AGAMOSPERMY IN *ARTOCARPUS HETEROPHYLLUS* LAM. (JAK FRUIT)

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ABSTRACT

A study was conducted to investigate the occurrence of agamospermy of *Artocarpus heterophyllus* Lam. Three controlled pollination treatments (selfing, out-crossing, without pollination) were used. Fruit-set, seed-set, mean seed weight and percentage germination of seeds following the three controlled pollination treatments and open pollination were assessed. Results revealed that *A. heterophyllus* has very high fruit-set and very low seed-set, with a mean of 3% of the flowers in an inflorescence producing seed as a result of open pollination. Pollination did not appear to be a prerequisite for fruit- and seed-set in *A. heterophyllus*, with 53% of inflorescences isolated with pollen-proof bags producing syncarps. Dissection of these ripened syncarps produced seeds although the number of seeds per syncap was significantly lower than in open pollination. Further, seeds were not found in the inflorescences where the top, bottom or lateral half of stigmas had been shaved, and the rest normally pollinated, in contrast to those inflorescences, which were completely shaved. These results suggest the occurrence of facultative agamospermy in *A. heterophyllus*. The mean seed weight of agamospermically produced seeds was not significantly different from that of open-pollinated seeds. Seed germination started within a week after sowing and was completed in approximately 35 days in seeds collected from all treatments. The overall germination success of agamospermic seeds was high (93%) and not significantly different from other pollination treatments.

KEY WORDS: Agamospermy, *Artocarpus heterophyllus*, Jak fruit, Controlled pollination, Seed-set, Fruit-set.

INTRODUCTION

Many plants reproduce both sexually and asexually, although sexual reproduction in plant species enhances genetic diversity due to the random exchange of genes (Asker and Jerling, 1992; Richards, 1986). Asexual reproduction of plant species may occur by vegetative propagation or apomixes and agamospermy. In flowering plants the term apomixes is commonly used to mean Agamospermy (Asker and Jerling, 1992). However, agamospermy is the asexual formation of embryos and seeds, without the occurrence of fertilization, which are genetically identical to the female parent (Asker and Jerling, 1992; Richards, 1986). The presence of agamospermy in a species may, therefore, increase the opportunity for the selection of desirable individuals based on the characteristics of the mother plants without compromising the genetic identity of seeds (Hanna, 1991; Hanna and Bashaw, 1987; Richards, 1990). Parthenocarpy, the formation of fruits without pollination, is important, particularly in plantations of fruit trees to reduce the unreliability of fruit-set due to lack of pollination (Asker and Jerling, 1992; Sedgley and Griffin, 1989).

Artocarpus heterophyllus Lam. (Jak fruit) in the family Moraceae is native species to the rain forests of the Western Ghats of India and the Malaysian Archipelago (Acedo, 1992). It has been introduced to other South and Southeast Asian countries, including Sri Lanka and considered as a naturalized species (Acedo, 1992). In Sri Lanka, it is an important multipurpose tree, mainly used as a fruit, timber and fodder tree species (Pushpakumara, 2006). Despite the importance of *A. heterophyllus*, little is known about its reproductive biology. It is a monoecious species and the flowers are grouped into male and female inflorescences (Morton, 1965; Pushpakumara, 2006). The female inflorescence consists of 5,695 ± 52 individual female flowers (Pushpakumara, 2006), and develop to a compound fruit technically termed as syncarp. The syncarp consists of core and fruitlets (true fruits) with a stiff rind and spines (Pushpakumara et al., 1997). Although parthenocarpy has been reported for other species in the genus *Artocarpus*, such as *A. altilis* (Brantjes, 1981) and *A. integer* (Sakai et al., 2000), little is known about the occurrence of parthenocarpy or agamospermy in *A. heterophyllus*. The objective of this study was to investigate the occurrence of agamospermy in *A. heterophyllus*.

MATERIALS AND METHODS

The study was conducted at the University of Peradeniya Experimental Station (UPES), Dodangolla, Sri Lanka (latitude 7°15' N; longitude 80°45' E; altitude 387 m amsl). The climate of the area is classified as humid tropical with a mean annual rainfall of 1,563 mm, mean relative humidity of 79% and mean day time temperature of 29 °C, and major dry periods from February to March and June to September. At UPES, there is a mixed-aged stand of 326 flowering age *A. heterophyllus* trees of varying diameter at breast height, from 15-90 cm and in height from 6-24 m, covering approximately 80 ha, was used as the experimental population.

Controlled pollination experiments were carried out on 12 trees in two successive years (1993/94 and 1994/95 flowering seasons) to obtain evidence for the presence/absence of agamospermy and/or parthenocarpy. On each tree, about 30 female inflorescences were isolated with pollen-proof bags (30x45 cm made from a transparent film with small perforations (<1 mm), code PBSPF-3, PBS International, Salter Road, Eastfield Industrial Estate, Scarborough, North Yorkshire YO11 3 UZ, UK) (see Plate 1A) 10 days before observation of stigmas or at stage 3 of female inflorescence development as suggested by Pushpakumara (2006). Controlled pollinations (selfing and outcrossing (see Plate 1B)) were carried out at the stage of maximum receptivity (stage 5 of female inflorescence development), using fresh pollen, by alternatively brushing the male and female inflorescences with a fine brush. From each tree, fifteen to twenty five inflorescences at the same stage were also labeled and allowed for open-pollination. The inflorescences isolated with pollen-proof bags were used as without pollination treatment and observed periodically and bags were removed once the tips of all the stigmas of the inflorescence were no longer receptive at the stage 6. The development of the inflorescences after pollination treatments and those labeled for without pollination and open pollination treatments, were periodically monitored.

Once the resultant syncarps matured (stage 8), seed-set in each syncarp was calculated. As the spine of the rind of the syncarp is formed from the tip of the perianth (the stigmatic protrusion; Acedo, 1992), it was assumed that each spine developed from a single flower with a single functional ovule. Spines of syncarp were counted using a marker pen and a hand held tally counter assumed as the number of ovules per inflorescence. Fruit-set was calculated by dividing the number of syncarps produced by the total number of female inflorescences labeled for open and controlled pollination. Each syncarp was then opened and the number of seeds counted. Seed-set was calculated by dividing the number of seeds developed by the total number of syncarp spines (ovules). Since seed-set data were expressed as a percentage ovules developed to seeds, they were subjected to Box-Cox transformation (Venables and Ripley, 1994). Transformed seed-set data were subjected to a nested, unbalanced; two-factor analysis of variance (Sokal and Rohlf, 1995).

Fruit and seed production without pollination and fertilization was also tested by shaving off the immature stigmas of inflorescence (entire inflorescence-fully shaved, lateral half, top half or bottom half (Plate 1C) shaved), using a sharp knife, on 42 randomly selected female inflorescences on five trees. These inflorescences were also periodically monitored and once resultant syncarps matured, they also opened and both the shaved and unshaved areas observed separately for seed-set.

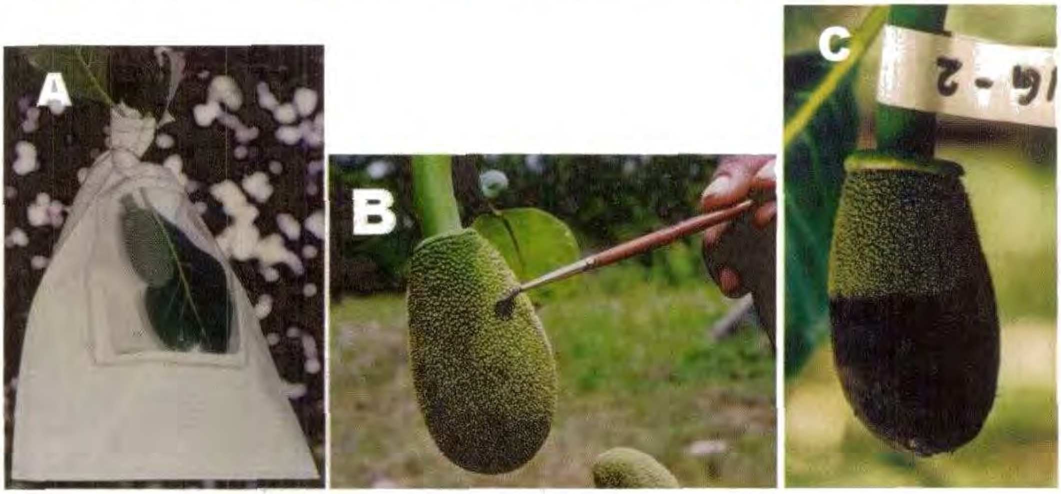

Plate 1. Isolation of Female Inflorescences with Pollen-Proof Bags (A), Controlled Pollination (B) and Shaving of Stigma to Test Agamospermy (C) in *Artocarpus heterophyllus*.

At least three syncarps from each pollination treatment were randomly selected from the 12 trees used in controlled pollinations and 50 seeds from each syncarp were randomly selected, weighed, and sown in a sand-bed in the nursery at UPES. Germinated seeds were counted and the presence and number of multiple seedlings also recorded. The general linear model (GLM) procedure was used to test the effect of pollination treatments on seed weight nested within trees, whilst germination percentage data were arcsine-square root transformed before undertaking GLM analysis (Sokal and Rohlf, 1995).

RESULTS AND DISCUSSION

In *Artocarpus heterophyllus*, the fruit-set expressed in terms of number of mature syncarps as the percentage of female inflorescence produced was high and ranged from 84-100%. However, seed-set was very low; and only a few flowers (ovules) in the inflorescence (mean of 3% from open pollination and 6% from selfing and cross pollination) developed into seeds (Table 1). Self and cross pollination significantly increased seed-set compared to open pollination (Table 1; Plate 2). Seventy two (53%) of the 135 inflorescences isolated with pollen-proof bags as treatment of without pollination produced syncarps. Dissection of ripened syncarps isolated with pollen-proof bags also produced seeds, although the number of seeds was significantly lower than in open-pollinated syncarps (Table 1; Plate 2).

Table 1. Effects of pollination treatments on seed-set, seed weight and germination percentage in Artocarpus heterophyllus.

Pollination Treatments	Seed set (Mean ± SE)	Seed set (Mean ± SE)	
		50 seed weight (g)	Germination (%)
Inflorescences isolated with pollen proof bags	0.41 ± 0.01 (N=72)[a]	729 ± 11.6 (N=40)[a]	92.4 ± 0.80 (N=40)[a]
Open pollination	3.01 ± 0.06 (N=208)[b]	707 ± 0.93 (N=62)[a]	93.1 ± 0.68 (N=56)[a]
Selfing	6.81 ± 0.92 (N=105)[c]	511 ± 0.82 (N=70)[b]	93.1 ± 0.71 (N=70)[a]
Outcrossing	6.492 ± 0.98 (N=124)[c]	519 ± 1.03 (N=82)[b]	92.9 ± 0.69 (N=82)[a]

Notes: N = number of syncarps assessed. Means in each column followed by different superscript letters are significantly different at $p < 0.05$. Pollination treatments were replicated in 12 trees. Means are of 50 seeds, extrapolated for 50 seeds for the case of seeds produced from inflorescences isolated with pollen-proof bags.

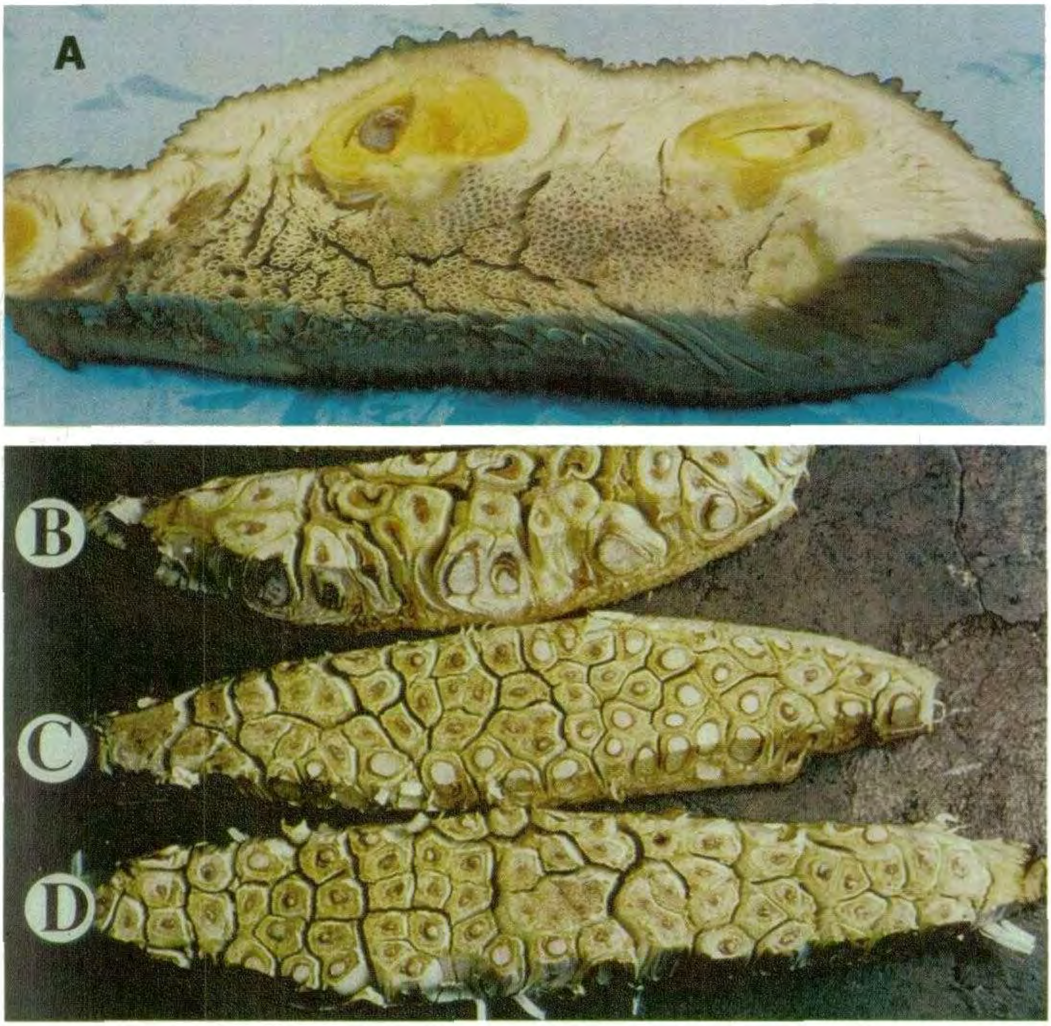

Plate 2. Effects of pollination treatments on fruit development and seed-set in *Artocarpus heterophyllus*.

Note: A = only a few seeds (less than 20 seeds per syncarps) were produced following isolating female inflorescences with pollen-proof bags to test for parthenocarpy and agamospermy (magnification x 0.5). B = Seed production following open pollination (magnification x 0.3). C = Seed production following self pollination (magnification x 0.3). D = Seed production following cross pollination (magnification x 0.3).

Three out of 12 (25%) fully shaved inflorescences developed as syncarps, and dissection showed similar seed-set to syncarps produced after isolation of inflorescences with pollen-proof bags. Syncarps were also produced (46%) following shaving of the lateral, top or bottom half of stigmas on inflorescences. However, a similar level of seed-set as in open-pollinated inflorescences was found only in unshaved areas whereas no seed was observed in shaved areas. Thus, mature syncarps following shaving of top, bottom or lateral half of stigmas of inflorescences were asymmetrically developed (Plate 3). Seeds produced from inflorescences isolated with pollen-proof bags had the highest mean weight based on 50 seeds, although their mean weight was not significantly different from that of open-pollinated seeds (Table 1).

Seed germination started within a week after sowing and was completed in approximately 35 days. The overall germination success of seeds was high (93%). Pollination treatments had no significant effect on percentage germination of seeds (Table 1). Only seven open-pollinated seeds (0.06%) produced two seedlings per seed whilst only two seeds produced from inflorescences isolated with pollen-proof bags (0.06% of such seeds) produced multiple seedlings.

Plate 3. Effects of shaving of lateral half of inflorescence on fruit formation in *Artocarpus heterophyllus.*

The results of the inflorescences isolated with pollen-proof bags suggest that *A. heterophyllus* is able to produce syncarps and seeds without pollination and fertilization, exemplifying agamospermy. However, these results should be interpreted with caution, since the species is wind-pollinated (Pushpakumara, 1997). It is possible that pollen was already on inflorescences before isolation, and therefore contamination cannot be completely ruled out. Further, there are some useful indicators associated with apomictic species, such as multiple seedlings per seed, multiple stigma and double or fused ovaries (Hanna and Powell, 1973; Kaur et al., 1978), that were not observed in this study. Only 0.06% and 0.6% of total and agamospermic seeds germinated produced two seedlings per seed, whilst all ovaries observed consisted of a uni-locular ovule and a single stigma (Pushpakumara, 2006; Pushpakumara et al., 1997). Although non- receptive stigmas from inflorescences were not observed for pollen grains in this study (results not shown), none of the stigmas collected from inflorescences isolated with pollen-proof bags had either pollen grains or grains with tubes (Pushpakumara et al., 1997), and all inflorescences isolated with pollen-proof bags and the few inflorescences that were completely shaved to remove stigmas consistently produced very few seeds. This evidence suggests that the likelihood of pollen contamination was low.

Seeds were not found in the inflorescences where the top, bottom or lateral half of stigmas had been shaved, and the rest normally pollinated, in contrast to those inflorescences, which were completely shaved. These suggest that agamospermy may be facultative. The development of below-ground syncarps has been reported for *A. heterophyllus* (Acedo, 1992: Gupta et al., 1987); although details of the seed production in such syncarps were not reported, they could be agamospermic. Since *A. heterophyllus* seeds germinate hypogeally (Soepadmo, 1991), they presumably exhibit below-ground separation of cotyledons that may allow below ground growth and elongation of the epicotyls. In such case, it may be possible to produce an inflorescences below the ground level and developed to a syncarp, depending on compactness of soil. Most apomictic reproduction occurs among polyploids, particularly those with odd numbers of chromosomes (Asker and Jerling, 1992; Richards, 1990). Although *A. heterophyllus* has an even number of chromosomes ($2n=4x=52$), it is presumed to be an autotetraploid species (Acedo, 1992; Pushpakumara et al., 1997).

Fruit and seed production without pollination and fertilization has been reported for some other tropical tree species such as *Shorea* spp. in Malaysian dipterocarp forests, *Clusia* spp., *Garcinia* spp., *Bombacopsis glabra*, *Cirtus* spp., *Mangifera indica* in South East Asia and *Stemonoporus oblongifolius* in Sri Lanka although our knowledge of the frequency of agamospermy in tropical tree species is limited (Baker, 1959; Ha et al., 1988; Hanna and Bashaw, 1987; Kaur et al., 1978; Maguire, 1976; Murawski and Bawa, 1994; Richards, 1990). Although few detailed studies have been conducted, the occurrence of agamospermy indicates that it may have a role in breeding of tropical trees. Furthermore, sexual outcrossing and facultative agamospermy has been suggested as the best mating model, as heterosis is not compromised in agamospermy as it is in selfing, and the species remains evolutionarily active despite agamospermy (Brown, 1989; Ha et al., 1988). In contrast to autogamy that propagates inbreeding and homozygosis, apomixis stabilizes the status quo of a species where genotype of an individual remains the same in its progeny.

The ability in *A. heterophyllus* to reproduce agamospermically may provide an opportunity for multiplying selected individuals under given ecological conditions in a tree improvement program because the resultant progeny may be genetically identical to the maternal parents. However, it is important to clarify the nature of agamospermy before utilization of agamospermically produced seeds. Detailed anatomical observation and molecular analysis (Hanna and Bashaw, 1987) are required to confirm this form of reproduction in *A. heterophyllus* and its relative importance in normal conditions of open pollination. Then, even a small-scale farmer can make their own seed material simply by bagging inflorescence before receptivity with paper bags.

CONCLUSIONS

A. heterophyllus had very low seed-set (3%) as a result of open pollination. Pollination is not a prerequisite for fruit and seed-set in *A. heterophyllus* suggesting the occurrence of agamospermy.

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