

Causes of Low Viability and Techniques to Improve Germination of Sago (*Metroxylon sagu* Rottb.) Seeds

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Portion of the B.S. Biology Thesis of Katherine A. Cutab

Abstract

The cultivation of sago, a species of palm growing in the wild in Mindanao and known for its high starch yield, is constrained by its low propagation rate. Seed propagation, though desirable, is difficult with sago because it tends to produce seedless or parthenocarpic fruits and its seeds have a low germination. To address this problem, we studied the cause of seedlessness and poor germination of sago fruits and explored hydro-priming as a way of mitigating poor germination. Confirming the result of previous studies, six fruit lots of sago, involving a total of 18,566 fruits collected from four towns and three provinces in Mindanao, Philippines, showed a consistently high incidence of seedlessness, with only 504 (2.7%) of the fruits bearing seeds. The biggest lot (7,630 fruits) was 100 per cent seedless. Among fruits with seeds, studies of the effects of hydro-priming (cycles of soaking and drying) and water replacement during soaking treatments show that only the hydro-priming treatment has a significant effect on germination and incidence of normal seedlings. The highest germination percentage of 36.7% was obtained from seeds subjected to two cycles of hydro-priming without water replacement after 125 days. The control (continuous soaking) had only 8.3% germination. Likewise, the highest percent of normal seedlings (31.7%) was obtained with two cycles of hydro-priming without water replacement. The control had only 1.67% normal seedlings. The present studies support the following conclusions: (a) parthenocarpic fruit development is common among the sago palms studied, and (b) seed germination and production of normal seedlings are enhanced by hydro-priming without water replacement. The first conclusion is a confirmation of similar reports in literature, while the second conclusion is unique for this study.

Keywords: hydro-priming, *Metroxylon*, parthenocarpy, sago, seed germination

Introduction

Sago palm is a versatile plant commonly found in New Guinea and parts of Southeast Asia (Flach and Rumawas, 1996). In the swamp regions, sago is superior to rice (the common crop in this environment) in productivity, utility and ecological impact (Rijksen and Persoon, 1991). Sago is an inexpensive raw material for a multi-product microbial conversion system (Abdul-Aziz, 2002; Vikineswary, et al. 1994). Under good conditions, the yield varies from at least 15 t to possibly 25 t of dry starch/ha. This is higher than that of any other starch crop (Flach, 1997).

There is very little effort to cultivate sago in the Philippines, unlike in Malaysia and Indonesia where commercial plantations are already established. Where it is cultivated at all, suckers or off-shoots are the most commonly used material for propagation. However, the survival rate of transplanted suckers is generally low (Omori et al., 2001). Thus, sago plantations in Riau, Indonesia and Sarawak, Malaysia have used botanical seeds (Ehara et al., 1998).

Seed propagation is constrained by the common observation that fruits collected from the wild seldom germinate. This could be attributed to seedlessness and parthenocarpy (Flach, 1997; Flach and Rumawas, 1996). It has been assumed that sago palm is mainly a cross-pollinator because viable seeds are usually collected from palms that flower simultaneously and physically close to each other.

Considering the potential of the sago palm and the vast areas in South-East Asia suitable for its cultivation, its commercial cultivation is inevitable. When this materializes, seed propagation will likely be desired as an alternative to vegetative propagation. Seed propagation is also important for the purpose of preserving the diversity of the species. This study examines the phenomenon of poor germination in sago fruits collected in Mindanao, Philippines, and explores hydro-priming as a way of mitigating it.

Hydro-priming or soaking and drying of seeds is one of several methods of seed enhancement practiced by the seed

industry (Taylor et al., 1998). It reduces the time between seed sowing and seedling emergence and promotes synchronization of emergence (Parera and Cantliffe, 1994).

Materials and Method

Collection and Characterization of Fruits

All of the fruits were collected near residential areas, from palms that were cultivated in the usual small isolated clumps in the backyard or edge of croplands. It is difficult to find trees with mature fruits from wild stands because these are cut for starch extraction by gatherers as soon as they start to flower. Otherwise, they are so heavily defoliated as leaves are collected for roofing purposes, that they are unable to undergo sexual reproduction. Consequently all but one of the fruit lots were collected from spineless trees, because villagers prefer to plant spineless trees near their homelots. In the wild, the common type is the spiny, apparently because wild animals are unable to eat them when they are young.

Mature sago fruits were collected from six sites (Figure 1) in four towns of Mindanao. A mature sago fruit has achieved a full size and is characterized by an all straw-colored husk (Alang and Krishnapillay, 1985) or partially straw-colored and partially yellow-green to straw color. The source palms were at the senescence stage with withered leaves. The source palms were observed for distinguishing characters such as presence of spines on the petioles and leaves. The immediate environment of the fruit-bearing palm was also observed for presence of other fruit-bearing palms.

To determine if the fruits had seeds, fruits were pierced with a sharp needle. Fruits that were easily penetrated by the needle were seedless, while those that offered resistance (due to the hard testa) were seeded. Measurements of fruit diameter, length, weight, volume and density were also taken. Fruit diameter and length were measured using a caliper, while volume was measured using the water displacement method. Density was obtained by dividing the weight by volume. The seed-bearing fruits collected from Bunawan, Agusan del

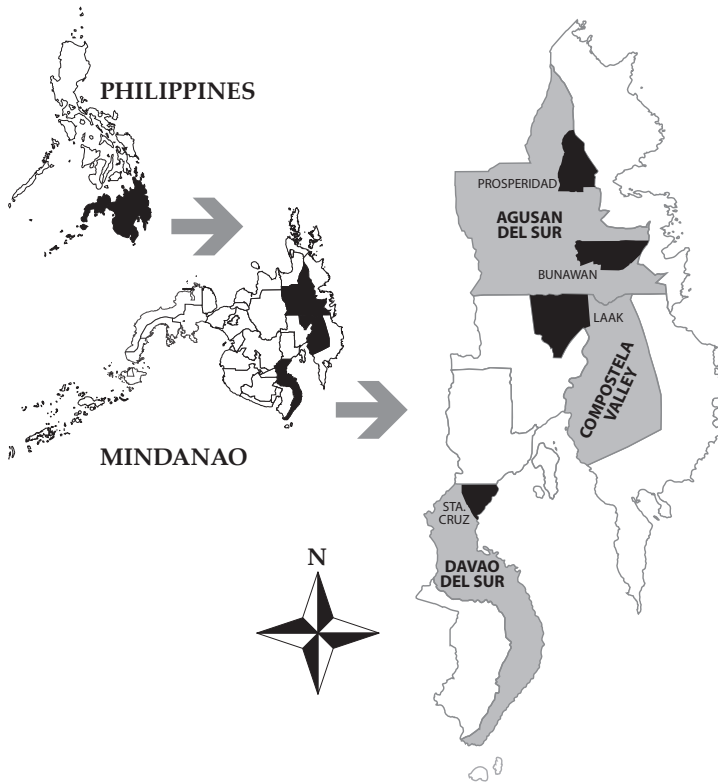


Figure 1. Four towns in Mindanao where sago fruits were collected

Sur were used in the germination experiment. This fruit lot was the only one of the six lots collected with a fairly high percentage of seeded fruits (19.8%). The seeded fruits were de-husked to facilitate water imbibition since the exocarp of sago fruit is thick and waxy. To eliminate microbiological factors that might interfere with the conduct of the study, the dehusked samples were soaked in 5% commercial Chlorox (sodium hypochlorite) for 20 minutes and rinsed three to five times.

Treatments for Improving Germination

Two factors were studied in a 3 x 2 factorial experiment with three replicates and 20 seeds per replicate. The factors were: hydro-priming and water replacement during the soaking

cycle. There were three levels of hydro-priming: A0 (soaked in water continuously for 15 days, control), A1 (one cycle of three days soaking in water and three days air-drying, then soaked continuously for 9 days), and A2 (two cycles of three days soaking in water and three days air-drying, then soaked continuously for three days). On the other hand, there were two levels of water replacement during the soaking cycle: B1 (water not replaced during the soaking period), and B2 (water replaced everyday).

Moist seeds resulting from the above treatments were kept in 8 x 16 cm polypropylene bags punched with 48 holes with 6 mm diameter for four months. The experiment was conducted in a dark room, and germination was monitored every day. Germinated seeds were planted in plastic pots and seedlings were also observed. Temperature of the germination room at 2:00 PM was 27.80 ± 0.82 °C and the relative humidity was $88.20 \pm 6.03\%$.

Seedling Evaluation

Seedlings were classified as normal or abnormal. The normal seedlings have the following characteristics: all essential structures are well developed, complete, well-proportioned and healthy; may show certain defects of the essential structures, but do not show any other unsatisfactory or unbalanced development; may show evidence of decay or discoloration but it can be clearly seen that this is due to external factors such as fungi, while all the other essential structures are normal. The abnormal seedlings are characterized by: coleoptile with damaged or broken tips; split, stunted, constricted or missing primary roots and shoots; and decayed primary structures (Tabora, 1998).

Data Gathering and Analysis

Germination was considered to have commenced upon the emergence of the coleoptile. Germination was observed over a four-month period, when it has stabilized at maximum level for most treatments. Seeds that did not germinate after this period were cut open and their endosperms and embryos

examined. Data on percent germination, percent normal seedlings and percent decayed embryos were subjected to arc sine transformation before statistical analysis. The data on percent fresh embryos were subjected to square root transformation. For the seedling height and number of leaves, descriptive statistics were used (mean and standard deviation). Quantitative data on characterization of fruits were subjected to T-test. For the rest of the data, two-way ANOVA was done to test for significant differences and DMRT was used for mean separation.

Results and Discussion

Fruit Collection

Description of collection sites and incidence of seedlessness of fruits are shown in Table 1. The seeds were of approximately uniform stage of full maturity except the La Suerte sample, which was relatively immature. All of the mother plants except the Kidawa sample were close enough to other trees of approximately the same stage of maturity, thus, they could easily have been cross-pollinated by insects.

Description of Fruits

Mature fruits are yellow-green to straw in color. Close examination of the vertical section of the seeded fruit reveal a brown-black sarcotesta indicating maturity. Fruits with seeds have a horse-shoe shaped white endosperm (Figure 2a), unlike the seedless (parthenocarpic fruits (Figure 2b).

In seeded fruit, the grain-sized embryo is situated at the proximal portion of the endosperm. The seedless fruits however, have whitish mesoderm indicating immaturity. In addition, a brown-black sarcotesta is concentrated in the central portion (Figure 2b). These fruits have underdeveloped endosperms and are devoid of embryo.

Table 1. Description of sources and incidence of seedlessness of sago fruits used in the study

Description	Source					
	Kidawa	Tayobo	La Suerte	Tagbayangbang	Tagabuli	Tuban
Date of collection	6 June 2002	12 July 2002	12 July 2002	17 October 2002	15 December 2002	22-23 December 2002; 17 January 2003
Location	Kidawa, Laak, Comval Province	Tayobo, Bunawan, Agusan del Sur Province	La Suerte, Prosperidad, Agusan del Sur Province	Tagbayangbang, Bunawan, Agusan del Sur Province	Tagabuli, Sta. Cruz, Davao del Sur Province	Tuban, Sta. Cruz, Davao del Sur Province
Coordinates	125033° E 7056° N	12600° E 8010° N	125052° E 8034° N	12600° E; 8010° N	125024° E, 6048° N	125024° E 6024° N
Site description	Edge of a rice paddy	Eastern periphery of Agusan marsh, edge of paddy rice field along national highway	Edge of a rice paddy along national highway	Edge of a corn field	Along national highway, periphery of Tagabuli bay	Within a coconut farm
Mother palm description	Spineless, all leaves are dry	Spineless, all leaves are dry	Spineless; leaves are brown but not completely dry	Spineless; all leaves are dry	Spineless, all leaves are dry	One spiny and three spineless; all leaves are dry
Location of nearest fruit- bearing tree	More than 1 kilometer	One spineless palm ~100 m away	Two spineless palms within 100 m; the closer one was ~70 m away	10 spineless sago palms on their reproductive stage; withered stage (same as source of seed) ~20-30 m away, flowering stage ~70 m away, ~100-150 m away also on their fruit- bearing stage	One spineless palm ~20 m away from first tree; one spiny palm ~40 m away from first tree	Fruit-bearing palms within the vicinity became the source of fruit
Number of fruits	2875	1899	279	7630	2175	3708
Number with seeds	1	377	1	0	37	88
% with seeds	0.035	19.85	0.36	0	1.70	2.37



Figure 2. Vertical section of seeded sago fruit revealing horse-shoe shaped endosperm and grain-sized embryo (A); seedless fruits (B)

Fruit Measurements

From the seed lot with the highest number of seeded fruits, it was possible to make a quantitative comparison between seeded and seedless fruits. As shown in Table 2, the seeded fruits are bigger than parthenocarpic fruits in diameter, height, weight, volume and density. This is expected because seeds produce hormones (auxins or gibberellins) that control fruit growth and development (Gillaspy et al., 1993). Genes for parthenocarpy have been isolated from diverse sources and used for genetic engineering of commercially important food species as eggplants (Acciarri et al., 2002) and tomatoes (Rotino et al., 2005). Sago could be a potential source of useful parthenocarpic genes, because it is able to produce full-size fruits in spite of seedlessness.

Table 2. Characterization of sago fruits taken from Tayobo, Agusan del Sur. (n=40)

Parameter	Seeded	Seedless	t-test, $\alpha=0.05$
Diameter (mm)	50.48 $\pm$ 1.27	45.48 $\pm$ 1.76	tc=13.71 > 1.645
Height (mm)	48.56 $\pm$ 1.89	43.37 $\pm$ 2.04	tc=11.02 > 1.645
Weight (g)	49.55 $\pm$ 5.34	30.00 $\pm$ 5.40	tc=15.20 > 1.645
Volume (ml)	58.75 $\pm$ 5.8	43.20 $\pm$ 5.63	tc=11.33 > 1.645
Density (g/ml)	30.84 $\pm$ 0.06	0.70 $\pm$ 0.10	tc= 7.09 > 1.645

Effect of soaking and drying on seed germination

The pattern of germination of seeds subjected to various treatments is shown in Figure 3. A clear advantage of the treatments over the control is discernible, both in terms of rate and extent of germination. On the 125th day, percent germination of the hydro-priming treatments is significantly higher than that of the control (Table 3). Water replacement and interaction between water replacement and hydro-priming do not show any significant effect.

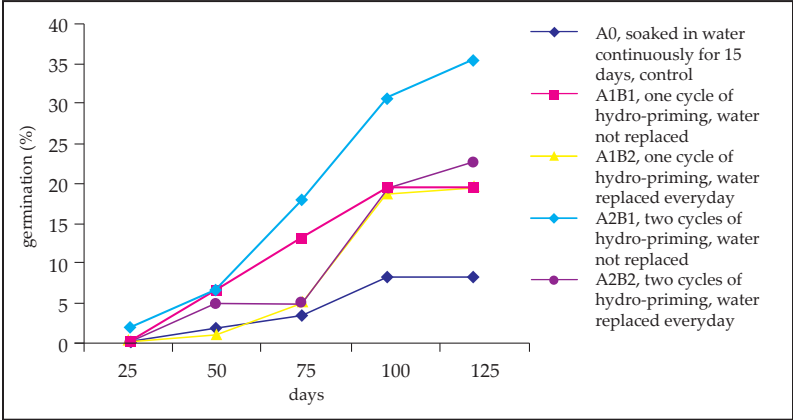


Figure 3. Percent germination of sago seeds recorded every 25 days as affected by hydro-priming and water replacement

Table 3. Percent germination of sago seeds after 125 days as affected by hydro-priming and water replacement during soaking

Treatment	Control, seeds soaked continuously for 15 days	A1, one cycle of hydro-priming	A2, two cycles of hydro-priming	Mean
B0, water not replaced	8.33	20.00	36.67	21.67 ^{ns}
B1, water replaced everyday	–	20.00	23.33	21.67 ^{ns}
Mean	8.33 ^b	20.00 ^a	30.00 ^a	

Means with the same letter are not significantly different at 5% level.
CV=29.84%

Effect of soaking and drying on incidence of normal seedlings

Seedlings were observed for eight months to determine if the treatments affected seedling characteristics. Table 4 shows that the soaking and air-drying treatments give a significantly higher percentage of normal seedlings compared to the control. Water replacement and interaction between soaking and air-drying do not show any significant effect. The normal seedlings have well developed leaves, leaves exhibited pigmentation at the juvenile stage (Figure 5). The abnormal seedlings exhibit stunted growth, deformed primary leaves and very thin primary roots.

Table 4. Percent normal sago seedlings after 8 months as affected by hydro-priming and water replacement during soaking

Treatment	Control, seeds soaked continuously for 15 days	A1, one cycle of hydro-priming	A2, two cycles of hydro-priming	Mean
B0, water not replaced	1.67	16.67	31.67	16.67 ^{ns}
B1, water replaced everyday	–	12.50	23.33	17.92 ^{ns}
Mean	1.67 ^b	14.59 ^a	27.50 ^a	

Means with the same letter are not significantly different at 5% level.
CV=30.16%



Figure 5. Normal (A) and abnormal (B) sago seedling, approximately two months old

Characterization of seeds that did not germinate

Seeds that failed to germinate after 125 days were cut to examine the condition of the embryo and endosperms. Some of the embryos were observed to be still fresh, perhaps indicating that they may still germinate given more time. Statistical analysis show that the two cycles of hydro-priming with water replaced everyday give a significantly lower (zero) incidence of fresh embryos and high incidence of decayed embryos compared to the other treatments. Comparisons are shown in Tables 5 and 6.

Table 5. Percent fresh embryos of non-germinated sago seeds after 125 days as affected by hydro-priming and water replacement

Treatment	Control, seeds soaked continuously for 15 days	A1, one cycle of hydro-priming	A2, two cycles of hydro-priming	Mean
B0, water not replaced	5.00 ^a	5.00 ^a	8.33 ^a	6.11
B1, water replaced everyday	–	14.17 ^a	0.00 ^b	7.09
Mean	5.00	9.59	4.17	

Means with the same letter are not significantly different at 5% level.

CV=36.06%

Table 6. Percent decayed embryos of non-germinated sago seeds after 125 days as affected by hydro-priming and water replacement

Treatment	Control, seeds soaked continuously for 15 days	A1, one cycle of hydro-priming	A2, two cycles of hydro-priming	Mean
B0, water not replaced	51.67 ^a	40.00 ^{ab}	28.33 ^b	40.00
B1, water replaced everyday	–	38.33 ^{ab}	48.33 ^a	43.33
Mean	51.67	39.17	38.33	

Means with the same letter are not significantly different at 5% level.

CV=7.39%

General Discussion

Six lots of sago fruits from different sources reveal a dominant number of seedless compared to seeded fruits, consistent with the theory of parthenocarpy (Flach, 1997), a condition where fruits set and mature without seed development and fertilization of an egg. Considering that the sago inflorescence consists of perfect and male flowers in a 1:1 ratio (Tomlison, 1990), seedlessness could result from failure of self-pollination or failure of self-fertilization when no other source of pollen is available. These can be caused by one or a combination of the following factors: 1) protandry – male flowers open before most hermaphrodite flowers open (Flach and Rumawas, 1996), 2) flower abortion before anthesis (Kiew, 1977; Utami, 1986; Torres, 2004), 3) abnormal pollen of male and perfect flowers (Torres, 2004), and 4) self-incompatibility (Flach and Rumawas, 1996).

Some types of parthenocarpy require pollination; others do not. Seedlessness may occur because the embryo aborts (Salisbury and Ross, 1992) before the fruit matures. In some species, pollination stimulates fruit development (Hopkins, 1999), but fruits mature without the pollen tube reaching the ovule (Kozlowski and Pallardy, 1997). It is especially common among fruits that produce many immature ovules (Salisbury and Ross, 1992). In the case of sago, self-pollination likely occurs but this does not lead to fertilization either because the pollens are abnormal (do not germinate *in vitro*) or normal pollens fail to produce pollen tube in the stigma even as they germinate *in vitro* (Torres, 2004).

The synchronization of pollen dispersal and stigma receptivity among sago flowers in the same inflorescence plays an important role in favoring cross-pollination (Jong, 1995). Flower opening of an inflorescence takes 30 days for male flowers and 50 days for the complete ones. Staminate flowers opens up to 2-4 weeks ahead of the complete flowers. The male flowers shed pollen to a large extent before the complete ones become receptive, but some overlaps occur. The abnormal complete flowers remain open for a period of three months. Their stamens shrivel quickly and the pollen grains appear abnormal.

Jong (1995) claimed that cross-pollination in sago is obligatory. Alang and Krishnapillay (1985) predicted a high proportion of seeded fruits when adjacent sago trees flower at the same time. They added that if there are no visible sources of pollen other than the tree itself or possible clones of the same tree, self-pollination is favored resulting to incidence of seedless fruits. Seedlessness is explained by incompatibility between pollen and stigma during self-pollination, which leads to abortion of ovules.

Results of our study showing a very low incidence of seeded fruits even when the mother trees were in close proximity with other fruit-bearing palms, trees that could have flowered at the same time as the mother tree, are not necessarily in conflict with these studies. It is possible that the neighboring trees were clones of the mother tree, considering that the trees were cultivated and the favored method of propagation of sago is the use of suckers.

While the simplest explanation for the low germination of fruits of sago is that they are predominantly seedless, seeded fruits also have germination problems. We addressed this problem by testing two methods for enhancing germination: soaking in water and hydro priming (cycles of soaking and drying). The water soaking treatments were inspired by the claim of Ehara et al. (2001) that there is a need for continuous soaking of seeds in water with constant replacement of water, to leach water-soluble inhibitors. However, we failed to show the benefit of water replacement, possibly because the fruits we used were already in an advanced maturity state and they no longer contained water-soluble inhibitors.

Hydro-priming was found to be more effective in enhancing germination of sago seeds. The treatment mimics conditions prevailing in the natural environment of sago. On rainy days, fruits that fall to the ground are completely soaked in water; and on sunny days they are subsequently exposed to dry conditions. Hydro-priming has been used in a wide range of orthodox-seeded crops such as maize, wheat, cotton, bittergourd, watermelon, mungbean and tomato, resulting in enhanced germination and initial seedling growth (Rasco, 1974;

Murungu et al., 2004; Rashid et al., 2003; Lin et al., 2005; Demir and Mavi, 2004; Giri and Schillinger, 2003). However, this is the first report that hydro-priming could be beneficial in recalcitrant seeds such as sago. Two explanations can be offered: a) soaking and drying may have broken dormancy, resulting in faster germination and higher germination percentage, and b) soaking and air-drying may have stimulated seedling growth faster than harmful microorganisms could infect them. In the field, these responses can enhance survival and production of normal seedlings. The delay in germination resulting from the control treatment, coupled with the high humidity of the germination medium, could render them physiologically old at the time of germination. The consequences may be: 1) seeds fail to germinate because their embryos are dead, or 2) weak embryo gives a weak seedling, which can easily succumb to environmental stresses in the field. With orthodox seeds, hydration during the priming cycle has been associated with increased RNA and protein synthesis (Fu et al., 1988), faster embryo growth (Dahal et al., 1990), and repair of deteriorated seed parts (Karssen et al., 1989).

Flach and Rumawas (1996) reported that sago seeds usually germinate within three weeks. On the other hand, Ehara et al. (1998) said that germination occurs in 1-2 months. Our data (Figure 4) indicate that the germination curve does not begin to rise beyond 5 per cent until approximately 50 days, and does not begin to flatten out until approximately 100 days. This pattern is associated with dormancy.

Seeded fruits that did not germinate within 125 days were examined to determine possible reasons behind their failure to germinate. Seeds with fresh embryo (associated with all treatments, except that subjected to two cycles of soaking and drying and daily water replacement) were, likely, still dormant. The relatively low incidence of decayed embryos in treatments that were subjected to soaking and drying is consistent with the observation that these treatments promote germination of viable seeds. Apparently, most of the viable seeds germinated when subjected to soaking and drying, thus these treatments gave a low incidence of decayed embryos. The effect of water

replacement was not so strong as to influence the effect of soaking and drying on germination. Water replacement was apparently more important in influencing embryo decay of seeds that failed to germinate. This explains the strong interaction effects of water replacement and soaking and drying on the incidence of fresh, as well as, decayed embryos, among seeds that failed to germinate. Apparently, replacement of water was a significant factor in promoting embryo decay when combined with two cycles of soaking and drying or a longer aggregate soaking period.

Summary and Conclusions

Six lots of sago fruits from different locations in Southern and North Central Mindanao reveal that the extent of seediness varies from zero to 19.8% depending on the presence of neighboring palms that could be a potential source of pollen. Among the possible reasons for failure of self-pollination that resulted in a high incidence of parthenocarpic fruits, we are inclined to support the self-incompatibility theory because incidence of seedlessness was commonly observed, even when the mother palm was surrounded by potential sources of pollen in the presence of ubiquitous pollinator bees. For most of the fruit samples collected, pollen supply was apparently not a constraint, even if lack of synchrony between pollen dehiscence and stigma receptivity occurs within an inflorescence. There were neighboring palms of the same stage of reproductive maturity in the fruit collection sites. However, it is likely that the neighboring palms were clones of the mother palm, thus self-incompatibility of pollen and stigma could still occur.

Germination of sago fruits and incidence of normal seedlings were consistently highest in seeds subjected to hydro-priming. Hydro-priming may have broken dormancy and stimulated seedling growth faster than the microorganisms can infect the young plant tissues. The high incidence of abnormal seedlings in slow germinating seed lots could be due to the seeds getting physiologically old at the time of germination.

Seeds that failed to germinate but showed fresh embryos (associated with all treatments except those subjected to soaking and drying and daily water replacement), may still be dormant at the end of the 125-day study.

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