

CHROMOSOME NUMBERS AND POLLEN STAINABILITY OF THREE SPECIES OF PACIFIC ISLAND BREADFRUIT (*ARTOCARPUS*, MORACEAE)¹

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Chromosome numbers were determined for 48 accessions of breadfruit (*Artocarpus altilis*, *A. mariannensis*, and *A. camansi* [Moraceae]) from 16 Pacific Island groups, Indonesia, and the Philippines. *Artocarpus camansi* and *A. mariannensis* exhibit counts of $2n = 56$; $2n = 56$ (diploidy) and $2n = 84$ (triploidy) were observed for *A. altilis*. Most diploid cultivars of *A. altilis* were seeded, but two cultivars with reduced seed number were observed. Micronesian accessions included putative interspecific hybrids between *A. altilis* and *A. mariannensis*. The majority of these accessions were seedless diploids, but triploid putative hybrids were also observed. Pollen stainability was shown to correlate with the degree of seediness.

Key words: *Artocarpus altilis*; *Artocarpus camansi*; *Artocarpus mariannensis*; breadfruit; chromosome number; pollen stainability; Moraceae; Pacific Islands.

Breadfruit is an important staple food crop in the Pacific islands where two species, including numerous cultivars, are recognized and grown (Parham, 1966; Ragone, 1995, 1997). *Artocarpus altilis* (Park.) Fosberg [synonyms *A. communis* J. R. & G. Forster, *A. incisus* (Thunb.) L. f.] is the most widely distributed and exhibits great variability. This species includes both seedless and seeded forms with seeded types most common in the western South Pacific. Seedless cultivars are most common in Micronesia and the eastern islands of Polynesia. *Artocarpus mariannensis* Trécul is always seeded and grows wild on the uplifted rock islands of Palau and the limestone ridges of the Mariana Islands. A few cultivated trees can be found growing throughout the islands of Micronesia, especially on coral atolls. It is not found in Melanesia or Polynesia other than the islands of Tokelau and Tuvalu. Fosberg (1960) suggested that introgression between *A. altilis* and *A. mariannensis* may have occurred in Micronesia, and there are many cultivars that have characteristics of both species.

A third species, *A. camansi* Blanco, is indigenous to the Philippines and probably New Guinea and the Moluccas. It is also found in cultivation throughout southeast Asia. This seeded species, commonly known as breadnut, has been introduced to many areas of the tropics where it is now widespread, especially in the Caribbean, parts of Central and South America, and coastal West Africa. It is rarely seen in the Pacific with the exception of a few trees in Hawaii, Samoa, Tahiti, and the Marquesas that were introduced in the past 35 yr, usually by immigrants from the Philippines. A few cultivated trees are found in Kolonia, Pohnpei, and a single tree was recently observed in a yard in Koror, Palau; otherwise it is not found in Micronesia.

Breadfruit trees are monoecious and normally cross-pollinated, with the small, powdery pollen grains spread by the wind (Jarrett, 1959; Brantjes, 1981). The fruit is a highly specialized syncarp (Jarrett, 1976). The perianths of individual flowers are fused together except at the base, forming a cavity that contains the true fruit and its enclosed seed (Reeve, 1974). As the fruit develops, this area of fusion grows vigorously and

becomes fleshy at maturity, forming the edible portion of the syncarp.

Seedless cultivars may lack developed ovules or may have numerous minute abortive ovules surrounding the core of the syncarp. Few-seeded cultivars usually have one or several normal or aborted seeds. Since many cultivars of breadfruit are seedless, it has been inferred that fruit development is due to parthenocarpy (Barrau, 1976), and Hasan and Razak (1992) showed that the fruits of breadfruit develop normally without pollination.

Little cytological work has been done on the genus *Artocarpus* and even less on the many cultivars of Pacific Island breadfruit. Counts of $2n = 56$ have been reported for *A. chaplasha* Roxb., *A. elastica* Reinw., *A. gomezianus* Wall. ex Trécul, *A. heterophyllus* Lam., *A. integer* (Thunb.) Merr., and *A. lakoocha* Roxb. (Habib, 1972; Hans, 1972; Mehra and Gill, 1974; Chen, 1993; Oginuma and Tobe, 1995). Data available for seeded breadfruit basically indicate diploidy with $2n = 2x = 56$; seedless cultivars are commonly triploid with $2n = 3x = 84$ (Jarrett, 1959; Barrau, 1976). These numbers are based on counts by Janaki-Ammal (1955) of $2n = 56$ for a seeded *A. communis* and counts by Nishiyama and Kondo (1942) of $2n = 54$ for seeded and $2n = 81$ for seedless *A. communis*. The latter authors acknowledged that their counts were suspect because of the poor preparations of their material.

The purpose of this study was to determine the chromosome numbers of *A. altilis*, *A. mariannensis*, and *A. camansi* and to clarify the basis of seed abortion and sterility in breadfruit.

MATERIALS AND METHODS

A collection of 155 breadfruit accessions from Indonesia, the Philippines, and 16 Pacific Island groups (Chuuk, Cook Islands, Fiji, Hawaii, Kiribati, Mariana Islands, Marquesas Islands, Palau, Pohnpei, Rotuma, Samoa, Society Islands, Solomon Islands, Tokelau, Vanuatu, and Yap) growing at the Magoon Horticultural Facility of the University of Hawaii in Honolulu was used in this study. Of these, 124 accessions are now growing in a breadfruit germplasm collection at the National Tropical Botanical Garden's Kahanu Garden in Hana, Maui. Forty-eight accessions were surveyed; seedless, seeded, and few-seeded accessions were selected randomly.

Chromosome counts were based on meristematic cells obtained from root

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TABLE 1. Species, provenance, accession numbers, local names, chromosome counts, and degree of fertility for 48 accessions of breadfruit.

Species Accession number	Provenance	Local name	Ploidy	Fertility
<i>Artocarpus camansi</i>				
531	Philippines	Kamansi	diploid	normal
282	Society Is. (from Indonesia)	Kamansi	diploid	normal
<i>Artocarpus mariannensis</i>				
309	Marianas	Dugdug	diploid	normal
292	Palau	Chebiei	diploid	normal
<i>Artocarpus altilis</i>				
326	Chuuk	Mei chon	triploid	sterile
096	Cook Is.	Paea	triploid	sterile
103	Cook Is.	Atu	triploid	sterile
147	Fiji	Samoan	diploid	normal
464	Fiji	Samoan	diploid	normal
489	Fiji	Karawa	diploid	normal
530	Hawaii	Ulu	triploid	sterile
311	Marianas	Lemae	triploid	sterile
216	Marquesas	Mei puou	triploid	sterile
220	Marquesas	Mei kiiahi	triploid	sterile
222	Marquesas	Mei maoui	triploid	sterile
286	Palau	Meriar	triploid	sterile
370	Pohnpei	Mein we	triploid	sterile
372	Pohnpei	Mei aroape	triploid	sterile
374	Pohnpei	Mei toahid	triploid	sterile
007	Samoa	Aveloloa	diploid	normal
013	Samoa	Unknown	diploid	normal
019	Samoa	Ulu sina	diploid	normal
433	Samoa (from Cook Is. ^a)	Enua	triploid	sterile
439	Samoa (from Rotuma ^a)	Rauulu	diploid	normal
123	Samoa (from Solomon Is. ^a)	Toro	diploid	normal
426	Samoa (from Solomon Is. ^a)	Kukumu tasi	diploid	normal
443	Samoa (from Vanuatu ^a)	Manang	diploid	normal
453	Samoa	Puou	diploid	partial
541	Samoa	Ulu ea	triploid	sterile
256	Society Is.	Huero	diploid	partial
236	Society Is.	Hamo	triploid	sterile
268	Society Is.	Apu	triploid	sterile
272	Society Is.	Apuapua	triploid	sterile
526	Vanuatu	Forari2	diploid	normal
<i>Artocarpus altilis</i> × <i>A. mariannensis</i>				
322	Chuuk	Mei on	diploid	sterile
331	Chuuk	Faine	diploid	sterile
333	Chuuk	Mei nifa	diploid	sterile
363	Chuuk	Mei chocho	diploid	normal
287	Palau (from Yap)	Unknown	triploid	sterile
290	Palau	Midolab	triploid	sterile
365	Pohnpei	Lipet	diploid	sterile
373	Pohnpei	Meipohnsakar	diploid	sterile
375	Pohnpei	Mein patak	diploid	sterile
385	Pohnpei	Mei kole	diploid	partial
379	Pohnpei	Mei kole	triploid	partial
041	Tokelau	Ulu afa	diploid	normal
301	Yap	Luthar	diploid	normal
303	Yap	Yuley	diploid	sterile

^a Accessions were collected from the South Pacific Commission breadfruit collection at Vailima, Samoa.

tips from three sources: germinated seeds, roots growing from 15 cm long sections of mature roots, or from airlayers made on the stems of potted plants ~2 cm in diameter and <1 m in height. Roots from all three sources were prepared in the same manner. Root tips of 3–4 mm length were excised and pretreated in a saturated solution of paradichlorobenzene (PDB) at 20°C for 2 h and fixed in Carnoy's fluid (3:1 of 95% ethanol and glacial acetic acid) for 24 h at 37°C before hydrolysis in 1 mol/L HCl for 7 min at 60°C. The root tips were stained in Feulgen solution for 1.5 h at room temperature, and slide preparations were made by squashing root meristems in a drop of 2% acetocarmine stain and Hoyer's solution. Chromosome numbers were determined from cells at mitotic metaphase using a Zeiss phase contrast microscope. Documentation was made with camera lucida drawings, and photo-

micrographs were taken with Kodak Technical Pan film and Ektachrome slide film at 1000× magnification.

Pollen fertility was assessed by observing the percentage of pollen grains that stained uniformly with acetocarmine. At least 500 pollen grains per accession were scored.

RESULTS

Chromosome numbers determined for 48 accessions are summarized in Table 1. Chromosome counts are estimated as diploids ($2n = 2x = 56$) or triploids ($2n = 2x = 84$). The small size of the numerous chromosomes made it difficult in

TABLE 2. The degree of pollen stainability for seeded, few-seeded, and seedless accessions of breadfruit.

Degree of seediness	Species	Accession number	Ploidy	Pollen stainability (%)
Many	<i>A. mariannensis</i>	292	diploid	96
	<i>A. mariannensis</i>	313	—	91
	<i>A. camansi</i>	531	diploid	91
Few	<i>A. altilis</i>	453	diploid	78
	<i>A. altilis</i>	147	diploid	71
	<i>A. altilis</i>	110	diploid	67
None	<i>A. altilis</i> × <i>A. mariannensis</i>	290	triploid	16
	<i>A. altilis</i>	541	triploid	7

many cases to precisely determine counts as $2n = 56$ or $2n = 84$, but it was possible to readily distinguish between diploid and triploid levels.

The chromosome number for *Artocarpus mariannensis* (accessions 292 and 309) is $2n = 56$ (Ragone, 1991, 1997). The report of $2n = 54$ by Nishiyama and Kondo (1942) for a seeded breadfruit from the island of Rota in the Northern Mariana Islands was probably erroneous. They identified their specimen as *A. communis*, but since seeded forms of this species are not known to occur in the Mariana Islands, the count was probably based on *A. mariannensis*. The chromosome number for *A. camansi* (accessions 282 and 531) is $2n = 56$ (Ragone, 1991, 1997).

Twelve seeded accessions of *A. altilis* were verified as diploids ($2n \approx 56$). Two accessions (256, 453) with fruits that typically yield only one to several viable seeds were also diploids. Diploid counts were also obtained for three seeded accessions from Tokelau and Micronesia (041, 301, and 363) and seven seedless Micronesian accessions (303, 322, 331, 333, 365, 373, and 375). Based on morphological characters and isozyme phenotypes (Ragone, 1991), these 10 accessions are putative crosses between *A. mariannensis* and *A. altilis*.

Chromosome counts of $2n \approx 84$ were obtained for 20 accessions. These were all seedless with the exception of accession 379 from Pohnpei which has fruits that rarely contain one or two seeds. This cultivar, and two triploid seedless Micronesian cultivars (287 and 290), are also putative crosses between *A. altilis* and *A. mariannensis*.

The degree of pollen stainability for seeded, few-seeded, and seedless accessions is shown in Table 2. These initial studies show that the degree of seediness in breadfruit cultivars is correlated with pollen stainability. Triploid cultivars have the lowest pollen stainability, averaging from 7 to 16%, and the pollen grains are typically malformed, clumped, and poorly stained. These facts were previously noted by Tri Sunarto (1981), who showed that a seeded form had the highest pollen grain stainability (99%), while a few-seeded form had medium stainability (45%), and a seedless form had low stainability (6%).

DISCUSSION

Seedlessness in breadfruit has been attributed to sterility due to triploidy (Jarrett, 1959; Barrau, 1976; Simmonds, 1979), and this seems to be the case for those cultivars with $2n \approx 84$. Jarrett (1959) also suggested that the failure of breadfruit to set seed may be due to genetic factors other than polyploidy. Since breadfruit trees are propagated by vegetative means, each cultivar is a clone within which the primary source of

variation is presumably somatic mutation. Repeated vegetative propagation of breadfruit clones permits the accumulation of somatic mutations affecting reproductive fertility, which may be deleterious in nature, but which are maintained and perpetuated as curiosities or useful variants by human selection. Reduced seed number in some diploid cultivars is probably a byproduct of the practice of clonally propagating these plants using root shoots or sections of roots.

Pollen sterility is also a factor contributing to reduced fertility and seed production in some diploid cultivars. In areas such as eastern Polynesia the majority of cultivars and trees are seedless triploids, which produce very little viable pollen. In Tahiti, for instance, breadfruit cultivars with seeds are so exceptional that the name for the only known seed-producing cultivar is 'Huero' (256), which means "with a seed." 'Huero' only occasionally produces one to several seeds. The proximity of seeded cultivars, which generally produce abundant pollen, results in an increased number of seeds in typically few-seeded cultivars. This has been observed in the NTBGB breadfruit collection at Kahanu Garden.

Seedless diploid cultivars are also found in Micronesia. All of these appear to be interspecific hybrids between *A. mariannensis* and *A. altilis*, based primarily on shared morphological characters. Seven of the 11 diploid putative hybrids were seedless or had reduced fertility. The hybrid nature of Micronesian diploids may be largely responsible for their sterility, as with many other interspecific hybrids in which insufficient homology between genomes results in meiotic abnormalities, embryo lethality, or disruption of normal embryo/endosperm relations (Stebbins, 1971; Simmonds, 1979).

In Micronesia, where *A. mariannensis* produces abundant pollen, some of the hybrids may derive from crosses between diploid *A. mariannensis* and triploid *A. altilis* clones, which have overlapping distributions (Fosberg, 1960). The hybrid progeny of such crosses could have additional sterility problems resulting from aneuploidy or sterility mutations derived from the triploid *A. altilis* parent.

Regardless of the cause(s) of sterility in breadfruit, the development of few-seeded and seedless fruits had significant benefits for Pacific Islanders who relied on breadfruit as a staple crop. The development of fruits with reduced seed numbers yielded a greater proportion of edible fleshy tissue and resulted in a shift from using this species as a nut crop (breadnut) in western Melanesia to a starch crop (breadfruit) eastwards. Since breadfruit is a seasonal crop typically available for just a few months of the year, methods had to be developed to deal with and use seasonal surpluses to provide food during the annual and often extended periods of scarcity. The method developed was that of fermentation and storage in pits. The importance of fermented breadfruit, especially in Samoa, Tonga, the Marquesas, Society Islands, and Micronesia, was a critical element in a preference for seedless cultivars that drove selection and perpetuation of seedless cultivars.

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