International Journal of Advanced Biochemistry Research

Crop improvement in citrus: A review

Susmita Das, Arti Sharma and Shaik Manshad

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(7): 485-492
www.biochemjournal.com
Received: 20-05-2024
Accepted: 26-06-2024

Susmita Das
(1) Ph. D. Scholar, Division of Fruit Science, Sher-e Kashmir University of Agricultural Science and Technology of Jammu, Jammu and Kashmir, India
(2) Assistant Professor, Department of Horticulture, School of Agriculture, Lovely Professional University, Punjab, India

Arti Sharma
Professor, Division of Fruit Science, Sher-e Kashmir University of Agricultural Science and Technology of Jammu, Jammu and Kashmir, India

Shaik Manshad
M.Sc. Fruit science, School of Agriculture, Lovely Professional University, Punjab, India

Corresponding Author:
Arti Sharma
Professor, Division of Fruit Science, Sher-e Kashmir University of Agricultural Science and Technology of Jammu, Jammu and Kashmir, India

DOI: https://doi.org/10.33545/26174693.2024.v8.i7Sf.1536

Abstract

Citrus, comprising the genera Citrus, Fortunella, and Poncirus within the Aurantioideae subfamily, plays a critical role in global horticulture. Among these, the Citrus genus predominates in consumption, encompassing varieties such as Pummelos (*C. maxima*), Citrons (*C. medica*) and Mandarins (*C. reticulata*), originating from distinct geographic regions that serve as foundational stocks for cultivated varieties. Additionally, Fortunella (kumquats) and Poncirus (*P. trifoliata*) contribute significantly to rootstock breeding due to their biotic resilience. In India, Citrus exhibits extensive genetic diversity, comprising 24 species and numerous varieties, crucial for socio-economy and health, necessitating robust conservation strategies. Key techniques like *in vitro* conservation and cryopreservation are pivotal for safeguarding endangered species such as *C. indica* and *C. ichangensis*. Flowering in Citrus, influenced by environmental cues, underscores adaptations to subtropical and tropical conditions, influencing commercial cultivation and breeding goals. Breeding programs focus on enhancing scion traits (e.g., seed lessness, fruit quality) and rootstock attributes (e.g., disease resistance, soil adaptability), utilizing methods such as mutation breeding, polyploidy induction, somatic hybridization, and genetic transformation. Molecular tools are essential for genetic diversity assessments and phylogenetic insights, facilitating advancements in breeding strategies. The integration of these approaches highlights multifaceted strategies in citrus improvement, ensuring sustainability and resilience in the face of evolving agricultural challenges. This abstract synthesizes current knowledge on citrus taxonomy, genetic diversity, breeding methodologies, and conservation efforts, emphasizing their collective significance in citrus improvement and global cultivation practices.

Keywords: Crop improvement, citrus, Poncirus

Introduction

Citrus refer to all species of three sexually compatible genera, Citrus, Fortunella and Poncirus, within the tribe of Citrate of the subfamily Aurantioideae. *Citrus* genus contains the majority of the consumed species while Fortunella genus includes some cultivars (kumquats). *Poncirus* genus is monospecific (*P. trifoliata*) and plays a central role in rootstock breeding because of its tolerances to many biotic constraints. *Citrus* genus contains, according to the different taxonomies, between 16 (Swingle and Reece, 1967) [86] and 156 species (Tanaka, 1961) [88]. Phenotypical data (Barret and Rhodes, 1976) [5] as well as molecular analysis (Herrero *et al*., 1997; Nicolosi *et al*., 2000; Ollitrault *et al*., 2003) [97, 61] clearly demonstrated the existence of three basic taxa (*C. maxima*—pummelos, *C. medica*—citronsand *C. reticulata*— mandarins) that have originated all cultivated Citrus. The differentiation between these sexually compatible taxa is explained by an initial foundation effect in three distinct geographic zones and subsequent allopatric evolution. The Pummelos originated in the Mlay Archipelago and Indonesia, the Citrons evolved in northeastern India and the nearby region of Burmaand China and the Mandarins were diversified over a region including Vietnam, Southern China and Japan (Scora, 1975; Webber, 1967) [76, 94]. The other cultivated species (*C. aurantium*—sour orange, *C. sinensis*—sweet orange, *C. paradisi*—grapefruit, and *C. lemon*—limon) have likely appeared by recombination among the basic taxa (Nicolosi *et al*., 2000; Ollitrault *et al*., 2003) [61, 66]. For *C. aurantifolia* (Mexican lime), a fourth original taxa (*C. micrantha*) were involved in combination with *C. medica* (Nicolosi *et al*., 2000) [61].

For propagation of healthy Citrus plants, it is imperative that at young age scions of choice are grafted on vigorous rootstocks that are selected for disease resistance and cold hardiness to avoid very long juvenile periods and allow production of better-quality fruit (Pena *et al*., 1995a, b; Seguin and Pena, 2001) [69, 70, 77]. Carrizo citrange and Rangpur Lime are very popular and most widely used rootstocks nowadays due to their high vigor and vitality and tremendous efforts have been made to further improve their genotype by biotechnological methods (Moore *et al.*, 1992; Pena *et al.*, 1995a; Cervera *et al.*, 1998b; Navarro *et al.*, 2004) [52, 69, 10, 70]. In nature, Citrus seedlings produce trees and fruits identical to the parent tree because of nucellar polyembryony, but in general practice, conventional breeders make use of vegetative propagation by means of clonal selection. Breeding usually emphasizes on selection of genotypes obtained by spontaneous or induced mutagenesis, which is the oldest breeding method for cultivar improvement. Traditional approach of tree breeding involves the selection of trees with desirable phenotype followed by their integration into breeding programs (Kaneyoshi *et al.*, 1994) [37].

Genetic Resources

Citrus is one of the most economically important fruit crops of India. It is believed to originate in Southeast Asia. India has an enormous diversity of Citrusgenetic resources, both in cultivated and wild types, comprising 24 species, one subspecies and 78 varieties, including wild, endangered and endemic species (Sharma *et al*., 2004; Malik *et al*., 2013) [78, 50]. Some of the wild species namely *C. indica*, *C. ichangensis*, *C. latipes*, *C. macroptera*, *C.assamensis*, *C. megaloxycarpa* and *C. medica* have special importance in Indian germplasm being of use in socio-economy and health of local people (Malik *et al*., 2012) [49], besides utility in Citrus industry and improvement programmes and therefore, need sustainable and inclusive management efforts. Management of Citrus genetic resources, some of which are still growing as natural wild and semi-wild populations as recorded from Citrus Gene Sanctuary in Garo Hills (Singh, 1981) [80], would require adoption of complementary conservation strategies. Within the genus Citrus, a species-specific conservation strategy is to be developed based on extent of genetic diversity available, mechanism of propagation, reproductive biology of species and present biological status of the species. In situ conservation is, therefore, recommended to conserve the available genetic diversity of such economically important species in the best possible way. For genetic improvement and genotype conservation, elite plants are conserved in field gene banks at various horticultural organizations in India. As per the IUCN norms, seven Indian Citrus species namely *C. indica*, *C. macroptera*, *C. latipes*, *C. assamensis*, *C. ichangensis*, *C. megaloxycarpa* and *C. rugulosa* are endangered as indicated by threat perception analyses (Singh and Singh, 2003; Malik *et al*., 2013) [82, 50]. Managing genetic resources of Citrus where species or genotypes are scattered amongst various stakeholders of National Agriculture Research System (Indian Council of Agricultural Research and State Agriculture/Horticulture Universities), State Horticulture Departments and Ministry of Environment and Forest (custodian of forest and wild flora), is far more challenging.

An alternative to the maintenance of germplasm is conservation *in vitro* (Engelmann, 1997) [18]. In Citrus, there exist some procedures for cryoconservation of embryogenic callus and embryos (Duran-Vila., 1995; González-Arnao *et al.*, 2003) [69, 70, 17]. Cryopreservation is the only current alternative for long-term conservation of species of Citrus, and this method has been extensively attempted using seeds and a wide range of other explants like zygotic embryos, embryonic axes, shoot apices, embryogenic callus, cell suspensions and somatic embryos (Malik *et al*., 2003; Malik and Chaudhury, 2006) [48, 47]. *In vitro* conservation and cryopreservation have been attempted at NBPGR and on a limited scale at National Botanical Research Institute, Lucknow. *In vitro* long-term preservation has been attempted in *C. aurantifolia* and in *C. grandis* using shoot apices (Chaturvedi, 2002) [12]. In *C. aurantifolia*, root cultures retained their ability to regenerate shoot buds when tested after three years of storage and regenerated plants with a normal diploid chromosome count. Similarly, shoot cultures of *C. grandis* were reportedly preserved for more than 32 years (Chaturvedi, 2002) [12]. In the case of highly recalcitrant seed species and wild, rare and endangered species like *C. ichangensis*, *C. indica*, *C. macroptera*, *C. megaloxycarpa* and *C. latipes*, *in vitro* conservation and cryopreservation ofshoot tips from *in vitro* plantlets are being attempted as alternative methods at NBPGR.

Flowering in Citrus

Citrus species are perennial trees requiring a long juvenile period of several years before the first flowers emerge. Subsequently, flowering becomes an annual process in many relevant Citrus varieties, broadly dependent on seasonal and climatic conditions. In subtropical regions, the major bloom occurs during the spring flush, after flower induction on quiescent buds by a low temperature period during winter time (Moss, 1969; Nebauer *et al*., 2006; Valiente and Albrigo, 2004) [54, 60, 90]. However, under tropical conditions, sprouting and flowering may occur over the whole year although the main bloom still takes place during the spring (Monselise, 1985; Reuther and Rios-Castano, 1969; Spiegel-Roy and Goldschmidt, 1996) [51, 73, 82]. It is generally believed that in these areas, water stress caused by drought periods in regions with a dry season substitutes the low temperature requirement of Citrus as the major flower induction agent (Cassin *et al*., 1969; Reuther and Rios-Castano, 1969) [9, 73]. Similarly, water deficit in subtropical climates has also been proved to increase the ratio of reproductive shoots and the total number of flowers (Southwick and Davenport, 1986) [95]. Different common practices and treatments affecting flower production and commercially usedto alleviate alternate bearing include pruning, girdling, defoliation, nitrogen fertilization and gibberellin application (Agusti, 2003; Guardiola *et al*., 1982) [1, 29]. Interestingly, gibberellins play an inhibitory role on citrus flowerbud induction and differentiation, as in many other woody trees.

Breeding Methods and Techniques

Citrus breeding programs have different objectives for scions and rootstocks. For scions, breeding programs aim mainly to improve specific traits depending on the target group, i.e. Oranges, Mandarins, Pummelos, Grapefruits or Lemons. Due to the low genetic diversity found within Oranges, Clementines, Satsumas, Lemons and Grapefruits, detecting or inducing mutations in elite cultivars are the main source of variability. In these groups, seedlessness, expanding the harvest period and reducing the furanocoumarin content (for Grapefruits) are principal breeding objectives. Regarding Mandarins and Mandarin-like hybrids destined for fresh consumption, breeding programs are directed to obtain easy-peeling, high-quality fruits (seed lessness, attractive fruit color, good sugar/acid ratio and flavor), good postharvest behavior, disease resistance and expanding the ripening season (earlier and later-ripening cultivars). More recently, there is increasing interest in fruits with high levels of beneficial compounds such as the health-promoting anthocyanins, which has become an objective in some citrus breeding programs (Navarro *et al*., 2015; Rapisarda *et al*,. 2003, 2008; Russo *et al*., 2014) [55, 72, 75]. Citrus growers are confronted worldwide with increasing biotic constraints, and therefore breeding programs are also focused on obtaining new cultivars resistant to diseases.

The main objectives in rootstock-breeding programs are resistance or tolerance to biotic (nematodes, Phytophtora, viruses, HLB) and abiotic stresses (flooding, drought, salinity, iron chlorosis), adaptation to different soil conditions (acid, calcareous) and the influence of early higher yield and fruit quality in the scions. In addition, some programs are aimed at obtaining dwarf rootstock to adapt Citrus to modern intensive cultivation techniques.

Mutation breeding

Selection of spontaneous mutants is the oldest and the most efficient breeding method in Citrus and most of the varieties cultivated worldwide arose from this process (Aleza, 2015) [55]. Since 1935, various mutagenesis agents, mainly gamma irradiation, have been used to obtain new cultivars (Aleza, 2015) [55]. The main advantage of this method is the preservation of the genetic background of the original cultivar and the modification of only one or a small number of agronomical traits. Another advantage of this technology is its simplicity (it is not necessary to have previous knowledge of gene control traits), rapidity (resulting trees will not display juvenile phase) and inexpensiveness. The main disadvantages are the large populations needed to find desirable stable mutations and the frequent chimeric status of the mutations. Star Ruby cv. Grapefruit was the first commercial cultivar obtained by irradiating seeds of cv. Hudson Grapefruit; later, Rio Red cv. Grapefruit was obtained by irradiation of Ruby Red cv. Grapefruit (Hensz, 1971) [31]. This technique is mainly used for obtaining diploid low-seeded genotypes and there are many examples of recently released seedless cultivars like Nulessin and Nero from cvs. Clemenules Clementine (López-García *et al*., 2015) [44]; Mor and Murina (Bermejo *et al.*, 2012; Vardi *et al.* 1993) [6, 92]; Murcott tangor and Orri from Orah Mandarin (Vardi *et al*., 2003) [91]; and Tango from Nadorcott tangor (Roose and Williams, 2007) [74].

Nearly all mutation breeding work aimed at inducing seedlessness has been conducted with citrus, reflecting the commercial importance of this trait and the relative ease with which it can be manipulated. The progenitors of cultivated Citrus were intrinsically fertile, producing abundant flowers highly attractive to pollinating insects, copious quantities of pollen and very large numbers of seed per fruit. It is not surprising then that mutagenesis, with its well-established detrimental effects on fertility, has been used so successfully in a crop where seedlessness is commercially desirable. However, the extent to which seed number is reduced following irradiation can vary enormously within the same genotype treated under identical conditions and breeding programs invariably develop a number of putative low-seeded selections from each progenitor genotype. These low-seeded selections are then subject to broader testing before the best cultivar can be identified and commercialized. It has proven very easy to obtain statistically significant reductions in seed number, even with quite small mutation breeding populations. However, achieving complete seed lessness or even very low seed numbers has proven far more difficult, except perhaps in highly parthenocarpic Lemons and Mandarins (Williams and Roose, 2010) [96], pointing to the importance of having strong parthenocarpic traits within breeding populations. Some successes with mutation breeding (Smith, 2006) [83] have resulted in cultivars that are still too seedy for many markets, particularly as consumers increasingly demand truly seedless fruit. More research is needed on techniques to increase the efficiency with which completely seedless accessions can be generated and mutation programs that combine commercial objectives with genomic analysis (Talon *et al*., 2004) [87] may help in this regard.

Polyploidy breeding

The potential of polyploidy as a means of achieving improved Citrus cultivars has long been recognized, various origins of Citrus polyploidy and their uses in crop improvement and genetic manipulations have been discussed (Lee, 1989) [42]. Polyploids such as triploids, tetraploids, pentaploids, hexaploids and octaploids are found spontaneously in Citrus. Well known are for instance tetraploid Hongkong wild kumquat (Longley, 1925) [43], triploid Tahiti lime (Bachi, 1940) [4] and tetraploid Triphasia desert lime (Esen & Soost, 1972) [19]. Triploids show more vigor than tetraploids and have thick round leaves. Only a low proportion of triploids have produced satisfactory yield so far (Soost & Cameron, 1975) [84]. However, triploids obtained from Citrus breeding were found the most resistant to low temperature. Tetraploids grow more slowly, are compact in habit and yield less than diploids of the same cultivar. Leaves of tetraploidsare broader, thicker and darker in color than leaves of diploids. Yields vary with selections that might have less economic value but are important as breeding material. Underdeveloped seeds are found with considerably high frequencies of polyploids in several polyembryoniccultivars of Sweet orange and Tangor (Wakana *et al.* 1981) [93].

Diploidy is the general rule in Citrus and its related genera with the basic chromosome number x=9 (Krug, 1943) [40]. However, some polyploid genotypes were detected early in Citrus germplasm, such as triploids (Lapin, 1937) [41] and tetraploids (Cameron and Frost, 1968; Lapin, 1937) [41]. The importance of ploidy manipulation in Citrus is reflected in three practical applications: (i) haploids for genome sequencing and obtaining double haploids (ii) triploids for obtaining seedless cultivars (iii) tetraploid to be used as parents in triploid breeding programs and for rootstock tree-size control. The selection of triploid lines is the classic route to develop seedless cultivars, as triploids are generally both male and female sterile. Thus, most of the trees of a triploid progeny under field evaluation should be seedless and consequently an efficient selection for other traits is possible. Moreover, larger fruit size associated with triploidy should correct the reduction of fruit size generally observed in seedless mutants of seedy cultivars. This strategy is being developed by several groups worldwide and new avenues have been opened by biotechnology.

Several methods have been developed for triploid Citrus creation (Aleza *et al*., 2010a; Navarro *et al*., 2003) [2, 58].

One of them exploits natural events of polyploidization such as 2*n* gametes, using embryo rescue and flow cytometry to select triploids in 2*x* X 2*x* crosses. Second meiotic division restitution (SDR) has been proposed for diploid mega gametophyte development in Clementine (Luro *et al*., 2004) [46], while reported first meiotic division restitution (FDR) in Sweet oranges. The classic strategy is to cross diploid monoembryonic females with tetraploid males. Such tetraploid plants can be found in apomictic seedlings (natural doubling of the chromosome stock of nucellar cells) or they are created by somatic hybridization (Grosser *et al*., 2000) [28]. Recently, tetraploid monoembryonic lines have been obtained by colchicine treatment of shoot tips grafted *in vitro* (Juarez *et al*., 2004; Navarro and Juarez, 2007) [36, 57].

Somatic Hybridization

Somatic hybridization in Citrus is performed by the fusion of protoplasts derived from leaf mesophyll with protoplasts derived from embryogenic callus. In Citrus, it has not yet been possible to regenerate plants from leaf protoplasts. Protoplasts isolated from embryogenic callus or leaf protoplasts that incorporate the mitochondrial genome from callus protoplasts are the only ones that have the ability to produce embryos and subsequently, plants (Kobayashi *et al.*, 1991; Grosser and Gmitter, 2005; Guo *et al*., 2007) [39, 27, 30].Various limitations presented by complicated reproductive biology of citrus can be successfully overcome through somatic hybridization by generating inter-specific and inter-generic allotetraploid somatic hybrids of desired cultivars for scion as well as rootstock development (Grosser *et al*., 1988; Ohgawara *et al.,* 1994; Grosser *et al.,* 1996) [26, 63] that can be utilized in breeding programs.

Somatic hybridization is now involved in five primary strategies to develop improved Citrus varieties. For scion improvement, the primary strategy is to produce allotetraploid breeding parents by combining complementary elite scion varieties. Pollen from such hybrids can be used in interploid crosses with selected monoembryonic diploid females to produce seedless triploid hybrids for selection (Grosser and Gmitter, 1996) [25]. A second way for producing improved scions is to produce triploids directly by haploid + diploid protoplast fusion (Deng *et al*., 1992a; Ollitrault *et al*., 1997, 1998b, 2000a) [15, 65, 67, 68]. Two strategies being employed for rootstock improvement are: (1) to produce somatic hybrids of complementary rootstock parents that have potential for improved disease resistance, tree size control, and horticultural performance (Grosser and Gmitter, 1990; Grosser *et al*., 1996a; Ollitrault *et al*., 1998a) [34, 67, 25] and (2) to produce wide hybrids of Citrus with related genera to broaden the germplasm base, including sexually incompatible or difficult to hybridize Citrus relatives (Grosser *et al*., 1996b; Guo and Deng, 1999) [25]. A final option is the production of Citrus somatic cybrids, which may have potential in both scion and rootstock improvement.

First instance of production of Citrus somatic hybrids and cybrids via electrochemical protoplast fusion was provided by Olivares-Fuster *et al.,* (2005) [64], where protoplasts of Sweet orange and Mexican lime were induced to undergo fusion in presence of polyethylene glycol (PEG) and electric impulses of direct current and exhibited high rates of embryogenesis. First somatic hybrid in citrus was produced between *Citrus sinensis* and *Poncirus trifoliata*was intergeneric in nature (Ohgawara *et al.,* 1985) [62]. Somatic hybrid between Caipira Sweet orange, a blight tolerant variety and Rangpur lime, a potential drought tolerant rootstock in Brazil was developed by PEG mediated fusion for use as a vigorous rootstock (Gloria *et al.,* 2000) [23]. Hybrid between 'Hamlin' sweet orange and Rangpur lime has also been produced in the same way (Louzada *et al.*, 1992) [45].

Molecular Breeding

Characterization and assessment of diversity is essential for the identification of distinct genotypes, for deciphering genetic relationships including parentages and for efficient management and utilization of germplasm. Morphological characterization is still the basic and initial step for diversity assessment before employing any other advanced methods. Although the Citrus genus is very complex involving multitude of species and their hybrids, but still the morphological characters especially the fruit and leaf characters are useful for visual scoring and distinguishing accessions within a species. It allows simple grouping of accessions, development of core collections, identification of gaps in collection, identifying specific germplasm for breeding programmes etc. On the other hand, molecular markers are of utmost importance for characterization studies in *Citrus* sp. because of its complex taxonomy and phylogeny. Studies at molecular level including the use of markers (RAPD, ISSR, AFLP, SSR's) and sequence analyses of rbcL and matK gene region of chloroplast DNA (Uchoi *et al*. 2016) [89] has been undertaken to infer the phylogenetic relationships between different species. DNA studies using as well as SSR's have been considered as almost ideal markers for genetic diversity analysis because of their reproducibility, multiallelic nature, co-dominant inheritance, relative abundance and good genome coverage. These markers are popular tools in genetics and breeding because of their relative abundance compared to other molecular marker types, high degree of polymorphism (number of variants), and easy assaying by PCR (Zhu *et al*. 2012) [99].

Genetic Transformation

Genetic transformation allows the introduction of specific traits into known genotypes without altering their elite genetic background. This applies to all Citrus species, and particularly to improved commercial species such as *C. sinensis* (Sweet orange), *C. limon* (Lemon) and *C. paradisi* (Grapefruit)and Mandarin groups such as Clementines and Satsumas, where due to their highly heterozygous and complex genetic structure; genetic transformation should be considered as the most promising tool for improvement. Genetic transformation methodologies in Citrus are based either on somatic embryogenesis (Hidaka *et al*., 1990; Fleming *et al*., 2000; Duan *et al*., 2007) [34, 21, 16] or more commonly organogenesis from *in vitro* growing seedling explants or internodes from greenhouse-grown plants (Moore *et al*., 1992; Pena *et al*., 1995) [52, 69, 70].

The genetic transformation procedure involves two major processes. The first is the incorporation of the foreign gene of interest into the plant genome while the second entails the regeneration of the transformed cells into whole transgenic plants (Singh & Rajam, 2009) [79]. The success of the genetic transformation technique depends on an effective and reliable procedure as efficiencies are often low. Several techniques such as polyethyleneglycol (PEG)-mediated direct uptake of DNA by protoplast (Kobayashi & Uchimaya, 1989) [38], particle bombardment (Yao *et al*., 1996) [97] and *Agrobacterium*-mediated transformations (Hidaka & Omura, 1993) [33] have been developed and used with various *Citrus* spp. However, the latter transformation system is now the most commonly used method because it has been proven most successful with higher transformation efficiencies resulting in the production of transgenic plants (Pena *et al*., 2007; Singh & Rajam, 2009; Yu *et al*., 2002) [71, 79, 98]. A very important step for utilization of genetic transformation for Citrus breeding was the successful transformation of mature plant material to overcome the juvenile stage (Cervera *et al*. 1998, 2008) [10, 11].

Shoot tip grafting

Morel and Martin (1952) [53] for the first time demonstrated that virus free plants of Dahlia could be recovered from infected plants through shoot tip culture. This technique, commonly referred to as meristem culture, has been applied to produce virus free plants of different species (Hollings, 1965) [35]. The technique of shoot tip grafting (STG) is a method in which the shoot tips about 0.2 mm was grafted on to aseptically grown, etiolated two-week-old seedlings, was developed by Murashige *et al.* (1972) [59] and later on Navarro *et al.* (1975) [59] improved the shoot tip grafting technique by inverted-T method of incision. Chaturvedi and Sharma (1988) [13] reported that shoot tip grafting is the last resort for virus elimination as shoot meristem of Citrus could not be grown successfully through *in-vitro* culture. STG had been employed in disease free germplasm exchange, physiological and histological studies of graft-incompatibility. In addition, shoot tip grafting of imported bud material is also effective in reducing the risk of introducing disease from one country to another. Shoot tip grafting is simply based on the principle that virus pathogens do not always invade newly developing cell tissue as it is rapidly dividing and expanding.

Conclusion

Citrus, a diverse genus within the Aurantioideae subfamily, plays a crucial role in global horticulture. Varieties such as Pummelos, Citrons, and Mandarins have origins in distinct geographic regions. Fortunella (kumquats) and Pon cirus (*P. trifoliata*) are essential contributors to rootstock breeding due to their biotic resilience. In India, the genus displays extensive genetic diversity, encompassing 24 species and numerous varieties, necessitating effective conservation methods. Conservation efforts, including *in vitro* conservation and cryopreservation, are vital for safeguarding endangered species such as *C. indica* and *C. ichangensis*. Breeding programs aim to enhance scion traits like seed lessness and fruit quality, alongside improving rootstock attributes such as disease resistance and soil adaptability. Techniques employed include mutation breeding, polyploidy induction, somatic hybridization, and genetic transformation. Molecular tools play a crucial role in assessing genetic diversity and providing phylogenetic insights, thereby advancing breeding strategies. In summary, this review highlights the collective importance of taxonomy, genetic diversity assessment, breeding methodologies, and conservation strategies in advancing Citrus improvement and global cultivation practices.

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