Citrus Breeding

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1 Introduction

With a total world production of 105.4 million tons, citrus is one of the world's most important fruit crops (FAO 2006). Its importance to agriculture and the world's economy is demonstrated by its wide distribution and large-scale production (Soost and Roose 1996). It is grown throughout the tropical and subtropical regions of the world where the winter temperatures are sufficiently moderate for tree survival and enough water is available for its growth (Gmitter et al. 1992). The best fruit quality is achieved under subtropical conditions. The most significant citrus-producing regions are in the Americas (Brazil, USA, Argentina, and Mexico primarily), the Mediterranean basin (Southern Europe, Southwest Asia, and North Africa), Asia (including China, India, and Japan) and South Africa. Citrus industries in many production areas generate substantial regional revenue. Brazil, USA, China, Mexico, and Spain are the five largest citrus producers in the world (Table 1, FAO 2006). Sweet orange is grown on about 3.6 million ha in 114 countries with an approximate production of 64 million tons (t) with Brazil being the largest producer. The world production of grapefruits and pummelo is 4 million t and is grown in 74 countries on about 264,000 ha. USA is the largest producer of grapefruits and pummelo. China produces 38% of 18 million t of mandarins and their hybrids produced in the world. Lemons and limes are produced in 94 countries on about 0.8 million ha with a production of approximately 7.7 million t with Mexico being the largest producer. Brazil and Florida (USA) produce citrus fruit primarily destined for the juice or concentrate market, while China, Mexico, Spain, and California (USA) produce primarily fresh-market fruit. Citrus is valued as a fresh fruit and is also processed into juice, or added to dishes and beverages.

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Table 1 Top ten *Citrus*-producing countries in the world

Country	Production (000 MT)
Brazil	20,576
China	10,395
USA	14,985
Mexico	6490
Spain	5103
Italy	3285
Argentina	2430
Egypt	2688
Turkey	2450
South Africa	1683

(Source: FAO 2006)

Citrus is widely produced in dooryard plantings for personal and local consumption as well.

2 Origin and Domestication

The center of origin and diversity of citrus and its related genera is generally considered to be Southeast Asia, especially East India, North Burma, and Southwest China, possibly ranging from Northeastern India eastward through the Malay Archipelago, north into China and Japan, and south to Australia (Tanaka 1954; Webber 1967; Scora 1975, 1988; Gmitter and Hu 1990; Soost and Roose 1996). The oldest known reference to citrus appears in Sanskrit literature that dates to before 800 BC followed by descriptions in Chinese, Greek, and Roman literature (Webber 1967; Scora 1975). The first citrus fruit to arrive in Europe was the citron, followed by the sour orange, lemon, and sweet orange (Webber 1967). The colonial expansion of Europe introduced citrus to the rest of the world, including the Americas, South Africa, and Australia. Recent evidence supports the view that Yunnan Province in the Southwest China may be the center of origin as a diversity of species is found there (Gmitter and Hu 1990). Although the exact routes of dispersion of citrus from its origin are unknown, the network of rivers in the Yunnan Province area could have provided a natural route for dispersal to the south (Sauer 1993). Most scion and rootstock cultivars that are widely grown in the main commercial producing areas of the world originated as either chance seedling selections or bud sport mutations (Hodgson 1967). In recent decades, however, with increasing capabilities for genetic improvement of citrus afforded by new technologies, there have been increasing numbers of new cultivars released from breeding and genetic improvement approaches, and that trend will accelerate in the coming decade.

3 Botanical Aspects

3.1 Taxonomy

The genus *Citrus* belongs to the subtribe Citrinae, tribe Citreaea, subfamily Aurantioideae of the family Rutaceae. This genus may be further divided into two subgenera (*Citrus* and *Papeda*), based on leaf, flower, and fruit properties. The evolution of modern citrus cultivars and their diversity has been addressed (Swingle and Reece 1967). On the basis of morphological characteristics, studies have been carried out on the relationships between genera and species. This has led to the formulation of numerous classification systems. The most commonly used citrus classifications are by Swingle (Swingle and Reece 1967) and Tanaka (Tanaka 1977). In the genus *Citrus*, Swingle recognized only 16 species, whereas Tanaka recognized 162 species. The difference in these two systems reflected opposing theories on what degree of morphological difference justified species status and whether presumed hybrids among naturally occurring forms should be given species status (Soost and Roose 1996). A comprehensive phylogenetic study by Barrett and Rhodes (1976) evaluated 146 morphological and biochemical tree, leaf, flower, and fruit characteristics; they concluded that there were three biological species of the so-called edible citrus types, with several other wild species. However, Scora (1975) suggested that only three citrus types, citron (*C. medica*), mandarin (*C. reticulata*), and pummelo (*C. grandis*; now *C. maxima*), constituted valid species and viewed all others as introgressions of these ancestral forms. Recently, molecular marker studies have supported this hypothesis (Nicolosi et al. 2000). Lime (*C. aurantifolia*), *C. micrantha*, and *C. halmii* are also included in the list of 'true' citrus species by many researchers. Papeda is a group of *Citrus* species (*C. ichangensis, C. micrantha, C. latipes, C. celebica, C. hystrix, and C. macroptera*) having inedible fruit with acrid oil droplets in the juice vesicles. Understanding taxonomy, phylogenetic relationships, and genetic variability in citrus is critical for determining genetic relationships, characterizing germplasm, controlling genetic erosion, designing sampling strategies or core collections, establishing breeding programs, and registering new cultivars (Herrero et al. 1996). The phylogeny and genetic origin of important species of citrus has been investigated using molecular markers (Nicolosi et al. 2000; Moore 2001; Berkeley et al. 2006); however, these studies have not been able to clearly differentiate all the species. Hence, there is a need for additional taxonomic studies to further clarify the taxonomic distinctions.

3.2 Geographical Distribution

Citrus is commercially grown in the tropical and subtropical regions around the world, primarily between the latitudes of 40°N to 40°S, from equatorial, hot-humid climates through warm-subtropical and even cooler maritime climates (Spiegel-Roy and Goldschmidt 1996). The warm, humid semitropical climate enables the production of large quantities of fruit suitable for processing and is also suitable for grapefruit production. The cool, coastal valleys are suitable for the production of lemons. High-quality sweet oranges are grown in the intermediate valleys, which have semi-arid, subtropical climates. The desert valleys have hot, arid climates suitable for the production of grapefruit and certain types of lemons and mandarins. There is some overlap in the types of fruits produced in the different growing areas (CDCGC 2004).

3.3 Morphology

Citrus plants are small to medium sized, spreading, evergreen trees with thorny shoots, growing to about 2–15 m tall. Distinctive growth habits, ranging from spreading to upright to weeping, are observed among various species and cultivars. Most species are single-trunked with very hard wood (Manner et al. 2006). The main branches diverge from the trunk at 60–120 cm above the ground, depending on whether they are seedling trees or grafted to rootstocks, which is the most common commercial production approach (Schneider 1968). The general branching system of cultivated varieties of citrus gives the top or crown of the tree a more or less spherical shape. Trees produced from seeds tend to have more thorns than trees produced from grafting (Manner et al. 2006). The stem is green, with unifoliate, alternate leaves. Leaf shape varies from lance-shaped to round, and the size varies from 4 to 10 cm in length. Some types of leaves possess more or less broadly winged petioles. Leaves contain citrus oil glands, which are released when crushed (Manner et al. 2006). Flowers are fragrant, borne solitary or in short cymes in the axils of the leaves or in small lateral or terminal inflorescences. The flowers are usually white but sometimes pink or purple pigmented (in lemon and citron, and their hybrids with other citrus), perfect with five thick, linear, strap-shaped petals and four- to five-lobed sepals. The petals alternate with the sepals (Schneider 1968). There are usually four times as many stamens as petals. The stamens are polyadelphous, cohering toward the bases in a few bundles. The yellow, four-lobed anthers surround the pistil at or near the level of the stigma (Spiegel-Roy and Goldschmidt 1996). The ovary is superior and composed of 6–14 carpels joined to each other and to a central axis (Soost and Roose 1996). The ovary has a prominent but usually deciduous style containing as many tubes as there are cells in the ovary. The fruit is a hesperidium berry. It is a true fruit arising through growth and development of the ovary, consisting of a variable number of united, radially arranged carpels (Spiegel-Roy and Goldschmidt 1996). The forms and sizes of fruits vary from globose to oblong and oblate. They are highly fragrant and full of flavor and juice. The outer rind is known as flavedo (exocarp and endocarp).

It is covered with tiny pockets containing aromatic oils. The albedo (mesocarp) covers the endocarp. The pulp (endocarp) is divided into 10–14 sections containing specialized structures, the juice vesicles (sacs). They are separated by thin septa (Manner et al. 2006).

4 Reproductive Biology

4.1 Flowering

Although seasonal conditions may cause citrus to bloom at various times, the main blooming period of citrus trees in subtropical climates is in the spring (Erickson 1968). The environmental and endogenous control of flower bud differentiation is quite complex and varies considerably from one species to another (Spiegel-Roy and Goldschmidt 1996). The induction of flower buds begins with a cessation of vegetative growth during the winter rest period in subtropics or dry periods in tropical regions (Davies and Albrigo 1994). Flowering shoots are most commonly produced in citrus on woody twigs of the previous year's spring flush but may also be borne on younger, summer flush twigs or on older wood (Spiegel-Roy and Goldschmidt 1996). The vegetative meristem undergoes histological and morphological changes to differentiate into a floral meristem (Davenport 1990). Cassin et al. (1969) demonstrated that cold or water stress are the primary inductive factors, with cold being the primary factor in subtropical climates and water stress in tropical climates. To induce a significant number of flower buds, temperature below 20°C or drought periods longer than 45–50 days are required (Cassin et al. 1969). Induction of flowering by low temperatures or water stress was correlated with an increase in leaf ammonia content (Lovatt et al. 1988). The low-temperature induction of flowering in citrus has been shown to be accompanied by a decrease in endogenous gibberellic acids. Carbohydrate levels have been suggested as playing a role in the control of flowering. Girdling healthy trees in the early fall, and the consequent accumulation of carbohydrates above the girdle, usually increases flowering on healthy, nonjuvenile trees (Goldschmidt et al. 1985). Citrus trees usually bloom heavily; however, a comparatively small percentage of flowers produce mature fruit, since many flower buds and flowers drop before fruit set (Erickson 1968).

4.2 Pollination and Fertilization

Pollen is of sticky, adherent type. Honeybees are very effective for cross-pollination, but wind is a minor factor in its transfer from flower to flower. Development follows the usual course for angiosperm pollen. Production of functional pollen varies tremendously among the various species as well as within the species. Several cultivars are pollen and ovule sterile. Most citrus cultivars are self-pollinated. The commercially important citrus species do not require cross-pollination generally (Davies and Albrigo 1994). Self-pollination can easily occur because of the proximity of anthers to stigma (Spiegel-Roy and Goldschmidt 1996). Some types are parthenocarpic, setting and maturing commercial crops of seedless fruit without fertilization and seed set. An exception to this is certain mandarin types and hybrids, which require cross-pollination (or in some cases self-pollination) to set fruit satisfactorily. Temperature also has a significant effect on pollination efficiency. The bee activity in the orchard is adversely affected when temperatures are below 12°C (Spiegel-Roy and Goldschmidt 1996). Even the pollen viability in some types, such as Satsuma mandarins, is dependent on the temperature (Soost and Roose 1996).

The germination and growth rates of the pollen grains which have landed on the stigma are enhanced at high temperatures (25–30°C). Low temperatures (<20°C) reduce or totally inhibited pollen germination. The arrangement and percentage of the planting of the pollenizer variety within the orchard are also important for successful pollination (Davies and Albrigo 1994). The pollen tube germinates and penetrates the embryo sac in the ovule. Fertilization occurs by fusion of a sperm (pollen) nucleus with an egg nucleus. Two microgametes are produced by the generative nucleus of the pollen. One microgamete fuses with the egg nucleus producing the zygote, while the other fuses with the two polar nuclei initiating the endosperm (Banerji 1954). Fertilization of the egg cell occurs 2 or 3 days after pollination under favorable conditions (Spiegel-Roy and Goldschmidt 1996).

4.3 Fruit Set

The term ‘fruit set’ is commonly used to describe the process through which the flower ovary adheres and becomes a fruit (Spiegel-Roy and Goldschmidt 1996). The appearance of a brown ring between the ovary and the style is the first sign of fruit set. The initial rate of fruit set, as observed soon after petal fall, is reduced markedly during the fruitlet abscission period. The percentage set expresses the ratio between the rather small, final number of fruit and the initial, very large number of flowers. Most of the commercially important cultivars produce around 100,000–200,000 flowers on a mature tree; however, the percentage of harvestable fruit is only 1–2% (Davies and Albrigo 1994). From flowering until 3–4 weeks postbloom, an initial drop period occurs. During this period, weak flowers and fruitlets with defective styles or ovaries, or flowers that did not receive sufficient pollination, abscise. The type of inflorescence and the position of individual flowers also affect fruit set. Most of the fruit set on leafless inflorescence drop and the crop is eventually borne on leafy inflorescence (Goldschmidt and Monselise 1978). The leaves of the leafy inflorescence have been assumed to play a role in provision of photosynthate, mineral nutrients, or hormones to facilitate persistence of the young fruit (Spiegel-Roy and Goldschmidt 1996). The better water transport capacity of leafy inflorescence shoots may be responsible for the higher rate of fruit set (Erner 1989). Depending on the variety grown and the growing area, fruit development may take 5–18 months. When the fruits have reached maturity but prior to harvest, preharvest drop occurs. Spraying with a combination of gibberellic acid and 2,4-D can retard this fruit drop.

4.4 Fruit Ripening

Fruit growth of most citrus cultivars follows a sigmoid pattern, which can be divided into three phases (Bain 1958). Phase I is the cell division phase in which all the cells of the mature fruit are produced and the cells differentiate into various tissue types. It may be assumed to commence at fruit set. The increase in fruit size during this phase is mainly due to growth of the peel. Cell division appears to terminate in all fruit tissues, except the outermost flavedo layers and the tips of juice sacs, within 5–10 weeks after bloom (Spiegel-Roy and Goldschmidt 1996). The peel reaches its maximum width at or soon after the end of phase I. Phase II is the cell enlargement phase and produces a rapid increase in the fruit size. The percentage of total soluble solids also increases during this phase. The rapidly expanding pulp exerts pressure outward on the peel, which stretches and becomes increasingly thinner. During phase III or the maturation phase, the color of the peel begins to change from green to yellow or orange. The color of the peel results from a combination of pigments including chlorophyll, carotenoids, anthocyanins, and lycopene. However, this external color change is a poor indicator of maturity; it is a function of climate more than fruit maturity. Fruit growth rate is primarily a function of temperature during each developmental stage with the highest mean temperatures providing the fastest fruit growth rates. Tree vigor also has a pronounced effect on fruit color. Vigorously growing trees produce more poorly colored fruit than slow-growing trees. Citrus matures slowly and once harvested, it does not continue to ripen. The maturity of the fruit is determined by gradual changes in juice content, and sugar and acid levels. As the fruit matures, the acid content decreases and sugar content increases. Most citrus fruits can be left on the tree without becoming overripe, though they do become senescent. Citrus fruit has two abscission zones, one at the base of the pedicel and other at the base of the ovary. Two major kinds of abscission may be discerned during fruit development. Fruitlet abscission is a self-thinning mechanism that adjusts the number of fruits to the tree's bearing potential (Goldschmidt and Monselise 1978). On the other hand, the shedding of the mature fruit may be regarded as a mechanism of seed dispersal (Spiegel-Roy and Goldschmidt 1996).

4.5 Polyembryony

The formation of multiple embryos is quite common in many citrus cultivars. They may be the result of multiple zygotic embryos, produced by the fission of one fertilized egg or from two or more functional embryo sacs in a single ovule (Bacchi 1943; Cameron and Garber 1968). However, the predominant cause of multiple embryo formation is nucellar embryony, the development of vegetative embryos from the nucellus. These embryos are the outgrowths of the nucellus and develop asexually by mitotic division of the cells of the nucellus. As the male gamete does not contribute to their formation, they are the product of vegetative reproduction having a genetic constitution identical to that of the female (seed) parent (Nageswara et al. 2008). This asexual reproduction is an important characteristic in citrus and has very important consequences for the evolution, breeding, and culture of citrus fruits (Frost and Soost 1968). Anatomical studies of open and controlled pollinations have indicated that adventive embryos are initiated autonomously and develop with or without pollination (Wakana and Uemoto 1987). However, pollination is essential for the stimulation of nucellar embryo development as they fail to develop without endosperm development. Early degeneration of endosperm also results in very poor seed development and, eventually, in poor development of adventive embryos (Wakana and Uemoto 1987). These nucellar embryos grow more rapidly than the zygotic embryo within the seed. One possible reason for this may be that the zygotic embryo is located unfavorably in the apex of the embryo sac (Toxopeus 1936; Iwamasa et al. 1970), receives fewer nutrients, and is more subject to crowding pressure; in addition, zygotic embryos derived from self-pollination in normally heterozygous cultivars could be associated with inbreeding depression expressed in zygotic embryos (Toxopeus 1936). If polyembryonic genotypes are used in crossing as female parent, several nucellar seedlings similar to the mother plant and very few or no hybrids are produced. This characteristic allows the selection of improved mutants, which have better yield efficiency and fruit quality than the parent. The satsuma varieties ‘Mihu’ and ‘Okitsu’ are nucellar selections of ‘Miyagawa’. The plants arising from nucellar seedlings are generally free of viruses. Citrus are almost universally propagated by budding onto seedling rootstocks (Xiang and Roose 1988). Uniformity of the rootstock genotypes is essential for reliable performance following budding and orchard establishment. Nucellar embryony allows fixing the genotype of a superior variety, and hence seed can be produced for many generations without loss of vigor or genotype segregation, circumventing any need to produce hybrid seeds for rootstock production (Garcia et al. 1999).

5 Breeding

Citrus (most frequently $2n = 2x = 18$, though higher ploidy levels occur spontaneously and have deliberately been produced) is vegetatively propagated. Selection of new citrus and related cultivars has been occurring for many years by selection of superior phenotypes from the wild for cultivation. However, systematic, mission-oriented breeding programs first began in Florida in 1893 with Swingle and Webber (Davies and Albrigo 1994). Since then, numerous programs have been developed worldwide with a variety of objectives. Due to its heterozygous nature, sexual hybridization to create new genotypes results in substantial variation of the characters in the progeny as they produce widely variant sexually derived progeny. Nucellar embryos, on the other hand, give rise to genetically and phenotypically uniform progeny. A long period of juvenility is characteristic of citrus seedlings and is evidenced by the presence of thorns, vigorous upright growth, delay in fruiting, and alternate bearing. It takes 5 or more years for the first flowering to occur in citrus seedlings. This long juvenile period of seedlings makes citrus breeding not only a difficult but a costly and land-intensive proposition.

There is a lack of knowledge regarding genetic mechanisms controlling the inheritance of agriculturally important traits. Only a few important traits show single gene inheritance (Furr 1969; Gmitter et al. 1992). Conventional hybridization has given rise to a few new citrus cultivars and rootstocks (Soost and Cameron 1975; Cameron and Soost 1984; Gmitter et al. 1992). Use of citrus cultivars and selections that give rise to only apomictic seeds as seed parents leads to the production of few or no hybrids as the apomictic seeds contain only asexual embryos. Due to inbreeding depression, crosses between closely related lines will produce primarily weak zygotic seedlings. Although it takes only minutes to effect a pollination, the difficult nature of citrus breeding lies in the elimination of undesirable hybrids and the evaluation of selections (Sykes 1987). Despite the fact that citrus breeding is very challenging, different breeding programs throughout the world have made significant progress in the application of conventional and modern approaches to genetic improvement and cultivar development. Important breeding goals exist in citrus with respect to both scions and rootstocks (Cameron and Frost 1968).

Conventional methods of breeding scion and rootstock cultivars are generally based on controlled crosses. To combine desirable traits from different selections, cultivars, or species in hybrid progeny, cross-pollination is carried out. After the hybrid fruits have matured, the seeds are extracted from the fruit and planted in the greenhouse. Once the seedlings have attained sufficient size, they are either grafted onto rootstocks or directly planted in the field for evaluation of their performance. The hybrids are evaluated for disease and pest resistance, stress tolerance, and overall growth characteristics during the juvenile period (Davies and Albrigo 1994), and subsequently for fruit characteristics of scions or rootstock traits of interest. Mutation breeding programs have also been established for the genetic improvement of citrus. Mutations have been induced by gamma rays and chemicals and the mutants analyzed for the desired traits (Hensz 1977; Hearn 1984; Deng and Zhang 1988; Deng et al. 1993; Gulsen et al. 2007).

5.1 Scion

The scion breeding programs are mainly aimed at improving the fruit color, size, shape, flavor, and yield, as well as low seed content, easy peeling, and disease resistance. The main breeding aims for scion cultivars vary with species and localities. The first step in scion breeding involves selection of parental types with favorable heritable characteristics/traits. Often those seed parents are selected that produce only zygotic progeny (Soost and Cameron 1975). Hence monoembryonic parents are preferred for scion breeding. It is traditionally achieved by controlled crossing. When there is a need to combine desirable traits/characteristics from different species, cross-pollination is carried out. The hybrid fruits are allowed to grow and harvested at maturity. The seeds are extracted from the fruit and planted in the greenhouse initially. Once the seedlings have attained sufficient size, they are then planted in the field where they are grown to fruiting and evaluated for their fruit characteristics. Once the desired scion hybrid is selected, it is budded onto different rootstocks to further evaluate its performance. The hybrids are also tested for biotic and abiotic stress tolerance, and their overall growth characteristics are also monitored. Multiyear and multilocation field trials are conducted to evaluate their performance with major emphasis on the fruit quality (size, shape, exterior rind characters, peel thickness, pulp characteristics, and seediness), and yield.

Most citrus cultivars have resulted from natural hybridization of well-adapted native cultivars, spontaneous mutation, bud sport mutations, or apomictic seedling mutants (Hodgson 1967). Many of the widely grown scion cultivar groups, such as sweet orange, grapefruit, lemon, and various clonal selections of certain mandarin cultivars such as ‘Satsuma’ and ‘Clementine’, originated as either bud sport mutations or apomictic seedling mutants. No cultivars of these have ever originated as sexually derived seedlings (Gmitter et al. 1992). They are not amenable to sexual hybridization as a genetic improvement strategy. Hence, selection of useful variations following induction via mutagenic treatment of seeds and axillary buds, from spontaneously occurring nucellar seed or bud mutations, or somaclonal variation (Grosser et al. 2003, 2007), have been the only effective approaches to cultivar development in these cultivar groups. The irradiated seeds of ‘Hudson’ grapefruit gave rise to ‘Star Ruby’ grapefruit, which had deep red flesh and reduced seediness (Hensz 1977). A low–seeded, grapefruit-like hybrid (USDA 77-19) was developed by

USDA citrus breeding program by irradiating the hybrid ‘USDA 75-8’ selected from a population of ‘Pearl’ tangelo × grapefruit (Chaparro 2003). A special objective of increasing significance is the breeding of grapefruit cultivars with low levels of acidity and less bitterness (Spiegel-Roy and Goldschmidt 1996). Trees propagated from irradiated buds of ‘Foster’ grapefruit gave a mutation in the acid metabolic pathway that resulted in low acid production and early fruit maturity (Yen 1987). Irradiated buds of ‘Kutdiken’ lemon have given rise to plants showing variations for fruit maturation time, flowering, branching habit, and thorniness (Gulsen et al. 2007). Seedlessness is a prime requirement for fresh fruit. Mutation breeding by irradiation of seeds and/or axillary buds has also given rise to seedless clones of normally seedy ‘Pineapple’ orange, ‘Duncan’, and ‘Foster’ grapefruit (Hearn 1984, 1985), ‘Monreal’ Clementine mandarin (Russo et al. 1981), ‘Eureka’ lemon (Spiegel-Roy et al. 1985, 1990; Miller et al. 2003), and ‘Kutdiken’ lemon (Gulsen et al. 2007). A seedless ‘Minneola’ tangelo has also been produced by mutation breeding (Spiegel-Roy and Vardi 1989). Irradiation of seeds of ‘Jincheng’ sweet orange has given rise to a seedless clone ‘Zhongyu No 7’ (Deng 2003). Irradiation of axillary buds of ‘Kutdiken’ lemon has also been used to obtain mutants resistant to *mal secco* (Gulsen et al. 2007). A seedless mandarin ‘Tango’ has been produced by irradiation of buds of W. Murcott (Roose and Williams 2006a). ‘Monreal verde’ is an almost seedless variety obtained by irradiating budwood of ‘Monreal’ Clementine (Nicotra 2001).

Mandarins are relatively easy to breed by crossing parents with good traits and selecting superior progeny. ‘Fairchild’ mandarin, a hybrid of ‘Clementine’ mandarin and ‘Orlando’ tangelo, has proven to be particularly well-suited to the California and Arizona deserts where it provides an early season fruit for the market (Furr 1964). ‘Encore’, a late-ripening variety, which originated from a cross between ‘King’ and ‘Willowleaf’ mandarins, was introduced in 1965 (Cameron et al. 1965). ‘Fallglo’ tangerine is a hybrid of ‘Bower’ and ‘Temple’ and was released in 1987 (Hearn 1987). USDA 88-2, a cross between ‘Lee’ and ‘Nova’ mandarins, is very juicy and easy-to-peel early season seedless mandarin with small to medium size fruit and is under evaluation for commercial potential. USDA 88-3 is a cross between ‘Robinson’ and ‘Lee’ mandarins and an early season mandarin; however, it is not considered for commercial purposes as the fruit is very seedy. ‘Tacle’ and ‘Clara’ seedless mandarins were obtained by crossing ‘Monreal’ Clementine and ‘Tarocco’ orange (Nicotra 2001). ‘Gold Nugget’, a seedless late-maturing diploid hybrid between ‘Wilking’ and ‘Kincy’, has been released (Roose et al. 2000; Roose and Williams 2003). ‘Daisy’, a hybrid between ‘Fortune’ and ‘Fremont’ mandarins, produces a medium-large, mid-season mandarin with an attractive dark orange rind with moderate peelability and sections. ‘Camel’ mandarin was a seedless hybrid of ‘Nules’ Clementine and ‘Willowleaf’ mandarin (Nicotra 2001). ‘Shasta Gold®’or ‘TDE2’ (a late maturing), Tahoe Gold®’ or ‘TDE3’ (mid-season maturing), and ‘Yosemite Gold®’or ‘TDE4’ (mid-late season maturing) are mandarin hybrids of ('Temple' tangor × 4*n* 'Dancy' mandarin) and 'Encore' mandarin that combine large fruit size, attractive deep orange rind color, rich fruit flavor, and the virtual absence of seeds (Roose and Williams 2006b). 'Winola' was a spontaneous triploid hybrid selected among a population of diploid hybrids between 'Wilking' mandarin and 'Minneola' tangelo (Nicotra 2001). 'Primosole', 'Simeto', 'Desiderio', 'Bellezza', 'Sirio', and 'Cami' are some other examples of seedless hybrids released (Nicotra 2001).

As large areas of citrus have low winter temperatures, several breeding programs also aim to incorporate cold hardiness (Spiegel-Roy and Goldschmidt 1996). Limequats (*Fortunella* × *C. aurantifolia*), citranges (*C. sinensis* × *P. trifoliata*), and citrumelos (*C. paradisi* × *P. trifoliata*) are some of the examples of intergeneric hybrids produced by controlled pollination with the objective of transferring cold tolerance to citrus. Although the cold tolerance was achieved, none of these intergeneric hybrids gained commercial acceptance as scion cultivars due to the transmission of negative fruit quality attributes along with cold tolerance (Gmitter et al. 1992). Reduced tree size without reduction in yield per unit volume is highly desirable as picking costs increase. Unusually early- or late-maturing new forms are always of interest, since they may fill a need in a pattern of production or marketing (Cameron and Frost 1968). Disease and pest resistance of the scion cultivars is desirable but difficult to achieve as gene sources are either not available or distantly related to the scion cultivar that recovery of acceptable cultivars is unlikely. Season of ripening, storage life, and adaptability to specific environments often determine the success or failure of new cultivars (Soost and Roose 1996).

Clonal selection can be used to isolate superior bud source strains of established cultivars and to eliminate propagation of undesirable budlines (Shamel 1943). Clonal selection within cultivar groups ('budline selection') has been useful for the development of improved strains of cultivar groups like *Satsuma mandarin* and navel sweet orange. Sexual hybridization will continue to receive attention for mandarin cultivar development because of increased numbers of improved monoembryonic breeding parents available for hybridization. Interspecific hybrids between mandarin-sweet orange (tangors) and mandarin-grapefruit (tangelos) have been developed by breeding programs (Gmitter et al. 1992).

5.2 Rootstock

The need for dependable new rootstocks is of primary concern as they affect all aspects of fruit quality. However, choice of rootstock is not usually based on fruit quality considerations alone; disease tolerance, soil type, and effects on yield are more often overriding considerations (Bevington 1987). Reduction of tree size without affecting yield or scion health is desirable (Soost and Roose

1996). The major objectives of rootstock breeding are aimed to control the tree size and to improve resistance and tolerance to biotic and environmental stresses such as citrus blight, CTV, *Phytophthora*, CVC, nematodes, cold, drought, salinity, and flooding. Rapid growth and lack of branching are desirable characters for convenient and economical nursery production of rootstock seedlings (Soost and Roose 1996).

Like scion breeding, rootstock breeding also involves controlled crossing. However, in rootstock breeding, highly polyembryonic species are selected as parents or at least one parent which is polyembryonic. Potential rootstocks showing favorable qualities are planted. Extensive multiyear and multilocation field trials are conducted to evaluate all aspects of rootstock performance. They are screened for resistance to various soil-borne diseases or stresses, compatibility with various scion cultivars, and effects on fruit quality and yield. Rootstock candidates are also screened for the seediness of fruit and the uniformity of seedling populations, as most citrus rootstocks are propagated from apomictic seeds.

Many commonly used rootstocks have not been products of planned breeding programs; rather they have been selected over time through grower experience. These include selections of various citrus species such as sour orange (*C. aurantium*), rough lemon (*C. limon*), Cleopatra mandarin (*C. reticulata*), Rangpur lime (*Citrus* × *limonia* Osbeck), and numerous others. Sexual hybridization has been used to produce genetically improved combinations of rootstocks. Carrizo and Troyer citranges (*C. sinensis* × *P. trifoliata*) and Swingle citrumelo (*C. paradisi* × *P. trifoliata*) rootstocks were selected from intergeneric hybrid progeny and were found to have *Phytophthora*, virus, and nematode tolerance inherited from *P. trifoliata*. Many other sexual hybrids have been made in efforts to exploit available genetic diversity for rootstock improvement in breeding programs around the world. A hybrid ‘US-852’ obtained from sexual hybridization of *C. reticulata* and *P. trifoliata* was found to exhibit outstanding effects on sweet orange fruit yield, producing fruit with high soluble solids on medium-size trees (Bowman et al. 1999). The IVIA in Spain has released four rootstocks, of which two (Forner Alcaide 5 and Forner Alcaide 13) were obtained by sexual hybridization between Cleopatra mandarin and *P. trifoliata*, one (Forner Alcaide 418) of Troyer citrange and *P. trifoliata*, and one (Forner Alcaide 517) of King mandarin and *P. trifoliata*. All the four rootstocks were resistant or tolerant to CTV and salinity. Forner Alcaide 5 was also found to be resistant to the citrus nematode and had good tolerance to calcareous soils and flooding. Forner Alcaide 418 was a dwarfing rootstock with good tolerance to calcareous soils and induced large fruits in the scion cultivar. Forner Alcaide 517 was also a dwarfing rootstock and had good tolerance to calcareous soils (Nicotra 2001; Forner et al. 2003). ‘X639’, a hybrid between ‘Cleopatra’ mandarin and *P. trifoliata*, has proved to be an excellent rootstock for lemons and mandarins; however, it is not resistant to nematodes and root pathogens (Miller et al. 2003).

6 Biotechnology

Traditional breeding approaches via sexual hybridization have not been useful for the genetic improvement of most of the citrus cultivars. Although spontaneous or induced mutations tend to be random and are not directed toward a specific target, they have resulted in varietal improvements. Application of the biotechnological sciences such as plant cell and tissue culture, genetic engineering, and genomics has the potential to unlock an entirely new round of genetic improvements for citrus crops. The genetic progress is impeded by barriers to sexual hybridization and the hybrid nature of important cultivated species. The scarcity of genetic information can be addressed and potentially mitigated by these biotechnological techniques (Gmitter et al. 1992).

6.1 Regeneration and Micropropagation

The direction for the genetic improvement of citrus is greatly impacted by the advances in plant cell and tissue culture. The amenability of citrus to be regenerated via organogenesis and somatic embryogenesis is the fundamental basis that makes possible much of the potential for these genetic advances. Plant regeneration systems are potentially useful for obtaining genetic change through cell transformation or mutagenesis. Organogenesis has been induced in vitro from various explants such as shoot meristems of seedling and mature trees (Barlass and Skene 1986; Omura and Hidaka 1992; Kotsias and Roussos 2001), nodal explants (Al-Khayri and Al-Bahrany 2001), stem internodes (Moore 1986), leaf sections (Chaturvedi and Mitra 1974; Huang et al. 2002), and root tissues (Sauton et al. 1982). In vitro culture of excised, fully developed embryos (Maheshwari and Rangaswamy 1958), early heart-shaped embryos (Rangan et al. 1969), globular embryos within undeveloped ovules of mature fruits (Starrantino and Russo 1980; Carimi et al. 1998), and immature embryos (Cavalcante-Alves et al. 2003) has also been used to recover plants. In vitro seedling explants have also been used for multiple shoot formation and/or regeneration (Normah et al. 1997; Yang et al. 2006). Regeneration has also been achieved by culturing thin sections of mature stem segments (Kobayashi et al. 2003; Soneji et al. 2007a)

Somatic embryogenesis is particularly attractive in citrus because many genotypes have the capacity for nucellar embryony (Soost and Roose 1996). Somatic embryogenesis has been induced directly in cultured nucelli (Rangan et al. 1969) and undeveloped ovules (Starrantino and Russo 1980; Gmitter and Moore 1986) or indirectly via callus formation (Kochba and Spiegel-Roy 1973; Button 1978; Koc et al. 1992; Tomaz et al. 2001; Kayim and Koc 2006). Embryogenesis has also been induced from endosperm-derived callus (Gmitter et al. 1990), juice vesicles (Nito and Iwamasa 1990), anthers (Chaturvedi and Sharma 1985;

Chiancone et al. 2006), styles (Carimi et al. 1995; Carimi et al. 1998; Calovic et al. 2003; D'Onghia et al. 2003), and pistil thin cell layers (Carimi et al. 1999).

6.2 Somaclonal Variation

The identification of valuable somaclonal variants holds great promise for cultivar improvement especially for the citrus species that are difficult to manipulate by sexual hybridization (Gmitter et al. 1992). Somaclonal variation has been observed in citrus plants regenerated from nucellar callus of monoembryonic 'Clementine' mandarin (Navarro et al. 1985). It is being exploited to identify sweet orange clones with improved traits such as fruit quality improvements across an extended season of maturity (Grosser et al. 2003). Preliminary observations of in vitro derived nucellar budlines of 'Navelate' sweet orange indicated that performance of two budlines may be superior to the others in terms of fruit quality and yield (Starrantino et al. 1990). Somaclones of 'Hamlin' and 'Valencia' have been obtained via regeneration of adventitious shoot buds, regeneration of secondary embryogenic callus via somatic embryogenesis, and/or regeneration from protoplast via somatic embryogenesis. Of these, early- and late-maturing somaclones, somaclones with fresh market potential, as well as somaclones with elevated soluble solids of 'Valencia' and 'Hamlin' with improved color are under field trial (Grosser et al. 2003). Callus lines have been selected for salt tolerance (Kochba et al. 1982; Spiegel-Roy and Ben-Hayyim 1985) and regenerated into plantlets. These plantlets lacked internodes and hence could not be propagated further (Ben-Hayyim and Goffer 1989). *C. limon* embryogenic culture lines resistant to 'mal secco' toxin were selected. These lines produced somatic embryos, which retained resistance to the toxin (Nadel and Spiegel-Roy 1987). 'Femminello' lemon somaclones have also been evaluated for tolerance to mal secco by artificial inoculation (Gentile et al. 2000). However, the field resistance in the mature plants has yet to be reported.

6.3 Ploidy Manipulation

Euploidy in citrus is represented by monoploids, diploids, triploids, tetraploids, pentaploids, hexaploids, and octaploids. Polyploid plants may offer considerable potential for cultivar improvement through exploitation of their horticulturally useful characteristics and as parents in breeding programs, particularly the triploid and tetraploid lines (Lee 1988).

Production of triploid hybrids is the most promising approach to obtain cultivars that do not produce seeds even with substantial cross-pollination (Navarro et al. 2004). Recovery of citrus sexual triploid hybrids ($3x = 27$) has been reported from $2n \times 4n$ (Cameron and Soost 1969), $4n \times 2n$ (Cameron and Burnett 1978), and 2*n* × 2*n* (Esen and Soost 1971) crosses. When the pistillate parent was used as tetraploid, 85% triploid progeny were recovered (Cameron and Burnett 1978). This high triploid recovery arises from normal sexual fertilization of the diploid female gamete with a haploid male gamete (Cameron and Burnett 1978). 'Shasta Gold®'or 'TDE2' (a late maturing), Tahoe Gold®'or 'TDE3' (mid-season maturing), and Yosemite Gold®'or 'TDE4' (mid-late season maturing) are triploid mandarin hybrids of tetraploid female parent ('Temple' tangor × 4*n* 'Dancy' mandarin) and diploid male parent 'Encore' mandarin that combine large fruit size, attractive deep orange rind color, rich fruit flavor, and the virtual absence of seeds (Roose and Williams 2006b). Compared to 4*n* × 2*n* crosses, 2*n* × 4*n* crosses gave rise to only 9% of triploid hybrids (Cameron and Soost 1969). 'Tacle' and 'Clara' seedless triploid mandarins were obtained by crossing diploid female parent 'Monreal' Clementine and tetraploid male parent 'Tarocco' orange, while 'Camel' mandarin was a triploid hybrid of diploid female parent 'Nules' Clementine and tetraploid male parent 'Willowleaf' mandarin (Nicotra 2001; Recupero and Tribulato 2003). 'Reale' isolated from a cross of 'Monreal' Clementine and *Fortunella hindisii* (4*x*) is interesting as an ornamental potted tree which is everblooming and early fruiting (Russo et al. 2003). In triploid progenies, the characteristics of many genes (fruit size, flavor, etc.) that are key factors for breeding programs seem to be strongly influenced by the tetraploid parent (Russo et al. 2004). In 2*n* × 2*n* crosses, the triploid embryos are originated by the fertilization of an unreduced diploid female gamete with a normal reduced haploid male gamete. Breeding triploids from 2*n* × 2*n* crosses eliminates the need for tetraploid parents, thereby overcoming the problem of aneuploidy which may result from irregular meiosis in the tetraploids (Esen and Soost 1971; Geraci et al. 1975). It is very difficult to regenerate plants by conventional methods from seeds with triploid embryos as these embryos are generally small in size, underdeveloped, or aborted. Embryo rescue has enabled breeders to rescue such genetically valuable embryos that otherwise would not develop into whole plants (Starrantino and Recupero 1982). Embryo rescue and culture in vitro are necessary because embryos that arise when diploid seed parents are crossed with tetraploid pollen sources do not undergo normal development. In normal (2*n* × 2*n*) crosses, the ratio of the endosperm chromosome set of maternal–paternal must be 2:1 for normal development of endosperm following hybridization. However, in 2*n* × 4*n* and 4*n* × 2*n* crosses, the ratio of the endosperm chromosome set of maternal–paternal is 2:2 and 4:1, respectively, leading to degeneration of endosperm. The imbalance between the embryo and endosperm ploidy (3:4 or 3:5) rendered seeds from such crossings incapable of germinating in vivo. Triploids have also been regenerated by in vitro culturing of hybrid endosperm (Gmitter et al. 1990). However, this method has not been adapted as a breeding strategy because it is species and cultivar dependent, and is far less efficient than creating triploid offspring by interploid hybridization. Regenerants of anther culture of *C. clementina* were found to be triploids

(Germana et al. 2005). Triploid citrus hybrids have also been obtained by electrofusion of a gynogenetic haploid cell line of Clementine and several diploid cultivars (Ollitrault et al. 2000)

Tetraploid progenies have arisen from all crosses of diploid and tetraploid parentage using monoembryonic seed parents. This may be due to nondisjunction leading to the doubling of chromosome number of one of the parents during cell division. Incorporation of colchicine in standard tissue culture media has made it possible to recover tetraploid plants of elite diploid selections or cultivars (Gmitter and Ling 1991; Gmitter et al. 1991). Tetraploids have also been produced by somatic hybridization (Grosser et al. 1998). The main uses of citrus tetraploids have been either as rootstock candidates combining complementary parents, or as parents in breeding programs aimed at producing seedless triploids (Cameron and Soost 1969; Cameron and Burnett 1978; Grosser et al. 1998). Tetraploids generally grow slowly, are more compact in habit, and also produce less fruits as compared to diploids of the same cultivar (Soost and Roose 1996). Tetraploid clones of rootstock cultivars may find utilization as dwarfing rootstocks (Gmitter et al. 1992). A dwarfing effect by tetraploids in a rootstock trial comparing diploids with corresponding autotetraploids has also been reported (Lee 1988).

Anther culture may give the breeders and geneticists the ability to recover plants of reduced ploidy level providing them with another useful tool for citrus cultivar improvement and genetic studies (Gmitter et al. 1992). This would facilitate the recovery of useful recessive alleles or mutations by haploid plant regeneration. Citrus (Chen et al. 1980; Germana et al. 1991; Germana et al. 1994; Germana and Reforgiato 1997; Chiancone et al. 2006) and *Poncirus* (Hidaka et al. 1979) anthers have been cultured in attempts to produce haploid plants. Colchicine treatment can be used for doubling of chromosome number to yield homozygous diploids. Such plants would facilitate considerable advancement in the understanding of citrus genetics and would offer plant breeders much greater control over phenotypic expression (Lee 1988). However, anthers from sour orange (Hidaka et al. 1982), sweet orange (Hidaka 1984), and lime (Chaturvedi and Sharma 1985) yielded only diploid regenerants. Nuclear fusions that were sometimes observed in the routes of microspore development were suggested to be the cause of diploidy in regenerants (Hidaka and Omura 1989). The production of diploid plants by culturing anthers of tetraploid somatic hybrids may also provide breeders with greater access to these unique genetic combinations. In *C. natsudaidai*, haploid seedlings have been obtained by irradiation (Karasawa 1971). Haploid plants and embryogenic calli of Clementine have been obtained after in situ parthenogenesis induced by irradiated pollen (Ollitrault et al. 1996). Haploid plantlet regeneration through gynogenesis of Clementine has been induced by in vitro pollination with pollen from a triploid plant (Germana and Chiancone 2001). Tri-haploids have been obtained by regeneration of anthers of *C. clementina* (Germana et al. 2005).

6.4 Somatic Hybridization

Somatic hybridization allows production of somatic hybrids that incorporate genomes of the two parents without recombination, thus avoiding the problem of the high heterozygosity in citrus (Navarro et al. 2004). In citrus, this technology has been extensively used and has many important implications (Grosser et al. 2000). The first successful protoplast isolations were reported as early as 1982 (Vardi et al. 1982), and the first citrus somatic hybrid was obtained between *C. sinensis* and *P. trifoliata* (Ohgawara et al. 1985). These results allowed the establishment of citrus breeding programs in several countries, both for scion and rootstock improvement (Grosser and Gmitter 1990). Somatic hybridization has provided a means of producing heterozygous tetraploid hybrids, which have incorporated complementary traits from donor parents. It has made production of hybrids from sexually incompatible or difficult to hybridize citrus relatives that possess valuable attributes possible, thus broadening the germplasm base available for rootstock improvement. Somatic hybrids have been developed, at the Citrus Research and Education Center, Florida, USA, from more than 150 parental combinations and are now in field trials to determine their potential in scion and rootstock improvement (Grosser et al. 2000). With the cost of production increasing over time, there is greater emphasis on reducing the tree stature to make orchard management and crop harvesting more efficient and also to bring young trees into economically valuable production earlier. In some cases, the somatic hybrids have combined desirable disease resistance and stress tolerance traits, and confer varying degrees of tree size control and precocity as well.

Somatic hybridization has also been used to create new tetraploid somatic hybrids that combine elite diploid scion material to be used as tetraploid breeding parents in interploid hybridization schemes to develop seedless and easy-to-peel new mandarin varieties (Grosser et al. 1998). Another approach to seedlessness is the transfer of cytoplasmic male sterility from Satsuma oranges to other elite but seedy scions via cybridization. This approach has the potential to make existing popular cultivars seedless, without altering the cultivar integrity in any other way. Creation of triploid citrus hybrids by electrofusion of haploid and diploid protoplasts is also promising (Ollitrault et al. 2000). Progress has also been made in the development of improved acid fruits (lemons and limes) and ornamental citrus, through somatic hybridization and the subsequent use of the hybrids in sexual crosses.

6.5 Transformation

Genetic transformation may provide an efficient alternative for citrus improvement, opening the way for the introduction of specific traits into known genotypes without altering their elite genetic background. The systems of transformation that have been used for genetic engineering experiments are dependent upon the fundamental abilities of in vitro regeneration. Genetic engineering has been applied to an increasing number of traits for citrus improvement. Gene constructs have been created for various types of CTV-derived genes (Gutierrez et al. 1997; Dominguez et al. 2000; Ghorbel et al. 2001; Fagoaga et al. 2006) and other genes from the naturally resistant *Poncirus trifoliata* (Soneji et al. 2007b), and have been inserted into citrus genomes in efforts to induce resistance to the CTV virus. A citrus blight associated gene has also been introduced into Carrizo citrange (Kayim et al. 2004). Genetic transformation and regeneration of mature tissues of citrus, which could bypass the juvenile phase, has also been attempted (Cervera et al. 1998). A gene for tolerance to salinity (HAL2) from yeast has also been introduced into citrus (Cervera et al. 2000). Genes that regulate vegetative and other behavior in *Arabidopsis* have been engineered into citrus resulting in altered growth habits and greatly reduced juvenility (Pena et al. 2001). Genes involved in metabolic pathway regulation, such as those in the flavonoid pathway, have also been introduced in citrus (Costa et al. 2002). The growing interest in manipulating carotenoid biosynthesis in plants is mainly related to human nutrition as precursors of vitamin A and natural antioxidants. Also, this kind of pathway manipulation holds promise of altering color and flavor development in citrus. *CS-ACS1* gene that controls the ethylene biosynthesis has also been introduced into citrus (Wong et al. 2001), and the transgenic lines that produced higher level of antisense ACS RNA repressed the increase in ACC content following chilling treatment. Attempts have also been made to introduce juice quality related pectin methylesterase gene into citrus (Guo et al. 2005). This exploitation of enzymes associated with ‘cloud separation’ may also offer a great promise of targeted trait modification and improvement of the responsible genes by genetic manipulation. The cDNA of the *Xa21*, a *Xanthomonas* resistance gene, has been introduced into citrus via protoplast cotransformation (Omar and Grosser, 2007). Study is underway to challenge these transgenics with canker at a state Division of Plant Industry quarantine facility in Gainesville, Florida, USA, to determine whether this gene has any potential in the improvement of citrus cultivars for canker resistance.

6.6 Genomics

Few studies have been carried out to understand the genetics of citrus. There is a lack of knowledge and understanding of the genetic mechanisms that control important traits such as disease resistance, cold tolerance, juvenility/maturity, and aspects of fruit ripening process (Gmitter et al. 1992). The entire field of citrus biology and genetics can be revolutionized by expanding the potential capabilities of genomics and bioinformatics to cultivar improvement through precise and targeted manipulations of the genome.

The rapid development of molecular marker technologies has made it possible to investigate gene expression, and has helped in construction and integration of genetic and physical maps of the economically important traits such as CTV resistance (Gmitter et al. 1996; Deng et al. 1997), nematode resistance (Ling et al. 2000), fruit acidity (Fang et al. 1997), and dwarfing (Cheng and Roose 1995). These genetic maps may provide the basis for early screening procedures, thus permitting breeders to make initial selection among very young progeny based on the phenotype predicted by their genotype at molecular loci known to cosegregate with a particular phenotype (Durham et al. 1992). It would be possible to improve the efficiency of conventional plant breeding by mapping the desired genes and carrying out selection not directly on the trait of interest but on molecular markers linked to genes influencing that trait.

Molecular marker technologies also provide tools to tag the genes of known phenotypes by developing localized molecular linkage maps. These are very essential for map-based cloning (MBC) approach and marker-assisted selection (MAS) breeding programs (Recupero et al. 2000; Asins 2002). Dominant trifoliate leaf, a morphological trait, was considered to be the earliest MAS marker and was used to distinguish zygotic hybrids from nucellar seedlings, but morphological characteristics cannot be used with varieties without such distinct traits. Hence, MAS is usually carried out with the help of biochemical and/or DNA-based markers. Markers such as isozymes, RAPDs, and EST-SSRs have been used for the identification of hybrids (Soost and William 1980; Nageswara Rao et al. 2008). DNA-based molecular markers may be used to select rootstocks that may contain many of the desired resistances to CTV (Gmitter et al. 1996), nematode (Ling et al. 2000), *Phytophthora,* etc. This will prove to be highly cost-efficient as compared to traditional greenhouse or field-screening approaches using inoculation, thus making the multitrait selection possible in a substantially shorter period of time. As more key genes for critical traits are identified and tagged with easy to score molecular markers, MAS will improve the efficiency of the breeding process using traditional hybridization and selection strategies.

MBC is also called as positional cloning. It is another approach to isolate gene(s), without prior knowledge of gene product, using tools of comprehensive genetics, genomics, and bioinformatics. MBC of genes for CTV resistance from *P. trifoliata* has provided target gene sequences (Gmitter et al. 1998; Deng et al. 2001) to develop CTV-immune scion and rootstock varieties (Soneji et al. 2007b). Cloning of genes regulating cold-stress tolerance (Jia et al. 2004) and generalized disease resistance pathways have been accomplished. These sequences have been engineered into citrus plants to test their ability to modify plant performance against these two globally important limitations to citrus production.

Studies are underway to unravel the resistance to citrus canker expressed in kumquat, a closely related genus to citrus (Khalaf et al. 2007).

Molecular markers have also been widely applied on phylogenetic and taxonomic studies in citrus (Herrero et al. 1996; Fang and Roose 1997; Fang et al. 1997; Bret et al. 2001; Berkeley et al. 2006). Some efforts have also been made in the areas of resistance gene candidates (Deng et al. 2000; Deng and Gmitter 2003; Bernet et al. 2004), satellites (Fann et al. 2001), microsatellites (Kijas et al. 1995; Ahmad et al. 2003; Chen et al. 2008), variations from fragment restriction (Liou et al. 1996), methylation (Cai et al. 1996), and individual gene expressions (Moriguchi et al. 1998; Shimada et al. 2005). Integration of genetic linkage maps with the physical maps is also required for efficient localization and isolation of the genes, to study the organization and evolution of the genome, and as an initial step for efficient whole genome sequencing. Plans have been implemented for an international collaboration to develop integrated genetic and physical maps of the citrus genome, with an intention to lead to a full genomic sequence of citrus. Along with this will come the ability to understand genetic and metabolic control of all critical aspects of the traits of economic importance.

7 Conclusions

Citrus can be grown throughout the world in tropical and subtropical areas. It is vegetatively propagated. The great wealth of citrus types and cultivars of today reflects the vast natural breeding options within citrus, as well as effective intentional human intervention. Although citrus breeding is very challenging, breeding programs throughout the world are making significant progress in the application of conventional and modern approaches to genetic improvement. Advances in plant cell and tissue culture also have major impacts on genetic improvement of citrus. Somatic hybridization and recovery of monoploids, triploids, and tetraploids have expanded the range of germplasm available to citrus breeders. Triploid induction through endosperm culture, triploid hybrid embryo culture, or fusion of haploid with diploid protoplasts would enhance the possibility of developing triploid seedless cultivars. The benefits offered by monoploid culture or homozygotes to breeding programs and to the understanding of citrus genetics are incalculable. Although the prospects of in vitro culture to develop somatic hybrids are complicated by ploidy differences and the unpredictable fertility of wide hybrids, it has helped in overcoming sexual incompatibility among citrus species and cultivars

Most of the critical goals for scion improvement, such as resistance to diseases or changes in fruit quality attributes (color, flavor, peelability, nutrient content, etc.), are difficult to approach in any practical sense by conventional breeding strategies. It will be through genomic research that an understanding of fundamental processes can be realized leading to the identification and cloning of candidate genes. Through genetic transformation, these candidate genes and their information will be exploited for the improvement of citrus. Genetic transformation promises to provide plant breeders with the ability to correct defects in existing elite cultivars by adding specific genes with little or no effect on other characters.

Selection of rootstocks is also critical. They vary greatly in their soil adaptability, relative susceptibility to diseases, viruses, interactions with scion, quality of fruit, size and vigor, and tolerance to soil-borne problems. Rootstock improvements will also be hastened and maximized through the application of new knowledge and tools developed from genomic technology. The knowledge and establishment of genomics and bioinformatics have provided efficient tools for tagging and cloning of important genes. It has also made the sequencing of the citrus genome plausible. Mapping and sequencing of a citrus genome would aid in elucidation of gene function, regulation, and expression. Advances in genomic science have a great impact in many respects, and will continue to provide new information and gene targets for manipulation.

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