Sexual and Nucellar Embryony in F1 Hybrids and Advanced Crosses of Citrus with Poncirus

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Additional index words: polyembryony

Abstract. Fourteen hybrid citrus progenies having polyembryonic Poncirus as a parent or grandparent were classified for their type of embryony, by seed dissection. Seedling populations from some of the hybrids were also studied. The ratio of polyembryonic (P) to monoeembryonic (M) individuals was nearly 1:1 among 52 F1 hybrids from P Citrus × P Poncirus. The ratio was about 1 P to 6 M among 121 F1's from 5 crosses of M Citrus with P Poncirus. Among 15 individuals from a backcross of Citrus × (Citrus × Poncirus) which was M × P, only 2 were clearly P. From backcrosses of M × M (?) parents, 28 individuals were M, 3 were probably M, and only 1 (a possible outcross?) was P. Results from the P × P and P × M crosses are in contrast to those from similar types of crosses within Citrus, where much higher proportions of P hybrids have usually been found. Results from the M × M (?) crosses probably support the concept that crosses between monoeembryonic strictly sexual taxa produce only monoeembryonic sexual progeny. A genetic basis for the inheritance patterns is discussed.

Some taxa in Citrus are entirely sexual in reproduction, while asexual (nucellar) embryony is common in many others. Among the latter, some produce both kinds of embryos in varying proportions, while others usually develop only nucellar ones. Cultivars such as 'Clementine' (C. reticulata Blanco), 'Wilking' (C. reticulata × C. nobilis Lour.), and apparently all those of pummelo (C. grandis (L.) Osbeck) have produced only sexual embryos, as have hybrids among such taxa (3, 4, 8). These strictly sexual cultivars are also typically monoeembryonic (M) except for occasional sexual twin or triplet embryos (1, 7). Hybrids from crosses between sexual parents and polyembryonic (P) partially asexual ones usually include both M and P individuals, often in ratios approaching 1:1. In a few such crosses, all tested hybrids have been P. Crosses of P × P parents can give rise to progeny which are M and strictly sexual (3, 4, 5).

Trees of the related monotypic genus Poncirus trifoliata (L.) Raf. are usually P and largely asexual in reproduction. However, 2 individuals bearing only M (and supposedly sexual) seeds have recently been reported (6). Both M and P progeny have been obtained in crosses between Citrus and Poncirus (2, 4, 5).

In an earlier study limited to the genus Citrus (8), a basis for the inheritance of nucellar embryony was suggested. This involved at least one major dominant gene, plus modifiers. The present paper provides additional data on the embryony of hybrids involving Poncirus, and considers the somewhat more complex inheritance which is implied.

Materials and Methods

Cultivars used in this study were all growing at the Citrus Research Center, University of California, Riverside. Two named clones of Poncirus, Rubidoux and Webber-Fawcett, were used as parents. Other cultivars of Table 1, not already taxonomically identified, were 'King' (C. nobilis Lour.); 'Ruby' orange (C. sinensis (L.) Osbeck); 2 pummelos (C. grandis (L.) Osbeck); 'Sukets' (C. paradisi Macf. × C. sinensis); C. ichangensis Swing.; C. tawamica Tan. & Shim.; C. volkameriana Ten. & Pasq.; and 'Yuzu' (C. ichangensis × C. reticulata?). The crosses involved 2 generations and were made in various years between 1956 and 1968. Most of the progeny trees were tested as original seedlings, since studies of nematode and phytophthora resistance were one purpose of the hybridizations. Seed samples from open pollination were usually collected from each tree in 2 or 3 separate years. For a few trees, tests were based on one year. Embryo counts on 50 seeds per tree per year were made under a dissecting microscope. Careful distinction was made between multiple embryos and single embryos with three or four cotyledons. Trees were classified as M if the number of seeds with multiple embryos averaged no more than 7% over all years. Trees with 8 or 9% multiples were classed as near monoembryonic (NM). Trees with 10% or more were classified as P, but most trees in this class had 20% multiples or more. For many trees, 50 additional seeds were grown in a greenhouse for one year to estimate further the degree of nucellar embryony.

Results

Numbers of P, NM, and M F1 hybrids obtained from crosses of Citrus with Poncirus are shown in Table 1, Groups I and II. In Group I, P × P parents, 27 P, 3 NM, and 22 M hybrids were identified, close to a 1:1 ratio of P to M. In Group II, which includes 5 crosses of P by-or-on M parents, there were 15 P, 9 NM, and 97 M hybrids. The proportion of M trees was high or very high in each cross.

Groups III and IV comprise hybrids from backcrosses of Citrus × Poncirus to Citrus. These populations varied widely in individual tree vigor. Most trees were slow to fruit and some have never fruited. Among 15 trees tested in Group III (M × P), 11 were M and only 2 were classified as P. In Group IV, the seed parents were all strictly sexual and were of course classes as M. The hybrid pollen parents had also been classified as strictly sexual by earlier studies. Among the 32 progeny trees tested, 28 were M and 3 were NM; 1 was clearly P. Excepting this tree, the ratio implies that the parents were indeed all sexual.

A few trees from cross number 11, not including the P tree, differed considerably in morphological characters from the other trees in the progeny. Isozyme profiles for four known loci (9) determined on each tree in the progeny showed that all of the genotypes could have been derived from crossing the indicated parents and could not have resulted from selfing the seed parent, 'Wilking'. However, the alleles present in the questionable individuals were also present in trees adjacent to the female parent and could have been acquired from them by accidental outcrossing. Thus, although there is no definite indication of outcrossing, the possibility cannot be eliminated.

Evidence was also obtained from seedling populations grown from many of the hybrid trees of Table I. These populations were classified for multiple seedlings and for morphological
uniformity. This method of determining nucellar embryony is of course less accurate than seed dissection. Classification of a hybrid as P or M usually agreed with that from seed dissection, but there were doubtful cases. Certain seed lots having as high as 7 to 9% multiple embryos by dissection gave no clear evidence of nucellar embryony in seedling tests. Conversely, a few seed lots which showed few or no multiples by either method still appeared to include some nucellar members among their seedlings. This was observed particularly in cross number 11.

Discussion

In an earlier study of *Citrus x Citrus* crosses (8) it was suggested that a single dominant gene might be responsible for polyembryony. A later study by Iwamasa et al. (4) supported this possibility in part, and included certain crosses of *Citrus* by *Poncirus*. However, our present data indicate that inheritance of the character in crosses which involve *Poncirus* may be more complex. All of the populations except those in Group I had high proportions of M plants. Plants classed as NM were few in Group I, and much less than 1:1 in Group III. In Groups II and III, the expected ratio would be close to 1:1. In Groups II and III, the expected ratio would be 1:6 in Group II and much less than 1:1 in Group III. In Group IV, the hybrid ratios nearly all fit the assumption that the pollen parents were completely sexual (M) and that M x M crosses always produced M progeny. The 1 clearly P hybrid in cross 11 could be an accidental outcross from a P source. Its tree and fruit characters were not definitive. None of the 3 NM hybrids averaged much more than 6% multiples over all years, and they could be genetically M.

Maisuradze (5) reported embryo counts in hybrids from crosses of P x P, P x M, and M x M parents. His larger populations from P x P and P x M within *Citrus* regularly contained both P and M individuals, and M x M crosses usually produced only M progeny. One exception was an advanced cross of *C. sinensis* x (*'Clementine' x 'Uvantin Mikan') from which 3 P and 1 M hybrids were listed. Both parents were classed as M, although monoembryony is rare among *C. sinensis* cultivars. Maisuradze did not report how many seeds per plant were examined or whether counts were made in more than one year. There was no mention of doubtful classifications between the P and M classes or of the limits used in defining the two. Thus, interpretation of his findings is difficult. He listed 4 populations of appreciable size from crosses of *Citrus* by-or-on *Poncirus*. Two crosses were P x P; they yielded ratios of about 2:1:1. The other two were M x M, with ratios of about 1P:3 M. These ratios show trends similar to those of the present study.

Overall, the progeny ratios imply that more than a single gene is involved. This was also suggested by Iwamasa et al. (4). The situation might be accounted for by 2 complementary dominant genes with *Poncirus* being heterozygous for both. The cross of M *Citrus* (p1p1 p2p2) with P *Poncirus* (P1p1 P2p2) should give a ratio of 1 P to 3 M, which approaches our results.
If some M Citrus were $P_1P_1$ $p_2p_2$, the cross would give 3 P:5 M. Various P Citrus cultivars could be either heterozygous or homozygous for one or both of the dominant genes, in which case populations of hybrid progeny would show a range of P: M ratios. A cross of 2 M Citrus cultivars, each carrying one of the dominant P genes, should sometimes produce P hybrids. Such hybrids could be represented among the exceptional cases mentioned above. Heterozygous polyembryonic Poncirus clones could give rise through occasional sexual selfs to monoembryonic progeny, which have been reported (6).

Dr. Piero Deidda in Sardinia (personal communication) obtained embryo counts on 47 hybrids of 'Clementine' $\times$ Poncirus. He reported 12 strictly M plants, 32 with 4 to 60% polyembryonic seeds, and three others highly polyembryonic. Thus the nature of some of these hybrids is uncertain.

Poncirus has shown several favorable characters as a parent for the production of new rootstock cultivars, but the relative scarcity of its hybrids which produce polyembryonic nucellar progeny is a handicap to its use.

**Literature Cited**


**Influence of Acclimatization on Carbohydrate Production and Translocation of Ficus benjamina L.**


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**Additional index words.** Chlorophyll, nutrition, shade, fertilizer, media

**Abstract.** Increasing shade decreased carbohydrate levels in Ficus benjamina leaves and roots during production. Increased shade during production decreased root carbohydrate during the interior holding phase and increased fertilizer during production increased root carbohydrate. Root and shoot carbohydrate was reduced during holding. Results of all variables correlated with keeping qualities.

Recent studies on light, medium, and nutritional requirements of foliage plants and acclimatization techniques have developed guidelines to prolong post production keeping quality of plant materials (4, 5).

Measurements of acclimatization generally have been limited to visual, subjective observations, but Conover (5) suggested a measuring system involving correlation of chlorophyll concentrations (mg/cm$^2$) during production with those during acclimatization. Shade and fertilizer levels which produce the least difference in chlorophyll content between production and holding times should optimize interior holding capabilities of plants (2). Whole plant light compensation points (LCP) were determined with occasional visual selfs to monoembryonic progeny, which have been reported (6).

Methods and Materials

A 3x3x2 factorial experiment in split-block design with 8 replications was initiated outdoors in Gainesville, Florida, April 16, 1976 to determine influences of 3 shade and 3 fertilizer levels and 2 media combinations on carbohydrate, chlorophyll and nutrient contents, and growth and quality of Ficus benjamina. The experimental unit consisted of a single plant/23.8cm plastic pot.

Shade levels of 65% and 37% light exclusion (about 45 and 81 klx, respectively) were provided by woven black polypropylene with unshaded blocks providing full sun treatments.