, characterization, curation, and distribution activities of citrus genebanks serve many of the needs of researchers in a wide range of scientific disciplines. Additionally, genebanks also are valuable resources for commercial citriculture interests globally, by providing a network to support the pathologically safe and validated distribution of desirable rootstock and scion cultivars to the many regions of the world where citrus production provides for livelihood of the many people engaged in the business of citrus, and for the nutritional and aesthetic benefit of citrus consumers. However, as the challenges raised by the global spread of deleterious insects and pathogens in existing or in new production regions increase, and as changes in climate and subsequent migration of growing areas occurs and abiotic limitations to

efficient, profitable, and sustainable production are encountered, genetic improvement takes on greater significance. In this context, perhaps one of the most valuable contributions of citrus genebanks is provision of genetic and allelic diversity to plant breeders (and others engaged in different approaches to genetic improvement) to address the newly arising threats. Genetic solutions generally have provided the most robust and sustainable answers to biotic and abiotic challenges in the many crop plants upon which humanity relies for sustenance; citrus is no different. Breeding is critical for the future of citrus production, and genebank resources are critical for providing the raw materials to craft the improved rootstock and scion cultivars of the future.

1.11.2 Priorities

Citrus trees in production conditions are almost always compound chimeric plants consisting of a scion grafted to a genetically distinct rootstock. Breeding priorities for improving scions or rootstocks are different, but there are some commonalities as well, particularly in the goals of improving disease resistance or tolerance. Given the continuing global spread of Candidatus Liberibacter species, primarily C. L. asiaticus (CLas, the presumed causal agent of HLB), and its most efficient vector, the Asian citrus psyllid (ACP, Diaphorina citri), and the enormous consequences it is bringing to citrus producers in many parts of the world (Bové, 2006; Gottwald, 2010; Wang, 2019; Graham et al. 2020), it is understandable that more research efforts have been directed at developing genetic solutions to this scourge than to any other breeding priority globally. These breeding activities are targeting both scions and rootstocks, seeking more tolerant or even resistant new cultivars, through a multitude of possible mechanisms (scion resistance or tolerance to the pathogen and/or the vector, boosting basal host defense responses to pathogen attack, etc., improving rootstock performance [greater ability to mine nutrients, or to suppress Clas populations in the roots]). It is beyond the scope of this document to review these activities in depth, but currently genetic strategies to overcome this disease are a top priority in the breeding and genetic improvement community, and diversity is needed to implement them successfully.

Regardless of HLB as a primary focus, there are breeding priorities that have long been in view, and work in these areas also continues. Rootstocks provide a means to manage biotic and abiotic stresses, and these challenges also have remained priorities for breeding programs globally. Abiotic stresses include conditions such as salinity, high soil pH, drought, and cold. Biotic stresses, including *Phytophthora* species, various nematodes, and CTV-declines associated with rootstock choices, can likewise have negative impacts and rootstock improvement can provide solutions to these. Many of the wild and unpalatable citrus species, such as *C. trifoliata*, *C. australasica*, and some types of *C. reticulata*, among others, have been valuable germplasm resources for such breeding goals. Rootstocks also can influence canopy size and structure, fruit yields, and fruit and juice quality, and these traits always are a part of long-term breeding program pipelines (Caruso, et al., 2020).

Scion breeding priorities vary among different production regions around the world, and according to the predominant scion type and the nature of the local or regional industry. As sweet orange remains the dominant type of citrus grown, regardless of whether for fresh market or processing, a pair of linked universal goals are extending the season of maturity through earlier or later maturing cultivars and improvements in fruit and juice quality attributes, such as color, size, flavor, and aroma. Mandarin production is increasing globally, and drivers of the breeding programs include season extension; improved appearance, size, and peelability; eating qualities; and absence of seeds in fruit. Seedlessness is becoming more important for lemon genetic improvements, as well as for pummelo. Grapefruit breeding targets include deeper red flesh, and improved eating quality. Overarching all the scion-specific breeding priorities is an interest in improving disease tolerance or resistance. Some of these disease problems affect multiple scion types, for example HLB, citrus black spot, and citrus canker. Other diseases are more specific to cultivar group and/ or production region, such as Alternaria brown spot affecting many mandarin cultivars, Witches' Broom disease of limes, and mal secco impacting lemons. Of course, productivity is another important priority that applies to all cultivar groups, as well as post-harvest performance improvements. There also exist many opportunities to improve the nutritive and phytonutrient content of citrus fruit and juice, to enhance the aesthetic, culinary, and health-promoting benefits of new citrus scion cultivars (Mattia et al., 2022).

1.11.3 Strategies

Breeding strategies for citrus are varied, depending on whether the goal is rootstock or scion genetic improvement. Rootstock breeding has more strategic options than does scion breeding. Historically, the major rootstocks used globally were selected by traditional growers based on the most used true species or derived hybrids, such as trifoliate orange, sour orange, Volkamer or rough lemon, Rangpur lime and *C. macrophylla*. However, many of these rootstocks have been supplanted in different regions by new options produced by systematic breeding program based upon sexual hybridization, followed by screening for desirable traits, and then by multilocation field trials (Caruso et al., 2020). Somatic hybridization via protoplast fusion has also been used to create new rootstock candidates that are tetraploids, and somatic hybrids have been used as breeding parents for selection of new candidates at the tetraploid level (Grosser and Gmitter, 1990 a, b; 2011). Whether sexual hybridizations are made at the diploid or tetraploid levels, or via somatic hybridization, rootstock improvement strategies can exploit much greater genetic and taxonomic diversity from genebank collections than can scion improvement, because palatability of hybrid fruit is irrelevant to rootstock selection. Knowledge development from phenotypic evaluation of materials in germplasm collections can help determine the most appropriate parents to use in hybridizations, or to develop molecular markers that can be applied for marker-assisted breeding.

Scion improvement strategies are more diverse in one sense but are also more restricted in another. Virtually all the cultivars within the sweet orange, grapefruit, Clementine, lemon, and satsuma mandarin groups have arisen as spontaneous or induced somatic mutations. Further, all the above except the Clementines reproduce from apomictic seeds, thus retaining the maternal genotype and phenotype. These types thus represent an extremely narrow slice of the genetic diversity that exists within the genus (Wu et al. 2014, 2018, 2021). Mutation breeding, either by selecting naturally occurring or radiation-induced bud sports, nucellar seedling variants, or in vitro derived somaclones (Germana, et al., 2020) is the most used strategy for these cultivar types. These efforts have been successful in developing new cultivars displaying primarily improvements in season of maturity and fruit quality attributes.

The goals of improved resistance to pests, diseases, or environmental stresses by introgression of genes or alleles from citrus or relatives outside of these groups are not possible. Sexual hybridization, if and when used, encounters various barriers to acceptance because of the narrow definition of these major groups for the market; essentially each cultivar group is nothing more than a collection of selected mutations that alter phenotype in relatively small ways while retaining nearly all the phenotypic characteristics of the original form. Recently, however, there have been possible alternatives suggested to improve sweet orange in particular, by selection of "sweet orange-like" hybrids that have enhanced levels of tolerance of HLB, and produce fruit that substantially resemble orange in appearance, chemistry, and flavor perception. Global discussions are taking place regarding changes to national and international

regulations that govern the limitations to cultivar-type identity (Stover et al. 2019). Should these arbitrary definitions be relaxed, then the opportunities for sexual hybridization in future scion breeding will be greatly enhanced.

Citrus and related genera are generally diploid with 2n=2x=18. There is at least one wild tetraploid form of a closely related genus (*Fortunella hindsii* Swing.), but polyploidy is uncommon in wild or un-selected citrus. Spontaneous tetraploid forms of many *Citrus* and *Poncirus* accessions have been identified over the decades, and may also be produced artificially (e.g. colchicine treatment, somatic hybridization). Triploids occasionally appear spontaneously from diploid-by-diploid crosses, and this is perhaps the origin of "natural" triploids such as large-fruited acid limes. Production of triploids by controlled diploid-by-tetraploid crosses is a technique used in some breeding programs to produce seedless progeny (Grosser and Gmitter, 2011; Soost and Roose, 1996).

New genome-based genetic improvement strategies are now being explored and will undoubtedly become more important in the future. These strategies include marker-assisted selection, genome selection based on genome-wide association studies (GWAS), and gene or genome editing using CRISPR technologies (Germana et al., 2020; Shimizu, 2020; Mattia et al., 2022).

1.11.4 Need for genetic resources

Citrus production globally truly faces an existential threat because of HLB's rapid spread in the past two decades, and the likelihood that it will continue to move into the relatively few regions where it has not yet been found. In addition to commercial implications, the spread of HLB also threatens the future of the limited native, wild citrus germplasm that still exists in some parts of Asia, Oceania, and Australia. Apparently, the interaction of CLas with citrus germplasm is a very recent phenomenon, and opportunities for natural selection in the broad germplasm pool are just now taking place. Therefore, much of the wealth of genetic diversity for many traits within the current global germplasm pool that can be found on our home planet is at risk of being lost forever on the wrong side of a CLas-controlled evolutionary bottleneck. It is imperative that gaps in collections and across taxa can be identified, so that what still exists can be collected and preserved for the future, before it is too late. This stark reality underlies the urgency of the task, and the critical importance of developing a truly global strategy that all nations can find the means of accepting and pursuing collaboratively, on behalf of all humanity.



2 RESULTS OF THE GLOBAL CITRUS COLLECTION SURVEY

In 2021, a survey was developed and widely distributed within the citrus genebanking community. This survey requested information about the composition, *ex situ* and *in situ* management, data available, health, security back-up, human resources, distribution and use, policies, and future development of citrus collections (Appendix 3). Surveys were distributed to collection contacts identified by personal sources, journal article authors, Genesys, and FAO WIEWS. Follow up reminders were sent to ask contributors to complete surveys. Survey results were downloaded, and duplicate submissions were removed.

A total of 43 unique survey responses was received from 27 countries (Figure 21). These included many major national and regional citrus genebank collections. Some breeding collections were also reported in cases when they represented the major citrus collection of a country, but breeding collections were not specifically targeted. Inventory data for seedling populations in breeding collections were not included in the tabulations. Of the 43 survey responses received, some collection responses from Cambodia, Taiwan, Thailand, Turkey, India, and Vietnam were limited to contact information and consequently were not included in further analyses (Appendix 2). Citrus organizations that provide clean plant materials to local citrus nursery industries for commercial production of trees, including AusCitrus (Australian Citrus Propagation Association Incorporated), and the California Citrus Clonal Protection Program and Florida Department of Agriculture and Consumer Services Bureau of Citrus Budwood Registration, in the United States, were not included. In some cases, sections of some submitted survey responses were incomplete and therefore not included in the analyses for those sections.

The citrus survey results provide novel information not previously available in Genesys and FAO WIEWS. Collection codes/identities were matched as closely as possible between the survey responses, Genesys, and FAO-WIEWS. Only five of the survey respondents are listed in the Genesys database (BRA020, CRI001, ESP025, ITA226, USA129; Appendix 1). Nineteen of the survey respondents are listed in FAO WIEWS,

although collection size information did not match (Appendix 1). The Embrapa Mandioca e Fruticultura Tropical Collection in Cruz das Almas, Brazil (BRA004) is a significant collection for which there are no data in the current survey. There were also some additional citrus collections listed in Genesys and FAO WIEWS, some of which have fewer than 10 accessions, that did not respond to the survey (Appendix 1). In some cases, collection contacts could not be identified. Results from a previous citrus collection survey that included 11 collections were published in 2015 (Roose et al., 2015). The previous survey did not include significant collections in Brazil, Spain, and Corsica, but it did include two collections at the National Citrus Breeding Center, Huazhong Agricultural University in Huazhong, Wuhan, China with 400 accessions and the Research Farm of Kinki University in Nishimitana, Kinokawa-City, Japan with 2019 accessions that did not respond to the 2021 citrus survey (Roose et al., 2015).

2.1 General information about collections

Citrus Collection Survey respondents represented collections that are large and small, highly diverse and less so, focused on breeding and public interests, and either publicly available or not (Figure 22). The oldest citrus collection that responded dates to was established in Florida in 1908 by the USDA Bureau of Plant Industry (currently a resource of the USDA-ARS Horticultural Research Laboratory in Fort Pierce, Florida). The University of California Citrus Variety Collection also dates to 1908, but its holdings are included in the response from the USDA-ARS National Clonal Germplasm Repository for Citrus & Dates. Other older collections include the 1928 collection at the Instituto Agronômico de Campinas/Centro de Citricultura Sylvio Moreira in Brazil and the 1930 Federal Research Centre the Subtropical Scientific Centre of the Russian Academy of Sciences in Russia (Appendix 2).

Survey respondents were asked what percentage of their collections was maintained in the field, greenhouse/screenhouse, in vitro, or as seeds, as well as the extent of collection duplication. Citrus collections are primarily maintained in the field or greenhouse/ screenhouse. Collections maintained in a clean state (see below) are mostly kept in protected environments. The two collections with significant in vitro components are Federal Research Centre the Subtropical Scientific Centre of the Russian Academy of Sciences, Russia and the Instituto Valenciano de Investigaciones Agrarias (IVIA), Spain. The extent of duplicate plantings varies considerably. Some collections have a single tree in the field for each accession, others have a partial greenhouse duplication, many have 2-5 trees in the field or in greenhouse pots for each accession. The USDA-ARS (USA) collects seeds for use and distribution, but not for long-term storage. EMBRAPA and INRAE also have some seed storage activities.

A total of 15,555 accessions are maintained in the 33 collections that responded with inventory information (Appendix 2), which is more than 4-fold the number of

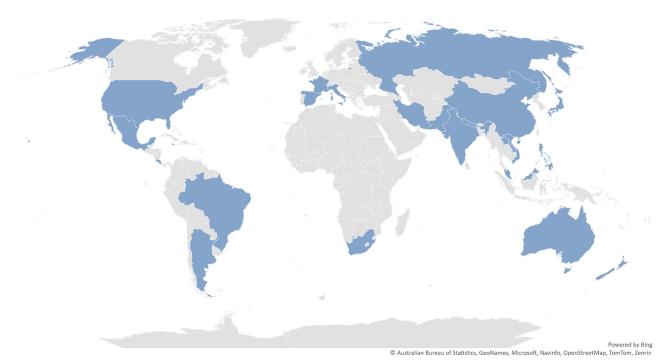


Figure 21. Map of countries that were represented by respondents to the Citrus Collection Survey.

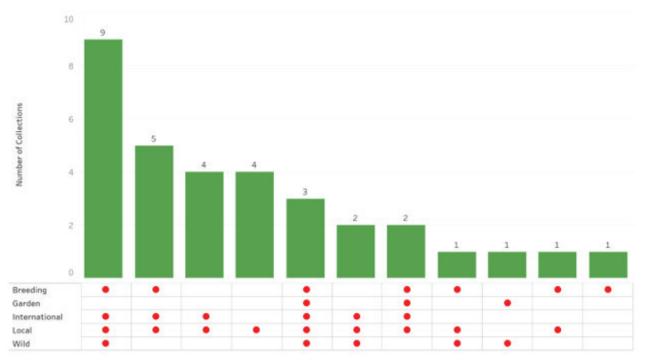
accessions recorded in either Genesys or WIEWS. Ten collections that responded to the survey have fewer than 100 accessions, and the largest collections are maintained by the Instituto Agronômico de Campinas/ Centro de Citricultura Sylvio Moreira in Brazil (1735 accessions); Citrus Research Institute, Southwest University in China (1700 accessions); USDA-ARS National Clonal Germplasm Repository for Citrus and Dates in the United States (1632 accessions); Institute of Fruit Tree and Tea Science (NARO, NIFTS) in Japan (1261 accessions); National Research Institute for Agriculture, Food and Environment (INRAE)-Corsica in France (1100 accessions); and Queensland Department of Agriculture and Fisheries in Australia (1000 accessions). These larger collections include significant numbers of accessions of local cultivars and wild species. Other collections also have hundreds of local cultivars and wild species accessions, including collections in India,

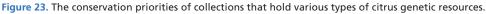
Laos, Nepal, Russia, South Africa, Spain, and Vietnam (Appendix 2).

The survey requested information about primary conservation priorities (international cultivars, local cultivars, crop wild relatives, breeding, and public gardens; Figure 23). Most collections focused on conserving breeding materials, international and local cultivars, and wild species. Fewer collections prioritized materials intended for gardens (Figure 23). The largest number of genebank accessions were commercial and local cultivars, followed by materials for breeding, seedlings, and rootstocks, with some wild materials (Figure 24) (Appendix 2). Survey respondents were asked to classify collection materials based on fruit types (mandarin, sweet orange, lemon, pummelo, grapefruit, hybrids, lime, sour orange, citron, kumquat, papeda, and finger lime) and results are sum-



Figure 22. Citrus in genebanks in A) China in 2008 (R. Krueger) and B) Plant Resources Center (PRC), Vietnam (G. Volk).





marized in Figure 25. The largest accession holdings across all the genebanks are mandarins and sweet oranges (Figure 25). Specific collection compositions are shown in Figure 26 and Appendix 2.

2.2 Collection management

2.2.1 Acquisition

Citrus collection managers must follow strict national and regional phytosanitary requirements when acquiring new materials either locally or from abroad. When budwood import is allowed, quarantine regulations require isolation, testing, and clean-up (often shoot tip grafting). Budwood can be transported across borders within Europe using a Phytosanitary Passport. Some countries have regulations in place that exempt budwood from accepted sources known to have high phytosanitary standards from some or all the regulations. This is particularly true of small countries lacking the resources to clean up citrus.

Most citrus collections are not currently focused on filling gaps. Some collections are interested in adding crop wild relatives, including *Merrillia, Wenzelia, Clymenia, C. micrantha*, and *C. macroptera*. One collection also expressed interest in acquiring additional rootstocks for managing HLB.

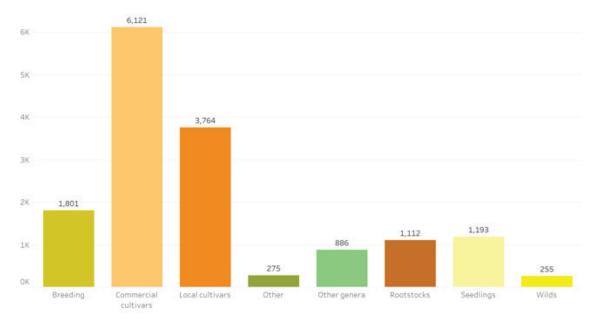


Figure 24. Number of ex situ citrus genetic resources reported in all survey responses that are classified in the listed categories.

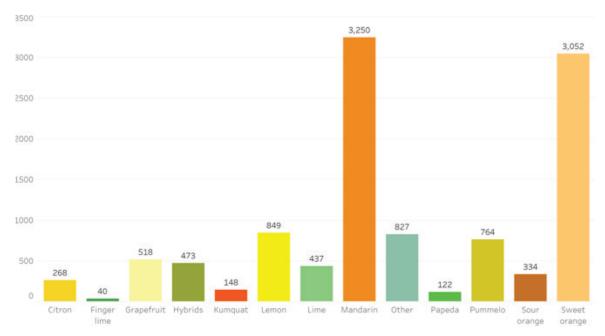


Figure 25. Number of ex situ citrus genetic resources reported in all survey responses that are classified as listed fruit types.

The survey requested information about wild species of citrus and Rutaceae that are found within respondent's borders. Survey responses are provided in Table 3—note this is not an exhaustive list of wild Rutaceae species. There are some efforts to conserve some native species in *ex situ* collections. Some *in situ* conservation occurs within national parks.

2.2.2 Collection health

Citrus collection health is a serious concern. Respondents were asked if pests and pathogens caused major, minor, or no effects on collection management (Figure 27). In all cases, most of the respondents stated that the effects of pests and pathogens were minor, although there were major effects for 5 to 10 collections. Listed scenarios included "Affecting trees in a wide range of accessions", "Affecting trees within specific accessions", "Causing annual losses of trees", "Incurring costs in pest and disease control", and "Preventing distribution" (Figure 27).

Survey respondents were asked which pests and pathogens threaten collections, as well as what testing was performed in their collections (Table 4). Responses reveal that tests are available for most threatening pests and pathogens, but many collections do not have the resources for regular testing. The extent of testing also varies widely, with some collections performing no testing, others just testing for CTV

 Table 3. Wild Citrus species and relatives that are native to countries with survey respondents

 alphabetized by country (first two columns) and taxon (third and fourth columns).

Country	Taxon	Country	Taxon
Australia	C. australasica	India	Aegle
Australia	C. australis	South Africa	Aegle
Australia	C. garrawayi	South Africa	Aeglopsis
Australia	C. glauca	South Africa	Afraegle
Australia	C. gracilis	India	Almost all citrus
Australia	C. inodora	South Africa	Atalantia
Australia	Clausena	South Africa	Balsamocitrus
Australia	Eremocitrus glauca	Australia	C. australasica
Australia	Evodia	Australia	C. australis
Australia	Faustrimedin	China	C. daoxianensis
Australia	Halfordia	Japan	C. depressa
Australia	Lunasia	Australia	C. garrawayi
Australia	Luvunga	Australia	C. glauca
Australia	Micromelum	Australia	C. gracilis
Australia	Murraya	China	C. hongheensis
China	C. daoxianensis	Philippines	C. hystrix
China	C. hongheensis	China	C. ichangensis
China	C. ichangensis	Australia	C. inodora
China	C. mangshanensis	Pakistan	C. jambhiri
China	Clausena	Nepal	C. jambiri
China	Fortunella classifolia	China	C. mangshanensis
China	Fortunella hindssi	Nepal	C. maxima
China	Fortunella japonica	Nepal	C. reticulata
China	Fortunella margarita	Japan	C. tachibana
China	Fortunella obovate	Nepal	C. x limon
China	Glycosmis	Pakistan	C. x limonia
China	Murraya	South Africa	Calodendrum
China	Poncirus trifoliata	South Africa	Citropsis
China	Severinia	Pakistan	Citrus aurantium
ndia	Aegle	Australia	Clausena
ndia	Almost all citrus	China	Clausena
ndia	Feronia	South Africa	Clausena
lapan	C. depressa	Vietnam	Clausena
Japan	C. tachibana	South Africa	Cuscuta

Country	Taxon	Country	Taxon
Japan	Murraya	Australia	Eremocitrus glauca
Japan	Phellodendron	South Africa	Eremocitrus glauca
Japan	Skimmia	Australia	Evodia
Japan	Zanthoxylum	South Africa	Fagaropsis
Nepal	C. jambiri	Australia	Faustrimedin
Nepal	C. maxima	India	Feronia
Nepal	C. reticulata	South Africa	Fortunella
Nepal	C. x limon	China	Fortunella classifolia
New Zealand	Leionema nudum	China	Fortunella hindssi
New Zealand	Melicope simplex	China	Fortunella japonica
New Zealand	Melicope ternate	China	Fortunella margarita
Pakistan	C. jambhiri	China	Fortunella obovate
Pakistan	C. x limonia	South Africa	Fragile
Pakistan	Citrus aurantium	China	Glycosmis
Philippines	C. hystrix	Australia	Halfordia
Philippines	Murraya paniculata	South Africa	Hesperethusa
South Africa	Aegle	New Zealand	Leionema nudum
South Africa	Aeglopsis	South Africa	Limonia
South Africa	Afraegle	Australia	Lunasia
South Africa	Atalantia	Australia	Luvunga
South Africa	Balsamocitrus	New Zealand	Melicope simplex
South Africa	Calodendrum	New Zealand	Melicope ternate
South Africa	Citropsis	South Africa	Microcitrus
South Africa	Clausena	Australia	Micromelum
South Africa	Cuscuta	Australia	Murraya
South Africa	Eremocitrus glauca	China	Murraya
South Africa	Fagaropsis	Japan	Murraya
South Africa	Fortunella	South Africa	Murraya
South Africa	Fragile	Philippines	Murraya paniculata
South Africa	Hesperethusa	South Africa	Oricia
South Africa	Limonia	Japan	Phellodendron
South Africa	Microcitrus	South Africa	Pleiospermium
South Africa	Murraya	South Africa	Poncirus
South Africa	Oricia	China	Poncirus trifoliata
South Africa	Pleiospermium	Vietnam	Ruta
South Africa	Poncirus	China	Severinia
South Africa	Severinia	South Africa	Severinia
South Africa	Swinglea	Vietnam	Severinia
South Africa	Teclea		Skimmia
South Africa	Toddalia	Japan South Africa	
South Africa		South Africa	Swinglea Teclea
	Toddaliopsus		
South Africa	Tricia	USA	Thamnosma
South Africa	Triphasia	South Africa	Toddalia
South Africa	Vepris	South Africa	Toddaliopsus
South Africa	Zanthozylum	South Africa	Tricia
USA	Thamnosma	South Africa	Triphasia
Vietnam	Clausena	Vietnam	Triphasia
Vietnam	Ruta	South Africa	Vepris
Vietnam	Severinia	Japan	Zanthoxylum
Vietnam	Triphasia	Vietnam	Zanthoxylum
Vietnam	Zanthoxylum	South Africa	Zanthoxylum

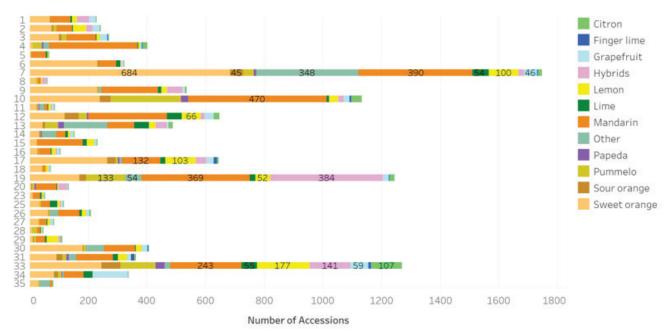
and/or HLB, and others (such as Instituto Valenciano de Investigaciones Agrarias, Spain (IVIA), Citrus and Subtropical Fruits Research Center, Iran, and Citrus Research International, South Africa) with comprehensive testing programs. Collections also vary with respect to if, and to what extent, they are maintained as cleaned-up plants. High percentages of cleaned-up plants are maintained by Embrapa Temperate Agriculture, Brazil (90%), INRAE, France (80%), IVIA (100%), Citrus Research International, South Africa (100%), Instituto Nacional de Tecnología Agropecuaria (INTA) -Concordia Experimental Station, Entre Ríos, Argentina (100%), Bodles Research Station Ministry of Agriculture and Fisheries, Jamaica (90%), and Plant Resources Center, Vietnam (100%).

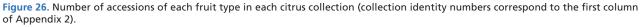
2.2.3 Safety duplication

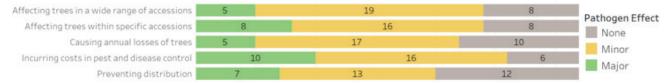
Collection back-up strategies vary. Some respondents stated that accessions are duplicated in other collections either within-country (Brazil, for example) or internationally. Some collections rely on greenhouse backups of field collections, and IVIA maintains *in vitro* collection back-ups. Some responses stated that accessions unique to specific collections are re-propagated as needed. Some collections cryopreserve recalcitrant seeds and/or embryos (INRAE, New Delhi) and over 400 accessions are cryopreserved as shoot tips by USDA-ARS. Barriers to collection back-ups include resources (funding, time), lack of skilled workers, facilities, orchard space, as well as international intellectual property rights (IPR) for commercial cultivars.

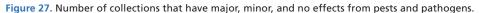
2.3 Collection use

Citrus collections are used on-site for several different purposes, with the most frequent uses being phenotypic evaluation, breeding and pre-breeding, propagation for resale, and plant and/or pathogen research. Collections were used for genomic characterization less frequently (Figure 28).









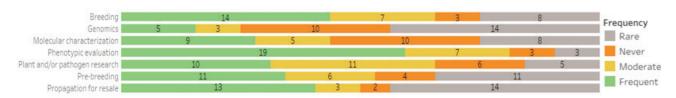


Figure 28. Number of collections that use materials on-site for listed purposes, and the frequency of those uses.

Table 4. Diseases and pathogens present in collections that responded to the survey. Disease and pathogens that threaten collections and are tested for within collections are identified.

Disease/Pathogen	Туре	Scientific name	Threatens collections	Testing methods available	Number of collections threatened
Citrus mites	Arthropod	Phyllocoptruta oleivora	х		4
African citrus greening	Bacteria	'Candidatus' Liberibacter africanus		х	
Citrus canker	Bacteria	Xanthomonas citri	х	х	7
Citrus Huanglongbing (HLB)	Bacteria	'Candidatus' Liberibacter asiaticus	х	х	13
Citrus stubborn	Bacteria	Spiroplasma citri	х	х	1
Citrus variegated chlorosis (CVC)	Bacteria	Xylella fastidiosa subsp. pauca		х	
Citrus brown spot	Fungus	Alternaria Brown Spot	х	х	1
Citrus black spot	Fungus	Phyllosticta citricarpa	х	х	2
Citrus fusarium	Fungus	Fusarium solani	х	х	1
Citrus gummosis	Fungus	Phytophthora spp.	х		1
Sour orange citrus scab	Fungus	Elsinoe fawcettii	х	х	1
Citrus stem-end rot	Fungus	Lasiodiplodia theobromae	х		1
Citrus sudden death	Fungus	Various sp.		х	
Mal secco	Fungus	Plenodomus tracheiphilus	х	х	1
Phytophthora root rot	Fungus	Phytophthora spp.	х		11
Sooty mold	Fungus	Various sp.	х		2
Verrucosis citrus scab	Fungus	Sphaceloma fawcettii	х		1
Charming caterpillar	Insect			х	
Citrus arrowhead scale	Insect	Unaspis yanoensis	х		1
Citrus leafminer	Insect	Phyllocnistis citrella	х		3
Citrus longhorned beetle	Insect	Anoplophora chinensis	х		1
Mealybugs	Insect	Pseudococcidae	х		1
Phoenix caterpillar	Insect			х	
White flies	Insect	Aleyrodidae	х		2
Witches broom disease of lime	Phytoplasma	Candidatus' phytoplasma aurantifolia		x	
Citrus bark cracking viroid (CBCVd)	Viroid	Cocadviroid Citrus bark cracking viroid		х	
Citrus bent leaf viroid (CBLVd)	Viroid	Apscaviroid Citrus bent leaf viroid		х	
Citrus dwarfing viroid (CDVd)	Viroid	Apscaviroid Citrus dwarfing viroid		х	
Citrus exocortis viroid (CEVd)	Viroid	Pospiviroid Citrus exocortis viroid	х	х	1
Citrus viroid V (CVd V)	Viroid	Apscaviroid Citrus viroid V		х	
Citrus viroid VI	Viroid	Apscaviroid Citrus viroid VI		х	
Citrus viroid VII	Viroid	Unclassified		х	
Hop stunt viroid (HSVd)	Viroid	Hostuviroid Hop stunt viroid		х	
Citrus cachexia viroid (xyloporosis)	Virus	Hostuviroid Hop stunt viroid	х	х	1
Citrus concave gum (CCGaV)	Virus	Coquvirus citri		х	
Citrus impietratura	Virus			х	
Citrus leaf blotch virus (CLBV)	Virus	Citrivirus Citrus leaf blotch virus		x	
Citrus leaf rugose virus (CLRV)	Virus	Ilarvirus Citrus leaf rugose virus		x	
Citrus leprosis (CiLV)	Virus	Cilevirus Citrus leprosis virus		x	
Citrus psorosis virus (CPsV)	Virus	Ophiovirus citri		x	
Citrus tatter leaf virus (CTLV)	Virus	Capillovirus Apple stem grooving virus		x	
Citrus tristeza virus (CTV)	Virus	Closterovirus Citrus tristeza virus	х	x	11
Citrus vein enation	Virus	Enamovirus Citrus vein enation virus	~	x	**
Citrus yellow vein clearing virus		Mandavirus Citrus Yellow vein clearing			
(CYVCV)	Virus	virus	х	х	1
Cristacortis	Virus			х	
Citrus yellow mosaic virus	Virus	Baadnavirus Citrus yellow mosaic virus		х	
Satsuma dwarf virus	Virus	Sadwavirus Satsuma dwarf virus		х	

2.3.1 Phenotypic evaluation

Most collections use the IPGRI *Citrus* descriptors for standardized phenotyping (IPGRI, 1999). Methods for phenotyping are also published as internal manuals and on websites (China; Japan; USDA, 2022) and within manuscripts (Volk et al., 2018; Caruso et al., 2016; Russo et al., 2020; Aparecida da Cruz et al., 2019, 2021; Stenzel et al., 2003; Tazima et al., 2013, 2014). Additional descriptor lists are published by the International Union for the New Varieties of Plants (UPOV) (2003, 2013).

2.3.2 Genotypic characterization

Citrus collections have been genotyped using a wide range of markers, including RAPD (Federici et al., 1998; Sanabam et al., 2018), simple sequence repeats (SSR; Barkley et al., 2006; Luro et al., 2008; Jannatil et al., 2009; Rohini et al., 2020; Mallick et al., 2017; Sanabam et al., 2018; Shahzadi et al., 2014), genotyping by sequencing (GBS; Ahmed et al., 2019), and single nucleotide polymorphism (SNP) arrays (Queensland; CREA, unpublished; Hiraoka, 2020). Whole genome sequencing has also been performed to assess genetic relationships and evolutionary history in citrus (Terol et al., 2015; Wu et al., 2014, 2018, 2021). Phylogenetic analyses have also been performed using chloroplast sequencing (Samarina et al., 2020; Carbonell-Caballero et al., 2015). Due to

Table 5. Citrus collections with databases available to the public

the non-uniform use of marker systems across collections and the incomplete coverage within collections, across-collection diversity and duplication assessments can not be performed with the existing datasets.

2.3.3 Data availability

Eleven collections have databases with information that is available to the public to some extent (Table 5). These databases are mostly available in English and often in other languages also. Citrus genomic data, including some generated from materials in genebank collections, are available in publicly available external databases including the <u>Citrus Genomic Variation</u>. <u>Database</u> (Li et al., 2020), the <u>Citrus Pan-genome-</u> <u>2breeding Database</u> (Liu et al., 2022), and the <u>Citrus</u> <u>Genome Database</u> (Staton et al., 2021). Collections that do not have publicly available databases usually store information on local databases and spreadsheets. Some collections with publicly available databases also maintain local databases.

2.4 Collection distribution

The Citrus Collection Survey asked respondents about the primary uses of their distributions. The most frequent uses are propagation for resale, certification programs, breeding, plant and/or pathogen research, phenotypic evaluation, and molecular characterization. To a lesser extent, collections are used for pre-

Name of organization with citrus collection	Country	Collection database	Avail- ability	URL	Language
Embrapa Temperate Agriculture	Brazil	Active Citrus Germplasm Bank (BAG-Citros-CPACT) Alelo Platform	Public, internal	LINK	Portuguese, English, Spanish and French
Citrus Research Institute, Southwest University	China	Chinese Crop Germplasm Resources Information System	Public	LINK	Chinese
Nueva Vizcaya State University (NVSU)	Philippines	CiTris (Citrus Genetic Resources Information System)	Public	LINK	English
Citrus and Subtropical Fruits Research Center	Iran	Database of Citrus and Subtropical Fruits Research Center	Public, internal	LINK	Persian - English
CREA-Research Center for Olive, Fruit and Citrus Crops	Italy	National Network on Plant Genetic Resources for Food and Agriculture	Public, internal	LINK	English and Italian
Instituto de Desenvolvimento Rural do Paraná – IAPAR/ Emater	Brazil	Embrapa Alelo Bag System	Public	LINK	Spanish, English and Portuguese
INRAE, UE Citrus	France	florilege	Public	LINK	French, some notes in English
Institute of Fruit Tree and Tea Science, NARO (NIFTS)	Japan	GeneBank Project, NARO Databases	Public, internal	LINK	English
Instituto Valenciano de Investigaciones Agrarias (IVIA)	Spain	GERMO and Excel	Public, internal	LINK	Spanish and Valencian
USDA-ARS National Clonal Germplasm Repository	United States	GRIN-Global	Public, internal	LINK	English
Plant Resources Center	Vietnam	Vietnamese plant resources database	Public, internal	LINK	English

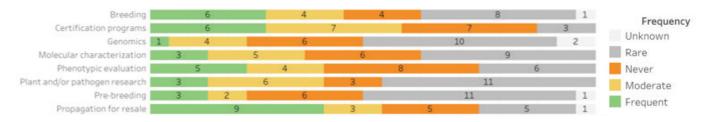
breeding and genomics (Figure 29). Most collections distribute materials for research purposes, and many materials are also distributed for breeding and commercial purposes. Sixteen collections distribute plant materials to the public (Figure 30). In total, about 3750 genebank accessions are distributed to about 350 users annually. The exception is the Citrus and Subtropical Fruits Research Center in Iran, which apparently functions as a nursery in support of the industry and distributes about 2 million grafted trees to 10,000 customers each year.

Most citrus collections do not have limitations on material use but there are some collections that only distribute for research (not commercial) purposes. IPR restrictions may be in place and there are also some limitations on distributions to the public. Agreements (material transfer, cooperative, consortium agreements) may also be necessary. Distributions are primarily within country, with a few collections for which more than 10% of their annual distributions are international (USDA-ARS, Queensland Department of Agriculture and Fisheries). Most of the large collections do not distribute internationally. Most citrus collections distribute materials without charges or on a cost-recovery (including shipping, sanitary certification) basis. Some citrus collections sell trees that have been propagated.

2.5 Human resources

The Citrus Collection Survey asked several questions about personnel, including if there is good retention of trained staff and if staff numbers and training are adequate. Most collections are limited with respect to staffing: some collections only have a manager and some field personnel; some are entirely without dedicated personnel. In some cases, positions are vacant, and some genebanks rely upon students to maintain collections and acquire data. Eleven collection managers responded that there is adequate retention of trained staff. Many respondents stated that staff training is needed, particularly with respect to molecular characterization.

There are also capacity needs with respect to collection re-propagation and greenhouse/screenhouse structure repair/replacement. Budgets range from reasonable institutional support to basic maintenance efforts supported from research grant proposals. Overall, additional financial resources were stated to be needed for citrus collection management. About half of the collections responded that resource inadequacies will result in a loss of germplasm in collections.



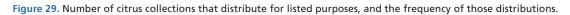




Figure 30. Number of collections that distribute citrus genetic resources to breeding programs, industry, the public, and to researchers.

Survey responses listed several organizations that provide opportunities for networking at national, regional, and international levels. For example, India citrus programs are part of an All India Coordinated Research Project (AICRP) on Fruits, the European Union has joint citrus projects, the Iberoamericana para la vigilancia de *Xylella fastidiosa* (IBER-SYFAS) is an Ibero-American effort focused on *Xylella*. The International Society of Citriculture, the International Society of Citrus Nurserymen, and the International Organization of Citrus Virologists are international organizations with wide membership and interest in germplasm collections, to ensure the future availability of citrus genetic diversity.

2.6 Future prospects

Survey respondents identified many ways in which collections could be improved. These included: building new, or renovating existing, facilities; establishing and maintaining clean plants; increased numbers of trained staff; developing secure back-ups (which could involve cryopreservation); and long-term commitments to genebank collections.

The final question on the survey was "What changes to the present situation would you consider to be essential for the long-term conservation of citrus at a global level?" The responses to this question were enlightening, ranging from increased cooperation and networks, secured collections and back-ups, internationally coordinated clean-plant programs, and improved characterization and identification of collections. Responses have been incorporated into the following section setting out a global strategy for the conservation of citrus genetic resources.



3 A GLOBAL STRATEGY FOR THE CONSERVATION AND USE OF CITRUS GENETIC RESOURCES

Healthy, secure citrus collections are critical for use in research and breeding programs aimed at improving fruit nutritional quality, abiotic and biotic resistance, yield, sustainable production, and farmer livelihoods on a global scale.

Citrus does not have a dedicated international research center and its diversity is currently maintained across local and national genebanks around the world. A global strategy for the conservation of citrus must consider citrus genetic resources at an international level and be inclusive of diverse collections that may not have a permanent funding base. Citrus genebanks around the world are collaborative, but not unified in their approach toward conservation, distribution, and use, resulting in a patchwork of disparate, though sometimes overlapping, activities. One very significant challenge for international collaboration is the difficulty in transferring citrus genetic resources across borders, and in some cases even within countries, due to pest and pathogen threats. Therefore, countries which support significant citrus production, breeding and research efforts must maintain collections that are available within all citrus producing regions of the country. These citrus collections are primarily maintained as field collections, which are costly and vulnerable. Vulnerabilities are due to a lack of skilled personnel, facilities, and financial resources; pest and disease pressures; changing institutional priorities; the need for training on technological

developments; and a lack of large-scale, coordinated international efforts. The difficulty in sharing and distributing citrus genetic resources across borders results in duplication of efforts and inefficiencies.

3.1 Structure of the Strategy

This Global Strategy for the Conservation and Use of Citrus Genetic Resources was developed by performing a literature review to summarize information about the crop's economic importance, conservation, and use. In addition, selected indicator metrics for citrus and apple (as a comparison) were identified (Appendix 4). The survey was developed and then distributed to citrus genebank collection managers around the world to gather additional key information about collections. This information was then summarized and provided in Section 2 of this document. Priority actions were developed based on the information acquired from the literature review and survey. The survey results and proposed priority actions were shared with the survey respondents to receive input from the international community. The strategy documents were updated with feedback from these interactions.

The Priority Actions set out below are supported by the findings of the survey and identify actionable activities that can be accomplished to address significant constraints if sufficient funding is available. Goal for global *ex situ* conservation of citrus: The diversity of citrus and its wild relatives is conserved and available in a disease and pathogen-free state in perpetuity in a secure, distributed network of genebank collections that provide collection data (passport, phenotypic, genotypic) in a standardized common information system.

3.2 Priority actions for the global *ex situ* conservation of citrus

Priority Actions were identified that seek to unify the citrus genebanking community with respect to sharing maintenance, inventory, and associated data through compatible online resources. With <u>funding</u>, Priority Actions will result in shared online resources, training opportunities, and standardized collection data as well as healthy, secure plant collections.

Priority Action 1. Increase citrus genebank community cooperation by establishing an international working group and developing/using a Citrus Community Information System (CCIS) for citrus and related genera. This shared community website could serve as a focal point for shared community information and outreach. It is critical that it have long-term financial support and community participation for the information to remain current and relevant.

It may have many, if not all, of the following features:

- Collaboration, funding, and training opportunities
- Information on relevant conferences
- Taxonomic information, including synonyms, and a method to easily "convert" among older and more recent citrus classification systems
- Standardized data formats for collecting and documenting accession information relating to
 - ° Passport information
 - Descriptor lists and data collection methods for phenotyping
 - ^o Genotyping
 - ° Disease testing and pathogen eradication
 - Shoot tip and seed preservation for long-term security
- Contact information for collection curation teams and inventories
- Collection availability information
 - What citrus genetic resources are available (within country or internationally) for which purposes and in what forms and health status
 - Information about *Citrus* and related genera that are conserved in situ
 - Whether collection materials are covered under the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA)

and thus available with an SMTA (Standard Material Transfer Agreement)

- Whether collection materials and/or in situ materials are available through the Convention on Biological Diversity
- How collection materials can be requested and acquired
- Standardized citrus collection data including taxonomy, passport, image, holdings and availability, phenotypes, genotypes, and digital object identifiers (DOI), with a mechanism to make information publicly available if permission is granted. Ideally there would be interoperability between the information system and citrus collection databases that provides information updates
- Links to published data in the literature, ideally gathered using artificial intelligence that mines literature for relevant information

A team of citrus collection managers should review available information system options, including the Citrus Genome Database and Genesys, to determine the feasibility of using/modifying existing resources to meet the needs of the international citrus genebanking community. Information should be stored in a way that facilitates cross-collection searches, identifies duplicate accessions (through DOI), and allows the user community to search for taxa, phenotypes, and genotypes of interest. This database should ideally be interoperable with genebank databases to ensure that records are always up to date. The database should have long-term funding support for necessary updates and service continuity. It should also have an advisory committee that includes citrus genebanking community members (among others) to ensure its applicability to this audience.

Priority Action 2. Support data collection and documentation efforts for citrus collections. Genotypic data could be acquired through a centralized genotyping center to ensure standardized data collection and interpretation. New cost-effective citrus SNP arrays must be developed (using newly available citrus genome sequences) to genotype the wide range of citrus collection diversity. Information collected and funded through the Global Strategy effort should be made publicly available through the information system in Priority Action 1. Provide financial resources to collect standardized phenotypic data at citrus genebanks and make information about how to access these resources widely available.

- Collect image and phenotype data for collections and make it publicly available
- Genotype collections to standardize collection identities, duplicates, overlaps using a common platform

Priority Action 3. Identify taxonomic gaps (cultivars and related genera) in citrus collections and fill gaps through collections and exchange. Compile taxonomic information about *in situ* and *ex situ* citrus collections, including international availability. Identify additional sources of citrus genetic resources and assess their availability. With financial resources, fill collection gaps through exploration and exchange to ensure the long-term *ex situ* conservation of citrus on a global scale.

Priority Action 4. Increase the health and security (backup) of citrus collections, particularly those that have vulnerable unique plant genetic resources.

- Carry out research and implement findings to control pests and eradicate pathogens from citrus collections
- Carry out research and implement findings for improved long-term storage (backup) of citrus seeds and shoot tips in genebanks

International and even within-country citrus exchange is dependent upon having plants that are healthy and pathogen-free. Clean plant networks are expensive to establish and maintain, but essential for genebank collections, breeding efforts, and nursery operations. Research is necessary to improve pathogen detection and clean-up procedures. Implementation will also require significant financial support because increased facilities and staff support will be necessary to implement clean-up efforts at many citrus genebanks.

It is critical to identify and financially support a system whereby citrus genetic resources can be backed up at secondary locations, which could include a combination of multiple field sites as well as long-term storage of seeds and/or shoot tips in liquid nitrogen. Currently, long-term preservation technologies are not available for all citrus related genera.

- Assess the feasibility of establishing multiple-location field backups of materials that are currently conserved at a single site.
- Determine the practicality, cost, and security of multiple vs. several vs. single cryopreserved backup locations. Consider overlaps and duplications among collections (based on genotypic data) when prioritizing backup efforts.

Priority Action 5. Provide training opportunities for the citrus genebanking community on a wide range of topics—through a combination of affordable in-person and online options. These training opportunities could include lectures/seminars, online courses, videos, eBooks, and/or in-person training. Information could be compiled and made available through the website proposed in Priority Action 1.

Priority Action 6. Develop, maintain, and distribute materials from a clean, secure international citrus collection at one or more locations that captures the fullest extent possible of the taxonomic diversity of citrus. A long-term goal for the international citrus genebanking community is to establish a well-documented genetically diverse, pathogen-free citrus collection that is available to researchers around the world. This collection may be maintained in an indoor (greenhouse or screenhouse) setting to minimize the introduction and transfer of pathogens between trees.

3.3 Success indicators

Success of the Global Strategy for the Conservation and Use of Citrus Genetic Resources is dependent upon having funding available to support personnel with citrus expertise specifically assigned to this effort. It requires long-term international cooperation to unite citrus genetic resource conservation efforts on a global scale. Success can be measured by implementation of activities listed within the Priority Actions, all of which will require financial support and cooperation. Most priority actions have specific items proposed that could be accomplished with sufficient funding and community engagement. Specific indicators of success should be established that align with funding levels to ensure that the funding received for projects is used appropriately.

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APPENDICES

Appendix 1. Listing of *Citrus* collections from FAO WIEWS and Genesys and their overlap with citrus survey respondents.

Collec- tion code	Country	City	Institute	Collection size in FAO WIEWS	Collec- tion size in Genesys	Re- sponded to survey
ALB020	Albania	Vlora	Agriculture Technology Transfer Center Vlore, Ministry of Agriculture and Rural Development	9	Genesys	
ALB026	Albania	Tirana	Plant Genetic Resources Centre, Agriculture University of Tirana		9	
ARG1219	Argentina	Concordia	Estación Experimental Agropecuaria de Concordia, Instituto Nacional de Tecnologia Agropecuaria	925		yes
ARG1228	Argentina	Las Talitas, Tucumán	Estación Experimental Agro-Industrial Obispo Colombres, Instituto Nacional de Tecnología Agropecuaria	395		yes
ARM005	Armenia	Yerevan	Institute of Botany, National Academy of Sciences of Armenia	1		
ATG007	Antigua and Barbuda	St. John's	Crop Research Unit, Central Cotton Station and Agricultural Experiment Station, Department of Ministry of Agriculture, Fisheries and Land	11		
AUS037	Australia	Dareton	Agricultural Research and Advisory Station, Department of Agriculture, New South Wales Agriculture	234		yes
AUS038	Australia	Gosford	Horticultural Research and Advisory Station, Department of Agriculture, New South Wales Agriculture	332		
AUS167	Australia	Adelaide, South Australia	Australian Pastures Genebank, South Australian Research and Development Institute		25	
AZE009	Azerbaijan	Guba	Fruit and Tea Growing Research Institute, Agrarian Science and Innovation Center	14	19	
BGD164	Bangladesh	Gazipur	Plant Genetic Resources Centre, Bangladesh Agricultural Research Institute	5		
BGR001	Bulgaria	Sadovo, Plovdiv District	Institute for Plant Genetic Resources "K.Malkov"	1		
BRA004	Brazil	Cruz das Almas, Bahia	Embrapa Mandioca e Fruticultura Tropical	811	642	
BRA005	Brazil	Vicosa, Mato Grosso	Departamento deFitotecnia, Universidade Federal de Viçosa	14		
BRA125/ BRA045/ BRA006	Brazil	Campinas, Sao Paulo	Instituto Agronómico de Campinas	2058		yes, duplicate?
BRA020	Brazil	Pelotas/RS	Embrapa Clima Temperado, Empresa Brasileira de Pesquisa Agropecuária		58	yes
BRA077 (BRA036, BRA199)	Brazil	Taquari, Rio Grande do Sul	Estação Experimental Fitotécnica de Taquari	398		yes
BRA037	Brazil	Piracicaba, Sao Paulo	Universidade de São Paulo, Escola Superior	1		
BRA044	Brazil	Londrina-PR	Area de Documentação, Instituto Agronomico do Paraná	400		yes
BRA067	Brazil	Vitoria, Espirito Santo	Empresa Capixaba de Pesquisa Agropecuária	30		
BRA125/ BRA045/ BRA006	Brazil	Sao Paulo	Centro de Citrocultura "Sylvio Moreira", Instituto Agronomico de São Paulo, Empresa Brasileira de Pesquisa Agropecuaria	2134		yes
BRA195	Brazil	Florianopolis	Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina	20		
BRA206	Brazil	Maceio	Universidade Federal de Alagoas	40		
BRB012	Barbados	Haggatts, St. Andrew	Soil Conservation Unit, Ministry of Agriculture and Rural Development	14		

Collec- tion code	Country	City	Institute	Collection size in FAO WIEWS	Collec- tion size in Genesys	Re- sponded to survey
CHN020	China	Chongqing, Sichuan Prov.	Citrus Research Institute, Chinese Academy of Agricultural Sciences	1880		yes
CHN052	China	Liantang, Nanning Jiangxi Province	Institute of Pomology, Jiangxi Academy of Agricultural Sciences	70		
CIV013	Cote d'Ivoire	Abidjan	Institut de Recherches sur les Fruits et Agrumes, Centre de Cooperation International en Recherche Agronomique pour le Developpement	78		
СОК003	Cook Islands	Avarua, Rarotonga	Ministry of Agriculture	19		
COL004	Colombia	Palmira, Valle del Cauca	Centro de Investigaciones de Palmira, Instituto Colombiano Agropecuario, AGROSAVIA	170		
COL029	Colombia	Rionegro, Antioquia	Centro de Investigación La Selva, Corporación Colombiana de Investigación Agropecuaria	169		
CRI001	Costa Rica	Turrialba	Centro Agronómico Tropical de Investigación y Enseñanza, CATIE		87	yes
CRI006	Costa Rica	Alajuela	Estación Experimental Agrícola Fabio Baudrit Moreno, Universidad de Costa Rica	30		
CRI008	Costa Rica	Guanacaste	Estación Experimental Enrique Jiménez Nuñez, Instituto Nacional de Innovacion y Transferencia de Tecnologia Agropecuaria	1		
CRI134	Costa Rica	Turrialba	CATIE - Jardín Botánico y Colecciones	23		
CRI137	Costa Rica	Alajuela	Estación Experimental Fraijanes - Universidad de Costa Rica	5		
CUB003	Cuba	La Habana	Instituto de Investigaciones en Fruticultura Tropical, Ministerio de la Agricultura	61		
CUB014	Cuba	Santiago de las Vegas-Ciudad	Instituto de Investigaciones Fundamentales en Agricultura Tropical, Ministerio de la Agricultura	96		
DEU109	Germany	Witzenhausen	Greenhouse for Tropical Crops, Institute for Production and Nutrition of World Crops, Kassel	16		
DOM002	Dominican Republic	Santo Domingo	Departamento de Investigaciones Agropecuarias, Secretaría de Estado de Agricultura	4		
DOM011	Dominican Republic	Santo Domingo	Jardín Botánico Nacional Dr. Rafael M. Moscoso, Secretaria de Estado de Medio Ambiente y Recursos Naturales	12		
DOM048	Dominican Republic	Bani	Centro Experimental de Frutales Baní, Instituto Dominicano de Investigaciones Agropecuarias y Forestales	10		
DZA005	Algeria	Blida	Station Expérimentale, Institut Technique d'Arboriculture Fruitière et de la Vigne	231		
ECU006	Ecuador	Portoviejo, Manabi	Estación Experimental Portoviejo, Instituto Nacional de Investigaciones Agropecuarias	28		
ECU018	Ecuador	Tumbaco, Pichincha	Granja Experimental de Tumbaco, Estacion Experimental Santa Catalina	136		
ECU021	Ecuador	Quevedo, Los Rios	Estación Experimental Pichilingue, Instituto Nacional de Investigaciones Agropecuarias	59		
ECU022	Ecuador	El Coca, Orellana	Estación Experimental Napo-Payamino, Instituto Nacional de Investigaciones Agropecuarias	1		
ECU023	Ecuador	Quito, Pichincha	Departamento Nacional de Recursos Fitogenéticos y Biotecnología, Instituto Nacional de Investigaciones Agropecuarias	84		
ESP025	Spain	Moncada, Valencia	Generalidad Valenciana. Consellería de Agricultura, Pesca y Alimentación. Instituto Valenciano de	430	421	yes
ESP172	Spain	Tacoronte	Cabildo Insular de Tenerife. Centro de Conservación de la Biodiversidad Agrícola de Tenerife, Cabildo Insular de Tenerife		14	

Collec- tion code	Country	City	Institute	Collection size in FAO WIEWS	Collec- tion size in Genesys	Re- sponded to survey
FJI002	Fiji	Seaqaqa	Seaqaqa Research Station, Ministry of Agriculture, Rural and Maritime Development and National Disaster Management	32		
FRA014	France	Montpellier Cedex 5	Centre de Coopération Internationale en Recherche Agronomique pour le Développement	1100		yes
FRA064	France	San Giuliano	Station de Recherches Agronomiques de Corse (INRA-CIRAD), Institut national de recherche pour l'agriculture, l'alimentation et l'environnement, Departement de biologie et amelioration des plantes	648		yes
FRA098	France	Saint-Pierre Cedex	Station de la Réunion, CIRAD-FLHOR, Departement des Productions Fruitieres et Horticoles	55		
FRA099	France	Le Lamentin Cedex 2	Station de la Martinique, CIRAD-FLHOR, Departement des Productions Fruitieres et Horticoles	169		
FRA201	France	Capesterre Belle-Eau	Station de la Guadeloupe, CIRAD-FLHOR, Departement des Productions Fruitieres et Horticoles	53		
GAB018	Gabon	Libreville	Institut de Recherches Agronomiques et Forestières, Ministère de l'Enseignment Supérieur et de la Recherche Scientifique	44		
GHA091	Ghana	Bunso, Eastern Region	Plant Genetic Resources Research Institute, Council for Scientific and Industrial Research	31		
GIN013	Guinea	Kankan, Haute Guinee	Centre de Recherche Agronomique de Bordo, Institut de Recherche Agronomique de Guinée	35		
GIN018	Guinea	Pia, Moyenne Guinee	Centre de Recherche Agronomique de Bareng, Institut de Recherche Agronomique de Guinée	35		
GRC016	Greece	Chania	Institute of Subtropical Plants and Olive Trees, National Agricultural Research Foundation, Directorate General of Agricultural Research, Hellenic Agricultural Organization-DEMETER	112		
HND002	Honduras	El Negrito, Yoro	Estación Experimental Guaymas, Dirección de Ciencia y Tecnología Agropecuaria	1		
HND007	Honduras	Tela	Jardín Botánico y Centro de Investigación Lancetilla, Escuela Nacional de Ciencias Forestales	75		
HND008	Honduras	La Ceiba	Centro Universitario Regional del Litoral Atlántico, Universidad Autónoma de Honduras	70		
IDN002	Indonesia	Bogor	National Biological Institute	56		
IDN028	Indonesia	Denpasar, Bali	University of Udayana	200		
IDN177	Indonesia	Malang, East Java	Indonesian Legume and Tuber Crops Research Institute	159		
IND001	India	New Delhi	National Bureau of Plant Genetic Resources, Indian Council of Agricultural Research	2		
IND024	India	Distt-Thrissur, Kerala	Regional Station Thrissur, National Bureau of Plant Genetic Resources	2		
IND034	India	Abohar, Punjab	Regional Fruit Research Station, Punjab Agricultural University	151		yes
IND065	India	Shillong, Meghalaya	Regional Station Shillong, National Bureau of Plant Genetic Resources	86		
IND216	India	Nagpur, Maharashtra	National Research Centre for Citrus, Indian Council of Agricultural Research	612		yes
IRN029	Iran	Karaj	National Plant Gene Bank of Iran, Seed and Plant Improvement Institute	305		
ITA040	Italy	Palermo	Centro di Studio per il Miglioramento Genetico degli Agrumi, Ministry of Agriculture and Forestry Policies	165		
ITA226/ ITA404	Italy	Acireale	CREA-Research center for Olive, Fruit and Citrus Crops (Acireale - CT)	261	578	yes

Collec- tion code	Country	City	Institute	Collection size in FAO WIEWS	Collec- tion size in Genesys	Re- sponded to survey
ITA236	Italy	Palermo	Istituto di Agronomia Generale e Coltivazioni Erbacee, Facoltá di Agraria, Univ. di Palermo, Viale d. Scienze	265		
JAM002	Jamaica	St. Catherine	Research and Development Division, Ministry of Agriculture and Fisheries	63		yes
JAM026	Jamaica	Port Antonio, Portland	College of Agriculture, Science and Education, Ministry of Education	4		
JAM027	Jamaica	Bog Walk Town	Citrus Growers Association Ltd.	131		
JPN003	Japan	Tsukuba-shi, Ibaraki-ken	Department of Genetic Resources I, National Institute of Agrobiological Sciences, National Agriculture and Food Research Organization	2118		yes?
JPN041	Japan	Kuchinotsu-cho, Minamikourai-gun, Nagasaki-ken	Fruit Trees Research Station, Kuchinotsu Branch Fruit Tree Research Station	195		
JPN043	Japan	Saga-shi, Saga-ken	Department of Horticultural Sciences, Saga University	208		
JPN176	Japan	Shimizu-shi, Shiuoka-ken	Fruit Trees Research Station, Okitsu Branch	1507		yes
KEN015	Kenya	Muguga	National Genebank of Kenya, Crop Plant Genetic Resources Centre - Muguga, Kenya Agricultural and Livestock Research Organization	34		
KEN041	Kenya	Mtwapa, Kikambala	Agricultural Research Centre - Mtwapa, Kenya Agricultural and Livestock Research Organization	33		
LBN060	Lebanon	Aakkar	Lebanese Agriculture Research Institute (Aabdeh Station)	69		
LBN061	Lebanon	Tyr	Lebanese Agriculture Research Institute (Tyr station)	11		
LCA001	Saint Lucia	Castries	Caribbean Agricultural Research and Development Institute	3		
LKA090	Sri Lanka	Dambulla	CIC Agri Business Centre	15		
LKA131	Sri Lanka	Peradeniya	Horticultural Research Unit 2, Horticultural Crops Research and Development Institute	33		
LKA152	Sri Lanka	Horana	Fruit Crop Research and Development Centre, Horticultural Crops Research and Development Institute	27		
MAR004	Morocco	Kenitra	Station Centrale de la Recherche sur les Agrumes, Institut National de la Recherche Agronomique	586		
MDG004	Madagascar	Antananarivo	Division Agronomique-Génétique et Amélioration des Plantes, Centre National de la Recherche Appliquée au Développement Rural	53		
MEX006	Mexico	Edo Mexico	Banco Nacional de Germoplasma Vegetal, Departamento de Fitotecnia, Universidad Autónoma de chapinga, Universidad Autónoma Chapingo (UACh)	20		
MEX008	Mexico	Col. San Rafael	Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP)	230		
MEX017	Mexico	Culiacan, Sinaloa	Centro de Investigaciones Agrícolas del Pacifico-Norte, INIA	66		
MLI099	Mali	Sikasso	Centre Régional de Recherche Agronomique de Sikasso/Fruits et Légumes	1		
MWI006	Malawi	Limbe	Bvumbwe Agricultural Research Station	36		
MYS117	Malaysia	Kuala Lumpur	Strategic Resource Research Centre, Malaysian Agricultural Research and Development Institute	5		
MYS142	Malaysia	Kuala Lumpur	Horticulture Research Centre, Malaysian Agricultural Research and Development Institute	20		

Collec- tion code	Country	City	Institute	Collection size in FAO WIEWS	Collec- tion size in Genesys	Re- sponded to survey
MYS193	Malaysia	Kota Bharu, Kelantan	Department of Agriculture, Kelantan	1		
NGA003	Nigeria	Ibadan, Oyo State	National Horticultural Research Institute	130		
NIC004	Nicaragua	Masatepe, Masaya	INTA Pacífico Sur (Masaya, Granada, Carazo, Rivas)	52		
NPL006	Nepal	Godawari, Lalitpur	National Citrus Research Programme	43		yes
NZL021	New Zealand	Auckland	HortResearch, Mt. Albert Research Centre, Batchelar Research Centre, Horticultural Food Research Institute of New Zealand	1		yes
PAN003	Panama	Panama	Facultad de Ciencias Agropecuarias-Chiriqui	16		
PER004	Peru	Huanuco	Universidad Nacional Hermilio Abad del Cusco, Centro K'Ayra	23		
PER034	Peru	Huaral	Estación Experimental Agraria Donoso, Instituto Nacional de Innovación Agraria	30		
PER054	Peru	Pucallpa, Coronel Portillo	Universidad Nacional de Ucayali	8		
PER113	Peru	Huanuco	Instituto de Desarrollo del Medio Ambiente	12		
PHL120	Philippines	Davao City	Bureau of Plant Industry-Davao National Crop Research and Development Center	108		
PHL130	Philippines	Laguna	Crop Science Cluster-Institute of Plant Breeding, College of Agriculture, University of the Philippines,	13		
PHL131	Philippines	Baguio City	Bureau of Plant Industry-Baguio National Crop Research and Development Center	80		
PHL180	Philippines	La Carlota City, Negros Occidental	Bureau of Plant Industry-La Granja National Crop Research and Development Center	1		
PHL446	Philippines	Batangas	Lipa Agricultural Experiment Station	10		
PLW004	Palau	Koror	Bureau of Agriculture	4		
PLW005	Palau	Nekkeng	Taiwan Technical Mission	5		
PNG001	Papua New Guinea	Keravat, East New Britain Province	Wet-lowlands Islands Research Programme, Keravat	8		
PRI410	Puerto Rico	Lajas	Estación Experimental Agrícola, Universidad de Puerto Rico	17		
PRT065	Portugal	Vila Real	Departamento de Protecção de Plantas, UniversidadeTrás-os-Montes e Alto Douro	1		
PRT102	Portugal	Funchal-Madeira	Banco de Germoplasma - Universidade da Madeira	1		
SDN002	Sudan	Wad Medani	Horticultural Research Section Agricultural Research Corporation	80		
SEN075	Senegal	Dakar	Unité de Recherche en Culture In-vitro, Laboratoire National de Recherches sur les Productions Végétales	62		
SLE015	Sierra Leone	Freetown	Njala University College	4		
SLV066	El Salvador	San Andres, La Libertad	CENTA - Programa de Frutales	29		
SUR008	Suriname	Paramaribo	Foundation for Experimental Gardens, Ministry of Agriculture, Animal Husbandry and Fisheries	26		
SUR020	Suriname	Paramaribo	Department of Agriculture Research, Marketing and Processing, Ministry of Agriculture, Animal Husbandry and Fisheries	48		
SYC001	Seychelles	Mahe	Grand Anse Experimental Centre, Ministry of Agriculture and Marine Resources	7		
THA019	Thailand	Bangkok	Department of Botany, Faculty of Science, Chulalongkorn University	531		
THA022	Thailand	Chantaburi Province	Plew Horticultural Experimental Station, Horticultural Research Institute Department of Agriculture	80		

Collec- tion code	Country	City	Institute	Collection size in FAO WIEWS	Collec- tion size in Genesys	Re- sponded to survey
THA056	Thailand	Bangkok	Horticultural Research Institute Department of Agriculture, Department of Agriculture, Ministry of Agriculture and Cooperation	375		
TTO001	Trinidad and Tobago	St. Augustine	Faculty of Agriculture, University of the West Indies	1		
TTO010	Trinidad and Tobago	Centeno	Central Experiment Station, Research Division, Ministry of Agriculture, Land and Marine Resources	156		
TTO019	Trinidad and Tobago	Curepe	Agricultural Services Division, Ministry of Food Production and Marine Resources, Ministry of Agriculture, Land and Fisheries	1		
TUN001	Tunisia	Ariana	Institut National de la Recherche Agronomique de Tunisie, Institution de la Recherche et de l'Enseignement Supérieur Agricole	51		
TUR001	Turkey	Izmir	Plant Genetic Resources Department, Aegean Agricultural Research Institute	785		
TUR005	Turkey	Icel	Alata Horticultural Research Institute, Ministry of Agriculture and Forestry	1		
TUR020	Turkey	Adana	Department of Horticulture, Faculty of Agriculture	741		
TUR060	Turkey	Antalya	West Mediterranean Agricultural Research Institute	1		
TWN001	Taiwan	Shanhua, Tainan	Asian Vegetable Research and Development Center, World Vegetable Center	1		
TWN002	Taiwan	Chia-yi	Chiayi Agricultural Experiment Station, Taiwan Agricultural Research Institute	159		
TZA014	United Republic of Tanzania	Mbeya	Agricultural Research Institute, Uyole, Ministry of Agriculture and Food Security	50		
URY002	Uruguay	Monevideo	Facultad de Agronomía, Universidad de la Republica Oriental de Uruguay	432		
URY010	Uruguay	Salto	INIA Salto Grande, Instituto Nacional de Investigación Agropecuaria	341		
USA108	USA	Mayaguez, Puerto Rico	Tropical Agricultural Research Station, Clonal Repository USDA/ARS	9		
USA109	USA	Riverside, California	Department of Botany and Plant Sciences, University of California, Riverside	591		
USA112	USA	Orlando, Florida	US Horticulture Research Laboratory, USDA/ ARS	306		yes
USA129	USA	Riverside, California	National Clonal Germplasm Repository for Citrus & Dates, USDA-ARS	376	1813	yes
UZB031	Uzbekistan	Tashkent Region	Uzbek Research Institute of Horticulture, Vine Growing and Wine Making named R.R.Shreder	52		
VEN132	Venezuela	Maracay, Estado Aragua	INIA - Centro Nacional de Investigaciones Agropecuarias	156		
VNM016	Vietnam	Vinh	Tropical Crops Experimental Station, Ministry of Agriculture and Rural Development	19		yes
VNM040	Vietnam		Phu Guy Fruit Research Centre, Vegetable and Fruit Crops Research Institute	209		
VNM079	Vietnam	Tiengiang	Southern Fruit Research Institute, Ministry of Agriculture and Rural Development	217		
ZAF004	South Africa	Nelspruit Transvaal	Citrus and Subtropical Fruit Research Institute, Agricultural Research Council	1005		yes

-> VINIDAAV										
FAO Col- lection Code	- Country	Name of organization with citrus collection	Organization city/state	Postal code	Collection owner	Website	Year	Type of organization	Treaty ²	Financing
ARG219	Argentina	Instituto Nacional de Tecnología Agropecuaria (INTA) - Concordia Experimental Station, Entre Ríos, Argentina	Concordia	3200	Instituto Nacional de Tecnología Agropecuaria (INTA)	https://inta.gob.ar/concordia	1994	Government	Yes	100% Government
ARG032	Argentina	Estación Experimental Agroindustrial Obispo Colombres	Tucumán	4101		www.eeaoc.org.ar	1961	Non- governmental organization (NGO)/ Nonprofit	Q	100% Private sector
AUS037	Australia	New South Wales Department of Primary Industries	/ New South Wales	2717	New South Wales Department of Regional NSW		2003	Government	No	85% Government; 15% Private sector
AUS022	Australia	Queensland Department of Agriculture and Fisheries	Bundaberg, Queensland	4670	The State of Queensland	www.daf.qld.gov.au	1960	Government	Yes	30% Government; 70% private
BRA077 (BRA036, BRA199)	Brazil	Secretaria da Agricultura, Pecuária e Desenvolvimento Rural	Porto Alegre/ Rio Grande do Sul	90130-060		www.agricultura.rs.gov.br/	2009	Government	No	100% Government
BRA125/ BRA045/ BRA006	Brazil	Instituto Agronômico de Campinas/ Centro de Citricultura Sylvio Moreira	Cordeirópolis	13490-970	Instituto Agronômico de Campinas - IAC, Avenida Barão de Itapura, 1481, CEP 13020-902 Campinas, SP	www.ccsm.br	1928	Government		20% Government; 10% Private sector; 70% Research Projects
BRA020	Brazil	Embrapa Temperate Agriculture	Pelotas, Rio Grande do Sul State	96090-575	Brazilian Agricultural Research Corporation (Embrapa)	www.embrapa.br/en/clima- temperado	2017	Government	Yes	100% Government
n/a	Brazil	Fundação Coopercitrus Credicitrus - Estação Experimental com Agronegócio de Bebedouro	Bebedouro/ São Paulo	14713-000		http:// fndcoopercitruscredicitrus. org.br/	1998	Non- governmental organization (NGO)/ Nonprofit	Yes	10% Government; 90% Private sector
BRA044	Brazil	Instituto de Desenvolvimento Rural do Paraná - IAPAR/Emater	Londrina/PR	86047-902			1980	Government	No	100% Government
CHN020	China	Citrus Research Institute, Southwest University	Chongqing	400712	Southwest University	http://english.cric.cn	1962	Public-funded university	No	100% Government
1 Year t 2 Is this	the collection collection su	Year the collection was established Is this collection subject to the International Treaty on Plant Genetic Resources for Food and Agriculture?	ant Genetic Reso	ources for Foo	d and Agriculture?					

Appendix 2. Citrus collections and their composition described by survey respondents.

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FAO Col- lection Code	Country	Name of organization with citrus collection	Organization city/state	Postal code	Collection owner	Website	Year¹	Type of organization	Treaty ²	Financing
CRI001	Costa Rica	CATIE	Turrialba, Cartago	30501	CATIE	www.catie.ac.cr	1994	Regional Research Center	Yes	100% Other
FRA014	France	INRAE, UE Citrus	San-Giuliano	20230	INRAE	www.inrae.fr/actualites/ diffusion-du-materiel-vegetal	1960	Government	Yes	90% Government; 10% Research projects
IND216	India	ICAR - Central Citrus Research Institute	Nagpur/ Maharashtra	440033		www.ccri.icar.gov.in	1992	Government	No	100% Government
IND034	India	PAU-Dr. JC Bakhshi Regional Research Station, Abohar	Abohar/ Punjab	152116	Punjab Agricultural University, Ludhiana, Punjab, India	www.pau.edu (website of PAU)	1959	Government	Yes	100% Government
IND080	India	Agricultural Research Station Sriganganagar, SKRAU, Bikaner	Sriganganagar	335001	Swami Keshwanand Rajasthan Agricultural University	http://arssgnr.org/	2010	Government	Yes	100% Government
IRN044	Iran	Citrus and Subtropical Fruits Research Center	Ramsar	4691733113	Horticulture Science Research Institute	https://icri.hsri.ac.ir/en-US/icri. hsri.ac/3733/page/home	1966	Government	No, 2025	100% Government
ITA226	ltaly	CREA-Research Centre for Olive, Fruit and Citrus Crops	Acireale / Catania	95024	Council for Agricultural Research and Economics (CREA)	www.crea.gov.it	1940	Government	Yes	100% Government
JAM002	Jamaica	Bodles Research Station Ministry of Agriculture and fisheries Jamaica	Old Harbour, St. Catherine	Old Harbour P.O	Ministry of Agriculture and Fisheries Jamaica		1997	Government	Yes	95% Government; 5% Private sector
JPN176 (JPN003?)	Japan	Institute of Fruit Tree and Tea Science, NARO (NIFTS)	Shizuoka, Shizuoka	424-0292		www.naro.go.jp/english/ laboratory/nifts/index.html	1985	Government	No	100% Government
JPN172	Japan	Faculty of Agriculture, Kagoshima University	Kagoshima	890-0065		www.kagoshima-u.ac.jp/en/	2005	Public-funded university	No	100% Government
LA0018	Lao PDR	National Agriculture and Forestry Research Institute	Saythany distrct, Viantiane capital, Lao PDR (Laos)		National Agriculture and Forestry Research Institute (NAFRI)	www.nafri.org.la	N/A	Government	Yes	MA
MYS031	Malaysia	Rimba Ilmu Botanic Garden and Herbarium of Universiti Malaya (KLU)	Kuala Lumpur	50603	Universiti Malaya	https://rimba.um.edu.my/	1974	Public-funded university	No	80% Government; 20% Other
MEX231	México	Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias	Tecomán, Colima	28930	INIFAP	www.inifap.gob.mx	1983	Government	Yes	100% INIFAP
MEX245	México	Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias	Tlapacoyan	37650		www.gob.mx/inifap	2005	Government	No	100% Government

FAO Col- lection Code	Country	Name of organization with citrus collection	Organization city/state	Postal code	Collection owner	Website	Year¹	Type of organization	Treaty ²	Financing
NPL006	Nepal	Nepal Agricultural Research Council	Dhankuta	44600	Nepal Agricultural Research Council	www.ncrpdhankuta.narc. gov.np	1961	Government	Yes	100% Government
NZL021	New Zealand	The New Zealand Institute for Plant and Food Research Limited (Abbreviated as PFR)	Palmerston North	4442		www.plantandfood.com	pre 1983	Other – a Crown Research Institute which is a Government owned company	°Z	100% Plant and Food Research
PAK019	Pakistan	University of Agriculture of Faisalabad, Pakistan	Faisalabad/ Punjab	38040		www.uaf.edu.pk	1962	Government	No	100% Government
PHL307	Philippines	Nueva Vizcaya State University (NVSU)	Bayombong, Nueva Vizcaya	3700		https://nvsu.edu.ph/	2017	Public-funded university	Yes	100% Nueva Vizcaya State University
RUS202	Russian Federation	Federal Research Centre the Subtropical Scientific Centre of the Russian Academy of Sciences	Sochi	354002		www.vniisubtrop.ru/	1930	Government	No, 2022	100% Government
ZAF004	South Africa	Citrus Research International	Uitenhage	6230	Citrus Growers' Association of Southern Africa	www.cri.co.za	1980	Non- governmental organization (NGO)/ Nonprofit	S	100% Private sector
ESP025	Spain	Instituto Valenciano de Investigaciones Agrarias (IVIA)	Valencia, Spain	46113	Autonomous Goverment of Valencia	https://ivia.gva.es	1975	Government	Yes	100% Government
USA112	United States	USDA-ARS Horticultural Research Laboratory	Fort Pierce, Florida	34945	USDA-ARS, Horticultural Research Laboratory	www.ars.usda.gov/ southeast-area/fort-pierce-fl/ us-horticultural-research- laboratory/	1908	Government	Yes	30% Government; 70% Grant funding
USA129	United States	USDA-ARS National Clonal Germplasm Repository	Riverside, California	92507	US Federal Government; University of California	www.ars.usda.gow/pacific- west-area/riverside-ca/ national-clonal-germplasm- repository-for-citrus/; citrus variety.ucr.edu	1987		Yes	70% Government; 30% UC Riverside
VNM049	Vietnam	Plant Resources Center	Hanoi	1000	Plant Resources Center	www.prc.org.vn	1996	Government	No	100% Government
VNM016	Vietnam	Research Center for Fruit Trees and Industrial Plants covered with Litmus	Vinh city	472000	North Central Institute of Agricultural Science and Technology		1996		Yes	60% Government; 40% Other

FAO Collection Code	Number of unique commer- cial citrus cultivars (including old ones)	Number of unique local culti- vars (#)	Number of unique related/ wild citrus species as grafted trees (#)	Number of unique wild relatives of other genera (<i>Poncirus</i> / <i>Microcitrus</i> / <i>Fortunella</i> etc.) as grafted trees (#)	Number of seedling trees of wild Citrus and other genera (Poncirus/ Microcitrus/ Fortunella etc.)	Number of breeding materials maintained clonally	Number of unique rootstock cultivars	Other (please specify)	Total number of unique acces- sions in collection
ARG219	196	12	0	8	0	0	12	0	228
ARG032	240	0	0	20	0	116	172	0	531
AUS037	250	50	15	10	1	-	90		350
AUS022	70	20	20	50	100	400	300	10	1000
BRA077 (BRA036, BRA199)	11	5		3	2	50	3		
BRA125/ BRA045/ BRA006	57		0	0	0	58			1735
BRA020	58								58
n/a	6	0	0	1	5	326	1		333
BRA044	538		6		9	51	5		
CHN020	1200	800	35	150	0	130	110		1700
CRI001	75	5		5					85
FRA014	253	338	1	2	0	90	97	10	1100
IND216	28	94	31		13	112	19		489
IND034	20	3	6	4	0	0	15	105	153
IND080	8	1				125	3		8
IRN044	122	90	0	0	1	14	17	0	122
ITA226				75	15		40		725
JAM002	40	12		12			10		74
JPN176 (JPN003?)	426	656	9	20	15	113	22	0	1261
JPN172	0	60	0	0	0	0	0	0	60
LAO018		300							
MYS031	2		4	10					
MEX231	17			18			19		54
MEX245	35	19	0	0	0	0	10	3	54
NPL006	120	500	20	150	100	144	17		144
NZL021	207	8	2		-		16		10
PAK019	10	2	0	0	0	4	2		18
PHL307	1	5	0	0	10	0	2	0	160
RUS202	76	48	19 0	3	300	0	3		160 444
ZAF004 ESP025	267	209 226	48	38	0	17	36 44		444
USA112	300	3	48	38	- 600	17	44		415
USA112 USA129	1022	86	27	272	16	29	47	35	1632
VNM049	22	212	7	5	7	29	47	30	338
VNM049 VNM016	22	212		5			0		0.0

FAO Collection Code	Sweet orange	Man- darin	Lemon	Lime	Grape- fruit	Pum- melo	Sour orange	Hybrids	Kum- quat	Finger lime	Citron	Papeda	Other
ARG219	71	68	18	4	20	0	1	43	2	0	1	0	0
ARG032	74	53	44	4	24	10	8	22	3	1	2	0	0
AUS037	100	100	12	3	25	15	10		2	2	3		
AUS022	10	300	5	5	5	30	2		7	204	15	5	20
BRA077 (BRA036, BRA199)	5	50	3	5		1			2				
BRA125/BRA045/ BRA006	684	390	100	54	46	35	45	20	4	2	13	9	348
BRA020	23	11	2	1		4		17					
n/a	232	60	4	12	6	1	4	3	3	0	3		
BRA044	232	192	21	10	9	2	3	53	3		4		9
CHN020	240	470	30	9	20	240	37	20	28	7	35	25	
CRI001	26	11	8	3	6	4	1	6	1			2	17
FRA014	120	268	66	49	31	27	47	12	6	2	17	7	
IND216	45	91	22	52	6	45	8	39			14	19	148
IND034	34	31	8	10	10	2	4	0	2	0	1	1	50
IND080	25	155	25	15	10								
IRN044	29	38	12	4	9	4	1	7	4	0	1	0	0
ITA226	265	132	103	16	27	8	28	36	6	9	6	7	7
JAM002	41	11	6	2	4	1	2	2	2	1	2		
JPN176 (JPN003?)	171	369	52	19	20	133	22	384	16	3	17	1	54
JPN172	5	70	3	2	2	10	3	30	5	0	2	5	0
LAO018													
MYS031				3									
MEX231													
MEX245	11	26	5	5	2						1		
NPL006	34	33	6	25	5	4		8	3	0	1	0	
NZL021	65	72	15	9	11	4	2		4		5		26
PAK019	30	25	8	3	9	1	2	2	2	1	1	0	
PHL307	7	11	4	2	0	17	0	2	1	0	1	1	0
RUS202	10	30	38	6	0	9	4	10	6		4	0	
ZAF004	182	107	19	4	20	9	1		2	1	2	0	61
ESP025	93	128	33	14	14	15	18		14	9	6	7	25
USA112													
USA129	245	243	177	55	59	121	64	141	17	10	107	31	18
VNM049	84	66		32	118	10	15		2	2	3	2	7
VNM016	30	8				2	2		1		1		37

Appendix 3. 2021 Survey of Citrus Collections

Survey of Citrus Collections

The Global Crop Diversity Trust is facilitating the development of the Global Conservations strategies for key crops, including citrus (https://www.croptrust.org/our-work/supporting-crop-conservation/conservation-strategies/). Dr. Fred Gmitter (U. Florida, USA), Dr. Robert Krueger (USDA), and Dr. Gayle Volk (USDA) are coordinating this effort for Citrus. One of the first steps in strategy development is to conduct a survey to determine the extent, availability, and security of citrus collections worldwide.

The information received in the survey, as well as that obtained through follow-up communications will be used to propose a written strategy that will be available for community review. Our goal is to complete the Global Conservation Strategy for Citrus by December 2021.

The Trust will seek to support the implementation of the Citrus strategy. We welcome survey participation from collections of all sizes and types. Our survey response deadline is July 1, 2021.

Thank you for participating in our survey. Your feedback is important. Please contact Dr. Gmitter (fgmitter@ufl.edu) if there are questions about either the survey or the resulting conservation strategy.

Survey of Citrus Collections

1. Survey respondent name

2. Survey respondent email address

3. Survey respondent relationship to the collection

4. Name of organization with citrus collection

5. Organization address

6. Organization city/state

7. Postal code

8. Country

9. Website

10. Curator/researcher responsible for the collection

Name	
Address	
Address 2	
City/Town	
State/Province	
ZIP/Postal Code	
Country	
Email Address	
Phone Number	

Survey of Citrus Collections

11. Is the organization holding the citrus collection:

- () 1) An independent organization
- 2) Part of a larger organization

Name and address of larger organization:

12. Type of organization

Government
Public-funded university
Private
Non-governmental organization (NGO)/Nonprofit
Individual
Other (please specify)

13. Is this organization the legal owner of the collection?

- 🕜 Yes
- 🔘 No

14. Is this collection subject to the International Treaty on Plant Genetic Resources for Food and Agriculture?

- Yes
- O No

If not, is there an anticipated date of entry into the ITPRGFA?

15. Who is financing the conservation of the collection and to what extent?

Government (%)	
Private sector (%)	
International/regional	
organization/agency (%)	
Other funding agencies	
(%) please specify	
(30) please specify	

16. Year the collection was established:

Survey of Citrus Collections

17. What are the primary conservation priorities of the collection (check all that apply)?

Internationally important cultivars	Breeding materials
Local cultivars	Public garden/arboretum
Wild materials	
Other (please specify)	

18. Are you a member of an international citrus network or research project?

\bigcirc	Yes
\bigcirc	No

Please specify:

19. Please indicate the number of accessions in the collection in the following categories:

Number of unique commercial citrus cultivars (including old ones):	
Number of unique local cultivars (#):	
Number of unique related/wild citrus species as grafted trees (#):	
Number of unique wild relatives of other genera (Poncirus/Microcitrus/Fortu nella etc.) as grafted trees (#):	
Number of seedling trees of wild Citrus and other genera (Poncirus/Microcitrus/Fortu nella etc.):	
Number of breeding materials maintained clonally:	
Number of unique rootstock cultivars:	
Other (please specify):	
Total number of unique accessions in collection:	

20. Approximate number of fruit type cultivars maintained clonally:	
---	--

Sweet orange	
Mandarin	
Lemon	
Lime	
Grapefruit	
Pummelo	
Sour orange	
Hybrids	
Kumquat	
Finger lime	
Citron	
Papeda	
Other (please specify)	

21. Percentage of accessions conserved in the following forms:

Field	
Greenhouse/screenhouse	
In vitro	
Seeds	
Other (please describe)	

22. Describe the collection on-site replication (i.e. number of trees per replicate, field and greenhouse, etc):

Survey of Citrus Collections

23. To what extent are pests or diseases having an effect on the collection?

	Major effect	Minor effect	No effect
Affecting trees within specific accessions	\bigcirc	\bigcirc	\bigcirc
Affecting trees in a wide range of accessions	\bigcirc	\bigcirc	\bigcirc
Causing annual losses of trees	\bigcirc	\bigcirc	\bigcirc
Preventing distribution	\bigcirc	\bigcirc	\bigcirc
Incurring costs in pest and disease control	\bigcirc	\bigcirc	\bigcirc
Comments			

24. What pathogens and diseases threaten the collection?

25. What pathogens are tested for in the collection?

26. What percentage of the clonally maintained accessions are pathogen tested?

27. What percentage of the clonally maintained accessions are "cleaned-up"?

Survey of Citrus Collections

28. Describe the back-up status of the collection (type of back-up: secondary collection elsewhere, cryopreservation, in vitro, etc and number of accessions backed-up):

29. What are the barriers to collection back-up?

30. What are the phytosanitary/quarantine requirements for receiving new materials?

31. What are the known collection gaps? Is there a timeframe for filling them?

32. Do you have wild Citrus/Poncirus/Fortunella/Microcitrus native to your country? Which species?

33. Do you have other wild genera/species of Rutaceae native to your country? Which taxa?

34. Are there efforts to conserve them in situ? Ex situ? Describe

Survey of Citrus Collections

35. On average, how many total accessions are distributed per year?

36. On average, how many unique recipients receive material per year?

% Rooted trees	
% Budwood	
% DNA	
% Tissue (leaf, etc)	
% In vitro	
% Fruit	
% Seeds	
% Flowers/Pollen	

37. Of the total number of accessions distributed, what percentage are the following:

38. Are your materials distributed:

	Yes	No
To researchers/scientists?	\bigcirc	\bigcirc
To breeding programs?	\bigcirc	\bigcirc
To industry?	\bigcirc	\bigcirc
To the public?	\bigcirc	\bigcirc
Other (please specify)		

39. Are there limitations on material use? If so, what are they?

40. What are the distribution costs to the recipient?

41. What types of agreements/permits are necessary for distribution?

42. Percent distribution to domestic recipients

43. Percent distribution to foreign recipients

44. Other comments regarding acquisition and distribution:

Survey of Citrus Collections

45. Are standardized methods used for phenotypic evaluations for the collection? Which ones? Please provide references.

46. Are standardized methods used for genotypic characterization of the collection? Which ones? Please provide references.

47. What is the name of the database used for documenting the collection?

48. What is the website URL for the database (if public)?

49. What language(s) is the website interface for the database?

50. The information/database is:

Public	
Internal	
Has both public and internal features	
Other (please specify)	

51. Information is available for:

	Public	Internal	Available by contacting the curator
Passport/source:			
Taxonomy:			
Images:			
Phenotypic data:			
Genotypic data:			
Pathogen status:			
Other (please specify)			

52. Are there key publications (peer reviewed, popular press, online, etc.) about the citrus collection? Please provide links/citations/website.

Survey of Citrus Collections

53. On site collection use:

	Frequent	Moderate	Rare	Never
Plant and/or pathogen research	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Molecular characterization	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Phenotypic evaluation	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Pre-breeding	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Breeding	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Genomics	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Propagation for resale	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Other (please specify)				

54. Use of distributed materials

	Frequent	Moderate	Rare	Never
Plant and/or pathogen research	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Molecular characterization	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Phenotypic evaluation	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Pre-breeding	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Breeding	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Genomics	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Propagation for resale	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Certification programs	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Other (please specify)				

Survey of Citrus Collections

55. Is there adequate retention of trained staff? Explain.

56. What type of constraints do you face?

Staff numbers (explain)	
Staff training (explain)	
Capacity to replant/maintain the	
collection (explain)	
Budget (explain)	
Other (explain)	

57. Will some of the above constraints result in a loss of germplasm?

58. Please describe the major needs or concerns influencing the long-term sustainability of the

collection

59. What changes to the present situation would you consider to be essential for the long-term conservation of citrus at a global level?

Appendix 4. Selected Indicator Metrics for citrus and apple (as comparison)

This appendix was written by Peter Giovannini and Felix Frey

Khoury et al. (2022) compiled a comprehensive dataset as part of a project funded by the International Treaty on Plant Genetic Resources for Food and Agriculture and with the collaboration Crop Trust, and implemented by the Alliance of Bioversity International and the International Center for Tropical Agriculture (CIAT). The aim was to introduce normalized, reproducible indicators to serve as an evidence base when prioritizing actions on the conservation and use of plant genetic resources for food and agriculture. The indicators encompass metrics associated with the USE of a crop (Global importance), the INTERDEPENDENCE between countries with respect to genetic resources, the DEMAND for genetic resources, the SUPPLY of germplasm by genebanks and the SECURITY of germplasm conservation. To generate the indicators, Khoury et al. (2022) collected a comprehensive dataset from multiple sources. We do not present those indicators here, but rather we present a small subset of the variables used in this study.

To put numbers into context, we compare citrus crops with apple (Table 1). Both crops are consumed as fruits and production as well as consumption are widely distributed throughout the globe. Citrus crops and apples are both perennial tree crops with a similar type of cultivation. Citrus crops span the three genera *Citrus, Fortunella* and *Poncirus,* and include multiple fruits, including oranges (*Citrus sinensis*), mandarins (*Citrus reticulata*), grapefruits (*Citrus paradisi*), lemons (*Citrus medica*), chinotto (*Citrus myrtifolia*), citron (*Citrus japonica*) (Tables 2 and 3). The apple, by contrast, is one species, *Malus domestica*.

The production of citrus crops is 146,797,247 tonnes/ year (average between 2015 and 2018) compared to 84,11,082 tonnes/year of apples. The quantity of food supply by citrus crops, i.e. the average global consumption, is about 16.3 Kg /cap/year, 83% higher than global apple consumption (8.87 Kg/cap/year).

Table 1. Selected metrics collected by Khoury et al. (2021) for citrus crops and apples, subdivided by indicator domain

Metric	Citrus (Sum/Range)	Apple	Citrus / Apple
Crop use			
Crop production (tonnes/year) (annual average 2015–2018)	146,797,247	84,113,082	1.75
Food supply (Amount consumed) [Kg/capita/year] (Sum across Citrus species)	28	8.87	3.16
Quantity exported globally [t/year] (Sum across Citrus species)	22,690,181	11,573,782	1.96
Number of publications between 2009–2019, including patents and citations, searching title of publication (Google scholar search hits) for genus ** (Sum across Citrus species)	22,100	4,280	5,16
Number of publications between 2009–2019, including patents and citations, searching title of publication (Google scholar search hits) for species *** (Sum across Citrus species)	4,569	1,930	2,37
Demand			
Accessions distributed from genebanks (Annual average 2015–2019) (Sum across Citrus species) – Plant Treaty Data Store	0	0	
Accessions distributed from genebanks (Annual average 2014–2019) (Sum across Citrus species) – FAO WIEWS	17,802	5,262	3.38
Variety releases in 5 years (2014–2018) (Annual Sum average across Citrus species)- UPOV	37.75	218.25	0.17
Supply			
Number of accessions in ex situ collections of genus ** (Sum across Citrus species)	9,938	44,789	0.22
Number of accessions in ex situ collections of species *** (Sum across Citrus species)	2,585	28,250	0.09
Accessions of the genus ** available through Multilateral System (MLS) directly noted in databases [%]	12%	42%	
Accessions of the species *** available through Multilateral System (MLS) directly noted in databases [%] (several species, range values shown)	6-21%	57%	
Accessions of the genus ** available through Multilateral System (MLS) indirectly by matching institute countries with party status [%] (Range)	86%	84%	
Accessions of the species *** available through Multilateral System (MLS) indirectly by matching institute countries with party status [%] (several species, range values shown)	87–100%	91%	
Security			
Accessions of genus ** safety duplicated in Svalbard Global Seed Vault [%] (Range)	0%	0%	
Accessions of species *** safety duplicated in Svalbard Global Seed Vault [%] (Range)	0%	0%	

Consumption of citrus and of apples as food are in a similar proportion to their global production. This is due to the fact that both crops are exclusively used as food source with no other obvious uses (e.g. feed, bioenergy, etc.). Export of citrus fruits is more important than export of apples. On average 22,690,181 t/year of citrus fruits are exported globally, whereas 11,573,782 t/year apples are exported.

The crop use metrics with respect to research were assessed using a manual search on Google Scholar, searching for the respective genus or species in the titles of publications, including patents and citations, between the years 2009 and 2019 (Khoury et al., 2022). Search hits on Google Scholar indicate the level of scientific interest in a crop. The genus names of the different citrus crops (Table 2) are found in 22,100 publication titles, which is about five times as many publication titles including the apple genus Malus. However, we must consider that the term "citrus" is not only used for the scientific genus name of citrus plants, but also as a common generic term for the different citrus crops, whereas Malus designates exclusively the genus of apple, and is only found in 4,280 publication titles. Publication numbers including the species names of citrus crops and apples are more comparable. The scientific names of the citrus crops (Table 2) appear in 4,569 publication titles, where Malus domestica is included in 1,930 publication titles. By this indicator, citrus research thus receives about 2.3 times as much attention as apple research.

DEMAND for germplasm is defined by various metrics (Khoury et al., 2022), including: (1) the number of distributions of accessions by genebanks, as an annual average between 2015 and 2019, drawn from the Plant Treaty's Global Information System; (2) the number of accessions distributed by national genebanks as reported to the FAO WIEWS system as an annual average between 2015 and 2019, (3) the annual average number of varieties released during the five years between 2014 and 2018, obtained from the International Union for the Protection of New Varieties of Plants (UPOV; www.upov.int).

According to the distribution metric from the Plant Treaty's Data Store, no citrus accessions were distributed between 2015 and 2019 compared to 107 accessions of apple. In the FAO WIEWS dataset 17,802 citrus accessions per year (Annual average 2014-2019) were distributed by genebanks, compared to 5262 accessions of apple per year. 37.75 citrus varieties per year were registered at UPOV during a five-year period, which represents only 17% of apple varieties registered in the same period (218 registered cultivars).

Khoury et al. (2022) illustrated the SUPPLY of germplasm by quantifying the number of accessions available in ex situ collections around the world, with respect to the crop genus and the most important species of the respective crop. They also assessed the number of accessions (again with respect to genus and species) available under the multilateral system (MLS) of the Plant Treaty. This MLS assessment was done first, directly, as noted (in MLS/not in MLS) in the public online databases Genesys, FAO WIEWS and GBIF. Secondly, the availability of accessions was assessed by considering whether the country hosting the institution that held the respective germplasm collection was a signatory to the Plant Treaty, as well as whether the crop was listed in Annex 1 of the Treaty; if both conditions were met, the accession was regarded as available via the MLS. According to these databases, global ex situ collections count a total of 9,938 accessions of the genera Citrus, Fortunella, Poncirus. However, in the citrus conservation strategy (see Chapter 2.1), 33 genebank reported through a survey a total of 15,555 citrus accessions. In contrast to citrus, the number of Malus accessions is higher, with 44,789 accessions, where 28,250 accessions are attributed to the species Malus domestica. Both apples and citrus crops are listed in Annex I of the Plant Treaty (FAO

 Table 2. Citrus crops, corresponding genus, species, FAO stat category and origin

Crop	Genus	Species	FAO stat category	Origin
Oranges	Citrus	Citrus sinensis, Citrus xsinensis	Oranges and mandarines	East Asia
Mandarines	Citrus	Citrus reticulata	Oranges and mandarines	East Asia
Grapefruits	Citrus, Fortunella, Poncirus	Citrus paradisi, Citrus xparadisi	Grapefruit	East and South-East Asia
Lemons and limes	Citrus	Citrus limon, Citrus aurantiifolia, Citrus xlimon	Lemons and limes	East Asia, South Asia
Citron	Citrus	Citrus medica	Citrus, Other	South Asia
Chinotto	Citrus	Citrus myrtifolia	Citrus, Other	South and East Mediterranean, South Eastern and South Western Europe
Kumquat	Citrus	Citrus japonica (Fortunella japonica)	Citrus, Other	South, East and South-East Asia

2009). Only 12 % of the citrus crop accessions by genus and 17% of citrus accessions by species are available under the MLS, as stated directly in databases; in contrast, 42% (by genus) and 57% (by species) of apple accessions, are available under the MLS. However, if counting accessions held in Party countries, depending on the genus and species, from 87 (grapefruit, citron and lemons and lime) to 100% (kumquat) of citrus accessions are available in the MLS, in contrast to 84 and 91% of apple accessions with respect to the apple genus *Malus* and the species *Malus domestica*, respectively. SECURITY of germplasm conservation is represented here by one metric: safety duplication at the Svalbard Global Seed Vault (SGSV). The numbers of accessions, by genus and species, safety duplicated were taken from the SGSV website (seedvault.nordgen.org) and divided by the total number of accessions stored in global *ex situ* collections (see above), with the result giving the percentage of germplasm that is safety duplicated. No citrus or apple accessions are safety duplicated at SGSV, because seed is not the usual mode of conservation for these crops.

Table 3. Table of indicator values for species within citrus crops

Metric	Oranges	Mandarins	Grape- fruits	Lemons and limes	Citron	Chinotto	Kumquat
Crop use							
Food supply (Amount consumed) [Kg/capita/ year]	11.7	1.1	1.9	1.56			
Percentage of countries consuming (being supplied with) crop *	99%	87%	86%	79%			
Quantity exported globally [t]	17,879,864	1,363,326	3,446,992	-			
Number of publications between 2009-2019, including patents and citations, searching title of publication (Google scholar search hits) for genus **	22,100	151					
Number of publications between 2009-2019, including patents and citations, searching title of publication (Google scholar search hits) for species ***	2,170	1,070	352	699	247	12	19
Demand							
Accessions distributed from gene banks (Annual average 2014-2019) FAO-WIEWS	14792	2993	0	0	0	0	17
Variety registered (UPOV) in 5 years (2014- 2018)	7	23	0.25	7.5	0	0	
Supply							
Number of accessions in ex situ collections of genus **	5,044	4,844	50				
Number of accessions in ex situ collections of species ***	1,305	403	193	539	126	13	6
Accessions of the genus ** available through Multilateral System (MLS) directly noted in databases	590	569	6				
Accessions of the species *** available through Multilateral System (MLS) directly noted in databases	217	64	29	112	7	2	1
Accessions of the genus ** available through Multilateral System (MLS) indirectly by matching institute countries with party status [%]	86%						
Accessions of the species *** available through Multilateral System (MLS) indirectly by matching institute countries with party status [%]	99%	87%	100%				
Security							
Accessions of genus ** safety duplicated in Svalbard Global Seed Vault [%]	0%	0%	0%				
Accessions of species *** safety duplicated in Svalbard Global Seed Vault [%]	0%	0%	0%	0%			0%

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