THE EVOLUTION OF CITRUS SPECIES - METHODS TO DEVELOP NEW SWEET ORANGE CULTIVARS

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Abstract. Citrus originated before recorded history. Citrus sinensis as the sweet orange began in 1765, though its origin is unknown. Hybridization between Citrus species and subsequent selection may have given rise to the orange. Cultivars likely resulted through selection of naturally occurring mutations over a long history. Selection of natural and induced mutations and hybridization are methods used to develop new orange cultivars. Hybridization is a more difficult method to accomplish orange cultivar improvement, but the magnitude of trait change is greater than is possible by selection of mutations. 'Ambersweet' is the first recorded orange cultivar developed through hybridization, but other hybrids very similar to the orange have been produced. Organoleptic or taste tests are essential to determine if a hybrid has juice that would be recognized as sweet orange.

Introduction

Citrus originated prior to recorded history. Although the orange is of more recent origin, its mode of development is unknown. Speculation and recent taxonomic and breeding research evidence suggest that the orange is of hybrid origin. It is likely that the current cultivars originated as naturally occurring genetic mutations of earlier orange types and were propagated as nucellar seedlings or by grafting. Methods that have been used to develop new orange cultivars include selection of naturally occurring mutations, inducement of mutations by irradiation, and controlled hybridization. 'Ambersweet' and other hybrids similar to orange have been developed. Hybridization offers potential for greater increments of progress in orange characteristics than the other methods, but is complex and long-term.

Historical review

The oldest known reference to Citrus appears in the Sanskrit literature in a collection of devotional texts dated prior to 800 B.C. The oldest Chinese reference to Citrus is contained in the book “Tribute of Yu” that was completed between 776 and 600 B.C. Citrus had been used by man for some time, perhaps before 700 B.C., though it was probably not described before 1000 B.C. The mandarin and the citron are among the earliest types mentioned. In 1179 A.D., Han Yen-Chih named and described 27 cultivars of the sweet-sour orange-mandarin group in his Chu Lu. The vast majority of Citrus and its wild relatives are native to southeastern Asia, New Guinea, Australia, the East Indian Archipelago, New Caledonia, and Melanisia. No Citrus nor citrus relatives have been native to Europe or the New World (Scora 1975). As a result of the extensive distribution and use, the orange has become the most important economic form of citrus grown worldwide.

I. Traditional botanical recognition of Citrus sinensis as a valid species.

Citrus sinensis (L.) Osbeck was used first in 1765 and currently is the most widely accepted species epithet for the orange. The most widely used classifications of Citrus species are the systems suggested by Swingle (1943) with 16 species, and Tanaka (1969) with 159 species. Swingle first used “sinensis” in his 1914 classification, and Tanaka first used “sinensis” in his 1924-1941 system. Fruit types within C. sinensis represent a fairly wide range from the navels to the bloods, and early to late maturing cultivars; some acidless forms have been identified, as well. All of the orange cultivars produce seeds containing multiple embryos; these usually do not include an embryo of sexual origin (with genetic contributions from both male and female parents). The asexual embryos are formed from cells of the nucellus tissue of the female plant, without the contribution from the male plant. Usually, the multiple asexual embryos and the attached cotyledons (food supply for each embryo) are more vigorous and crowd out the weaker developing sexual (zygotic, formed from the union of male and female genetic contribution) embryo. Thus, the sexual embryo usually/almost always fails to survive. Seedling plants from the asexual embryos are genetically identical unless a mutation occurs. The asexual (nucellar) seedlings are very vigorous in growth for up to 25 years in some cases, and this period is known as juvenility (thorns and delayed flower initiation). Fruits produced near the end of the juvenile period are larger and of poorer quality than those of the more mature mother plant. Swingle and Tanaka did not understand that apomixis (production of seeds containing asexual embryos) assured seedlings would be true-to-type genetically to the seed parent. This reproductive trait ensures a high degree of trueness-to-type and distinctiveness from other forms of citrus, a basis for species rank by Swingle and Tanaka. Webber and Swingle (1904) even named a genetically identical nucellar seedling of ‘Dancy’ tangerine (C. reticulata Blanco) as a new cultivar called ‘Weshart’.

II. Recent taxonomic studies of the relationships of Citrus species.

Citrus taxonomists have used very few of the multitude of characters in the genus to delineate species. Swingle used 14 and Tanaka seven of Swingle’s characters plus 10 others not used by Swingle. Barrett and Rhodes (1976) used approxi-
mately 200 characters in their studies of affinity relationships in cultivated Citrus and its close relatives. They found that C. sinensis is intermediate between C. reticulata (mandarin) and C. grandis (pummelo). They concluded that the C. sinensis biotype is predominately the C. reticulata genotype introgressed with genes from C. grandis. Scora (1975) concluded that C. sinensis developed by hybridization of C. reticulata and C. grandis with the morphological characteristics closer to the mandarin. Much of the confusion regarding classification within the genus Citrus is primarily due to repeated cross-pollination among genetically diverse types throughout time. Then, many of these types are preserved genetically unchanged by asexual seed reproduction. Because these types are distinct, and remain so from one generation to the next, Swingle and Tanaka called these separate species.

III. The theory of how orange cultivars evolved.

The original forms of C. sinensis originated by natural or man-made hybridization as described above, and probably did not result from F1 hybrids of C. reticulata and C. grandis. Frost (1943) believed that many of the cultivated forms of Citrus may have been reproduced almost entirely through nucellar embryos from remote antiquity. He concluded that the differentiation of horticultural cultivars of sweet oranges had taken place mainly by somatic change, or bud variation, without frequent recombination of genes in sexual reproduction. Frost did not have evidence to support his contention, however. As a consequence, the genetic base among the sweet oranges is very narrow, and cultivars probably differ by only a few genes or differences in genomic organization (e.g., due to inversions, translocations, etc.). The inability to differentiate orange cultivars by isozymes (Torres et al., 1978) and other analyses supports Frost’s conclusion. The almost exclusive use of vegetative propagation and the massive numbers of plants produced have combined to create large populations where even a rare mutation could occur. Hand harvest of individual fruits enhances the probability that a variant or mutant form will be recognized and desirable types propagated as new cultivars.

IV. Experimental evidence that C. sinensis is a hybrid, not a true species.

Seeds of orange cultivars rarely contain a sexual embryo that survives and produces a seedling. Therefore, resulting nucellar seedlings are genetically identical to the female parent. Phenotypically (appearance traits), the seedlings are identical, even though their genetic makeup is heterozygous (genetically diverse due to differences among the parents). Few references that document the production of confirmed hybrids between two orange (C. sinensis) cultivars can be found. Furr (1969) reported such a hybrid that was never confirmed, and Hearn (1977) reported the production of a population of hybrids between ‘Mediterranean Sweet’ and ‘Pineapple’ oranges. The zygotic orange hybrids reported by Hearn generally did not closely resemble C. sinensis cultivars. Many of them had leaf and general characteristics commonly found in C. reticulata and, sometimes, species with winged petals such as C. grandis, C. paradisi, or C. aurantium. This shows that the orange is a diverse hybrid resulting from at least some of these species. C. paradisi is grapefruit and could not have been part of the original orange because the grapefruit originated much later in the western hemisphere. Barrett and Rhodes (1976) and Scora (1975) speculated that hybridization was the method of origin of the original form known as the orange (C. sinensis). Frost (1943) speculated that there was an original form (hybrid) recognized as the orange, and during many years of culture mutations occasionally occurred and someone propagated them. It is believed that this is the method by which various orange cultivars originated. Hearn’s work supports this contention. Many of these mutant types have been preserved without change as cultivars through vegetative propagation (grafting) and through asexual (nucellar) seed reproduction, where the offspring are genetically identical to the female or seed parent.

V. Methods being used to develop new orange cultivars.

A. Selection of natural mutations.

Most of the orange cultivars have been developed by this method. Selection is largely based on fruit characteristics that are perceived as different and possibly advantageous. Most mutations are deleterious and ignored, but changes in peel or interior fruit traits, seed content, and maturity date have been recognized and saved by vegetative propagation. Many of these have been valuable and helped make the orange the most important type of citrus worldwide. While mutant types have been collected over many years, this technique is currently in use in organized research programs, and growers and marketers are still recognizing desirable mutant types. Although mutations, especially desirable types, rarely occur, the massive numbers of trees and their long life span offer substantial opportunity for mutations to occur and be recognized. Numerous selections of oranges, especially navel and ‘Valencia’ types, have arisen in this manner. ‘Midsweet’, ‘Sunstar’, and ‘Gardner’ oranges are examples of naturally occurring seedling cultivars developed in recent years (Hearn 1988).

B. Induced mutations.

Irradiation of seeds or buds with X-rays, thermal neutrons, or gamma rays has been used to induce mutations. Seedless forms (‘Star Ruby’ and ‘Rio Red’ grapefruit) of cultivars that normally produce seeded fruit were developed by irradiation (Hensz 1971, 1985). Other fruit traits may also change following irradiation. Irradiation is useful as it substantially increases the frequency of mutation occurrence. Hearn (1984) is using this technique with ‘Pineapple’ orange in attempts to produce seedless forms. Some of the seedless forms are being evaluated. Other new methods of cultivar development are being tried.

C. Hybridization.

Development of orange cultivars by hybridization has been attempted for at least 100 years. It has largely failed because new selections must be very similar to that of the existing orange cultivars. This means that extreme difficulty is faced in attempts to develop new orange cultivars through hybridization. F1 hybrids have not been developed that are similar to the orange, except for a limited number of zygotic hybrids of ‘Mediterranean Sweet’ X ‘Pineapple’ oranges produced by Hearn (1977). Multiple generation hybridizations in a few cases have resulted in hybrids similar to oranges. One
such hybrid by Hearn (unpublished) was developed by crossing 'Temple' X 'Orlando' tangelo (C. reticulata) hybrid ? X (C. paradisi X C. reticulata) and a selected seedling was then crossed with 'Hamlin' orange. One hybrid plant with fruit and juice characteristics very similar to orange was found. Flavonoid analyses from leaves by Tatum (unpublished) failed to distinguish this hybrid from orange leaves. The juice had poor color and the plant was judged unworthy of release. If new hybrids are not very similar to existing orange cultivars in fruit and juice characteristics, they probably will not be accepted nor recognized as an orange. Federal label laws on orange juice and orange juice products are a big factor here, too. Nonetheless, hybridization is the most likely method of incorporating significant improvements in new orange cultivars. Such traits include cold hardiness, improved juice and peel color, high pounds solids (sugar content), desirable peel traits, diversity of fruit maturity date, and improved disease resistance of trees.

Hybridization by backcrossing was attempted by Hearn several times by multiple methods, and inbreeding depression was substantial before any hybrids with fruit closely resembling oranges were produced (unpublished). The rapid rate of inbreeding depression confirmed the heterozygous makeup of the orange.

The 'Clementine' tangerine (C. reticulata) was crossed with 'Orlando' tangelo, and 10 selected hybrids were then crossed with an orange selection. About 5,000 hybrid seedlings were produced in 1964. Among the 712 hybrid progeny from one of the crosses was a hybrid with many orange characteristics. This hybrid was named 'Ambersweet' (Hearn 1989). No other hybrid from any of the crosses was judged similar enough to an orange based on fruit traits and juice taste to warrant extensive testing as an orange.

Testing was done with fresh fruit and juice to determine how similar 'Ambersweet' was to sweet oranges based on appearance and juice taste. Processing tests were initiated with the Florida Department of Citrus to determine if the fruit, juice, and flavor were similar to those of oranges (Barros et al., 1990). Essence fractions were analyzed by Moshonas et al. (1991) for qualitative and quantitative compositional content of volatile flavor and aroma of 'Ambersweet' fruit products. They were compared with those of similar products from the parent fruits, orange, tangerine, and grapefruit. All 21 constituents identified in 'Ambersweet' fresh juice were identical to those in fresh orange juice. All 30 constituents identified in 'Ambersweet' aqueous essence were identical to those in aqueous orange essence with no appreciable quantitative differences. Selected components of 'Ambersweet' peel and essence oils important in flavor were found to be identical to those in orange peel and essence oils with no quantitative differences.

Processing tests with 'Ambersweet' fruit were conducted in 1987 and 1989 by the Florida Department of Citrus using commercial type citrus juice extractors (Barros et al., 1990). Raw, pasteurized, and pumpout concentrated juices were made. All juice samples produced were subjected to analysis for 10 quality indicators. Flavor evaluations conducted used a 10- to 13-member experienced taste panel, with a 9-point hedonic scale, where 9 represents "like extremely", 5 is "neither like nor dislike" and 1 represents "dislike extremely." The positive flavor panel judgements indicated that the juice and concentrated juice of the 'Ambersweet' orange could be blended in commercial orange juice products satisfactorily without percentage limitations.

In 1992 Florida Department of Citrus scientists prepared samples of 100% 'Ambersweet' concentrate and a blend of 50% 'Ambersweet' and 50% 'Valencia.' These samples were presented to USDA inspectors in Florida and California and they received favorable ratings for flavor and color in each state (unpublished).

A petition was prepared by the Florida Department of Citrus using all of the data available from tests with 'Ambersweet' fruit and juices and submitted to the Food and Drug Administration to permit unlimited use of juice in orange juice products. Organoleptic test results were very important in the decision to approve the petition. The result of this experience is clear evidence that organoleptic tests are very important in showing that the taste of juice must be very similar to that of orange juice for approval of its use in orange juice products by the Food and Drug Administration and conformance with federal label laws. These factors must be addressed by anyone who attempts to develop improved orange cultivars by any form of hybridization. The selection of naturally occurring or artificially induced mutations of orange plants can avoid many of the obstacles encountered in market classification of orange hybrids, but organoleptic tests still would be important in the acceptance of any potential new orange cultivar. However, selection of mutant types places severe limits on the range of improvement variations that can be accomplished in comparison with hybridization techniques.

**Literature Cited**


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