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THE INVESTIGATION OF CITRUS FRUIT QUALITY. POPULAR CHARACTERISTIC AND BREEDING

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Abstract

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Citrus is the most economically important fruit crop in the world. In citrus, the concept of fruit quality comprises several other aspects intimately related to human health apart from physical attributes and diet components. Citrus is an excellent model to study fruit quality because of its peculiar fruiting, singular biochemistry and economical relevance. A citrus breeding programme starts with the selection of suitable parents and the planning of controlled crosses. Information on the breeding value of available parents and the heritability of specific characters is important in a plant breeding programme to aid the breeder in parent selection and the planning of controlled crosses. Major goals of variety breeding in citrus are mostly related to fruit quality, productivity and harvesting period. In a broad sense, citrus fruit quality includes many physical attributes like fruit color, fruit size, easy of peeling and seedlessness. These traits have become paramount in commercial citrus types and new cultivar being developed through plant breeding and selection of new sports. This paper focus on four main citrus characteristics that responsible for fruit quality and are the basis for judging the product acceptability by consumers. We also discuss the variety strategy for citrus quality improvement.

Keywords: citrus, fruit quality, plant breeding

INTRODUCTION

The term citrus originated from the Latin form of 'Kedros' a Greek word denoting trees like cedar, pine, and cypress. As the smell of citrus leaves and fruit is reminiscent of that of cedar, the name citrus has been applied to the citron. Linnaeus grouped all citrus species known to him in the genus Citrus. In Greek mythology citrus fruits are called hesperides. The suggested origin of the true citrus fruits is South East Asia, including South China, north-eastern India and Burma. Evidence from wild citrus in the area is still unclear. In many cases, seed has been spread long distances from the sites of origin and culture by birds, water streams, and human activity (Spiegel-Roy *et al.*, 1996). 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. Citrus species are essentially diploids ($2n = 2x = 18$). On the basis of morphological characteristics, studies have been carried out on the relationships between genera and species (Jain and Pryadarshan, 2009). 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 (Tadeo *et al.*, 2008). 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. With a total world production of 149.431 million tons, citrus is one of the world's most important fruit crops (FAO, 2015). Its importance to agriculture and the world's economy is demonstrated by its wide distribution and large-scale production (Soost and Roose, 1996).

There has been a strong international market demand for easy peeling type citrus fruit in recent years. These include seedless clementines in the US(United States) and EU (European Union) and satsuma mandarins in Asia and the U.K. The seedless and easy peeling characteristics of these types of citrus fruit provide a market advantage over traditional seeded type citrus. The world grapefruit market has shifted significantly toward seedless high-pigmented colored types. The US and EU market almost exclusively requires cultivars with a red pulp high in carotenoids. The Japanese market is another traditionally strong grapefruit market that is shifting to pigmented grapefruit, although the non-pigmented types still have a high demand. Other quality parameters important in international marketing of citrus fruit include peel firmness and juice content in lemons and limes. Depending on the needs of a specific region of the world, the objectives of citrus breeding programs may vary considerably. For example, some of the objectives may have high priority in some regions and low or no priority in others. However, breeding for quality is one of the important objectives in most regions (Khan, 2007).

Citrus Fruit Quality

Quality is a term that when applied to fresh fruit may convey a number of interpretations. Usually within the markets it refers to the external appearance of the product. Within the EU and the US, quality standards are applied to fresh fruit and vegetables. And products exported to them from countries outside Europe and the US must conform to essential standards. In these standards, fruit and vegetables according to their grade (i.e., extra, first, and second) are packed and graded so as to be virtually free of injury, blemishes, and diseases and to be uniform in shape, size, color, and maturity. Quality, however, does not only relate to appearance, but also some other characterization for example: taste, rich in nutrients, vitamins, seedlessness, odour, etc. must be considered (Jenks and Bebeli, 2011). Irrespective of the yielding potential of a newly developed cultivar, success in agriculture will be determined by the end-use quality of the saleable product. There are two main types of end-use quality: *Organoleptic* – consumer acceptance or preference of taste, size, texture, and colour. Although many people differ in their preference, there is often agreement within taste panels as to general preference towards certain levels of expression of these attributes, thus 'liking' some genotypes over others (even disregarding 'off-tastes'). *Chemical* – where quality is determined by chemical analyses of the harvestable product. This is perhaps easily understandable in saying, oil crops, where the quality of the oil can be determined with great accuracy by the determination of the oil fatty acid profile, or in the pharmaceutical (or farm-aceutical) industry where the quality of drugs are chemically determined. However, these are also true for fibre plants like cotton, and indeed fodder crops where protein content and digestibility are determined by analytical methods (Brown and Caligari, 2011). Overall, quality is what creates the demand for a product. It is the end-user who will mostly determine if that crop will be grown in future years. It is a very naïve breeder who ignores the fact that consumer preference is continually changing and that the quality standards of today may be superseded by a newset of standards in the future. It is, therefore, imperative to organize a breeding scheme to be flexible and to try to cover as many potential aspects of yield, quality, and other factors which may be important in the next two decades (Brown and Caligari, 2011).

The objectives of citrus fruit production and the methods applied to achieve it are different depending on the final destination of fruit, that is, the processing industry or the fresh market (Davies and Albrigo, 1994). The most appreciated features of citrus fruit for processing are related to internal fruit quality which is a function of flavour and palatability. Citrus fruit for processing must have no decay symptoms, elevated juice content, high level of TSS (total soluble sugars, mostly sucrose, glucose and fructose), low TA (titratable acidity, mostly citric acid) and minor amounts of bitter components such as the triterpene limonin and the flavonoid naringin. The external appearance of fruits, however, is not particularly important for processing although attractive internal colour in oranges is usually required. Citrus fresh fruit quality standards, on the other hand, are largely dependent on consumers' preferences that in addition may change substantially between countries. In general, considerable more emphasis is put in fresh markets on external than on internal fruit quality standards. Although citrus fruit must reach a minimum 'maturity index' (the TSS to TA ratio; in general, 7–9 for oranges and mandarins and 5–7 for grapefruits) to be marketable, the essential quality requirements of citrus fruit for fresh market are mostly cultivar characteristics such as fruit size and shape, brightly coloured and firm rind, and absence of surface blemishes (Tadeo *et al.*, 2008).

Mandarins, navel oranges, some common oranges, pummelos, and grapefruits are mainly for fresh markets; lemons are for both fresh and juice markets. Generally, both external and internal fruit quality are equally important in the fresh market. Suitable fruit shape, deep peel color, and smooth and shiny appearance are the most attractive external quality characteristics for the consumers. Easy peeling, pleasant flavor, seedlessness, and fragrance are the most desired internal quality parameters (Jenks and Bebeli, 2011).

Breeding Objectives

Objectives are the first of the plant breeder's decisions. Breeding objectives are manifold and vary from hardy cultivars, adapted to particular climatic and soil conditions, improved tree vigour and longevity, reduced tree size, fruit quality, industrial purposes, season of ripening and to disease and pest resistance.

The reasons for the low impact of traditional breeding of this major fruit crop are related to the peculiarities of citrus reproductive biology so the plant breeder for fully utilizing the variability in citrus, faced with some barriers include: the incompatibility, sterility and poly-embryony, facultative parthenocarpy, heterozygosity, and the prolonged juvenile period that occur in some varieties. In addition to this unusual reproductive biology, citrus also possess a rare combination of intriguing biological characteristics including non-climacteric fruit ripening, juvenility, dormancy, surprising root/shoot interactions, and several other specific tree traits. Overall, variety development is extremely slow and inherently costly because it requires many years and efforts to adequately evaluate fruit quality improvement. Furthermore, many of the commercial citrus types produce polyembryonic seeds through nucellar embryony. These circumstances and the narrow germplasm bases actually represented in citrus have precluded breeding as a strategy for cultivar development and improvement (Iglesias *et al.*, 2009).

Breeding efforts for the improvement of citrus fruit quality have been focused on traits that first attribute perceived by the consumers and make fruit more difficult to eat: peel and pulp color, fragrance, pulp taste, seedlessness, improvement of fruit size and easy peeling. Thus, we want a fruit that is seedless, can be eaten without peeling, is bite size, and does not spurt juice out when eaten. Hundreds of cultivars have been released to meet different market requirements.

Fruit Color

Color is the most important quality characteristic of fruits because it is the first attribute perceived by the consumers and is the basis for judging the product acceptability. The most important color changes in fruits are related to chemical, biochemical, and physicochemical mechanisms: (a) breakdown of cellular chloroplasts and chromoplasts, (b) changes in natural pigments (chlorophylls, carotenoids, and anthocyanins), and (c) development of enzymatic browning (Sinha *et al.*, 2012).

Fruit coloration is governed by several factors such as fruit maturity, tree nutrition, rootstock, cultivation practices, water availability, and temperature. Fruit color also depends on climatic conditions and groundcover in the orchard (Ladanyia, 2010). It is a unique phase in the life cycle of fruiting plants. it is one of the most important traits for both commercial and aesthetic value of fruit, mostly attributed to flavonoids, betalains, carotenoids, and chlorophylls. Color development in fruits is an important evolutionary trait and a major factor contributing to fruit quality and subsequent market value. Coloration encompasses several physiological and biochemical changes that happen through differential expression of various developmentally regulated genes (Pomar *et al.*, 2005; Torres *et al.*, 2010).

Major pigments that give color to citrus fruits are chlorophylls (green), carotenoids (yellow, orange, and deep orange), anthocyanins (blood red), and lycopenes (pink or red). During growth and maturation, especially in the immature stage, chlorophylls predominate in the peels of all citrus fruits. Due to the presence of chlorophyll, immature fruits are capable of photosynthesis, but they cannot make a significant contribution to the own nutrition (Ladanyia, 2010).

Animals and human cannot synthesize flavonoids and carotenoids by themselves, so these health beneficial substances must be obtained from their feeding on horticultural products, especially fruits. Modern technologies of genetic engineering or metabolic engineering may harness some microorganisms for industrial production of anthocyanins or carotenoids (Ajikumar *et al.*, 2008), but it is uncertain those products will be accepted by consumers. Thus, it is reasonable to endeavor to develop the health-benefiting colors of fruits. Flavonoids and betalains are water-soluble pigments, whereas carotenoids and chlorophylls are liposoluble.

Citrus is among the most consumed fruits in the world and is rich in flavonoids and carotenoids. To elevate the health-beneficial values and the attractive appearance, new varieties of citrus accumulating flavonoids and carotenoids in fruits have been brought to the world market continuously via breeding efforts, such as selection from bud mutation, seedlings and nucellar lines, or sexual hybridization. In general, a large percentage of new varieties have been selected from spontaneous bud sports in the field with improved fruit color caused by anthocyanin or carotenoids. Sexual hybridization programs have also been adopted to develop new varieties by taking at least one pigmented citrus as parent (Deng and Xu, 2011). The flavonoids are an important class of plant secondary metabolites, belonging to the broader family of plant phenolics. No specific role can be assigned so far to the flavonoids in the physiology of citrus. Citrus flavonoids have aroused much interest due to their organoleptic properties (Spiegel and Goldschmidt, 1996).

Over 5000 different flavonoids have been described to date and they are classified into at least 10 chemical groups (Sinha *et al.*, 2012). These are low-molecular-weight polyphenolic compounds that flavones, mostly yellow flavonols, flavanones, flavanols, isoflavones, colorless proanthcyanidins, and colorful anthocyanidins are particularly common in fruits (Le Marchand, 2002; Springob *et al.*, 2003). Citrus fruits contain numerous flavonoids with a typical pattern for each species, resulting good markers of the relative products and by-products. Most flavonoids are in the fruit albedo and flavedo. And they can be grouped in flavanones, flavones, and anthocyanins (Horowitz and Gentili, 1977). The most numerous are the flavanones which are, almost invariably, in the form of glycosides belonging to two groups: rutinosides and neohesperidosides (Tripoli *et al.*, 2007). The flavonoids may affect fruit quality characteristics such as texture, color, flavor, and the nutritional value of fruits (e.g., in apples) through their involvement in the formation of undesirable brown pigments following bruising or cutting, due to the enzymatic oxidation of endogenous phenolics into quinone's, which are then polymerized into brown products (Robards and Antolovich, 1997). Flavonoids increase the postharvest resistance of fruits to pathogens (Lattanzio, 2003), but may contribute to the formation of undesirable sediments in fruit juices and wine. Former investigation indicated that 'Citrin', a crude mixture of flavonoids prepared from lemon peel, was effective in reducing capillary permeability in man, in prolonging life, and mitigating tissue hemorrhaging due to scurvy in guinea pigs. Among the many other clinical applications proposed for flavonoids, it is worth noting its use, although of undemonstrated effectiveness, in the treatment of the common cold. The group of substances responsible for these effects was considered to be a vitamin, and was given the name 'Vitamin P'. Successive research was unable to demonstrate that flavonoids are necessary factors in the diet, and the term 'Vitamin P' fell into disuse, and was replaced by the term 'bioflavonoids' (flavonoids having a biological activity). Flavonoids have been extensively studied for antioxidant, anticancer, antiviral, and anti-inflammatory activities, effects on capillary fragility, and an observed inhibition of human platelet aggregation (Dugo and Di Giacomo, 2003).

Citrus flesh is the distinctive and edible tissue that contains anthocyanins, and these are sometimes visible as by the red blushes on the rind of blood orange. In variety development programs, more attention has been paid lately to flesh coloration improvement caused by anthocyanins to improve fruit health quality. So far, only blood orange cultivars, such as 'Budd', 'Moro', 'Tarocco', 'Sanguinello', and so on, have been reported to produce fruits colored by anthocyanins (Lee, 2002; Rapisarda *et al.*, 2009). Major anthocyanins in blood orange fruits and juice are cyanidin- 3-glucoside and cyanidin-3-(6′′-malonylglucoside). Additionally, minor anthocyanins and anthocyanin-derived pigments, such as 4-vinylcatechol adducts of cyanidin-3-glucoside, cyanidin-3-(3′′-malonylglucoside), delphinidin 3-glucoside, cyanidin 3-sophoroside, cyanidin -3-(6′′-dioxalylglucoside), have also been identified (Hillebrand *et al.*, 2004). Anthocyanins are the most prominent (Wheelwright and Janson, 1985) localized within the vacuole of the plant cell (Artes *et al.*, 2002) largest and most diverse group of plant pigments including: cyanidin, delphinidin, malvidin, peonidin, and their various glycosides and acylated derivatives, and these provide a wide range of colors including violet, red, pink, blue, magenta, black and that derived from the phenyl propanoid pathway. Anthocyanins are always produced during fruit ripening, but in some fruits such as nectarines (*Prunus persica* [L.] Batsch.), they are produced during the first stages of fruit development. The anthocyanin content of ripe fruit is affected by environmental conditions such as light and temperature (Faragher, 1983; Arakawa *et al.*, 1985). Most studies reported on anthocyanin biosynthesis mechanism are in apple and grape (Matus *et al.*, 2008), and there is no clear study reported on other fruits. It's probably due to some genes involved in anthocyanin biosynthesis having pleiotropy and being affected by many internal and external factors. Transgenic approaches have discovered a strong relationship between phenyl propanoid/flavonoid gene expressions for fruit skin coloration (Kayesh *et al.*, 2013). Investigation in grape indicated that higher phenol and anthocyanin content was directly correlated with higher antioxidant activities (Alcolea *et al.*, 2002). Anthocyanins are one class of flavonoid compounds that act as powerful antioxidants so are able to prevent oxidative damage to proteins, lipids, DNA, and other macromolecules caused by reactive oxygen species (ROS). Thus besides the classical use of inhibitors or the isolation of mutants for scavenge the toxic ROS, manipulation of the antioxidant enzyme levels in specific cell by genetic tools can influence the accumulation of ROS in given cellular compartments. Maintenance of a high antioxidant capacity to scavenge the toxic ROS has been linked to increased tolerance of plants to environmental stresses (Abouzari and Fakheri, 2015). Several methods are used to measure antioxidant activity. The most commonly used are those that use chromogen radical compounds to simulate reactive oxygen and nitrogen species. Anthocyanins are easily lost by lixiviation when the cell membranes break down. Also oxidation can play an important role in anthocyanin degradation catalyzed by light (Sinha *et al.*, 2012).

Carotenoids are lipid-soluble plant pigments, common in photosynthetic plants. The term carotenoid summarizes a class of structurally related pigments, mainly found in plants. At present, more than 600 different carotenoids have been identified although only about two dozen are regularly consumed by humans. The most prominent member of this group is β-carotene. Normally, carotenoids as efficient photoprotective agents, quench the excited state of chlorophyll, thus preventing the formation of singlet oxygen. Little is known about carotenoids in ROS detoxification in plants. However, overexpression of ß-carotene hydroxylase in Arabidopsis leads to increased amounts of xanthophyll in chloroplasts and results in enhanced tolerance towards oxidative stress induced in high light (Abouzari and Fakheri, 2015). The carotenoids of citrus fruits have been extensively investigated. The Citrus genus is remarkable for producing the largest number of carotenoids found in fruit. About 115 different pigments have been reported, including a large number of isomers, some of which might be formed during isolation. Each species and hybrid has a characteristic carotenoid complex which is responsible for its typical color; peel and pulp pigments reveal certain differences. In yellow citrus fruits (pummelo, grapefruit, lemon, lime), the total amount of carotenoids is low and most of these belong to the colorless carotenoids. Thus, in white Marsh seedless grapefruit the colorless phytoene and phytofluene account for 74% of the carotenoids of the peel. Orange-colored citrus fruits (orange, sour orange, mandarin) contain relatively large amounts of a complex mixture of carotenoids (Spiegel-Roy and Goldschmidt, 1996). There is a rapid synthesis of carotenoids in the chromoplast during ripening, which is accompanied by a simultaneous loss of chlorophyll. With ripening, total carotenoids increase in the peel as well as in the pulp. Rind of fruit is the region of higher carotenoid concentration, and 50–75 percent of the total carotenoids of oranges exists in the peel. (Ladanyia, 2010). Carotenoids are affected by pH, enzymatic activity, light, and oxidation associated with the conjugated double bond system. The chemical changes occurring in carotenoids during processing have been reviewed by several authors (Simpson, 1986; Rodriguez-Amaya, 1997).

In citrus, natural mutants with red peel and juice vesicles have been found mainly in Texas grapefruit. Mutation breeding using thermal neutrons has brought about a most significant change from a deeply coloured, highly seeded grapefruit to a seedless one. In another, more recent case, thermal neutrons and natural mutation have been instrumental in producing a mutant with an increased red colour from an established natural mutant with a good peel and flesh colour. Of the other fruit characteristics, mutants for low acidity are of considerable interest in citrus, and particularly in grapefruit (Jackson, 1987). In the apple, low acid types depend on a single homozygous recessive gene (Brown, 1975).

The world grapefruit market has shifted significantly toward seedless high-pigmented colored types. The US and EU market almost exclusively requires cultivars with a red pulp high in carotenoids. The Japanese market is another traditionally strong grapefruit market that is shifting to pigmented grapefruit although the non-pigmented types still have a high demand. Other quality parameters important in international marketing of citrus fruit include peel firmness and juice content in lemons and limes (Picha, 2006). 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.

Seedlessness

In some plants seedlessness is attributed to abortion of embryos produced by fertilization, a phenomenon known as stenospermocarpy, which requires both normal pollination and fertilization. Seedlessness can also result from parthenocarpy, wherein fruits set without fertilization. On the other hand, parthenocarpic fruit development may or may not require pollination, but most seedless citrus fruits require stimulation from pollination to produce fruit (Jenks and Bebeli, 2011). Certain environmental factors, i.e., low or high temperatures, chromosomal aberrations, chemical treatment, and some genetic factors, like gene(s) controlling meiosis process can also induce seedlessness (Lin *et al.*, 1984; Nuez *et al.*, 1986). On the other hand, self-incompatibility may be combined with trait(s) of parthenocarpy. High endogenous auxins levels and GAs arise in natural parthenocarpic citrus cultivars (George *et al.*, 1984; Talon *et al.*, 1992).

Among the different criteria of citrus quality, seedlessness is a major characteristic and is a highly desirable trait for fresh fruits. There are many desirable characteristics of seedless fruit, which include high quality and taste, greatly valued by both consumers and the processing industry. Commercial cultivars of bananas (*Musa acuminata*) and pineapples (*Ananas comosus*) are examples of seedless fruits. Seedless cultivars of citrus, grapes (*Vitis vinifera*), and watermelons (*Citrulus lanatus*) are highly valued by consumers. The presence of a large number of seeds in citrus fruits greatly hinders consumer acceptability, even if it has good organoleptic properties. Therefore, the development of seedless fruit cultivars has become a major goal for fruit breeders around the world and different approaches have been adopted by numerous researchers. Genes that induce seedlessness in fruit are being major objective in some quest (Koltunow *et al.*, 2000). Considerable breeding work has been done to develop new seedless cultivars. These consist of both orthodox and modern molecular breeding tools. Conventional breeding in citrus has important limitations due to the complex reproductive biology of these species and was also time exhaustive. Most genotypes are apomictic, and adventitious embryos develop directly from nucellar cells limiting or precluding the development of zygotic embryos. This limits the recovery of large sexual populations, and in practice, apomictic genotypes are avoided as female parents in many programs.

Several high-quality genotypes have pollen and/or ovule sterility and thus cannot be used as parents in breeding programs. Self- and cross-incompatibility are relatively common among many genotypes, also limiting the possibilities to select parents for specific crosses. Citrus has a long juvenile phase and in most species at least five years are required to start flowering and many more to completely lose the undesirable characters, such as thorns, associated with juvenility. These biological factors and the difficulty to manage field evaluation for large progenies and multigenerational breeding schemes are the main reason for the relatively low success of conventional breeding programs. The seedlessness of citrus varies among accessions and sometimes environmental conditions. 'Navel' orange and 'Satsuma' mandarins are usually seedless, but occasionally they produce seeds when pollinated. Information regarding genetic and molecular aspects affecting the gamete fertility and seed development is now increasing rapidly and will definitely enhance the potential of breeding for seedlessness through sexual crosses at diploid level.

Seedlessness in citrus mandarin cultivars can be induced by many factors, including female or male sterility, defective ovules and embryo-sac abortion, pollen self incompatibility, polyploidy, abnormal climate and application of plant-growth regulators, such as gibberellins. Many seedless citrus cultivars, especially seedless mandarin cultivars, originate from the bud mutation of seedy cultivars (Shen, 1997).

Easy of Peeling

Consumer appear to be less willing to spend time preparing fruit for consumption (i.e. peeling, slicing, removing seeds, etc.), but are willing to spend more for ready -to-eat (i.e."fresh-cut").

In citrus breeding, in addition to color and seedlessness, easy of peeling is another essential traits and selection objectives for fresh market cultivars (Stover *et al.*, 2005).

The combined form of albedo (mesocarp) and flavedo (epicarp) is called the pericarp, commonly known as the rind or peel. From the molecular point of view, pectin, cellulose, and hemicellulose are responsible for the adherence of the skin to the fruit (Whitaker, 1984). In some varieties of orange and mandarins, the albedo is very thin and difficult to separate from the flavedo, a fact affecting the enzymatic peeling. Enzymatic peeling is an alternative method for removing the rind of the fruits with lower water consumption and causing less contamination than the traditional methods. The presence of the navel hampers the diffusion of the enzymatic solution into the tissue and the irregularity of the segments decreases the quality of the final product, as could be verified in the Thomson variety (Pretel *et al.*, 2007).

Mandarins are an easily peeled citrus fruit due to the weak adherence between epicarp and endocarp. These fruits are widely cultivated in the world, especially for their fresh consumption although some varieties like Satsuma are commercialized advertised in syrup (Bayindirli, 2010).

A fairly large group of mandarin hybrids has been found and propagated, and also produced by breeding. These are treated in commerce in a manner similar to mandarin or 'easy peeling fruit'. Those originating from a cross between grapefruit and mandarin are called 'tangelos'. Grapefruits and Valencia oranges can be stored for three to five months and green lemons even longer, but many easy peeling, mandarin-like cultivars cannot be stored for longer than a few weeks. so long time of stored must be considered in breeding program for easy peeling (Spiegel-Roy and Goldschmidt, 1996).

Enzymatic peeling is an alternative method for removing the rind of the fruits with lower water consumption and causing less contamination than the traditional methods. The principle of the enzymatic peeling is based on the digestion, by an enzymatic preparation, of substances that found in cell walls of plants and responsible for the adherence of the skin to the fruit. Therefore, both pectinases and cellulases are needed for the enzymatic peeling. The cellulases are probably needed for the release of the pectins in the albedo, and the pectinases contribute to the hydrolysis of the polysaccharides of the cell wall.

Fruit Size

Size is of foremost economic significance for marketing of fresh fruit, particularly with small, easy-peeling cultivars like 'Clementine'. Fruit of the genus Citrus vary greatly in size and range from about 30 mm in diameter for kumquats (*Fortunella* spp.) to more than 300 mm in diameter for pummelos (*C. grandis*) (Ray, 2002). Fruit size in mandarins can vary from small to large, but most fruit have a size of between 50 mm and 80 mm in diameter.

Seed content also plays a role in fruit size. A linear relationship between fruit size and seed number is common in citrus which indicates a strong influence of seeds on fruit development. Consequently, fruit size is often sacrificed when seedless fruits are desired (Vithanage, 1991).

Seedless fruit are generally small and an increase in the seed content can increase the fruit size. Since fruit development is linked to the development of the ovule, it is possible that a hormonal stimulus from the seeds regulates fruit growth. Pollen has been found to play a role in fruit size and generally larger fruit with more seeds are produced if more pollen is applied to the stigma of the flower. Almost every possible agrotechnique has been tested in an attempt to increase fruit size. A large amount of photosynthate is required for fruit enlargement. Larger fruit are, therefore, found on leafy, compared to leafless, and branches due to the nearby leaves being potent suppliers of photosynthate (Ladaniya, 2010). Mechanical practices such as girdling and fruit thinning can be used to increase the amount of photosynthate available to the fruit, and therefore, increase fruit size. Girdling involves removing a ring of bark from the trunk or scaffold branches interfering with the downward phloem transport and preventing the escape of the photosynthate from the girdled area to other parts of the tree. Girdling during the fruit enlargement stage can increase the fruit size by up to 30%. Fruit thinning, where some of the fruit are removed, increases the leafy area per fruit making more photosynthate available to each individual fruit and thereby increasing the fruit size. A several-fold increase in fruit size may be obtained when girdling is combined with extreme fruit thinning.

Plant growth regulators have proved effective for increasing fruit set, checking fruit drop, thinning out, keeping the fruit on the tree for longer periods, increasing the postharvest life, and influencing fruit quality. The effectiveness of these plant growth regulators, however, is influenced by the climatic conditions and the citrus varieties to which they are applied. Plant growth regulators for example auxins can be used to manipulate fruit size. Fruit size increased with auxins in three ways: firstly by thinning the crop and increasing the growth of the remaining fruitlets; secondly when applied at the end of the first stage and beginning of the second stage of fruit development, they directly enhance fruit growth; and thirdly when applied to the stylar end of young fruit they enhance the growth of some fruitlets and increase late fruitlet abscission (Ladaniya, 2008).

Mineral nutrition is another factor influencing fruit growth and the macronutrient potassium plays an important role in fruit size. A potassium deficiency in the tree results in smaller fruit, and potassium sprays are used commercially to increase the fruit size. Several horticultural practices such as girdling the stems, spraying with plant growth regulators, and hand thinning of fruit are used to reduce the effect of this irregular bearing behavior (Saunt, 2000).

Intrinsic fruit size is probably a genetic trait while the fruit number reflects the tree's bearing limits. Even though the yield varies considerably between citrus cultivars, all cultivars have shown the fruit size to be inversely proportional to the number of fruit on the tree (Spiegel-Roy and Goldschmidt, 1996), with heavy bearing trees producing smaller fruit (Ladaniya, 2008).

In oranges, an inverse relation between size, soluble solids, acidity, and ascorbic acid content has been established. No appreciable difference in the contents of sugars, ascorbic acid, or naringin was found in the large and small fruits of Japanese Natsudaidai, but the small fruits were higher in total and amino nitrogen. It was reported that large and medium sized Satsuma mandarins contained higher percentage of soluble solids and titratable acid, and had a better color than the small mandarins (Ramana *et al.*, 2009).

Major Breeding Strategies

The basic features of any effective quality assessment in a plant breeding program is that they should be quick, cheap, and use very little material. These three criteria are important because (Brown and Caligari, 2011): 1) A plant breeding program will involve screening many thousands of breeding lines each year or growing cycle. 2) A plant breeder is often working against time. Many quality traits are assessed post-harvest and it is often important to make selection decisions quickly, before large-scale quality is determined. The development of new and improved citrus cultivars by conventional methods is a slow and costly process. It may take as long as 20–35 years or longer to release a new cultivar from the time of making the cross (Khan, 2007). 3) In most stages of a plant breeding scheme, there are only limited amounts of material available for testing. It is often the propagative parts of the plants (seeds or tubers) that are used for testing, and so a further complication is that many of the quality tests available are destructive of the very parts of the plant that are required to be grown to provide the next generation for selection. 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. Citrus breeding methods include bud and seedling mutation selection, mutagenesis, hybridization, and various biotechnologies (Deng and Xu, 2011).

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 first are evaluated for some important treats includes disease and pest resistance, stress tolerance, and overall growth characteristics during the juvenile period, then subsequently for fruit characteristics of scions or rootstock traits of interest.

Clonal Selection

Clonal selection can be used to isolate superior bud source strains of established cultivars and to eliminate propagation of undesirable budlines. 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 (Jain and Pryadarshan, 2009).

Use of New Self-incompatible Cultivars

Phenomenon of self-incompatibility is genetically controlled which prevents seed set in self-pollinated crops and produce functional gametes. Usually in condition of self-pollination, no properly developed pollen tubes were observed in the ovaries of self-incompatible plants (Ton and Krezdorn, 1967). The incompatibility system of citrus is of gametophytic in nature and incompatibility 'S' allele(s) are widely distributed; not only in self-incompatible cultivars but also in self-compatibles like satsuma mandarins and 'Dancy' grapefruit (Soost, 1968; Vardi *et al.*, 2000). Self-incompatible accessions have the ability to produce seedless fruits when cross pollination is prevented. However, those accessions may produce seedy fruits in mixed plantings particularly in present of pollinating insects. There is little information available on the inheritance of self-incompatibility in citrus, however, it has been found that hybrids between self-incompatible cultivars have also been self-incompatible and sometimes cross-incompatible (Soost and Cameron, 1975). Sterility can be classified into three types (Yamamoto *et al.*, 1995): self-incompatibility, female sterility, and male sterility. Gametophytic self-incompatibility is common in pummelo and is found in several mandarin and mandarin hybrid cultivars (Soost, 1968). Male and female sterility results in the complete inability to reproduce by means of seed and may be due to different genetic factors such as triploidy, sterility genes, and chromosomal abnormalities such as reciprocal translocations and inversions.

Some male-sterile and self-incompatible accessions cannot produce seedless fruits because of the lack of parthenocarpy. Thus, parthenocarpy is an indispensable trait for seedless fruit production, and this character seems to be widely present in citrus germplasm. Autonomic parthenocarpy, where seedless fruit is produced without any external stimulation (pollination), is the main type of parthenocarpy in citrus ('Navel' orange or 'Satsuma' mandarin), but stimulative parthenocarpy has also been reported (Vardi *et al.*, 1988). The importance of self-compatibility for achieving better crops, at least in certain seasons and environments, and for better orchard management (dispensing with lower value pollenizers), is well recognized. 'Clementine', 'Fortune' mandarin, and 'Afourer' tangor are good examples of such self-incompatible cultivars. However the list of self-incompatible cultivars is on the increase, and with the ancestry of many cultivars unknown, the presence of incompatibility in many progenies cannot be predicted. The main default of these cultivars is their ability to be cross-pollinated and to produce seedy fruits when they are cultivated in multicultivar orchards (Janick, 2008).

Among the citrus species and cultivars, the percentage of functional pollen is very different. Lemon and other orange cultivars have low amounts, Marsh grapefruit have very little pollen, while mandarin and pummelo produce mostly functional pollen. Satsuma mandarins and navel oranges are mostly pollen sterile and set parthenocarpic fruit. Cultivars with non-functional pollen usually show ovule abortion although Washington navel and satsumas have functional ovules. Pollen degeneration before meiosis is also encountered (Spiegel-Roy and Goldschmidt, 1996; Ray, 2002).

Use of Triploid Varieties

The first chromosome studies of the genus Citrus were reportedly conducted by Strasburger in 1907 who recorded the haploid number as eight. Diploidy with somatic chromosome number $2n = 18$ is the norm for Citrus and closely related genera. There are a high degree of morphological variability between species, and between varieties within the species. Various Citrus euploids have been reported. Monoploid plants of several Citrus species and of *Poncirus trifoliata* have been derived from pollen culture *in vitro*. Monoploid seedlings also arose after gamma irradiation of seeds. Triploids is one of the most common polyploids of citrus, discussed in this paper (Lee, 1988). Triploids have generally been considered to be an evolutionary dead end because they have very low fertility and tend to produce aneuploid gametes, due to problems of chromosome pairing during meiosis. Citrus species are consistently diploid, but a low frequency of triploid and tetraploid seedlings occur in their progenies. Triploids are particularly important because they are seedless. In the case of citrus, early cytogenetic studies have described triploid meiosis. Trivalent pairing and some univalents were observed and described a pre dominance of trivalents but also the presence of numerous bivalents and univalents as well as a great variation of the number of extra microspores in some genotypes. The selection of triploid lines has been, and is already, a very interesting way to develop seedless cultivars (Ollitrault *et al.*, 2008). Triploid vigour is reflected in the production of large fruits and, in the case of the triploid citrus genotypes, in seedlessnes (Cannell and Jackson, 1985). Indeed triploidy is generally associated with both male and female sterility. Thus, most of the trees under field evaluation present these characteristics, and an efficient selection can be carried out in other traits. Moreover larger fruit size associated with triploidy should correct the reduction of fruit size generally observed in seedless mutants of seedy cultivars. Triploids can produce haploid, diploid, or triploid gametes at low rates, which can lead to diploid, triploid, and tetraploid progenies (Otto and Whitton, 2000).

Several methods have been developed for triploid citrus creation: One of them exploits natural events of polyploidization such as 2n gametes, while the more recent strategies combine haplomethods such as androgenesis or induced gynogenesis, somatic hybridization for a direct synthesis of triploid hybrids, using embryo rescue and flow cytometry to select triploids in 2x × 2x crosses (Ollitrault *et al.*, 2008).

Second meiotic division restitution has been proposed for diploid megagametophyte development in clementine (Luro *et al.*, 2004) while Chen *et al.* (2008) reported first meiotic division restitution 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 are created by somatic hybridization (Grosser *et al.*, 2000). In the past this strategy was limited by the scarcity of tetraploid genitors. Today however, the pool of tetraploid genitors can be substantially enriched with new, highly heterozygous tetraploid genotypes obtained by somatic hybridization (Tusa *et al.*, 1996).

Another possible source is endosperm *in vitro* culture, which has also enabled the recovery of triploid plants. However, this technique seems poorly adapted for large scale breeding programs (Ollitrault *et al.*, 2008). Recently, tetraploid monoembryonic lines have been obtained by *in vitro* colchicine treatment of shoot tips grafted in vitro (Juárez *et al.*, 2004; Navarro and Juárez, 2007). While Wakana *et al.* (2005) obtained tetraploid branches by top-grafting shoots with sprouting axial buds treated with colchicine. In this way these authors avoid the juvenility of seedlings and have been able to use these tetraploids as parents two to four years after colchicine treatments. It appears that 4x × 2x crosses have been more successful in producing triploid embryos (Cameron and Frost, 1968; Soost and Cameron, 1975). It was proposed that the 5:3 ratio between endosperm and embryo ploidy obtained with these last crosses was more favorable than the 4:3 obtained in the reciprocal ones. These tetraploids open the avenue to 4x × 2x crosses with the maternal tetraploid Clementine. Tetraploid plants have also been regenerated by somaclonal variation from *C. limon* embryogenic callus obtained from ovules culture on medium containing 2, 4-D (Vardi and Spiegel-Roy, 1982). In the last 10 years, several new triploid cultivars resulting mostly from diploid × autotetraploid sexual crosses have been released in Italy, the USA, and Japan (Badenes and Byrne, 2012). The current ploidy manipulation programmes will result in an extensive range of triploids (seedless) citrus cultivars and will be utilized to combine trait of seedlessness and other characters, i.e., resistances against insect pest and diseases, quality traits or extended harvesting period.

French Agricultural Research Centre for International Development (CIRAD) has used somatic hybridisation between diploid and haploid lines. This method makes it possible to add, without recombination, a haploid genome to the whole diploid genome of high organoleptic quality cultivars. Somatic hybridisation is done by electrofusion of protoplasts isolated from haploid embryogenic callus lines of clementine, obtained from haploid embryos generated by induced gynogenesis, and callus lines or mesophyll protoplasts of diploid cultivars (Ollitrault *et al.*, 2008).

Mutation breeding by irradiation is also a suitable approach to pursue desired triploids because natural mutations and bud sprouts has often observed in citrus. Gamma irradiation was employed to obtain somatic mutations to study the evolution patterns of several citrus varieties and species. Although polyploidy often occurs naturally in citrus through spontaneous mutations, the condition of triploidy has occurred only one case of cultivated citrus ('Tahiti' Lime) (Recupero *et al.*, 2005).

Use of Mutation

Mutation induction has arisen as a useful technique to increase genetic variability in plant populations, not only in citrus, but also in other cultivars of commercial relevance. The International Atomic Energy Agency has registered more than 2000 different mutant cultivars officially released and obtained through irradiation mutagenic programs. Several studies have shown that irradiation induced a large number of functional variations in plants although the efficiency depends on the dosage, type of mutagen and species (Iglesias *et al.*, 2009). Mutation was conceived as a complementary method to conventional breeding. Spontaneous mutations have been reported in various citrus fruits (e.g. oranges, lemons, mandarins). Selection of spontaneous mutations is the oldest citrus breeding method and most of the varieties cultivated worldwide arose from this process. For the induction of mutational events in plant material the mutation breeder can choose between two groups of mutagenic agents, namely, chemical and physical mutagens. The latter have been used for many decades, whereas the use of chemicals is relatively recent, starting in approximately 1940 (Broertjes, 2012). Radiation is the most widely used type of mutagen for citrus. The two most commonly used radiation types are X-rays and γ-rays (generally from a ^{60}Co source). Dose is the amount of mutagen applied per unit time while exposure is the total absorbed radiation. Radiation has been found to induce many non-lethal chromosome aberrations that often affect more than one gene. Gamma irradiation has been the most common method of mutagenesis. It can induce a wide range of mutations (point mutation, chromosomes breaks, and rearrangements). The latter type is particularly interesting for seedless selection (Hearn, 1984) because translocations and inversions may cause sterility. Roose and Williams (2007) recommend exposure of budwood to 30–50 Gy to induce seedless mutations. Classical examples of cultivars obtained by irradiation programs are the Star Ruby red flesh grapefruit obtained by irradiation of seeds of Hudson grapefruit (Hensz, 1971), and more recently the low-seeded selection of Murcott, called 'Mor' mandarin produced by budwood mutation (Vardi *et al.*, 1993), and the seedless mandarin Tango produced by irradiation of Afourer (Nadorcott) budwood (Roose and Williams, 2007).

Chemical mutagens have been used less commonly in citrus than radiation (Broertjes, 2012). Different chemical mutagens induce different spectra of mutations. A very wide range of chemical

I: *Advantages and disadvantages of treating various tissue types with mutagens (Khan, 2007)*

Target tissue	Ease of use	Time to evaluation	Chimerism	Identification of mutants
Pollen	Good	Long	None	Difficult
Seed	Good	Long	Low	Varies
Embryogenic callus culture	Fair	Long	Low	Easy
Mature stem segment culture	Fair	Short	High	Easy
Budwood	Good	Short	High	Easy

mutagens are now available, but few of these have been evaluated in citrus.

The breeder can initiate a mutation breeding project with one of several different types of tissues, each with certain advantages and disadvantages (Tab. I). Choice of tissue may also depend on the target trait and type of mutagen to be used. Pollen irradiation has rarely been used in citrus. In versus treatment of buds has been widely used in citrus (Froneman *et al.*, 1996). A major advantage is that trees propagated from such buds will not display the delayed flowering and thorniness typical of seedlings. However, the probability of chimerism is high relative to treatment of single-cell systems (Khan, 2007). Mutant genotypes may be obtained that are improved in one trait and poorer in other characteristics. This imposes the need for large vegetative progenies, and the selection and observation of a larger number of mutants (Donini, 1982).

Nearly all work on inducing seedlessness through mutation breeding has been performed in citrus. The most important natural seedless mutants have been found in various citrus species (Spiegel-Roy *et al.*, 1985; Spiegel-Roy, 1990). Induced mutagenesis of seedy cultivars have been developed in several countries and gave good results for seedless line selection (Chen *et al.*, 1991; Froneman *et al.*, 1996; Uzun *et al.*, 2008). Seedlessness is usually, but not invariably, associated with high pollen and ovule sterility. However, the quality, economic value and marketability in citrus will increase with a decrease in the number of seeds per fruit.

In citrus, a pronounced parthenocarpic tendency is essential for satisfactory fruit set and yield. Problems of parthenocarpic fruit set and the size of the yield are often linked to seediness, at least with certain genotypes. Generally the seedless mutants should produce fruits significantly smaller than the parental seedy cultivar. These effects on the decrease in fruit size are in numerous cases not severe enough to detract from economic importance. The points to the need for making a larger number of selections from the same genotype when selection is performed for seedlessness. The time (years) required for final selection and assessment of the economic value will also have to be increased. Because the seedlings will be juvenile, it may be difficult to distinguish mutants from recombinants in the sexual progeny that result. Induction of seedlessness has been achieved by treating apomictic seeds with gamma rays (Hearn, 1984), seeds with thermal neutrons (Hensz, 1977), and scions with gamma rays (Hearn, 1986; Spiegel-Roy *et al.*, 1985). Somatic mutations have played an important role in the development of new cultivars, partly because apomixis is common in most Citrus species through the formation of nucellar embryos which derive from somatic cells of the nucellus. Seedlings may be of zygotic or non-zygotic (nucellar) origin. The percentage of seedlings that are of non-zygotic origin varies greatly, often being high to very high and very rarely zero (Soost and Cameron, 1975).

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, ‘Monreal’ Clementine mandarin, ‘Eureka’ lemon, and ‘Kutdiken’ lemon. A seedless mandarin ‘Tango’ has been produced by irradiation of buds of W. Murcott. ‘Monreal verde’ is an almost seedless variety obtained by irradiating budwood of ‘Monreal’ Clementine (Jain and Pryadarshan, 2009).

Natural mutants of improved color are of great significance in orange and grapefruit. Nucellar selection accompanied by thermal neutrons enabled the development of two mutants of great commercial significance and prospect. Star Ruby exceeds all natural and induced mutants in colour, it has been derived from a highly seeded cultivar (Hudson Red), with an intense internal and external colour. Rio Red, also seedless, has been derived from Ruby Red, a coloured, seedless mutant, and is now being ranked by most experts as second only to Star Ruby in color, with better possibilities of adaptation to the environment and cultural practices (Novak, 1991).

Scion Breeding

The scion breeding programs are mainly aimed at improving the fruit quality include: flavor, size, color, shape, 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. Mandarins are relatively easy to breed by crossing parents with good traits and selecting superior progeny. The seeds from mature hybrid fruits after extraction, separately planted in greenhouse carefully and after seedlings have attained sufficient size, they are then planted in the field. 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. 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).

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).

Rootstocks Breeding

Beyond productivity, the rootstock choice has a strong impact on fruit quality. It can affect the sugar and acid content, the fruit size, and the percentage of juice content. For example Poncirus and its hybrids are known to favor high quality of mandarin and orange production, while C. *macrophylla* is very productive but reduces fruit quality. Thus productivity and fruit quality of the associated scions are an essential criteria for rootstock selection. 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. The major objectives of rootstock breeding are aimed to control the tree size and to improve resistance and tolerance to biotic and abiotic stresses. 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 (Jain and Pryadarshan, 2009).

New Breeding Techniques

Conventional cross breeding can be considered foundation of citrus variety improvement. However, this method cannot be used to develop improved cultivars in many economically important citrus species such as sweet orange, grapefruit, and lemon due to barriers of sterility, self-and cross-incompatibility, and the widespread polyembryony. In addition to this, the heterozygosity makes the breeding for specific traits extremely difficult. Traditional breeding approaches via sexual hybridization have not been useful for the genetic improvement of most of the citrus cultivars. Therefore citrus variety improvement programmes have in the past relied on limited sources of genetic variation. In addition to conventional breeding they have included spontaneous mutations, irradiation of seed and budwood and importing germplasm from other locations.

Nowadays new directions have been opened by biotechnology. Modern biotechnologies based on *in vitro* cell, protoplast, tissue, and organ culture, and gene introduction, are being tried to improve the quality of existing cultivars and to produce new varieties. The modern tools of molecular biology should throw more light on the functions of enzymes, their pathways, and the genes controlling them. Using genetic engineering techniques, metabolic pathways can be modified with certain enzymes, forming desired metabolites and eliminating problems related to the seedless citrus cultivars, flavor and eating quality of fruits (Ollitrault *et al*., 2008). It is a pleasant surprise that in recent years several genetic, genomic, and proteomic tools and technologies have been quickly adapted by the citrus research community to address major challenges of this plant system.

Micropropagation

Application of the biotechnological sciences such as regeneration and micropropagation, somaclonal variation, somatic hybridization, ploidy manipulation, transformation, genomics has the potential to unlock an entirely new round of genetic improvements for citrus crops.

The responsibility of citrus to be regenerated via organogenesis and somatic embryogenesis is the fundamental basis that makes possible much of the potential for advances in plant cell and tissue culture.

Somatic Embryogenesis

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 and undeveloped ovules or indirectly via callus formation. Embryogenesis has also been induced from endosperm-derived callus, juice vesicles, styles, anthers and pistil thin cell layers (Jain and Pryadarshan, 2009).

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 (Jain and Pryadarshan, 2009).

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. In citrus, this technology has been extensively used and has many important implications. 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 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. 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. 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. A second way for producing improved scions is to produce triploids directly by haploid + diploid protoplast fusion. 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 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. A final option is the production of citrus somatic cybrids, which may have potential in both scion and rootstock improvement (Grosser *et al.*, 2000).

Transformation and Genomics

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 (Jain and Pryadarshan, 2009).

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). 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 programmes (Asins, 2002). 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. Molecular markers have also been widely applied on phylogenetic and taxonomic studies in citrus. 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 (Jain and Pryadarshan, 2009).

Molecular Markers

Molecular markers are very useful tools to help plant breeders in facing this and other issues: they allow the study of genetic variation, the search of new sources of agronomically important genes, the identification of cultivars and the efficient management of segregant generations during plant breeding programs through marker-assisted selection (Asins, 2002). A molecular marker is a genetic difference that may exist between two plants, visualized by means of biochemical methods and located at a certain chromosomal position. When the genotype for one or more marker loci is known for two plants, inferences can be made about the genotypes that may appear in the progeny. Isozyme analysis is the usual technique in the separation of zygotic from nucellar plants among progenies (Iglesias *et al.*, 2009).

Progress in the Citrus Genome Project coupled with recent advances in molecular biology techniques should aid the development of faster, targeted, and more efficient tools to expedite citrus breeding. For instance, DNA markers for traits associated with seedlessness, such as parthenocarpy, male and female sterility, and self-incompatibility, could be used as tools for directed breeding. These markers could be further used in map-based or candidate gene approaches for the cloning of genes that encode important traits, thus expanding the list of genes that can be transformed to induce seedlessness.

CONCLUSION

For consumers quality is a term that usually applied for the fresh fruit and within the markets refers to the external appearance of the product. Fruit color, fruit size, easy of peeling and seedlessness are paramount in commercial citrus types and new cultivars are being developed through plant breeding and selection of new sports. Therefore, conventional breeding of fruit trees will most likely continue to be a very important way to meet consumer demand for improved fruit quality. Triploidy is probably the most popular method for the development of seedless citrus cultivars. Fruit color is a unique characteristic in the life cycle of fruiting plants and is one of the most important traits for both commercial and esthetic value of fruit. Mutants are invaluable plant materials to help understand the relationship between genotype and phenotype and Mutation induction has arisen as a useful technique to increase genetic variability in citrus. Although citrus breeding is very challenging, breeding programmes 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. The various methods for obtaining varieties with best fruit quality may be applied to different types of cultivars/varieties (sterile, mono or polyembryonic) and lead to very different populations in terms of genetic segregations and recombinations. Most of the critical goals for scion improvement, such as resistance to diseases or changes in fruit quality attributes, are difficult to approach in any practical sense by conventional breeding strategies. 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. Biotechnology with different methods and strategy can be used to develop improved cultivars in many economically important citrus species.

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