Research Article

A Study of Cannonball Trees in Thailand: Hood Staminodes are Larger than Ring Stamens but only Germination of Staminal Ring Pollen can be Stimulated by Exogenous Sucrose

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Abstract
The size of the staminodes and ring stamen of *Couroupita guianensis* (cannonball tree) grown in the Nonthaburi province, Thailand as well as some characteristics of their pollen were investigated. The staminodes were clearly larger than the ring stamens. This finding is at variance with other previous studies. Viability staining showed that almost all the cannonball tree pollen from hood staminode were not viable but about 85% of the ring stamen pollen were viable. When both types of cannonball tree pollen were cultured on modified Mercado medium, hood staminode pollen did not germinate whereas the sucrose concentrations in the medium had a promotive effect on germination of ring stamen pollen. About 65% of the ring stamen pollen germinated on the medium supplemented with 20% sucrose. These pollen studies were in agreement with other similar studies on cannonball trees, suggesting that the relative sizes of the male reproductive organs of cannonball trees may not be related to fertility of their pollen.

Keywords: *Couroupita guianensis*, Hood staminode, Lecythidaceae, Pollen germination, Pollen fertility, Ring stamen, Sterile pollen

1 Introduction

Cannonball tree (*Couroupita guianensis* Aubl.) is one of the cauliflorous species in the Lecythidaceae family. The name of this plant came from the fruit size and shape that look like a cannonball. Cannonball tree is a good indicator for healthy ecosystem. It is frequently grown in the tropical and subtropical regions and currently considered as threatened medicinal tree species [1], [2]. Various parts of this plant had been used for several therapeutic advantages. Leaf, flower and fruit extracts showed anti-inflammatory effects, antioxidant and anticancer properties and antimicrobial activities, respectively [3]–[5].

Besides, cannonball tree flowers are of great scientific interest, for example, having two types of

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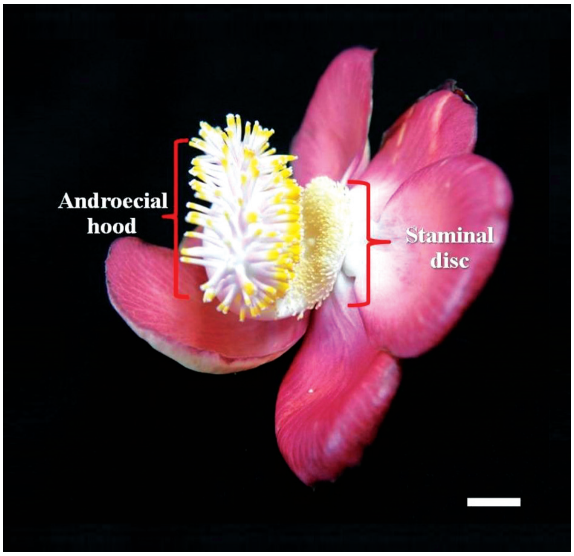

Figure 1: Position of staminal disc and androecial hood on natural cannonball tree flower (scale bar = 1 cm).

male reproductive organs, the hood staminodes and ring stamen. There is no study to examine the floral characters of the cannonball trees grown in different countries. Therefore, it is not known if variation in their floral characters might be present in different geographical regions although genotype-and-environment interactions in plants are not uncommon [6]. The objectives of the present study were to present findings about the characteristics of the hood staminodes and ring stamen of the cannonball trees grown in Thailand. Also, the shape, size, viability and *in vitro* germination of the two types of pollen produced were investigated.

2 Materials and Methods

2.1 *Plant materials*

Blooming cannonball tree flowers (*Couroupita guianensis* Aubl.) were collected from the Wat Sanghathan, Muang diatrict, Nonthaburi province, Thailand at about 9 A.M. in summer (April) as it was previously reported that the peak of cannonball tree flower anthesis presenting pollen was around this time of the day [7]. During harvest, the staminal disc and androecial hood (Figure 1) were gently separated and each placed into a plastic bag to avoid mixing up the two types of pollen therein.

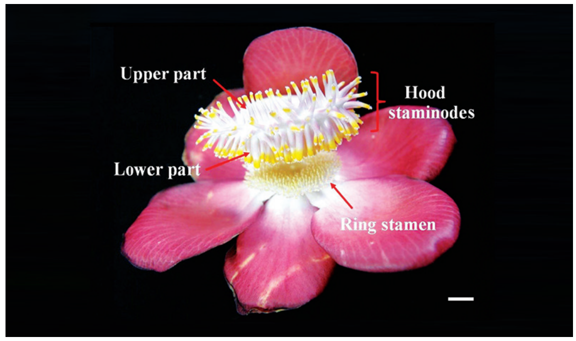

Figure 2: Position of ring stamen and hood staminode (upper part and lower part) on natural cannonball tree flower (scale bar = 1 cm).

2.2 *Determination of form, number and length of stamen*

Parts of the staminal disc and androecial hood were initially observed under a stereomicroscope (EMZ-TR, Meiji Techno Co., Ltd.) and photos were taken using a digital camera (Olympus C-760). Then, the ring stamen and hood staminode (Figure 2) were detached from staminal disc and androecial hood, respectively. After placing on the glass slide, ring stamen and hood staminode were observed under a light microscope (ML2000, Meiji Techno Co., Ltd.) and photos were also obtained using a digital camera. The number of ring stamen and hood staminode per flower were counted from randomly selected 30 cannon ball tree flowers whereas the length of ring stamen and hood staminode (upper and lower parts) were measured from 120 replications of each structure.

2.3 *Pollen shape, size and viability*

Ring stamen or staminal ring pollen and hood staminode pollen were separately removed from the respective structures, and placed on a glass slide for observation of their shape and size without any staining. Subsequently, both types of pollen were stained with 1% (w/v) acetocamine solution before their shape, size and viability were observed. Pollen stained red was considered to be viable pollen while unstained pollen was a non-viable one. Pollen observation before and after staining was made under a light microscope and shape of pollen had been estimated by using the P/E ratio [8]. Data from this experiment were collected from 30 replications.

2.4 *Observation of pollen germination*

Germination of both types pollen was assessed by using modified Mercado *et al*. medium [9] containing 0.1 mM boric acid and 1 mM $CaCl_2$ and different concentration of sucrose (0, 5, 10 and 20% w/v). This medium was adjusted to pH 5.7, gelled with 0.9% (w/v) agar and autoclaved at 121°C and 15 psi for 20 minute before use. Staminal ring pollen and hood staminode pollen were separately brushed over the surface of medium and were incubated at 25±2°C for 24 hours in the dark. The percentage of pollen germination and tube length were determined under a light microscope and obtained from 30 replications.

2.5 *Data analysis*

Statistical analysis of mean differences in number and length of ring stamen and hood staminode, pollen size and viability, pollen germination and tube length were performed using independent samples t-test or Tukey's test at $P < 0.05$ level.

3 Results

3.1 *Form, number and length of stamen*

The hood staminode of cannonball tree had longer filaments and anthers than the ring stamen as observed under a stereomicroscope and light microscope (Figure 3). The ring stamen had more stamens/flower (839.9±1.37) than the hood staminode (439.53±1.36 stamens/flower). The lengths of the ring stamen, upper and lower parts of the hood staminode were also found to be statistically different (2.84±0.31, 6.15±0.63 and 8.30±0.71 mm, respectively).

3.2 *Shape and size of pollen*

Before any staining and observed under a light microscope, the shape of both staminal ring and hood staminode pollen was prolate with P/E ratio about 1.7 (Figure 4). Besides, the staminal ring pollen appeared as monads while the hood staminode pollen remained as tetrads (Figure 5). After staining, both types of pollen had the same shape which was spheroidal (Figure 6). Additionally, the stained staminal ring pollen still appeared as monads and the stained hood staminode pollen remained as tetrads. The diameter of the ring stamen pollen was greater than that of the hood staminode pollen (Table 1).

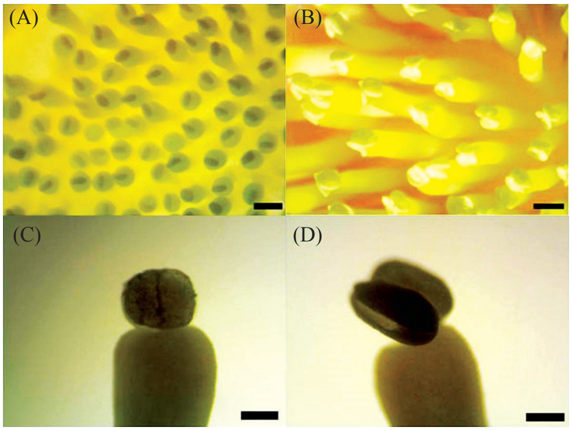

Figure 3: Male reproductive structures of cannonball tree: ring stamens on staminal disc (A), hood staminodes on androecial hood (B) anther on filament of ring stamen (C) and anther on filament of hood staminode (D). A and B: scale bar = 0.2 mm; C and D: scale bar = 0.1 mm.

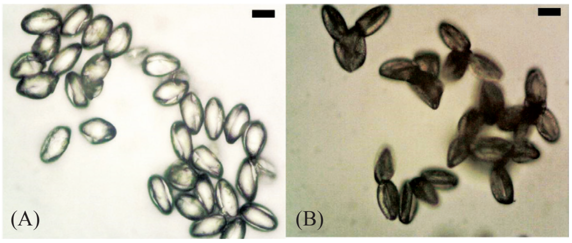

Figure 4: Morphology of staminal ring pollen (A) and hood staminode pollen (B) of cannonball tree before staining (scale bar = 20 µm).

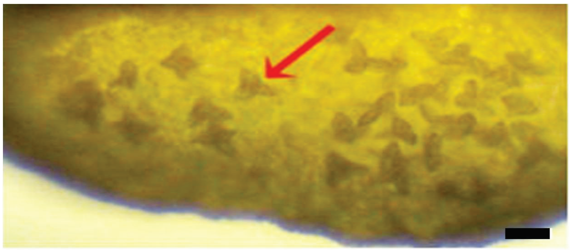

Figure 5: Pollen tetrads (arrow pointed) inside the anther from hood staminode of cannonball tree (scale bar = 20 µm).

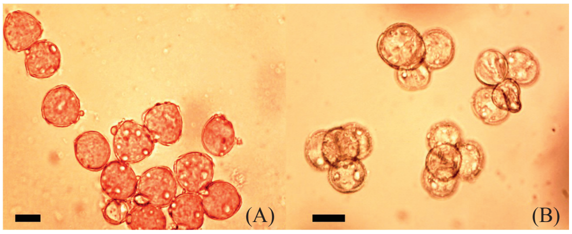

Figure 6: Morphology of staminal ring pollen (A) and hood staminode pollen (B) of cannonball tree after being stained with 1% (w/v) acetocamine solution (scale bar = 20 μm).

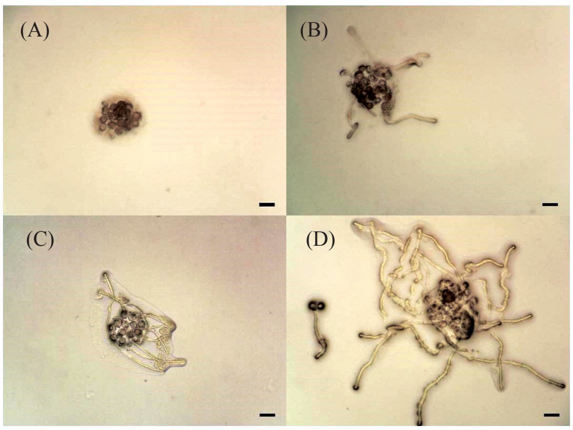

Figure 7: Staminal ring pollen of cannonball tree germinated on modified Mercado *et al*. medium (1994) [12] supplemented with 0% (A), 5% (B), 10% (C) and 20% (D) sucrose (scale bar = 50 μm).

3.3 *Pollen viability and in vitro germination*

A majority (about 85%) of the staminal ring pollen were shown to be viable but less than 1% of the hood staminode pollen were viable (Table 1). The effect of different concentrations (0, 5, 10 and 20%, w/v) of sucrose on the germination of the staminal ring pollen and hood staminode pollen was investigated. None of hood staminode pollen germinated on the different sucrose-containing media (Table 2). In the absence of added sucrose, the staminal ring pollen also showed no germination, but with increasing sucrose concentrations, pollen germinability and pollen tube lengths were considerably improved (Table 2). The highest percentage of germination (about 65%) of staminal ring pollen was achieved on medium supplemented with 20% sucrose (Figure 7).

Table 1: Viability and size of staminal ring and hood staminode pollen of cannonball tree after staining

Type	Viability (%)	Diameter Size (μm)
Staminal ring pollen	85.14 ± 9.80a	30.67± 1.56a
Hood staminode pollen	0.90 ± 2.39b	24.00 ± 2.14b

Data are means of 30 replications ± SD. Values marked by different letters in a column are significantly different ($P < 0.05$).

Table 2: Germination percentage and tube length of staminal ring and hood staminode pollen of cannonball tree grown on modified Mercado *et al*. medium [12] supplemented with various concentrations of sucrose

Type	Sucrose (%)	Germination (%)	Tube Length (mm)
Staminal ring pollen	0	0 ± 0d	0± 0d
	5	33.97 ± 13.79c	0.15±0.03c
	10	51.08 ± 13.66b	0.22±0.05b
	20	65.18 ± 12.01a	0.40±0.10a
Hood staminode pollen	0	0 ± 0d	0± 0d
	5	0± 0d	0± 0d
	10	0± 0d	0± 0d
	20	0± 0d	0± 0d

Data are means of 30 replications ± SD. Values marked by different letters in a column are significantly different ($P < 0.05$).

4 Discussion

Up to now, only a few angiosperm plant species are known to have two types of male reproductive organ: stamen and staminode in their flowers. Most zygomorphic flowers of Lecythidaceae contain staminal disc, ligule and androecial hood [10]. In the androecial parts, there are numerous stamens over the ring and hood structures but, in some plants of this family such as *Lecythis corrugata* and *L. pisonis*, anthers had not been found on the hood of the androecium [11], [12] suggesting that staminodes may or may not include pollen producing structure. For cannonball tree (*Couroupita guianensis* Aubl.), the floral organ consists of ring stamens and hood staminodes. Normally, staminodes are smaller than the fertile stamens [13] but, in the present study on the cannonball trees grown in Thailand, the size of the cannonball tree staminodes was clearly larger than the ring stamens (Figure 3). The reason for this variation is not known and remains to be elucidated. This result was, however, similar to the observations of some plants such as *Penstemon*, *Jacaranda* and *Digomphia* in which their staminodes were greatly developed, prominent and bigger than the stamens, and appeared to play a crucial role in the pollination ecology of those species [13], [14]. Interestingly, the number of cannonball tree ring stamens was found to be greater than that of the hood staminodes. It would be interesting to determine, in future research, if this might possibly influence the chance of pollination and fertilization of this plant.

In the case of cannonball tree, the anthers of the ring stamen or hood staminode produced pollen that were indistinguishable in shape. Nevertheless, the size of the staminal ring pollen was larger than hood staminode pollen which unlike the former were not viable (Table 1). Similarly, the staminode pollen of *Commelina coelestis* and *C. dianthifolia* had considerably lower viability than pollen of stamen [15]. Besides, it was interesting that pollen from hood staminode retained as groups of four pollen grains (tetrads) whereas pollen from ring stamen was monad. The type of tetrad formation or arrangement inside the anther from the hood staminode of cannonball tree was tetrahedral. Interestingly, aggregated pollen, especially tetrad, was commonly found in primitive angiosperm species such as *Typha latifolia* and Annonaceae [16], [17]. It was also likely that the anther of hood staminode but not ring stamen of cannonball tree may lack some enzymes such as callase or β-1,3-glucanase to free the pollen grains which is known to be required for degradation of callose to release pollen grains from tetrads in many plants [18].

Here, the present result suggested that hood staminode pollen were likely to be not fertile. This result corroborated the previous idea that in dimorphic flowers with two types of stamens, one of which is the feeding stamen producing fodder pollen for pollinators and another one was fertilization stamen giving normal pollen for safe gamete transport [19]. Thus, hood staminodes of cannonball tree are of colorful appearance and seem to be more attractive than ring stamen (Figure 2). For staminal ring pollen, there was no germination found on modified Mercado *et al*. medium [9] without added sucrose, suggesting that without a supply of energy, fertile pollen of cannonball tree was unable to germinate. When the concentration of this sugar increased, germination percentage and pollen tube length also rose in response. Furthermore, sucrose concentration obviously had an effect on the pollen tube growth of cannonball tree and the maximum length was observed on an artificial medium containing 20% sucrose. This was the same as in the study of sucrose effect on eucalyptus and okra pollen germination [20], [21]. Even so, the requisite optimum sucrose concentration in the artificial medium for pollen germination could vary among different plant species, for example, *Calotropis procera* ssp. *hamiltonii* and *Nymphaea nouchali* var. *versicolor* required 30% and 5% sucrose, respectively [22], [23].

5 Conclusions

Unlike previous studies, in the present study on the cannonball trees grown in Thailand, the size of the cannonball tree hood staminodes was found to be clearly larger than the ring stamens. The reason or this variation is not known. At present, the possibility that genotype-environment interactions might be associated with this remains to be investigated. It would be necessary to investigate further if this is evident also in the cannonball trees grown in other geographical regions. Nevertheless, the pollen produced by the hood staminodes were different from that produced by the ring stamens as the former were sterile and could not germinate even in the presence of promotive sucrose concentrations. These pollen studies were in agreement with other similar studies on cannonball tree not from Thailand, suggesting that the relative sizes of the male reproductive organs of cannonball trees may not be related to fertility of their pollen. Also, the sucrose requirement for *in vitro* germination of pollen seems to be the same from trees grown in other geographical regions. From a possible evolutionary perspective, the tetrad hood pollen, and not the ring monad pollen, resembled those produced by some primitive angiosperms.

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