CryoLetters 47(1), 1-15 (2026)

https://doi.org/10.54680/fr26110110112

PERSPECTIVE

# INDIAN CITRUS GERMPLASM IN THE FROZEN STATE: CONSERVING EXCEPTIONAL GENETIC DIVERSITY FOR POSTERITY, UTILIZATION AND FOOD SECURITY

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## Abstract

India has a rich genetic diversity of citrus, both cultivated and wild types, particularly in the northeastern and northwestern regions. Managing genetic resources of this enormous diversity is a challenging task. Citrus species show non-orthodox seed storage behavior and, therefore, are not easily conserved using conventional -20°C storage methods. Cryopreservation is the only feasible long-term ex situ conservation strategy of a diverse genus like citrus. This strategy not only protects valuable genetic material against threats such as diseases, climate change, and habitat loss, but also facilitates future research and breeding efforts to develop resilient and high-yielding citrus varieties. Although germplasm collection and conservation of citrus began >100 years ago, the long-term ex situ conservation of citrus genetic resources accelerated from the 1990s with the establishment of the cryogenebank at ICAR-National Bureau of Plant Genetic Resources, New Delhi, India. During the last three decades, germplasm of citrus has been collected from various parts of the country and protocols to cryopreserve seeds, embryos, embryonic axes and pollen have been successfully developed. The base collection of citrus germplasm at the National Cryogenebank has cryopreserved embryos and embryonic axes through different techniques, namely desiccation-fast freezing, encapsulation-dehydration, vitrification, or encapsulation-vitrification. Basic studies on seed physiology and storage behavior of diverse citrus species have been conducted, resulting in the successful development of cryopreservation protocols. These advancements have facilitated the cryobanking of over 1,245 accessions from 39 species of *Citrus* and of the related genus *Poncirus* in the National Cryogenebank.

**Keywords:** cryobiotechnology; cryogenebank; cryopreservation; cryoprotocol; non-orthodox seeds; vitrification.

## INTRODUCTION

Climate change has both direct and indirect impacts on agricultural production systems. Direct impacts include modification of physical characteristics, such as temperature and rainfall distribution, on specific agricultural production systems. Indirect effects are those that affect production through changes on other species such as pollinators, pests, disease vectors and invasive species. These indirect effects, which can play a major role in the loss of plant genetic resources, are much more difficult to assess and many are still unknown (1). Agriculture contributes about 18.8% to India’s gross value added (GVA) and is the largest employer of the workforce (2). Agriculture plays a central role in increasing food availability and incomes, supporting livelihoods and contributing to the overall economy (3), and it is a key factor to improve food and nutrition security. According to the World Bank's 2024 report (3), agricultural, cereal, and export price indices have each declined by 8%, 10%, and 9% respectively, resulting in significant economic losses for farmers. Urgent action is needed to reorient the long-term direction of agri-food systems. This reorientation should aim not only to enhance farm incomes but also to ensure better access to safe and nutritious foods. To enhance the country’s currency value, cultivating and conserving fruit crops presents a profitable opportunity. Fruits not only contribute to improved nutrition and income but also play a vital role in global food security. India boasts a rich diversity in fruit crops, which are abundant sources of vitamins, fiber, and essential antioxidants.

Citrus is an important fruit crop grown in the tropical and sub-tropical parts of the world. This economically valuable genus belongs to the family Rutaceae (sub-family- Aurantioideae/ Citroideae). The Indian sub-continent is recognized as the home to citrus and this fruit is an important part of day-to-day life of people as fruit, medicine, and has socio-religious significance since ancient times. The most ancient literature that mentions citrus, in any form, is Shukla Yajurveda. a Sanskrit devotional text older than 800 B.C. Specifically, Vajasaneyi Samhita, which is the part of the Yajurveda sacred book, includes the word Jambhila. The word ‘Jambhila’ resembles citron and lemon (4). Citrus has also been mentioned by different names in ancient Ayurvedic texts, namely, Dhanwantri (10th B.C.), Charak Samhita (1st century B.C.) and Sushruta Samhita (2nd century B.C.) (5). Therefore, citrus fruits have special significance in the Indian context and need attention for diversity management.

According to FAO, in 2021 citrus was the second most produced fruit crop in the world, with a production of 161.8 million tons on 10.2 million hectares (6). India's diverse climate makes it a great source of fruit crop biodiversity. After bananas and mangos, citrus is the third most important and extensively farmed crop in India. In India, citrus genetic resources are facing significant challenges, primarily due to climate change, habitat degradation, and spread of plant diseases. The northeastern and northwestern regions of India, known as centers of citrus diversity, are experiencing rapid habitat loss due to deforestation and urbanization. Similarly, in Uttarakhand, warming temperatures have led to a sharp decline in the yield of temperate fruits, including citrus varieties. According to IUCN norms, various citrus species such as *C. indica, C. macroptera, C. latipes, C. megaloxycarpa, C. assamensis, C. ichangensis* and *C. rugulosa* are at risk of extinction and need to be conserved urgently (5, 7). All these species are a potential source for improvement of biotic and abiotic stress tolerance in commercial citrus cultivars. Other than these, there are some intermediate seed storage behavior types likely that are not yet fully explored, mostly in species of the undisturbed area of the NE region. For in situ conservation of these wild species, India has set up a citrus gene pool sanctury at Nokrek Meghalaya (8). In India, citrus germplasm is also being maintained and conserved in field genebanks, established in various parts of the country. There is a total of 20 field genebanks in India, which hold about 1,500 indigenous and exotic collections (7). Of these the ICAR-Central Citrus Research Institute (CCRI), Nagpur sustains a maximum of 614 citrus accessions in its National Citrus Repository and Field Genebank. Exotic and indigenous species and rootstocks, such as *Poncirus trifoliata* and *Severinia* species are also maintained there (9). Other major centers that maintain citrus germplasm include: the ICAR-Indian Institute of Horticultural Research (IIHR) in Bangalore, Karnataka; the Central Horticultural Experiment Station in Chethali, Karnataka; the PAU-

Regional Research Station in Abohar, Punjab; the MPKV-Department of Horticulture in Rahuri, Maharashtra; the Citrus Experiment Station in Kalol, Maharashtra; the Citrus Research Station in Tirupati, Andhra Pradesh; the Horticultural Experiment Station in Periyakulam, Tamil Nadu; the Citrus Experiment Station in Tinsukia, Assam; and the ICAR-NBPGR Regional Stations in Bhowali and Shillong (7). The plants conserved in field genebanks and greenhouses are at higher risk of various biotic and abiotic stresses. Consequently, they require high maintenance due to regular intercultural operations, making the process cost intensive. Citrus, being a hardy crop with a long juvenile phase, is difficult to maintain in field genebanks as during the establishment period the plant goes through various growth stages that are vulnerable to disease and pest infestation. The routine maintenance of trees in the field genebank is more expensive than any other conservation method (10).

Storage of seeds/zygotic embryos/ embryonic axes in genebanks and cryogenebanks has been the most preferred method for genetic conservation because the material is easy to handle and is accessibility. Moreover, the presence of a high degree of polyembryony in many citrus species provides the opportunity to conserve the original genotype as seed despite high levels of heterozygosity in them. Leaving aside the commercial cultivars or clones of citrus, most of the wild species and rootstocks are seed propagated. Also, there are no reports of seed transmitted viruses in citrus, which gives an extra impetus for conserving citrus germplasm in the form of seeds. Seeds of many citrus species show non-orthodox storage behavior and, therefore, do not preserve well under conventional -20°C storage conditions (11). In such a case, cryopreservation is the only alternative for the long-term conservation of such species. It has been extensively attempted using seeds and a wide range of other explants such as zygotic embryos, embryonic axes, shoot apices, embryogenic callus, cell suspensions and somatic embryos. Citrus cryopreservation began in the late 1980s and early 1990s, with pivotal work by Sakai and co-workers who first applied vitrification to preserve nucellar cells of navel orange (*C. sinensis*) (12). Throughout the 1990s, researchers extended these techniques to embryonic axes (e.g., *C. madurensis*) via vitrification protocols combining preculture, loading, PVS2 treatment, and liquid nitrogen immersion, achieving survival rates up to 82.5% (13). Gonzalez-Arnao et al. (14, 15) pioneered encapsulation-dehydration protocols to cryopreserve citrus shoot apices, ovules and somatic embryos. The first successful cryopreservation of commercial citrus shoot tips came in 2004, when a vitrification method enhanced with glutathione enabled shoot-tip survival across multiple cultivars (16). Building on this, the USDA-ARS and UC Riverside developed PVS2 vitrification combined with micrografting, achieving average regrowth rates of ~50–60% for diverse citrus germplasm (17).

Confirming the seed storage behaviour of citrus species is essential for handling seeds and embryonic axes. Citrus species are reported to be mainly intermediate and recalcitrant, i.e., non-orthodox (18, 19). However, some studies showed that seeds of *C. aurantifolia* and *C. aurantium* were tolerant to desiccation and freezing (20). Also, seeds of several *Citrus* species, viz. *C. grandis* and *C. reticulata, C. sinensis, C. madurensis, C. limon, C. latipes, C. macroptera, C. indica* and *C. cavaleriei* were reported to have both intermediate and recalcitrant seed storage behaviour (7, 21, 22, 23, 24 ,25).

Cryostorage of citrus zygotic embryos has been shown to be a successful and reliable method for a diverse range of citrus species accessions, retaining ≥ 70% viability after 6 to 8 years and comparable to the original viability (21). Therefore, the ex situ conservation of embryos and embryonic axes in cryogenebanks can support the conservation of citrus genetic resources. Realizing the importance of ex situ conservation via cryopreservation, and after analyzing all the pros and cons of other options for citrus germplasm conservation, ICAR-NBPGR decided to establish a base collection of citrus germplasm as seeds, embryos, embryonic axes and pollen at the National cryogenebank in New Delhi.

## COLLECTION MANAGEMENT

### *Collection of germplasm*

The mission programme to collect, characterize and conserve citrus germplasm of India from its various parts was initiated in 1997. Exploration and collection trips were accordingly planned to citrus growing areas especially in the east, northeast, northwest, south

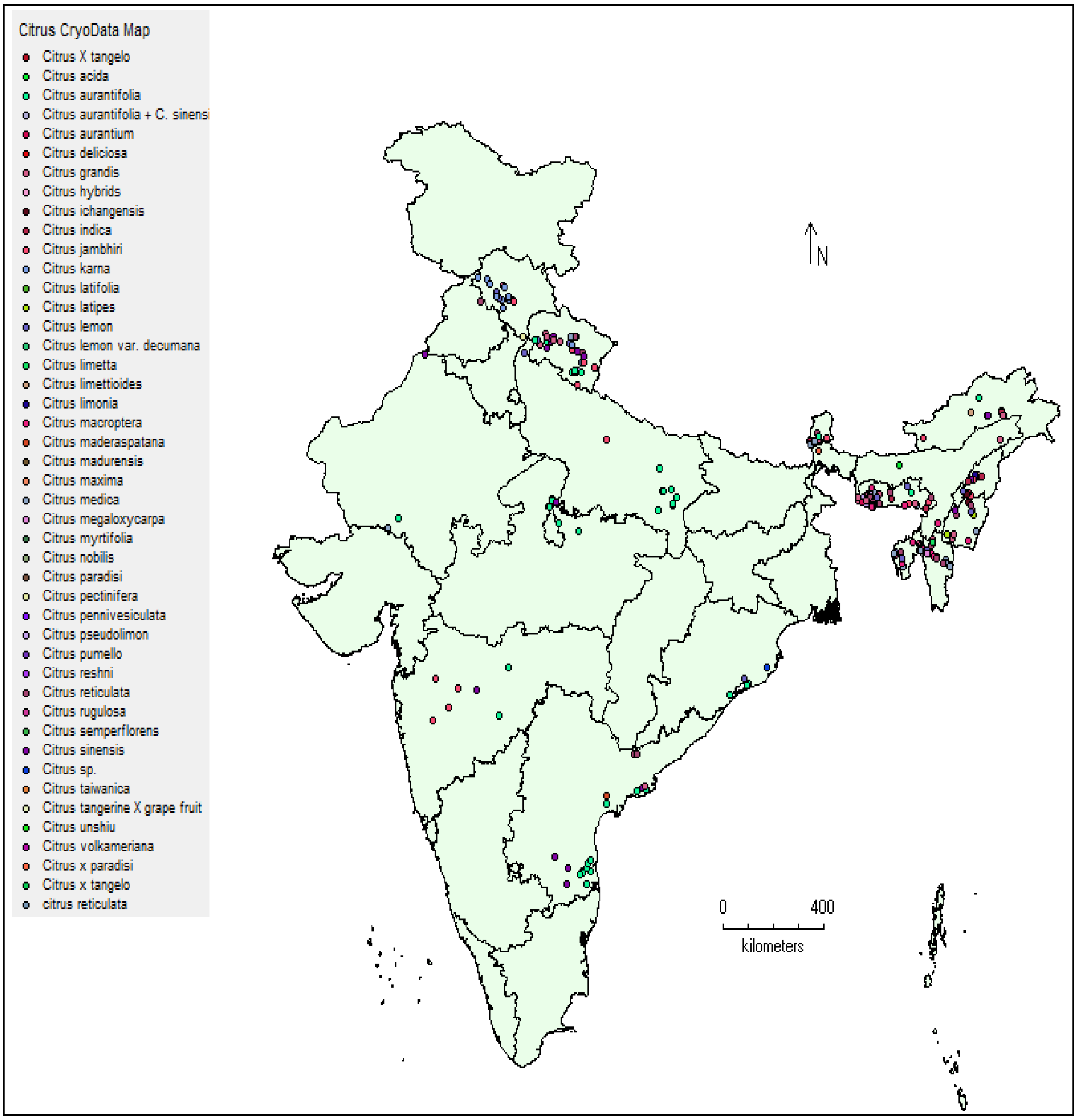


**Figure 1.** Map of India showing collection sites of citrus species for exploration and conservation.

and central parts of India. During each year, two or three explorations of 10-15 days were planned based on reported citrus diversity in these areas. During the last 20 years, about 30 explorations were undertaken in the different parts of the country and about 1,750 accessions were collected (Fig. 1). Major emphasis was given on diversity-rich areas of northeast India comprising eight States and from these areas the most accessions were collected (Fig. 1). Surveys and collections were made from a variety of sources: natural wild, semi-wild, backyards, farmers' fields, orchards, field genebanks, botanical gardens, etc. For all the collections, passport data information, as designed by ICAR-NBPGR, was collected and indigenous collection (IC) numbers were allotted to the collected germplasm. Most of the explorations were planned to coincide with fruiting season. Preferably whole fruits or freshly extracted seeds were collected and brought to the laboratory in New Delhi for further experimentation.

***Seed storage behaviour and cryobanking***

Basic studies on seed morphology, seed moisture, seed germination and desiccation-freezing tolerance were undertaken. The most suitable explant to be cryopreserved was based on seed morphological and seed physiological parameters. In most cases, cryopreservation of zygotic embryos was preferred. However, in the case of large seeded species, embryonic axes were cryopreserved. Suitable protocols were developed for zygotic embryos and excised embryonic axes for cryopreservation of specific species and an optimized protocol was followed for further cryogenebanking (7, 21, 22, 23, 24, 26, 27). Embryos, embryonic axes and pollen were cryopreserved in cryovials (1, 2 or 5 mL) as per the size and quantity of explants. Cryovials were manually labeled using cryomarkers to include crop name, Indigenous or Exotic Collection Number (IC/EC number) or collector number, date of cryostorage and, most importantly, the location in the cryotank. Cryovials were loaded on cryo-canes and placed in the cryo-canisters. These cryo-canisters were finally positioned at the designated place in the cryotanks. Cryotanks make MVE model XLC1830 of 1000 L capacity were used for long-term cryogenebanking of samples in the vapour phase (-170-180°C). Standard procedure was followed for cryogenebanking and maintenance of cryopreserved germplasm in the cryotanks (Table 1) (28).

***Retesting of cryostored germplasm***

Cryopreserved embryos, embryonic axes and pollen were randomly retested for viability after 8-10 years of cryostorage. Viability was tested following standard recovery methods optimized for the specific explant (7, 21,28).

## CURRENT HOLDINGS OF GERMPLASM

***Evolution of the collection***

Cryopreservation activities at ICAR-NBPGR started in 1986 at a pilot scale only. At NBPGR, cryogenebanking using extra-large capacity cryotanks was started in 1996. The initial focus was on the optimization of cryo-conservation protocols for non-orthodox seed species, including citrus species. Most of the partially desiccated embryos/ embryonic axes of citrus species survived well after cryopreservation, retaining original germinability (%). This high recovery of genetically stable plantlets with normal growth prompted the establishment of a citrus base collection in the National Cryogenebank at ICAR-NBPGR, New Delhi, India. The first sample of citrus zygotic embryo was cryopreserved in 1991. However, a systematic mission mode collection and cryobanking

**Table 1**. Cryopreservation studies on citrus species conducted at ICAR-NBPGR, New Delhi.

| Species | Explant | Cryopreservation method / survival / regeneration response | Reference |
|---|---|---|---|
| *C. indica* Tanaka | Embryonic axes (EA) | Air-desiccation freezing / 90% / 64%<br>Encapsulation-dehydration / 85% / 55%<br>Vitrification / 80% / 52% | (27) |
| *C. macroptera* Mont. | Embryonic axes | Air-desiccation freezing / 87%<br>Encapsulation-dehydration / 62%<br>Vitrification / 92% | (26) |
| *C. latipes* Tanaka | Embryonic axes | Air-desiccation freezing / 64%<br>Encapsulation-dehydration / 45%<br>Vitrification / 80% / 77% | (26) |
| *Citrus jambhiri* | Embryo and axes | Air-desiccation freezing / 70% (Embryo), EA: 63% | (21) |
| | Embryonic axes | Air-desiccation freezing / 40-50%<br>Encapsulation-dehydration / 30-60%<br>Vitrification / 25% | (29) |
| *C. grandis* | Embryo and axes | Air-desiccation freezing / ~ 75% | (21) |
| *C. reticulata* | Embryo | Air-desiccation freezing / ~ 75% | (21) |
| *C. medica* | Embryo | Air-desiccation freezing / > 75% | (21) |
| *C. aurantifolia* | Embryo | Air-desiccation freezing / > 65% | (21) |
| *C. cavaleriei* H. Lév. ex Cavalerie | Embryo and axes | Air-desiccation freezing / ~50%<br>Encapsulation-dehydration / 40%<br>Vitrification / 40% | (23) |

programme for Indian citrus genetic resources was launched in 2001. Since then, more than 1,500 citrus collections have been made mostly through crop specific targeted explorations, and procurement from the field genebanks of various organizations.

To date, ICAR-NBPGR has cryobanked a total of 1,245 citrus accessions that were collected from 21 states encompassing most of the regions of India, including North Eastern India (786 accessions), North West India (292 accessions), Central India (70 accessions), South India (82 accessions) and East India (15 accessions) (Fig. 2). These 1,245 accessions belong to 36 species, hybrids and indigenous cultivars that were cryobanked in the form of

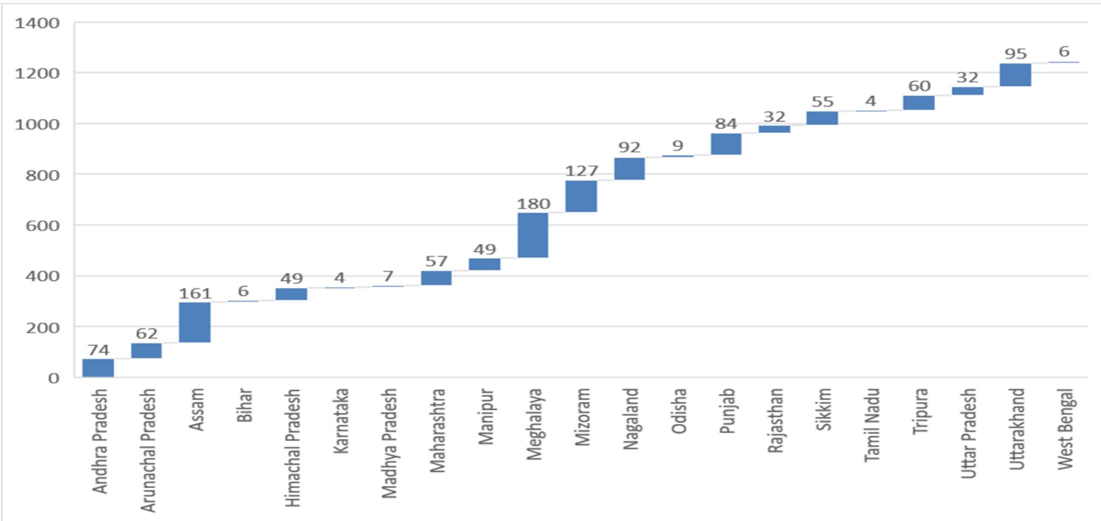


**Figure 2.** Geographic distribution by state of citrus germplasm conserved at the National Cryogenebank. Value over bar represent number of cryo-conserved accessions.

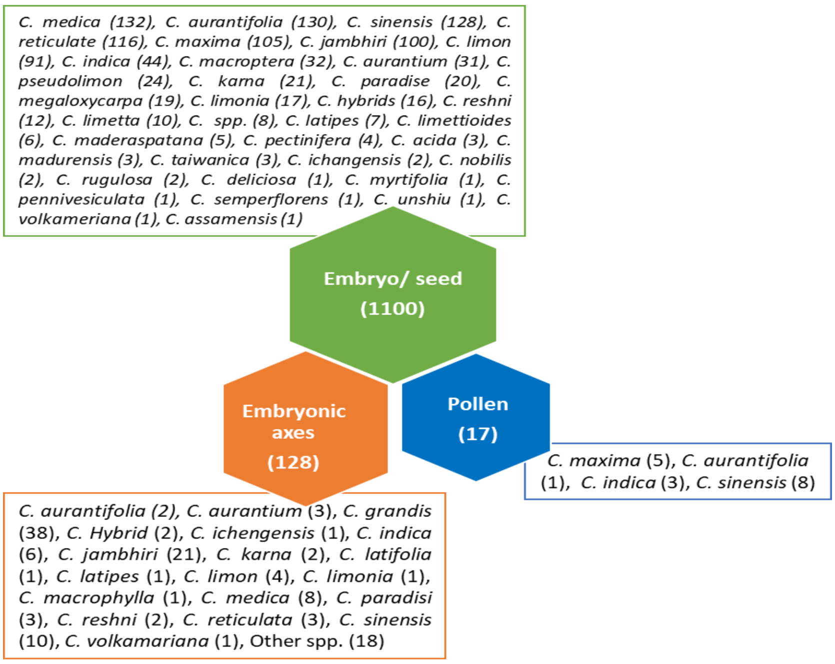


**Figure 3.** Conservation forms of Indian *Citrus* germplasm at the National Cryogenebank. Status of germplasm in Cryogenebank. Value in parentheses is number of accessions.

seeds, zygotic embryos, embryonic axes and pollen (Fig. 3). Here we provide a detailed analysis and value of this diverse inimitable citrus collection. The USDA-ARS National Plant Germplasm System (NPGS) also makes similar efforts to conserve and manage a collection of 1,522 citrus genetic accessions, including various cultivars and crop wild relatives, in field genebanks. Of these, 540 accessions are preserved as pathogen-free duplicate clones in a screenhouse at the National Clonal Germplasm Repository for Citrus and Dates (NCGRCD) in Riverside, California, with additional backup through cryopreservation methods (30).

***Explants cryopreserved***

As citrus produces non-orthodox seeds, the preferred explants are zygotic embryos, embryonic axes and pollen. Eleven hundred accessions from 33 species have been cryostored in the form of seeds and embryos. In addition, 128 accessions of embryonic axes belonging to 18 species, mostly large seeded species including recalcitrant seed species *C. cavalarie*, have been cryostored. Pollen cryopreservation of 17 accessions has also been undertaken, including that of rare and endangered wild species *C. indica* and three other species (Fig. 3).

The citrus cryogenebank collection at ICAR-NBPGR totals 1,245 accessions belonging to diverse citrus groups. The number of accessions, species and extent of diversity that is cryopreserved in each group have been analyzed and described under the following sections (Fig. 4).

***Taxonomic grouping of conserved germplasm***

1. Acid Group

*Citrus* species of this group are highly important as far as economic importance and genetic diversity in India. Overall, 577 accessions of the Acid Group, from 14 species, have been cryobanked (Fig. 4). Major collections belong to citron (*C. medica*), lemon (*C. limon*), hill lemon (*C. pseudolimon*), rough lemon (*C. jambhiri*), karna khatta (*C. karna*), sour lime (*C. aurantifolia*), rangpur lime (*C. limonia*) and sweet lime (*C. limetta* and *C. limmettoides*).

<u>Citron (*C. medica*).</u> Citron is unique, historical and a highly diverse species in India with much indigenous socio-economic and religious importance (31). It has been used for centuries in medicine for its antioxidant, anti-inflammatory, antimicrobial, antiviral, and anti-hyperglycemic properties. Some of the important natural variants / cultivars of citron have either become extinct or are at the verge of extinction due to various reasons. Therefore, urgent conservation efforts are required both for in situ and ex situ conservation. One hundred and forty diverse accessions of *C. medica* from 12 states of India have been cryobanked. However, a major of the collection (i.e. 125 accessions) is from the northeast Indian states namely Arunachal Pradesh (n=7), Assam (n=36), Manipur (n=18), Meghalaya (n=8), Mizoram (n=25), Nagaland (n=16), Sikkim (n=5) and Tripura (n=7) while 16 accessions are from other states [Uttarakhand (n=7), Karnataka (n=3), Punjab (n=4) and Tamil Nadu].

<u>Sour lime (*C. aurantifolia*)</u>. Sour lime or acid lime is a very important crop for India with vast variability and wide cultivation in almost all the states. It is a highly cross-pollinated crop and therefore, varied phenotypes have been reported. There are various cultivars � such as Kagzi, Akale lime, Pramalini, Chakradhar, Vikram, Phule Sharbati, Sai Sarbati, PKM-1, Tenali, Balaji, Arunachal lime, Atol etc. � from Maharashtra, Uttarakhand, Andhra Pradesh, Punjab, Arunachal Pradesh, Meghalaya and other states. A total of 132 accessions of *C. aurantifolia* are conserved in the cryogenebank. These accessions belong to 18 states of India namely Andhra Pradesh (n=43), Arunachal Pradesh (n=5), Assam (n=3), Bihar (n=2), Himachal Pradesh (n=4), Madhya Pradesh (n=2), Maharashtra (n=17), Meghalaya (n=7), Mizoram (n=2), Nagaland (n=1), Odisha (n=5), Punjab (n=5), Rajasthan (n=2), Sikkim (n=1), Tamil Nadu (n=2), Tripura (n=2), Uttar Pradesh (n=18), and Uttarakhand (n=11).

<u>Rough lemon (*C. jambhiri*)</u>. Rough lemon is one of the important rootstock species used by the citrus breeders for crop improvement programmes in India. It is highly vigorous, early maturing, provides good yield, has a well-developed and strong root system and is tolerant to drought, exocortis viroid and tristeza virus. It is distributed mainly in the North eastern region of India and also in some parts of Uttarakhand. Vast variability has been reported in the rough lemon accessions collected from the various parts of India (32). Important cultivars cryopreserved are Jamir, Naity Jamir, Jatti Khatti, Jullandhuri Khatti, Gole Nimbu, Soh-myndong, Soh-Jhalia, Esteus Rough, etc. A total

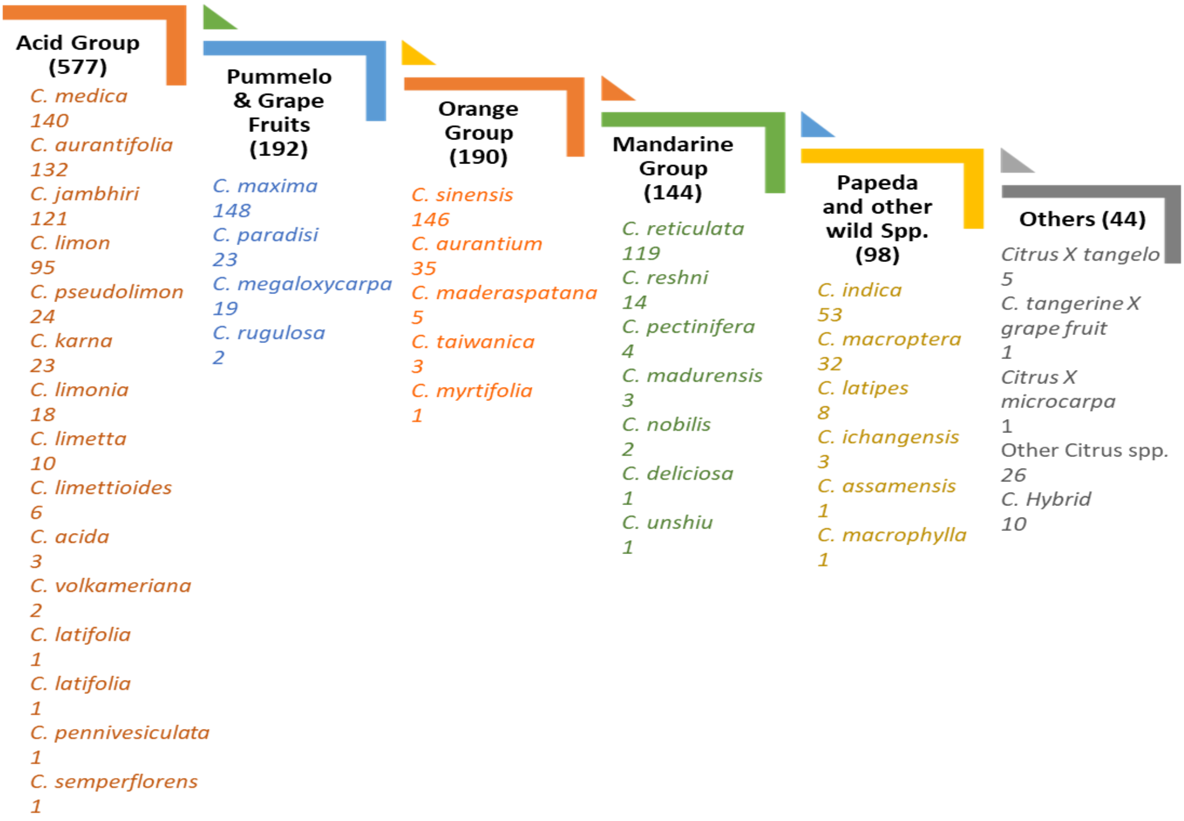


**Figure 4.** Taxonomic group classification of citrus germplasm conserved in the National Cryogenebank. Value in parentheses is number of accessions.

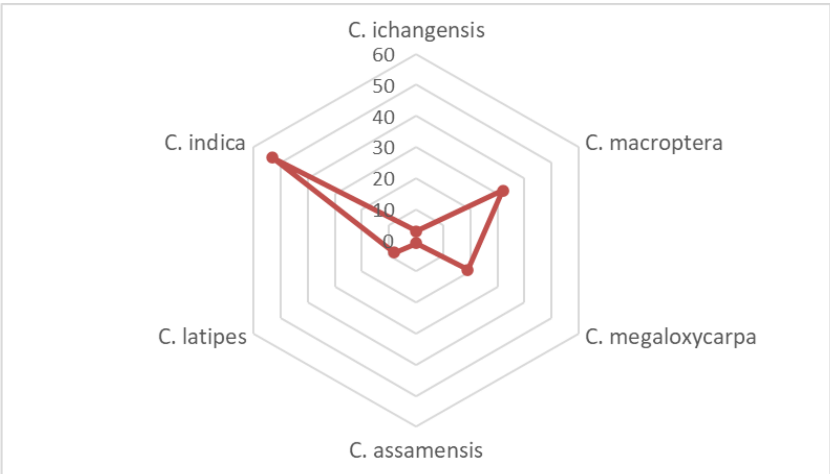


**Figure 5.** Citrus wild species germplasm conserved in the National Cryogenebank.

of 121 accessions of *C. jambhiri* are conserved in the cryogenebank. These accessions belong to 17 states namely Andhra Pradesh (n=1), Arunachal Pradesh (n=8), Assam (n=20), Himachal Pradesh (n=10), Madhya Pradesh (n=2), Maharashtra (n=5), Manipur (n=1), Meghalaya (n=15), Mizoram (n=6), Nagaland (n=9), Punjab (n=9), Rajasthan (n=2), Sikkim (n=13), Tripura (n=3), Uttar Pradesh (n=2), Uttarakhand (n=14) and West Bengal (n=1).

*Lemon and hill lemon (C. limon and C. pseudolimon).* *C. limon* Burm. is the most popular lemon to grow due to its wide adaptability, frost resistant nature and high content of phenols, flavonoids (hesperidin, limocitrin and diosmin) and essential oils. Lemons are an important source of vitamin C, citric acid, iron and phosphorous. Lemon is grown throughout the country and vast diversity is reported (33). Important types are Assam lemon, Khasi lemon, Nimbu, Baramasi lemon, Bhadri lemon, Kagzikalan, Sher-phang, etc. In the hilly regions of Himachal Pradesh and Uttarakhand popular lemon is hill lemon or galgal described as *C. pesudolimon*. Similarly in northeast India, *C. pseudolimon* is known as Chinara. Lemons are very popular and consumed raw and pickled. Due to localized importance of lemon cultivars in various regions and high market demand, it is important to protect and conserve the existing diversity. There is a total of 95 accessions of *C. limon* and 24 accessions of *C. pseudolimon* cryopreserved from almost all the citrus growing states. Cryopreserved accessions represent 17 states of India namely Arunachal Pradesh (n=4), Assam (n=8), Bihar (n=4), Himachal Pradesh (n=16), Madhya Pradesh (n=1), Manipur (n=2), Meghalaya (n=12), Mizoram (n=11), Nagaland (n=6), Odisha (n=1), Punjab (n=8), Rajasthan (n=2), Sikkim (n=1), Tripura (n=8), UP (n=3), Uttarakhand (n=7), West Bengal (n=1). *C. pseudolimon* accessions mainly belong to Meghalaya, Uttarakhand and Himachal Pradesh.

In the Acid Group, other citrus species cryopreserved are *C. karna* (23 accessions), *C. limonia* (18 accessions), *C. limetta* (10 accessions), *C. limettoides* (6 accessions), *C. volkameriana* (2 accessions) and *C. pennivesiculata* (1 accession).

2. Orange Group

The Orange Group is another commercially important citrus group and sweet orange (*C. sinensis*) is the dominating species in India. Large scale cultivation of sweet orange is undertaken in Andhra Pradesh, Telangana, Maharashtra, Punjab, Madhya Pradesh, Karnataka, Assam, and other northeast States. Important sweet orange cultivars being grown in these states are satgudi, mosambi, nucellar, phule mosmabi, sonamitri, Malta, mitha chakola, chakola tenga, tasi, jaffa, blood red, Valancia, etc. There are total of 190 accessions successfully cryopreserved at the National cryogenebank. The group includes 146 accessions of *C. sinensis* belonging to 14 states of India namely Maharashtra (n=25), Punjab (n=22), Assam (n=20), Uttarakhand (n=18) Andhra Pradesh (n=9), Arunachal Pradesh (n=9), Rajasthan (n=8), Manipur (n=4), Meghalaya (n=5), Nagaland (n=4), Mizoram (n=9), Uttar Pradesh (n=4), Sikkim (n=3) and Tripura (n=3). *C. sinensis* varieties such as blood orange, mosambi, sathgudi, are generally known for their high juice content and high total soluble solids (TSS): acid ratio which is a valuable trait for improving the commercial importance of the crop.

<u>*Sour orange*</u>. Another important species in the Orange Group includes sour orange (*C. aurantium*) which is also known for its medicinal and anti-inflammatory properties due to the presence of flavonoids. A recent phylogenomic classification suggested a new taxonomic name for the sweet orange, *C.* × *aurantium* var. sinensis L., based on fresh data from a whole-genome analysis. This is because both the sour orange and the sweet orange are derived taxa that share the same ancestors. A total of 35 accessions of *C. aurantium* have been cryobanked successfully. Important local cultivars of sour orange cryopreserved are Karun jamir, serenga, kampil, chinotto and bampsim and exotic cultivar smooth flat Seville. These 35 accessions belong to nine states namely Andhra Pradesh (n=4), Assam (n=1), Karnataka (n=1), Meghalaya (n=14), Nagaland (n=6), Punjab (n=2), Rajasthan (n=2), Sikkim (n=4) and Tripura (n=1). In addition to the above mentioned, three more species have been cryobanked: *C. taiwanica*, *C. myrtifolia* (originated as a mutant from sour orange) and *C. maderaspatana*. There three are not very commonly grown by Indian farmers but can be used in research programme in quality enhancement and stress tolerance.

3. Mandarin Group

In India, the major share of grown citrus fruits belongs to Mandarin Group. In 2021-2022, the production of mandarins was 6.265 million tons in India. The important species include *C. reticulata, C. nobilis, C. reshni, C. deliciosa, C. unshiu, C. pectinifera* and *C. madurensis*. Mandarins generally require high humidity and rainfall (34) due to which they are mostly distributed in North Eastern region of India and also in some parts of Maharashtra, Punjab, Haryana, Rajasthan, Uttarakhand, Tamil Nadu, Karnataka and Andhra Pradesh. It is preferred as a table fruit owing to its easy peeling, juice quality attributes and high demand of juice. Consequently, the germplasm of various species and cultivars of mandarins needs to be conserved for quality enhancement and improvement programmes. There are 119 accessions of *C. reticulata* cryobanked. These belong to Khasi mandarin, Nagpur mandarin, Sikkim mandarin, Coorg mandarin, Darjeeling mandarin, Soh-niamtra, santara, orange east India, butwal, laddu and exotic cultivars/ hybrids namely kinnow mandarin, Fremont, ponkan, dancy, honey, wilking, feutrel early, king, afourer, fortune, pearl tangelo, minneola tangelo, dweet tangor, Orlando tangelo, Sampson tangelo, and Thornton tangelo. In *C. reshni* (Cleopatara mandarin), 14 accessions collected from various states have been cryopreserved. Other cryobanked species belonging to this group are *C. nobilis* (king mandarin) (2 accessions), *C. madurensis* (3 accessions), *C. pectinifera* (4 accessions) and one accession each in *C. deliciosa*, *C. unshiu*. The major areas covered for collection comes from 13 states of India namely Arunachal Pradesh (6 accessions), Assam (11 accessions), Maharashtra (n=6), Manipur (n=7), Meghalaya (n=14), Mizoram (n=19), Nagaland (n=12), Punjab (n=11), Sikkim (n=10), Tripura (n=7), Uttarakhand (n=11), Rajasthan (n=2) and West Bengal (n=1).

4. Pummelo and Grapefruits Group.

<u>*Pummelo (C. grandis or C. maxima)*</u>. This species is found growing wild in several states of India with maximum diversity in the northeastern states. Pummelo fruits have immense socio-economic and religious importance in some parts of India, especially in Bihar and eastern Uttar Pradesh. Its fruits are the largest among the commercially grown citrus in the world with a lot of diversity in the shape, size, colour and taste of fruit. In contrast, grapefruit is a natural cross between pummelo and orange and cultivated in the selected pockets in Punjab, Uttarakhand, and Northeast India. Modern pummelo varieties have evolved from their ancient ancestors primarily through centuries of selective breeding based on taste and appearance. However, the species found in the northeastern hill region of India remains wild and is not commercially utilized due to its bitter and sour flavor profile. Instead, these local cultivars are used by the local people for medicinal purposes. For this group, a total of 192 accessions have been cryo-conserved including 148 accessions of *C. maxima* (pummelo), 23 accessions of *C. paradisi* (grapefruit), 19 accessions of *C. megaloxycarpa* (sour pummelo) and two accessions of *C. rugulosa* locally known as attani. These cryopreserved accessions come from 10 states of India namely Arunachal Pradesh (n=6), Assam (n=27), Mizoram (n=20), Himanchal Pradesh (n=4), Manipur (n=6), Meghalaya (n=30), Punjab (n=3), Sikkim (n=13), Tripura (n=3) and Uttarakhand (n=16).

5. Citrus Wild Species Group

Crop wild relatives (CWR) play an essential role in crop improvement programmes due to their wider adaptation and beneficial traits, which are generally not present in commercial cultivars. Conservation of wild citrus species is challenging due to the limited understanding of the genetic and environmental factors contributing to their endangered status and risk of extinction. Some of the wild relatives of citrus today exist as only an individual tree (35). In order to conserve and maintain the rare, endangered and threatened germplasm, and for the sustainable utilization of this genetic reservoir of rare genes, it is necessary to ensure long-term conservation by cryobanking. Loss of citrus genetic diversity due to human interventions, climate change, deforestation, shifting cultivation and preference of farmers for growing more economically viable species are causing serious threats to this unique citrus diversity. Six wild citrus species - namely *C. indica, C. macroptera, C. latipes, C. assamensis, C. ichangensis* and *C. megaloxycarpa* - are rare and endangered. Besides, several local cultivars of cultivated species are endemic to northeast India. *C. indica* and *C. latipes* offer resistance to both environmental and biological challenges commonly encountered by cultivated citrus cultivars (36). Conservation of some of the wild accessions also provides a missing link in citrus phylogeny. Genetic diversity of these species has been collected from the natural populations by conducting various exploration and collection programmes in different regions of India, especially in the northeast region. From there, 53 accessions of *C. indica*, 32 of *C. macroptera*, eight of *C. latipes*, three of *C. ichangensis* and one accession of *C. assamensis* have been collected and cryopreserved (Fig. 5).

<u>*C. indica*</u>. This species is found naturally growing as wild in the Garo Hills of Meghalaya (14). Recent reports on the presence of *C. indica* in the East Siang and Upper Siang valleys of Arunachal Pradesh and Dailong village in Manipur’s Tamenglong district are exciting. This clearly indicates the need for more extensive surveys and explorations in northeast

India for indigenous plant species. The discovery of new locations of these species not only enhances access to existing diversity and new populations with inherent variations due to genetic and edapho-climatic factors but also provides very interesting information on diverse and localized socio-economic, cultural and religious use of these wild species in different states of north-east India. Maximum diversity was found in two populations namely Daribokge in East Garo Hills and Rongwak in South Garo Hills. Only a limited number of plants are observed at locations such as Chandgre, Mandalgre, Sasatgre, Nokrek Peak, Dura Kalakgre and Rengsangre in and around the Nokrek Biosphere reserve (7). An extensive and targeted collection programme by ICAR-NBPGR to collect this rare species has led to collecting and cryopreservation of 53 accessions of *C. indica*, of which six are from Arunachal Pradesh, 32 from Meghalaya, and 13 from Nagaland, Assam and Manipur.

*C. macroptera*. This species is commercially cultivated at a very small scale by the local people of Mizoram and Meghalaya and locally it is commonly called as Satkara or Hatkara (7). In northeast India, local communities use the fruit as folk medicine for the treatment of gastritis, kidney stone and diabetes (7). This species is widely cultivated now in almost all the states of northeast India. Targeted exploration and field trips for this important species have led to the collection and cryobanking of 32 accessions of *C. macroptera* in the cryogenebank. These accessions are from Meghalaya (n=7), Mizoram (n=8), Manipur (n=2), Assam (n=2), and 10 accessions from Tripura.

*C. latipes (khasi papeda)*. This species is mostly reported from Khasi hills of Meghalaya and is found in a semi-domesticated state. Because of its tolerance to harsh and cold environments, this wild species provides vital genetic components that may help cultivars become more stress-tolerant. It is highly tolerant and imparts tolerance against Huanglongbing (HLB) bacterial disease (36). Despite being a potential rootstock, its availability is very limited. Currently, eight accessions of *C. latipes* have been collected and cryopreserved, which could be used in future breeding programmes.

*C. ichangensis (Ichang Papeda)*. This species was previously known as *C. cavaleriei* H. Lev. *ex Cavalerie*. It is also a wild and endangered species of NE India that requires urgent preservation of its genetic resources (23). It is only grown in the forest near Khonoma village in Naga Hills and some other parts of Nagaland. Fruits are inedible due to their scanty pulp and the very large number of seeds (7). Another very less well known, underutilized and endangered species is *C. assamensis*, also called Adajamir. It is a rich source of nutrition and has increased antioxidant properties (37)

***Status of Citrus germplasm from Northeastern (NE) India.***

The northeastern (NE) region of India comprising Assam, Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Nagaland, Tripura, and Sikkim, is known as the home and reservoir of various cultivated and wild citrus species (38, 39, 40). This area is renowned for its richness in citrus germplasm, with reports indicating the origin of 17 citrus species, 52 cultivars, and seven probable natural hybrids. Therefore, this area has been recognized as a treasure trove of citrus genetic resources (5, 31).

In this region, many citrus species originated and later dispersed to various parts of the world, leading to significant advancements in the citrus industry. Six wild citrus species (*C. indica, C. macroptera, C. latipes, C. assamensis*, *C. ichangensis*, and *C. megaloxycarpa*) are considered rare and endangered. Besides, several local cultivars of cultivated species are endemic to this area. The unique combination of diverse soil types, physiographic features, and climatic conditions in this region foster extensive variability among citrus species (41).

Citrus genetic diversity collecting from northeastern part of India was taken up intensively by Tanaka, Bhattacharya and Dutta (42, 43, 44) between the 3rd and 5th decades of last century. During this period, most of the new citrus species were identified, collected and documented from this region. Since then, no significant information has been added to our knowledge about the occurrence of additional citrus species from this part of India. However, extensive recent surveys and explorations during the last 2-3 decades by ICAR-NBPGR enumerated the loss of some species / cultivars from various pockets where they were earlier reported to occur.

Also, several new local types and new locations of already existing species and unknown citrus types were reported from northeast India (21, 22, 26, 45).

ICAR-NBPGR, in collaboration with ICAR-Central Institute of Citrus Research (earlier National Research Centre for Citrus), has taken up a comprehensive programme on the collection and conservation of Indian citrus germplasm. During last several years, systematic explorations have been undertaken to collect, characterize and conserve the citrus genetic diversity from different states of north-east India. This structured programme on revisiting the citrus genetic resources of India, especially northeast India, is now building on the pioneering work and monograph published nearly seven decades ago (44). During these

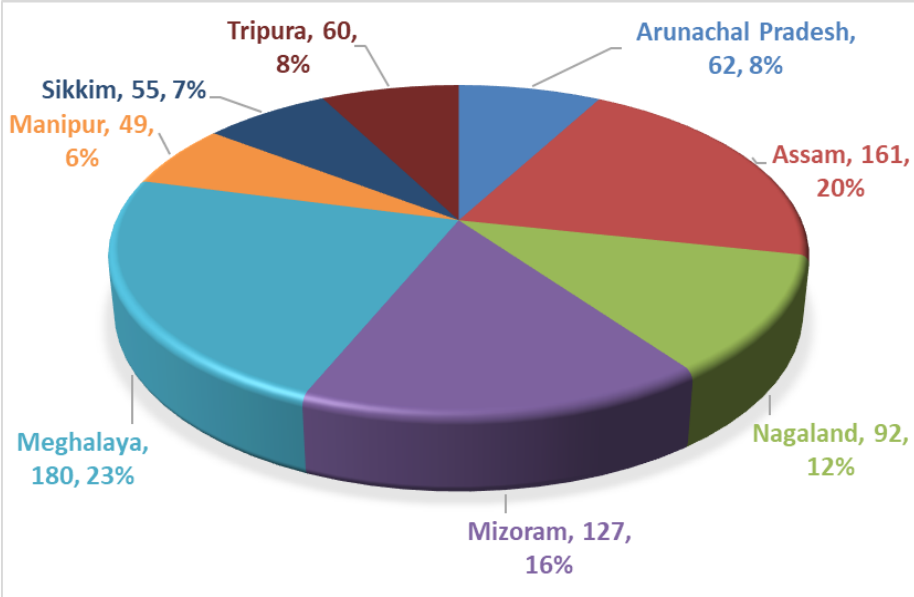


**Figure 6.** Geographic distribution by state of northeast Indian citrus germplasm conserved at the National Cryogenebank.

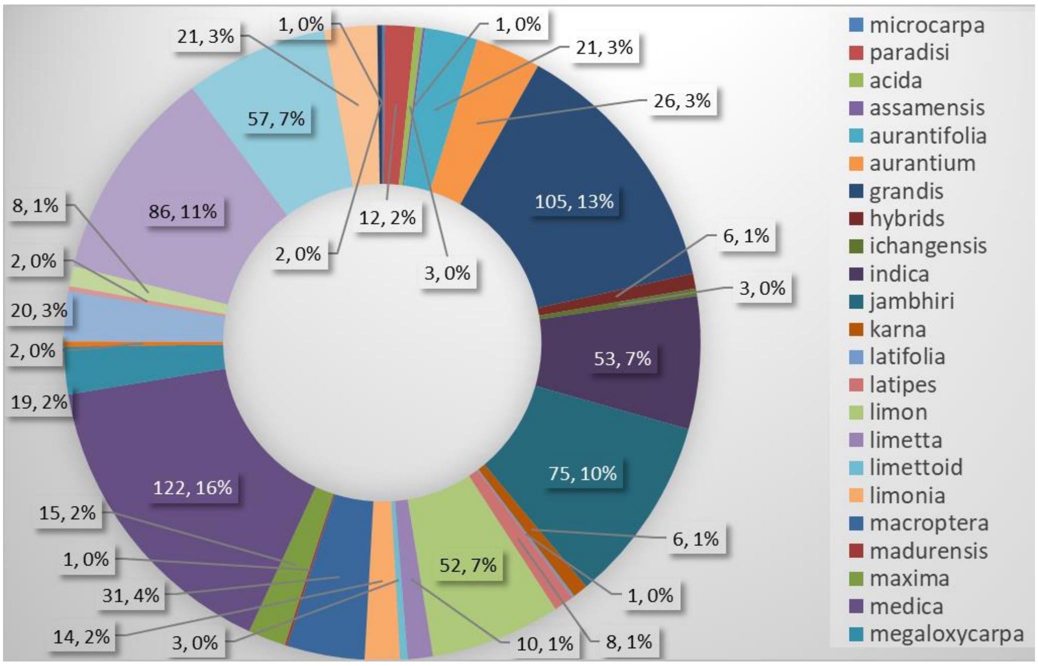


**Figure 7.** Citrus germplasm from northeast India conserved in the National Cryogenebank. Colour key indicates the species names.

explorations, collections have been made of a vast diversity of various citrus species representing important rootstocks, mandarins, acid lime, pummelo, grapefruit and sweet oranges along with indigenous wild species (21, 26, 45). A major emphasis was placed on collecting the diversity of wild, semi-wild and endangered citrus species such as *C. indica*, *C. assemensis*, *C. ichangensis*, *C. megaloxycarpa*, *C. macropteraand*, *C. latipes* from the entire northeastern Indian region. As a consequence, over the past four decades, significant diversity of cultivated, wild, rootstock, and several intermediate types of citrus germplasm have been systematically collected through various explorations. Out of 1,245 accessions, 786 accessions were collected from eight different states of northeast India (Fig. 6). Major collections are from Meghalaya (23%) followed by Assam (20%), Mizoram (16%) and Nagaland (12%). The germplasm collection from NEH region represents rich species diversity, with 31 different species currently conserved in the National cryogenebank (Fig. 7).

## FUTURE PROSPECTS

The management of citrus germplasm is highly important for citrus improvement programmes and for the conservation of valuable genetic diversity. Exploration, collection, characterization and conservation of diverse citrus germplasm has been undertaken by ICAR-NBPGR and other ICAR institutions and State Agricultural Universities of India, but still more systematic efforts are required. Conservation of citrus germplasm is of prime concern due to its increasing vulnerability. Climate change is constantly threatening the habitat of wild, endangered and endemic species. Reflecting the high importance of a strong interaction and coordination between different stakeholders, the Global Citrus Germplasm Network was developed in 1997 to undertake citrus germplasm conservation throughout the globe. Citrus has also been recognized as an Annex 1 crop by the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) (46). It is believed that extensive exploration, collection, conservation and documentation of citrus species at natural and suitable locations is necessary to sustain the genetic diversity of the species. Some key areas for future attention are:

- Despite considerable research and the significant commercial importance of cultivated citrus species, many aspects of their origin and domestication history remain unresolved (47, 48).
- There is a need to revisit citrus genetic resources of Northeast India to explore its vast existing diversity and distribution, especially of ambiguous taxa.
- Explorations, particularly in remote and challenging areas, should be targeted to collect rare germplasm from new sites.
- Characterization, evaluation, taxonomic studies, domestication trends and existing bottlenecks need to be addressed through extensive research and use of biotechnological tools.
- It is time to recognize the true value of the available genetic diversity, which can be further exploited for citrus improvement programmes.
- Citrus genetic resources in the region require urgent conservation efforts, utilizing a complementary approach that includes both in situ and ex situ methods. Ex situ conservation such as field genebanks, clonal repositories and cryobanking, would address the immediate needs. However, dynamic in situ conservation including on-farm conservation, gene sanctuaries, and national parks, is essential, particularly for wild and semi-wild species, to ensure their conservation and natural evolution.

**Acknowledgements:** We acknowledge the support and encouragement received from the Director, ICAR-NBPGR, New Delhi. We thank and recognize the contributions of the Late Dr. Rekha Chaudhury, Ex OIC and Principal Scientist, TCCU, ICAR-NBPGR for her contribution in pioneering the cryogenebanking of germplasm. We thank the research, technical staff, students and scientists associated with the collection and cryogenebanking of citrus germplasm at ICAR-NBPGR and other institutions for their help and support. We recognize the efforts of the farmers and field staff of various Field Genebanks from where germplasm was procured. Funding support from DBT Project "Collection, Conservation, and Morpho-Phenological Characterization of Citrus germplasm of North East Region" is acknowledged. Actions within this study were also supported by the MoU between ICAR-NBPGR, India and the Royal Botanic Gardens, Kew, UK.

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