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Interspecific Hybridization in and Chromosome Numbers in Persea¹

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Wild relatives of the commercial avocado, *Persea americana*, have been introduced into California for use in breeding work or as possible rootstocks (4, 6). Several, including *P. borbonia*, the "swamp bay" of southeastern United States, arc tolerant of very high soil moisture conditions and may be resistant or immune to the root rot disease of the avocado. Others are indigenous to various environments, such as high altitude, and may be useful in breeding for cold resistance or in meeting problems of disease and culture.

This is to report successful hybridization between *Persea americana* and wild species related to the avocado and the chromosome numbers of four *Persea* species.

MATERIALS AND METHODS

Plant materials were from the University of California collection at Los Angeles. *Persea americana* trees used as female parents were all of the Guatemalan race. Species hybridization involved two inedible near-relatives of the avocado, *P. floccosa* and *P. nubigena*, and the more remotely related *P. borbonia*. The chromosome number for *P. longipies*, a willow-leafed near relative is also included. These species are described by Schroeder (4). Hybridizations resulted from hand pollination of flowers that were isolated from large flying insects by gauze bags or screen cages. Emasculation was assumed not necessary because of the dichogamous nature of the flowers (5) and the rapidity with which pollen germinates under favorable conditions (within ten minutes, author).

Pollen mother cells were examined after the flowers were fixed in Carnoys 6:2:2, stored in 70 per cent alcohol and smeared with steel needles in propiono-carmine stain.

HYBRIDIZATION

Sixty-four flowers of the Hass avocado variety were pollinated in 1951 with *P. floccosa* pollen and one hybrid plant was obtained from the single fruit that matured. The seedling was easily identified as hybrid shortly after germination because the plumule was covered with thick pubescence similar to *P. floccosa* seedlings. Hybridity was confirmed as the seedling developed. The leaves had pronounced pubescence, strong red pigmentation, particularly in the veins and petioles, and the narrow-lanceolate leaves characteristic of *P. floccosa*. Clonally propagated scion material from this hybrid has shown marked vigor in growth. Additional *P. americana* x *P. floccosa* hybrid seedlings were obtained from 1952 pollinations. These seedlings could also be readily identified as hybrids because of the above characteristics.

¹Received for publication July 29, 1953. ²Formerly University of California, Los Angeles Three seedlings resulted from *P. americana* x *P. nubigena* pollinations in 1952. They could not be clearly distinguished from *P. americana* seedlings while small. This was to be expected since small *P. nubigena* seedlings are very similar to those of *P. americana*. Several immature seeds from the reciprocal mating were obtained in 1951, but only one of them germinated and it failed to survive.

There is some evidence that pollen from *P. floccosa* and possibly *P. nubigena* effect seed set less readily on *P. americana* plants than does *P. americana* pollen. Table 1 contains a summary of the recorded 1952 pollinations and fruit set on three Guatemalan avocado varieties: Hass, Dickinson, and Anaheim. Totals include all pollinations, but individual figures for only the five most frequently used pollen sources are given for each variety. The percentage values in the third column are probably high by as much as one percentage point, since a number of individually marked pollination were not recorded due to tag drop.

Cross	Total - pollinations	Fruit harvested	
		Number	Per cent of total pollinations
Hass × Rincon	122	4	3
Hass X Lyon	111	8	7
Hass X Mexicola	86	16	19
Hass X Zutano	79	10	13
Hass $\times P$. nubigena	23	2	9
All Hass ^a	447	45	10
Dickinson × Zutano	309	36	12
Dickinson $\times P$, floccost	265	9	3
Dickinson × Mexicola	196	21	11
Dickinson $\times P$, nubicena	87	Ô	Ô
Dickinson × Fuerte	33	ĩ	3
All Dickinson ^a	954	72	8
Anaheim × Zutano	465	36	8
Anaheim $\times P$, floccosa	268	6	$\tilde{2}$
Anaheim × Mexicola	231	25	11
Anaheim × Fuerte	185	17	9
Anaheim $\times P.$ nubigena	65	1	2
All Anaheim ^a	1,398	94	7
All Pollinations ^a	2,799	211	8
All $\times P$, floccosa	533	15	3
All $\times P$, nubigena	175	3	2
All × Mexicola.	513	62	12
All × Zutano	853	82	10

*Includes miscellaneous crosses not shown in table.

Pollinations involving *P. floccosa* in 1952 were confined to the Anaheim and Dickinson varieties, and in both instances the set was very low in comparison to crosses with avocado varieties that were involved in comparable numbers of pollinations (Table 1). Only 15 fruits matured from a total of 533 *P. floccosa* pollinations, while 46 and 72 fruits matured from 427 and 774 pollinations with Mexicola and Zutano pollen, respectively, made at the same time on the same varieties (3 vs. 11 and 9 per cent).

Data for *P. nubigena* are less suggestive of differential functioning of pollen, although the mean set is only 2 per cent compared with the total mean percentage set of 8. The number of pollinations is relatively small and the variation is consequently large. Two of the 23 pollinations on the Hass variety were effective, whereas none of the 87 pollinations made on the Dickinson variety were effective and 1 of 65 pollinations on the

Anaheim variety set a fruit. Day to day fluctuation in the flowering behavior appears to be an important factor in fruit set (3). Such fluctuations are of minor importance in one season, when several hundred hand pollinations are made over a number of days.

Table 2 summarizes the initial results of the 1953 pollinations of two avocado varieties with *P. borbonia* pollen. There is evidence that this species may not be sexually compatible with the avocado or only slightly so. The set of 2 shown in the table may or may not be hybrids and they may or may not mature, since only 80 to 90 per cent of the initial set noted in July, 1952 reached maturity. The *P. borbonia* pollinations were made under reasonably favorable conditions, as evidenced by the fair to good set resulting from intervarietal pollinations of *P. americana* during the same period. *P. borbonia* pollen germinates and the tube begins to penetrate the styles of *P. americana* as rapidly *as avocado* pollen. Several hundred pollinations using *P. borbonia* as the female parent, resulted in no set.

TABLE 2.—Summary of Recorded Pollinations and Initial Set Resulting from 1953 Hass and Anaheim $\times P$. borbonia and Comparable Intervarietal Avocado Pollinations.				
Cross	Total – pollinations	Initial set		
		Number	Per cent of total pollinations	
× P. borbonia. × 21 avocado varieties. All pollinations.	$1,749 \\ 1,802 \\ 3,551$	2 75 77	$0.1 \\ 4.0 \\ 2.0$	

CHROMOSOME NUMBERS

Figures 1a, 1b, 1c and 1d are photomicrographs of meiosis I meta-phase plates of *Persea borbonia, P. floccosa, P. longipies,* and *P. nubigena,* respectively. All have 12_{II} as does *P. americana.* The meiotic chromosomes are too small for ready morphological analysis.



FIG. 1. Chromosomes at metaphase of meioiss I, left to right, *P. borbonia*, *P. floccosa*, *P. longipies* and *P. nubigena* each 12₁₁. × 750.

DISCUSSION

Experimental evidence has been presented which indicates that two wild *Persea* species are sexually compatible with *P. americana*. It should be possible to transfer useful genes from the wild relatives to the avocado for use in fruit production or rootstocks. This can evidently be done directly with closely related species such as *P. floccosa*, *P. nubigena*, and possibly others. Direct transfer may be feasible on a limited

basis with more remote species such as *P. borbonia* if a great number of pollinations are made. It might be necessary to approach the problem indirectly with species such as *P. borbonia*, locating first a mutually compatible species, hybridizing *P. borbonia* with such a species, and then crossing the hybrid or a derivative with the avocado.

Graft-compatibility relationships of these species and the avocado (4) are similar to the apparent sexual-compatibilities reported here. Both *P. floccosa* and *P. nubigena* are graft compatible to a considerable degree with the avocado. Significantly, attempts by Mr. Frolich to reciprocally bud and graft *P. borbonia* and the avocado have failed to result in any growth of the bud or scion.

There is a possibility that species hybrids or immediate derivatives themselves may be useful for varieties or rootstocks. However, it will probably he many years before genes derived by direct or indirect hybridization with the wild species are available in suitable varieties or rootstocks, even if it is assumed that problems of sexual-sterility or incompatibility will not seriously interfere.

There is no evidence of a polyploid or aneuploid series in *Persea*, since the chromosome number for five of the dozen or more classified .species is constant. Bowden (2) previously reported the chromosome number for *P. pubescens* or *P. carolinensis* which was evidently *P. borbonia*.

SUMMARY

Seedlings have been obtained following pollination of avocado flowers with pollen of two closely related species, *Persea floccosa* and *P. nubigena*. The former, x *P. floccosa*, are clearly hybrid while there is some question about the latter. There is some evidence that the pollen of the two related species is less effective in the setting of seed on *P. americana* than is *P. americana* pollen. Attempts to hybridize a more distant relative, *P. borbonia*, with the avocado have yielded only two fruits set in. over 1700 pollinations and neither is known to he hybrid.

The chromosome number of 12n is reported for *P. borbonia*, *P. floccosa*, *P. nubigena* and *P. longipies*.

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