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REVIEW ARTICLE

Genetic Diversity and Characterization of Pummelo (*Citrus maxima*) Species in Asia: A Review

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Abstract

Pummelo [*Citrus maxima* (Burm.) Merr.] is one of the three true citrus species and a primary progenitor of several cultivated citrus fruits. Despite its wide distribution across Asia, particularly in Southeast Asia and Northeast India, systematic efforts toward its genetic characterization and improvement remain limited. Significant morphological variability exists among pummelo genotypes; however, the extent and structure of underlying genetic diversity are not fully understood due to insufficient integration of molecular and genomic studies. This lack of comprehensive genetic information restricts effective germplasm utilization, conservation, and breeding for desirable traits such as disease resistance, fruit quality, and environmental adaptability. Therefore, the present review aims to critically examine current research on genetic diversity and characterization of pummelo using morphological, biochemical, and molecular markers, including SSR, RAPD, AFLP, SNP, and next-generation sequencing approaches. This review also highlights existing research gaps, particularly in underexplored diversity hotspots such as Northeast India, and emphasizes the importance of integrating genomic tools for precise genetic characterization. Understanding the genetic variability and population structure of pummelo is essential for germplasm conservation, parent selection, and the development of improved cultivars through marker-assisted breeding. The review underscores the need for comprehensive genomic studies and coordinated conservation strategies to facilitate genetic improvement and sustainable utilization of pummelo genetic resources in Asia.

Keywords: Breeding, *Citrus maxima*, Germplasm characterization, Genomics, Genetic diversity, Molecular markers

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Introduction

Pummelo [*Citrus maxima* (Burm.) Merr.; syn. *Citrus grandis* L.], a tropical fruit species, is native to Southeast Asia (Nazeer *et al*., 2022). Karyotype analysis identifies it as one of the three true citrus species, alongside *C. medica* and *C. reticulata* (Hynniewta *et al*., 2011). Historically, citrus cultivation began with the selection and preservation of individuals from wild populations, marking the early stages of citrus cultivar development (Barrett, 1977). Pummelo is also the progenitor of hybrids such as grapefruit (*C. paradisi*) and tangelo (*C. reticulata* × *C. maxima* or × *C. paradisi*). In recent years, there has been increasing demand for pummelo, especially in warmer climates where growing other sweet citrus fruits is not feasible (Paudyal, 1999). The three primary components of the fruit are the mesocarp (white, spongy albedo peel), the endocarp (fruit pulp), and the exocarp (flavedo, green peel) (Sharma *et al*., 2024) (Fig. 1). Pummelo fruits display variations in shape, size, and flavour, with peels ranging from green to yellow and pulp colours from pale yellow to pink or red (Kore *et al*., 2017). Rich in antioxidant flavonoids, potassium, phosphorus, and folic acid, pummelo offers significant nutritional value.

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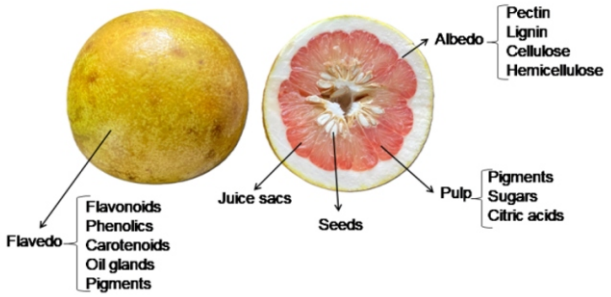


Fig. 1: Anatomy of Pummelo.

Pummelo has become widespread across various countries, including Chile, Thailand, Cambodia, India, Malaysia, Indonesia, Bangladesh, Japan, and Vietnam. In Asia, pummelo cultivars are categorized into three groups: Thai cultivars, regarded as the best tropical varieties; Indonesian cultivars, which are highly diverse and occasionally of lower quality; and Chinese cultivars, adapted to subtropical conditions (Makkumrai *et al*., 2021). Despite its significant commercial potential, pummelo has yet to gain the attention it deserves in India. The crop is primarily grown in family gardens across several regions, with little focus on its improvement or large-scale cultivation. *Citrus maxima* cultivars show a wide range of morphological variability. This variability can be attributed to the fact that Pummelo itself is a natural non-hybrid species (Kaur *et al*., 2019).

Pummelo also shows considerable genetic diversity due to its wide eco-geographical distribution across India, as well as the prevalence of spontaneous mutations and natural hybridization (Roy *et al*., 2014). Understanding the genetic diversity and phylogeny of pummelo cultivars enhances the effectiveness of germplasm characterization and its use in breeding programs. Moreover, genetic diversity within and between populations is essential for conserving plant genetic resources, ensuring their continued availability for future use (Quedraogo, 2001).

Morphological traits are often used as primary markers for germplasm management and play an important role in distinguishing variations or subspecies within a taxonomic group (Gitonga *et al*., 2008; Paganova, 2009). Given the complexity of citrus germplasm's phylogeny and taxonomy, molecular markers are vital for its accurate characterization. DNA-based markers offer an effective approach to distinguishing closely related species and genotypes (Susandarini *et al*., 2020). These markers help identify genotypes, assess relatedness between accessions, and trace the inheritance of economically valuable traits. Evaluating genetic diversity within germplasm is crucial for effective breeding (Vinu *et al*., 2013) and for assessing the adaptability of pummelo in diverse environments (Lowe *et al*., 2004). Investigating genetic differences and relationships between breeding materials is key to advancing crop development efforts.

Distribution in Asia

Pummelo, a large citrus fruit indigenous to Southeast Asia, is considered the ancestor of grapefruit (Nicolosi and Khan, 2007). Top pummelo-producing countries include China, Japan, India, Fiji, Malaysia, Vietnam, and Thailand (Bhende and Pavithra, 2020), with China being the largest producer and exporter, while Thailand is known for its high-quality fruits and ranks among the top ten exporting nations (Table 1) (Makkumrai *et al*., 2021).

Table 1: Common Pummelo cultivars in China and Thailand

Country	Cultivar	Reference
China	Shatian, Guanxi	Zhang *et al*., 2017
	Honey Pink, Honey White Huangjin, Sanhong, Dongshizou	Vivian *et al*., 2019 Zhu *et al*., 2020
Thailand	Thong Dee, KaoYai	Vivian *et al*., 2019; Rosales *et al*., 2015
	Tab Tim Siam, Kao Numphueng, Kao Tangkwa	Rosales *et al*., 2015

In India, pummelo is cultivated in home gardens across all states, with the North-East region (Singh and Singh, 2003; Roy *et al*., 2014) exhibiting the highest diversity, followed by Bihar and Bengal (Fig. 2). Pummelo is cultivated in Bangladesh, Cambodia, Chile, India, Indonesia, Japan, Laos, Malaysia, Philippines, Thailand and Vietnam (Orwa *et al*., 2009) and is also grown in China (Vinning and Moody, 1997; Zhang and Peng,

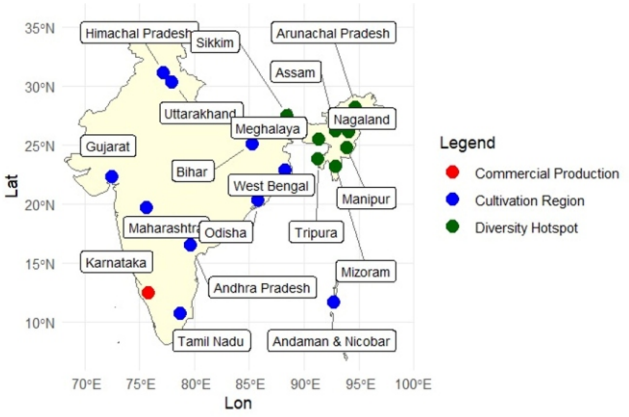


Fig. 2: Pummelo distribution in India. Source: Singh and Singh, 2003; Roy *et al*., 2014.

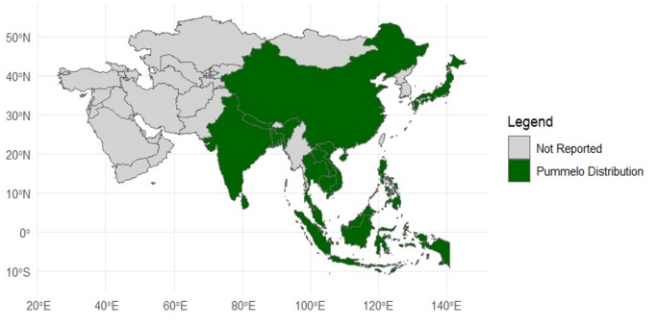


Fig. 3: Distribution of Pummelo (*Citrus maxima*) in Asia.
Source: Vinning and Moody, 1997; Zhang and Shaolin, 2000; Orwa *et al*., 2009; Roy *et al*., 2014; Kore *et al*., 2015; Singh *et al*., 2015a.

2000), Sri Lanka and Nepal (Fig. 3). The NEH region of India is part of the Himalaya and Indo-Myanmar biodiversity hotspot, indicating its rich crop genetic resources, particularly citrus, which is considered their place of origin (Hazarika, 2012). Among citrus species, pummelo [*Citrus maxima* (Burm.) Merr.] synonymous with *Citrus grandis* (L.) Osbeck, also referred to as the Chinese grapefruit, pomelo, or shaddock, showed wide diversity in the region. It is classified as either pigmented (or pink) or common (or white) pummelo (Rymbai *et al.,* 2025). In the North-eastern region of India, approximately 17 species, 52 cultivars, and 7 potential hybrids of citrus have been reported (Singh, 1990). Some of these species are native to the region, as documented by Bhattacharya and Dutta (1956), including *Citrus limon* Burm., *C. medica* Linn., *C. jambhiri* Lush, *C. inchangenis* Swing, *C. lalipes* Tanaka, *C. macroptera* Montr., *C. assamensis* Dutta and Bhattacharya, *C. indica* Tanaka, *C. aurantium* Linn., *C. karna* Raf., *C. aurantifolia* Swing, *C. limetta* Risso, *C. nolulis*Lour., *C. sinensis* Osbeck, *C. grandis* L., and *C. megaloxycarpa* Lush. The region is also home to several naturally occurring hybrids, such as *Soh-Khyllah* No. 1, *Soh-Khyllah* No. 2, *Soh-Khyllah* No. 3, Hashkhuli, Sarbati, Nieholsray, and Dewa tenga (Shadeque, 1989). Recent studies from the North Eastern Himalayan (NEH) region of India have further highlighted the substantial variability present in pummelo germplasm. Rymbai *et al*., (2025) evaluated different pummelo genotypes from Meghalaya and reported significant variation in morpho-biochemical traits, antioxidant properties, and fruit pigmentation. The study revealed considerable differences among genotypes in terms of fruit size, peel thickness, pulp colour, total soluble solids, acidity and antioxidant activity, indicating the presence of diverse genetic resources within the region. Variations in pigmentation and antioxidant content also suggest differences in secondary metabolite composition, which may be associated with genetic factors and environmental adaptation. Such diversity is particularly important for identifying superior genotypes with improved nutritional quality, stress tolerance, and consumer acceptability. The findings emphasize that the NEH region represents a valuable reservoir of pummelo genetic diversity and highlight the importance of systematic characterization and conservation of these genotypes.

Importance of Pummelo in Agriculture

Pummelo is increasingly being recognized as more than just a traditional fruit grown in backyard orchards. It represents a crop with strong agricultural promise, particularly in regions where farming conditions are challenging. The literature consistently highlights its adaptability and resilience. As reported by Roy *et al*., (2014), pummelo is easily grown in inferior, marginal and backyard orchards and is considered as an excellent tree for wasteland development in arid and semi-arid regions. These qualities make it especially valuable for small and marginal farmers who often cultivate crops under low input conditions. Its ability to perform well in diverse agro-ecological environments positions it as a practical option for climate-resilient and sustainable horticulture.

Beyond its adaptability, pummelo also stands out for its nutritional richness. Fayaz *et al*., (2020) describe it as rich in potassium, fibre, and calcium and containing significant levels of vitamins A, B, and C. Such a nutrient profile supports its role in improving dietary diversity and promoting health-conscious consumption. In addition to fresh consumption, pummelo is processed into juices, squashes, marmalades, candies, and other value-added products. This versatility enhances its commercial relevance and opens opportunities for small-scale processing enterprises and rural entrepreneurship. In many communities, different parts of the plant are also used in traditional practices, reflecting its broader socio-cultural and economic importance.

A particularly important dimension of pummelo is its genetic richness. Previous studies (Singh *et al*., 2015a; Singh *et al*., 2015b; Alam *et al*., 2016; Kore *et al*., 2017; Haque *et al*., 2022 and Angami *et al*., 2022) consistently report substantial morphological and molecular variability across different regions of India. This diversity has significant practical implications for crop improvement. The availability of diverse genotypes allows breeders to select for desirable traits such as larger fruit size, improved pulp quality, sweetness, better shelf life, and tolerance to pests, diseases, and environmental stresses. Moreover, pummelo holds

special significance in citrus improvement programs, as it contributes valuable genetic material for developing improved cultivars of related citrus species. Its role in the evolutionary background of citrus further emphasizes its importance as a genetic resource.

Genetic Diversity in Pummelo

Genetic diversity plays a crucial role in the enhancement and breeding of pummelo, providing the variation necessary to develop cultivars with improved traits. Studies have shown that pummelo germplasm exhibits significant genetic variation, which can be effectively analyzed using molecular markers such as Simple Sequence Repeats (SSR) and Random Amplified Polymorphic DNA (RAPD) (Wang *et al*., 2019). This genetic variation is essential for breeding programs focused on developing cultivars with desirable characteristics, including enhanced disease resistance, greater adaptability to various environmental conditions, and improved fruit quality (Swarup *et al*., 2021).

Through the use of molecular markers, researchers have identified distinct genetic clusters within pummelo variants, offering valuable insights for breeding strategies. These clusters enable the selection of optimal parent combinations to maximize desirable traits in progeny (Yong *et al*., 2006). Additionally, the characterization of pummelo genotypes using both morphological traits and molecular markers has uncovered several promising parent combinations, providing a foundation for future breeding efforts (Haque *et al*., 2022). It is essential to preserve and strategically utilize pummelo's genetic diversity, especially in addressing current agricultural challenges. Issues such as the need for crop development in response to climate change and the growing demand for high-quality fruit that meets consumer preferences highlight the importance of this diversity. By maintaining a broad genetic base, pummelo breeding programs can continue to evolve, developing cultivars that are resilient, high-yielding, and well-adapted to both environmental and market changes.

Genetic Characterization in Pummelo

Genetic characterization plays a crucial role in understanding the genetic diversity and improving breeding strategies for pummelo (*Citrus grandis*). A variety of molecular tools and techniques have been utilized to analyze the genetic structure and variability of pummelo germplasm, offering significant insights into its genetic makeup. These methods have been essential in identifying distinct pummelo cultivars and hybrids, assisting in their conservation and enhancement. Recent advances in molecular marker analysis have provided deeper insights into the genetic diversity and evolutionary relationships of pummelo. A comprehensive study by Luro *et al.,* (2025) using SSR, SNP, and InDel markers to analyze 58 citrus accessions revealed substantial genetic diversity within pummelo cultivars and confirmed its distinct genetic identity compared to grapefruit and other citrus species. The study identified 20 true pummelo cultivars and demonstrated that pummelo exhibits higher allelic diversity and varietal differentiation due to its allogamous reproductive system and diversification through sexual hybridization. Genetic relationship analysis further showed that pummelo accessions formed a distinct cluster, clearly separating them from grapefruit and hybrid citrus types, thereby confirming their unique genetic lineage and evolutionary significance.

A range of molecular markers, including AFLP (Amplified Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), and SSR (Simple Sequence Repeat), have been widely employed for genetic characterization of pummelo. Among these, SSR markers have proven particularly valuable due to their high polymorphism and ability to detect genetic variation at a finer scale. SSR analysis has revealed significant genetic differences among pummelo cultivars, with an average of 9.85 alleles per locus (Yong *et al*., 2006). These markers are instrumental in assessing genetic diversity, identifying parent-offspring relationships, and improving breeding efficiency.

RAPD and AFLP markers have also been extensively used for analyzing the genetic variability within pummelo populations. These markers help in assessing genetic relationships and diversity, enabling the identification of distinct genetic groups and the classification of pummelo accessions into different clusters (Liu *et al*., 2005). Studies utilizing these molecular markers have shown a remarkable level of genetic variation within pummelo germplasm, which is crucial for breeding programs focused on improving traits such as disease resistance, fruit quality, and environmental adaptability (Wang *et al*., 2019).

Recent advancements in genomic technologies, such as next-generation sequencing (NGS), have provided deeper insights into the genetic landscape of pummelo. NGS techniques allow for the sequencing of entire genomes, facilitating the identification of genes associated with key traits, such as disease resistance and fruit quality. These technologies offer a more comprehensive understanding of pummelo's genetic

structure, paving the way for marker-assisted breeding programs and the identification of novel genetic traits (Wang *et al*., 2019; Swarup *et al*., 2021).

The genetic data obtained from molecular markers have been pivotal in guiding pummelo breeding programs. Molecular markers enable breeders to select parents with desirable traits, such as improved fruit yield, disease resistance, and tolerance to environmental stresses. Furthermore, genetic characterization through molecular techniques aids in maintaining the genetic integrity of pummelo cultivars and preventing the loss of genetic diversity in breeding populations (Swarup *et al*., 2021). By incorporating molecular data into breeding efforts, pummelo cultivars can be developed to meet the growing demand for high-quality, resilient fruits in diverse agricultural conditions.

The use of molecular markers and advanced genomic technologies has significantly advanced the understanding of pummelo's genetic diversity and provided valuable tools for breeding and conservation. By continuing to employ these methods, researchers can further unlock the genetic potential of pummelo, ensuring the development of superior cultivars with enhanced traits that meet both agricultural and consumer demands.

Genetic Diversity Studies in Pummelo

The study of pummelo (*Citrus grandis* Osbeck) genetic diversity has yielded valuable information about the species' variability, which is crucial for both genetic improvement and conservation. Several molecular markers, such as AFLP (Amplified Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), and SSR (Simple Sequence Repeat) markers, have been used to measure genetic variation within pummelo germplasm. With an average of 9.85 alleles per locus, SSR markers have shown substantial levels of polymorphism among them, indicating notable genetic variation among cultivars (Liu *et al*., 2005; Yong *et al*., 2006). The existence of detectable genetic variety among accessions has also been supported by RAPD and AFLP investigations, which have found significant variation across pummelo populations (Liu *et al*., 2005).

Pummelo germplasm has been further divided into discrete genetic groupings by cluster-based investigations, which have included well-known cultivar varieties like Shatian and Wendan. According to Wang *et al*., (2019), the Sichuan Basin has been recognized as a significant hub for pummelo diversity, highlighting its importance in breeding and germplasm preservation efforts. When taken as a whole, these findings show that pummelo has structured genetic variation that can be used to benefit crops.

Nevertheless, a number of studies indicate that overall molecular divergence within the species may be quite mild, despite the significant phenotypic variation shown across pummelo cultivars (Ahmed *et al*., 2019). A significant unsolved problem in pummelo research is the apparent disparity between a wide range of morphological diversity and relatively little molecular distinction. It calls into question whether the marker systems used, in particular low-density SSR and RAPD markers, offer enough genome-wide resolution to fully capture genetic variation. Moreover, the majority of diversity evaluations have depended on geographically constrained sampling and a small number of loci, which may have understated population structure and allelic richness throughout the species' range.

Interpretation is further complicated by the potential that certain phenotypic differences result from localized selection or somatic mutations rather than widespread genetic divergence (Ahmed *et al*., 2019). Therefore, even though previous research has confirmed that pummelo has genetic variability, high-density marker systems and more extensive sampling techniques are needed to gain a more thorough understanding of the species' population structure and evolutionary dynamics.

Conservation and Utilization of Pummelo Genetic Resources

The preservation of pummelo genetic resources is critical to ensuring the availability of diverse genetic materials for future agricultural advancements. SSR markers have revealed substantial genetic variation across multiple pummelo cultivars, with an average of 9.85 alleles per locus, highlighting the importance of these resources for breeding and conservation. However, citrus species face unique challenges in germplasm conservation due to their refractory seeds, which have a limited storage viability of approximately one year, and their general practice of clonal propagation (Kaur *et al*., 2019). Furthermore, hybridization between different citrus species can lead to fertile F1 hybrids, which may not always accurately represent the maternal parent. As a result, most citrus germplasm is conserved in the form of living trees in field genebanks or controlled environments like greenhouses and screenhouses.

India is recognized as a center of origin for various citrus species, with a diverse array of wild and cultivated types found in the Northeast and Northwest regions (Singh and Singh, 2006). The country's citrus germplasm is a rich source of genetic material for agricultural

improvement, but this diversity is increasingly threatened by agricultural activities and deforestation (Malik *et al*., 2014). Conservation efforts, including field genebanks, cryopreservation, and exploratory missions, are essential to gather and describe germplasm. Although India holds a substantial collection of citrus accessions, it faces challenges such as disease and pest management, lack of funding, and a shortage of skilled personnel. International collaboration through a global citrus germplasm network is crucial for the long-term preservation and sustainable use of these genetic resources (Roose *et al*., 2015).

In situ Conservation

In-situ conservation involves preserving plant species within their natural habitats, allowing them to evolve and adapt to environmental changes. This approach is essential for maintaining the genetic diversity of pummelo (*Citrus maxima*) and protecting it from environmental stressors. Furthermore, studies suggest that homestead gardening, through family farming, is a valuable strategy for preserving pummelo diversity, as seedling progenies often show significant variation in fruit traits, which can be harnessed for cultivar improvement and livelihood security (Singh *et al*., 2015b).

India's first "Citrus Gene Sanctuary," established in 1981 at the Nokrek Biosphere Reserve in Meghalaya, stands as a landmark for *in situ* conservation (Singh, 1981). The sanctuary, covering 10,266 hectares, enjoys a unique microclimate with tropical and temperate conditions, making it a suitable habitat for a variety of citrus species. Despite the sanctuary's success in preserving citrus species, challenges remain, such as slow regeneration rates and increased human interference (Hazarika, 2024).

Ex situ Conservation

Ex situ conservation of pummelo is effectively achieved through in vitro slow growth techniques and global genebank collections, with multiple studies demonstrating successful long-term preservation and genetic stability. The evidence base includes three primary in vitro conservation studies with concrete results. Tyas (2012) tested two approaches: MS medium without sucrose achieved 30.7 months conservation with 75% shoot elongation inhibition, while MS + sorbitol 2% + paclobutrazol 7.5 ppm achieved 22.2 months with 65.1% shoot length inhibition. Kaur *et al*., (2019) demonstrated successful micropropagation with 86% germination and confirmed genetic stability through SSR profiling of regenerated plantlets. Dewi *et al*., (2016) found MS + 20 g sorbitol/l optimal for pummelo cv. Srinyonya conservation. At the global scale, Volk *et al*., (2023b) surveyed 43 *ex situ* citrus collections across 27 countries, with the six largest maintaining 1,000-1,735 accessions each, including pummelo germplasm. Additionally, Singh *et al*., (2015) documented conservation through homestead farming practices in India, identifying superior clones for commercial potential. Among the largest collections are those at the Citrus Research Institute in China, Instituto Agronômico de Campinas in Brazil, and the USDA-ARS National Clonal Germplasm Repository in the United States (Volk *et al*., 2023a) (Table 2). These collections are essential for maintaining citrus diversity, including both native cultivars and wild species. In India, the Central Institute of Citrus Research (CICR) in Nagpur, along with its regional stations, preserves citrus accessions tailored to various agroclimatic conditions, ensuring the continued conservation of pummelo and other citrus species (Tripathi *et al*., 2018).

The advancement of biotechnology has significantly enhanced conservation efforts by introducing innovative techniques that increase the effectiveness and efficiency of preserving plant genetic resources. One such method is in-vitro culture, which enables the preservation of plant tissues in a controlled environment. This approach is particularly valuable for species whose seeds are difficult to store using traditional seed preservation methods. Another key biotechnological breakthrough is cryopreservation,

Table 2: Citrus accessions in various institutions

Institution/Collection centre	Country	Number of Accessions
Instituto Agronômico de Campinas/Centro de Citricultura Sylvio Moreira	Brazil	1,735
Citrus Research Institute at Southwest University	China	1,700
USDA-ARS National Clonal Germplasm Repository for Citrus and Dates	United States	1,632
Institute of Fruit Tree and Tea Science (NARO, NIFTS)	Japan	1,261
National Research Institute for Agriculture, Food and Environment (INRAE)-Corsica	France	1,100
Queensland Department of Agriculture and Fisheries	Australia	1,000

Source: Volk *et al*., (2023a).

where plant tissues are stored at extremely low temperatures, halting all metabolic processes and preserving the genetic material in its original state. These techniques have been successfully applied to various crops, including pummelo, where micropropagation and in-vitro conservation protocols have been developed using shoot tip and nodal section explants. Studies have shown that these methods can maintain pummelo germplasm without causing genetic alterations, thus preserving the integrity of the plant's genetic makeup (Kaur *et al*., 2019). Cryopreservation methods for embryogenic callus and embryos are available for citrus species (Duran-Vila, 1995; González-Arnao *et al*., 2003). Given that cryopreservation can be used with a variety of explants, including seeds, zygotic embryos, embryonic axes, shoot apices, embryogenic callus, cell suspensions, and somatic embryos, it is currently the most effective long-term conservation method for citrus species (Malik *et al*., 2003; Malik and Chaudhury, 2006).

At institutions like ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, and, to a lesser extent, the National Botanical Research Institute, Lucknow, attempts have been made to implement cryopreservation and in-vitro conservation (Malik *et al*., 2025). Shoot apices have been used in efforts to preserve *C. aurantifolia* and *C. grandis* in vitro for extended periods. After three years of storage, root cultures of *C. aurantifolia* were still able to produce shoot buds, and the regenerated plants maintained a normal diploid chromosome number. Similarly, *C. grandis* shoot cultures have been successfully preserved for over 32 years (Chaturvedi, 2002). Additionally, in-vitro conservation and cryopreservation of shoot tips from in-vitro plantlets are being explored at ICAR-NBPGR as alternative techniques for conserving resistant seed species and wild, rare, and endangered citrus species such as *C. ichangensis*, *C. indica*, *C. macroptera*, *C. megaloxycarpa*, and *C. latipes* (Srivastava *et al*., 2024).

The global ex-situ conservation of citrus aims to preserve and ensure the availability of citrus and its wild relatives for the long term, free from disease and pathogens, through a distributed and secure network of gene bank collections. These collections provide collection data (genotypic, phenotypic, and passport) in a standardized information system (Volk *et al*., 2023).

Genomics of Pummelo and Its Applications

The genome of pummelo (*Citrus maxima*), one of the three true citrus species, has been sequenced and extensively analyzed, providing vital insights into citrus evolution, domestication, and breeding. As an ancestral species to many cultivated citrus fruits, pummelo plays a central role in understanding the genetic makeup and hybrid origins of modern citrus varieties. One of the landmark studies by Xu *et al*., (2013) reported the draft genome of sweet orange (*Citrus sinensis*) and traced its hybrid origin to pummelo and mandarin, emphasizing the importance of pummelo genomic data. Further advancing this, Wu *et al*., (2014) sequenced multiple genomes of mandarin, pummelo, and orange, uncovering the complex admixture patterns that occurred during citrus domestication. These studies revealed that pummelo maintains a relatively stable and less admixed genome, making it a valuable reference for citrus genetics.

A high-quality draft genome of wild pummelo was published by Wang *et al*., (2017), enriching the available genomic resources and enabling the identification of key gene families involved in fruit development, aroma, disease resistance, and stress tolerance. These genomic resources are accessible through public databases such as NCBI, the Citrus Genome Database (CGD), and Phytozome, serving as powerful tools for genetic research, molecular breeding, and conservation efforts in citrus. The sequencing of pummelo's genome has provided valuable resources for advancing research in citrus genetics and breeding.

Genomic Approaches in Pummelo Breeding: GWAS and Genomic Selection

Recent research has utilized Genome-Wide Association Study (GWAS) to investigate the genetic basis of various important traits in pummelo, such as fruit size, disease resistance, and fruit quality. GWAS has enabled the identification of specific genetic markers linked to these traits, which can be used for marker-assisted selection in breeding programs. For instance, a study by Liu *et al*., (2020) in *Citrus* species, including pummelo, identified several loci associated with fruit quality characteristics such as fruit weight and sweetness. By linking these traits to specific genomic regions, the study provided valuable information for improving fruit quality through genetic improvement. Additionally, Yu and Jiang (2022) conducted a GWAS study focused on identifying markers related to disease resistance in pummelo, particularly against citrus canker, a major threat to citrus production. These results contribute to the development of disease-resistant varieties of pummelo through the application of genomic markers.

Genomic selection, a more advanced form of marker-assisted breeding, has also been explored in pummelo. This method involves using genome-wide markers to predict the breeding value of individuals in a

population, allowing for more accurate selection of superior genotypes. A study by Minamikawa *et al*., (2017) in *Citrus* demonstrated the potential of genomic selection for improving complex traits such as yield and quality in citrus breeding, including pummelo. The use of genomic selection has the potential to speed up the breeding process, making it possible to create improved pummelo cultivars with desirable characteristics more efficiently.

Constraints and Opportunities

The genetic characterization of pummelo is an important area of research that presents both significant opportunities and challenges. Despite the considerable morphological diversity observed among pummelo varieties, studies have revealed relatively low levels of genetic variation at the molecular level (Ahmed *et al*., 2019). This apparent discrepancy highlights the need for comprehensive genetic analyses to better understand the species' diversity and support its conservation and improvement. Somatic mutations, which provide observable phenotypic diversity without significantly changing the underlying genetic structure, are probably the cause of this seeming paradox. These restrictions underline the difficulty of precisely determining genetic variability and the necessity of trustworthy molecular methods for proper characterization. In order to overcome these obstacles, scientists have been using molecular markers increasingly. Simple Sequence Repeat (SSR) markers in particular have been very successful in assessing genetic diversity and assisting in breeding initiatives. The value of SSR markers in detecting genetic variation, comprehending genotype connections, and aiding in the selection of desired phenotypes was shown by studies by Yong *et al*., (2006) and Haque *et al*., (2022). Additionally, the resources available for genetic study and breeding have increased due to recent developments in genomic technologies. De novo transcriptome sequencing, for example, has made it possible to identify a large number of SSR and SNP markers, offering useful resources for in-depth genetic study and marker-assisted selection (Liang *et al*., 2015).

Notwithstanding these technological developments, several obstacles still stand in the way of the efficient conservation and application of pummelo genetic resources. Regulations pertaining to intellectual property have made it more difficult for nations and organizations to share germplasm, which has reduced access to priceless genetic resources. Furthermore, even if there are standards for exchanging germplasm, their application is frequently hindered by budgetary constraints, especially in underdeveloped nations. Attempts to maintain genetic variation are made more difficult by a lack of financing for biodiversity protection. Therefore, to support inclusive and participatory approaches for germplasm conservation and breeding, research institutions, policymakers, and local people must work together more closely to develop effective conservation plans (Gepts, 2006). By encouraging coordinated conservation, germplasm management, and international cooperation, global programs like the Global Strategy for the Conservation and Use of Citrus Genetic Resources have offered crucial foundations for tackling these issues. However, problems including disease susceptibility, environmental adaptability, and the gradual degradation of germplasm resources make it challenging to manage citrus germplasm collections (Barbora *et al*., 2020). These difficulties emphasize how crucial it is to put into practice efficient conservation and management plans in order to protect genetic resources.

Looking ahead, an integrated approach combining genomes, transcriptomics, and phenomics will be very beneficial for future studies in pummelo genetic characterization. Breeding programs will be more accurate and effective thanks to such thorough methodologies, which will allow for a deeper understanding of genetic variation, gene function, and trait expression. Furthering genetic research and promoting germplasm interchange will also be greatly aided by the creation of more sophisticated molecular markers and the bolstering of global cooperation (Haque *et al*., 2022). It will take ongoing work to identify genetic diversity, create reliable molecular markers, and put conservation plans into action if pummelo cultivars are to be improved over the long run. To protect genetic resources and create superior cultivars that can withstand new threats, including disease pressures, climate change, and changing consumer tastes, ongoing research and conservation efforts are crucial. In the end, improvements in molecular characterization and conservation will give breeding programs a solid genetic basis, allowing for the creation of hardy, high-yielding cultivars and improving the sustainability and financial feasibility of pummelo farming, especially in areas where it is a significant agricultural and economic activity.

Conclusion

Pummelo represents an important ancestral citrus species with substantial genetic diversity and significant potential for crop improvement, conservation, and breeding. The wide variability observed in morpho-

logical, biochemical, and molecular traits reflects its diverse eco-geographical distribution, particularly in Asia and Northeast India, which serves as a major reservoir of genetic resources. Molecular marker technologies, including SSR, SNP, RAPD and next-generation sequencing, have greatly enhanced the understanding of genetic relationships, population structure, and cultivar differentiation in pummelo. These tools provide valuable support for germplasm characterization, conservation, and the selection of superior genotypes for breeding programs. However, despite its importance, pummelo remains underutilized and requires greater research focus, particularly in genomic characterization and conservation strategies. Future efforts integrating advanced genomic tools, systematic germplasm evaluation, and conservation approaches will be essential to fully exploit pummelo's genetic potential and develop improved cultivars with enhanced productivity, adaptability, and fruit quality, thereby ensuring sustainable cultivation and utilization of this valuable citrus species.

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