Hybridization of *Citrus* and Related Genera

H. C. Barrett[1]

Intergeneric hybridization has not received much attention from plant breeders and the literature on this subject is rather meager. Successful results from intergeneric hybridization have been rare. Almost without exception, this strategy has been used only after attempts to solve breeding problems by the use of more conventional strategies have failed. Let us examine some facts about *Citrus* and its relatives that must be considered in determining if intergeneric hybridization is an appropriate breeding strategy for solving citrus breeding problems.

Intergeneric hybridization is based on a simple concept. The genus *Citrus* with its vast array of forms and cultivars has great genetic variability or diversity. The relatives of *Citrus* also have great genetic diversity, some of it a different kind of diversity than that found in *Citrus*. Intergeneric hybridization is the key process by which this different and previously unavailable genetic diversity of the *Citrus* relatives can be added to the genetic diversity of *Citrus* and made available for use by citrus breeders.

If the *Citrus* relatives are an additional source of desirable diversity, why would citrus breeders, only as a last option, turn to them in the search for donors of specific traits? There are numerous reasons for doing so, such as 1) intergeneric crosses are usually much more difficult to make and if seed is obtained at all, the seed yield is often low or it may be nonviable; 2) if hybrids are obtained, the progenies ultimately available for selection are often few in number due to lethals, abnormal recombinants, or poor field survival; 3) the surviving hybrids may not flower, those that do may have very long juvenile periods or may be completely to partially ovule or pollen sterile.

The most important reason for reluctance to use this germplasm source has to do with the undesirable traits brought into the breeding lines by these donors. The related genera are often wild biotypes that possess a whole array of undesirable traits and trait complexes that will be introduced into their progenies along with the sought-after desirable traits. Breaking linkages between desirable and undesirable traits or concentrating diffuse desirable traits from multiple donor sources will require a very long and often tedious program of complex crossing and selection.

PREPARATION AND PROBLEM ANALYSIS

Citrus breeders need the best preparation they can obtain or devise if they expect to make efficient use of the time and resources available to them. Information on genetic and plant breeding theory and basic technique abound in textbooks. Other kinds of specialized information are also needed to develop efficient strategies to deal with each breeding problem and to help in minimizing the inevitable, time-consuming mistakes of unproductive crosses.

In the author's judgment, the most fundamentally important information needed is a thorough and detailed knowledge of the nature, properties, and relationships of the variation that occurs in the germplasm of the various taxa that will serve as parents. The breeder needs all the information on morphology, anatomy, geographic dis-

[1]U.S. Department of Agriculture, Agricultural Research Service, 2120 Camden Road, Orlando, Florida 32803.

tribution, cytology, physiology, pathology, biochemistry and any other facts about these taxa that he can obtain. Cultivars are the basis of citriculture and variation has produced every cultivar in existence. Without variation the citrus industry simply could not exist. Variation is the only known mechanism by which permanent future improvement of citriculture can be brought about.

The presence or absence of specific variations in plant populations is the basis for the separation of species and genera in the classification of the subfamily Aurantioideae. Taxonomy attempts to examine this diversity of a seemingly disorderly assemblage of differences that we call variation and seeks to bring some kind of order to our understanding of how one plant with its set of variations might be related to another plant with its set of variations.

Citrus breeders or anyone seeking an understanding of the relationships of the variation in *Citrus* and its relatives from published systematics face a formidable problem. Taxonomists and other factors have combined to create almost complete disagreement and near chaos in the taxonomic interpretations of this group of plants. The cultivated taxa of *Citrus* belong to a genus whose interpretation has been enormously complicated by their long history of domestication and cultivation. The ability of most of these taxa to hybridize and at the same time to reproduce by apomixis, i.e. they are facultative apomicts, has made their relationship very difficult to comprehend. It is regrettable that most taxonomists who have struggled with *Citrus* taxonomy did not understand the effects of facultative apomixis on population samples and its relationship to genetic heterozygosity. As a consequence, this situation has made it almost impossible for any of them to develop a meaningful concept in the genus *Citrus* or a taxonomic understanding of their relationship as a group.

Additional details and experimental data dealing with the taxonomic interpretation of *Citrus* and its near relatives may be found in Barrett and Rhodes (1).

A hybridizing program will require a germplasm collection to provide as complete a sampling of the variation present in each taxa as can be obtained. For the wild or uncultivated relatives, variation samples should be collected throughout the natural range of each taxa. This would preferably be done by an experienced citrus breeder or breeder-taxonomist team. All information and data on growth habit, response to environment, parasites, ecology, reproduction, morphological diversity and other often-more-subtle facts should be collected at the same time as the living germplasm. Herbarium specimens of each accession collected for future reference should also be made. Cultivated *Citrus* taxa exist in many experiment stations and should be relatively easy to collect.

The next essential step is the choice of a convenient location where all of this germplasm may be grown in the same environment for comparative cultivation. It is only by growing both cultivated *Citrus* taxa and the taxa of related genera contemporaneously in the same environment that comparable, unbiased data of their biological properties and responses can be obtained. The author would strongly stress the need to preserve this germplasm for future use. None of us can predict the future of citriculture with certainty, but this germplasm should be retained for study by techniques still to be discovered in order that it might help solve problems presently unrecognized or that have not yet arisen.

Careful thought must be given to planning and designing the breeding strategies that will be effective in producing the anticipated end product of intergeneric hybridization. The initial step in this process is determining what adverse condition or conditions affect the plant and/or its development cycle in the area of intended culture. The breeding system of each potential taxa donor source, the taxonomic relationships of the taxa, the mating sequence for genotype building, other citrus breeders' experiences, if any, with the problem or donor sources are only a few examples of the many factors that have an important bearing on the decisions that go into planning and designing breeding strategies. It also should not be forgotten that these strategies will be carried out over a protracted period of time and that changes or modifications of breeding strategies could become necessary.

CITRUS RELATIVES – GENERAL ATTRIBUTES

Because of time limitations, the attributes of *Citrus* and its diverse forms will not be discussed here. Only a few of the more important attributes of four genera, *Eremocitrus, Fortunella, Microcitrus* and *Poncirus,* will be discussed.

Eremocitrus glauca (Lindl.) Swing., the desert lime, is a native of the semiarid regions of eastern Australia. Under cultivation, bearing-age, budded trees are medium small, 2 to 5 m high, evergreen, with narrow, linear, grey-green leaves, and pendulous branches. *E. glauca* has a pronounced rest-period dormancy and has survived a freeze of −12.2°C without injury. The small, white flowers appear in mid-March at the same time *Citrus* blooms. The very small, 12- to 15-mm diameter, greenish yellow fruit mature about 10 weeks later or in late May. The very juicy, acid fruit drop from the tree as soon as they are mature. Soluble solids have ranged from 13.4 to 14.3% with 4.0 to 5.27% acidity in juice samples. The small, wrinkled seeds are monoembryonic. Almost all seed sources tested here have produced very slow-growing, multiple-stemmed plants that do not appear to be well adapted to the sandy soils of central Florida. The juvenile period is very long—10 years or more.

Fortunella margarita (Laur.) Swing., the oval kumquat, has served as the chief source of *Fortunella* attributes in our hybridization work. It is from China and is known only under cultivation. Bearing-age, budded trees are evergreen, small, 3 to 5 m high, dense and bushy with thornless branches. Rest-period dormancy is very pronounced. *F. margarita* is somewhat hardier than *Citrus* but much less hardy than *Eremocitrus.* A freeze of −12.2°C caused complete leaf kill and limbs frozen back to lower trunks. Under Florida conditions, *F. margarita* is remontant with the first small, white flowers appearing 3 to 4 weeks after *Citrus* blooms. Mature fruits are yellowish orange, small, obovate, 20 × 35 mm. The fruits have sparse flesh, sparse acid juice with sweet, edible, adherent peel. Juice samples are in the range of 9 to 10% soluble solids and 5.6 to 6.2% acidity. The small seeds are monoembryonic, but both zygotic and nucellar seedlings are produced. Young seedlings grown in the glasshouse are very sensitive to copper deficiency and may suffer heavy mortality unless copper supplements are given. *F. margarita* is fully compatible with *P. trifoliata* rootstock but shows budunion crease and twig dieback symptoms of incompatibility with most common *Citrus* rootstocks, including most hybrids of *Poncirus trifoliata.* The juvenile period is slightly shorter than that of *Citrus.*

Microcitrus. Our hybridization work has used *M. australasica* (F. Muell.) Swing. and *M. australis* (Planch.) Swing., both natives of eastern Australia's rainforest, Sydney hybrid—a hybrid of these two species that originated under cultivation, and *M. papuana* H. F. Winters—native to eastern New Guinea. The Australian species and Sydney hybrid are generally similar in attributes except for fruit shape and some slight differences in leaf shape and relative size. Under cultivation *M. australasica,* the finger lime, is a small, shrubby evergreen, 4 to 6 m high, with a dense, thorny crown. The branches have very short internodes and very small leaves. The flowers are small, purple-tinged, and the fruits are medium small, long cylindroidal, 27 × 90 mm. On different trees, they may be green, yellow, purplish red or nearly black in skin color. The vesicles are noncoherent with very acid, sour juice. Seeds are small and monoembryonic. Trees are very tender to cold. Some clones are resistant to burrowing nematodes, *Radopholus similis* (Cobb) Thorne, and *Phytophthora parasitica* Dastur. The juvenile period is somewhat shorter than for *Citrus. M. australis,* the round lime, has small, globose fruit borne on a somewhat more vigorous tree. The Sydney hybrid has attributes generally intermediate between its parents. The fruit is shorter and broader, 30 × 65 mm, than the fruit of *M. australasica. M. papuana* is a small, bushy shrub, 1.5 to 2.5 m high. It has small, white flowers and is remontant flowering. The fruit are shaped similar to *M. australasica* but they are smaller, more slender and elongated, about 15 × 75 mm. *M. papuana* is even more tender to cold than the other *Microcitrus* species. It is also quite susceptible to burrowing nematode and *Phytophthora. M. papuana* has a very short juvenile period. Fruit can be produced on young, glasshouse-grown seedlings in 1 year's time from planting of the seed.

Poncirus trifoliata (L.) Raf., the trifoliate orange, is a native of central and northern China. Under cultivation bearing-age trees are small, 4 to 5 m high, dense and thorny with 3-foliolate leaves. *P. trifoliata* is deciduous and has been known to survive cold to at least −26°C. The flowers are white, below medium to very large. Under Florida conditions they may appear before, during, or after citrus bloom, possibly depending on the duration of the chilling period during the winter dormancy. *P. trifoliata* normally has a single flowering period but may flower remontantly following drought stress and intermittent rains, a condition that may occur fairly often in central Florida. The small, 35 to 50 mm diameter, globose, yellow, pubescent fruits begin to mature in September. The inedible fruit are bitter, acrid, very acid, and very unpleasant in taste. Samples of juice ranged from 12.1 to 17.6% soluble solids and 4.16 to 7.5% acidity. The large, smooth seeds are polyembryonic. *P. trifoliata* has high resistance to citrus nematode, *Tylenchulus semipenetrans* (Cobb), to *Phytophthora parasitica,* and to citrus tristeza virus. It is sensitive to high chloride levels and to excess lime in soils. The juvenile period is slightly shorter than that of *Citrus.*

GERMPLASM DONORS – PERFORMANCE AND BREEDING VALUE

Eremocitrus—This genus was used as a trait source of 1) tree cold hardiness, 2) very early fruit maturity, and 3) pronounced rest-period dormancy. The objective was to produce improved scion varieties by hybridization with *C. grandis* (L.) Osbeck, *C. paradisi* MacFayden, and *C. sinensis* (L.) Osbeck. In the sweet orange crosses, results thus far would rate *Eremocitrus* as a good donor for the traits named. A few of these seedlings were outstanding in cold resistance with foliage and branches undamaged after −12.2°C. All seedlings that have fruited have had pronounced rest-period dormancy and were early maturing. There were negative aspects as well. The majority of the seedlings have been pollen sterile, polyembryonic, and sparse-seed producers. Fruit size has been small and the acidity has been too high for dessert-type fruit. There have been some significant tree damage and loss from *Phytophthora* and herbicides. Hybridization with pummelo and grapefruit was much more difficult. Many crosses were attempted before compatible combinations were found. Nonbearing trees of these F_1 hybrids growing in the field appear to be vigorous and healthy, but evaluation is incomplete.

Crosses were attempted with *C. aurantifolia* (Christm. & Panz.) Swing., *C. aurantium* L., *C. limon* (L.) Burm. F., *C. medica* L., and *C. reticulata* Blanco, but the hybrids produced were too few for reliable evaluation. Many crosses were attempted between *Eremocitrus* and the genera *Fortunella*, *Microcitrus* and *Poncirus*. The objective in most cases was to produce genetic bridges. The cross of *Eremocitrus* and *Poncirus* was very difficult to make and succeeded only after repeated efforts. F_1 hybrids were eventually produced but all died in the seedling stage. A few hybrids of *Eremocitrus* and *F. margarita* were produced. Some F_1 hybrids were outstanding in cold hardiness and survived −12.2°C without damage. All had a very pronounced rest-period dormancy. Only sparse crops of very early-maturing fruit have been produced. The fruit are very small, light yellow, highly acid with edible peel, and sweet-tasting peel oil. All progenies of *Eremocitrus* crossed with *M. australis*, Sydney hybrid, and *M. papuana* were nearly as cold tender as the *Microcitrus* parent. They have been repeatedly killed back and heavily damaged by freezes.

Fortunella—*F. Margarita* was hybridized with *M. papuana* for the purpose of producing genetic bridges. All of these F_1 hybrids have suffered heavy freeze damage or were killed back to ground level on several occasions. *Fortunella* was hybridized with *Poncirus*, but all resulting hybrids died when still small seedlings. Several seedlings were produced from a recent cross of *F. margarita* and *C. ichangensis* Swing., but only one vigorous seedling has survived in the field. It has not yet flowered. *Fortunella* appears to be a good donor for pronounced rest-period dormancy and desirable peel oil traits.

Microcitrus — Numerous attempts have been made to produce F_1 hybrids of *Microcitrus* and *Poncirus*. The objective was to produce improved rootstocks that would combine the traits of burrowing nematode and *Phytophthora* resistance from the *Microcitrus* donor source with the citrus nematode, tristeza virus, *Phytophthora*, and cold resistance of the *Poncirus* donor source. Crossing attempts of *M. australasica*, *M. australis* and *M. papuana* with *P. trifoliata* either failed completely or if seeds were produced, they failed to germinate. Only the cross of Sydney hybrid and *Poncirus* produced viable seed and living progeny. These hybrids were extremely variable in vigor — from vigorous to many stunted, slow-growing weak types. Only two of the vigorous seedlings flowered sparsely. Repeated efforts to cross these F_1 hybrids with a wide range of parents never produced any seed. Perhaps more varied and persistent efforts with other clones of *Microcitrus* and *Poncirus* might produce better results. On the basis of performance to date, *Microcitrus* would rate as a poor donor source for the several valuable attributes that this genus possesses.

Poncirus — This genus, as noted in the foregoing discussion, was used in hybridization attempts with all of the *Citrus* relatives, but only the crosses of *Poncirus* and *Citrus* produced valuable F_1 progenies. *Poncirus* was used as a trait source for 1) *Phytophthora* resistance, 2) citrus nematode resistance, and 3) tristeza virus resistance in a program objective to produce improved rootstocks. In a concurrent program objective to produce improved scion varieties, *Poncirus* was the trait source for 1) tree cold hardiness, 2) deciduousness, and 3) tristeza virus resistance. The various cultivars of *C. grandis, C. paradisi, C. sinensis,* and to a lesser extent *C. reticulata* were the sources of *Citrus* germplasm traits. Evaluation for *Phytophthora* resistance has been the most advanced of the rootstock objectives. *Poncirus* has had a record of variable performance as a donor as both *Phytophthora*-resistant and -susceptible hybrids have been produced in the same progeny.

For the scion variety objectives, *Poncirus* has been an excellent donor for tree cold hardiness. Hundreds of F_1 hybrids of the four named *Citrus* species and *Poncirus* subject to $-12.2°$ C freeze survived with nearly complete leaf kill but no other visible damage. As a donor of deciduousness, *Poncirus* has been only fair. Partly or semi-deciduous F_1 hybrids were the general rule in all *Citrus* crosses. Only the crosses with *C. reticulata* produced a few completely deciduous F_1 hybrids. *Poncirus* has had a variable performance record as a donor of tristeza virus resistance. Both susceptible and resistant F_1 hybrids were produced in the same progeny.

There were many negative features found in all of the hybrids. The worst examples were the extremely thorny trees and the horrendous quality of the fruit. In all crosses and progenies where *Poncirus* has been one of the parents, the fruit of every F_1 hybrid has been acrid, bitter, highly acid and inedible. In the various crosses of *Poncirus* and *C. grandis,* the latter appeared to be a good donor for relatively large fruit size and monoembryony. In the other *Citrus* species crosses, their performance as donor sources was mixed or obscure. Fruit size was more variable but generally averaged smaller, and most hybrids were polyembryonic.

Because of time limitations, only a few comments will be made on advanced crosses derived from F_1 intergeneric hybrids. Details on these and other crosses, especially as they relate to breeding cold-hardy scion cultivars, may be found in Barrett (2). An F_1 hybrid of *C. paradisi* X *P. trifoliata* backcrossed to *C. sinensis* has produced several BC_1 selections that appear to be cold hardy and produce fruit closely resembling *C. sinensis* in fruit morphology, size, color, soluble solids, acidity, and flavor. Some of the same seedlings appear to be resistant to citrus tristeza virus also. The most complex cross now in the field is derived from three genera: *Citrus, Eremocitrus* and *Poncirus,* and from three species of *Citrus: grandis, paradisi* and *sinensis.* A few of these seedlings have given a negative reaction when challenged by citrus tristeza virus, but nothing is known of their fruit traits. We may reasonably expect that some unique recombinations of traits may appear in these and other complex intergeneric hybrids in the near future.

Literature Cited

1. Barrett, H. C., and A. M. Rhodes. 1976. A numerical taxonomic study of affinity relationships in cultivated *Citrus* and its close relatives. Syst. Bot. 1(2):105-136.
2. Barrett, H. C. 1981. Breeding cold-hardy citrus scion cultivars. Proc. Int. Soc. Citriculture 1:61-66.

ERRATUM

In paper by B. C. Strik in V39(1): p. 7 right hand column, line 20 should read: "However, in other cultivars, long days may be necessary for flower bud initiation (6)."