Investigating the possible cause of sterility in *Cananga odorata* var. *fruticosa* (Annonaceae)

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Abstract

Cananga odorata var. *fruticosa* (Annonaceae) is apparently sterile as no fruiting and pollen tube germination was observed in controlled pollination. From the results of in-vitro pollen tube germination and microtome sectioning, it was found that possible reasons of sterility include pollen defects such as poor development and the failure in pollen release caused by failure in endothecium rupture.

Introduction

Cananga odorata has bisexual flowers with a pistillate (female) phase followed by the staminate (male) phase (Deroin, 1988).
Cananga odorata var. *fruticosa* is the dwarf variety of *Cananga odorata* which blooms with scented flowers and is popularly grown in Southeast Asia (Jin et al., 2015). It was mentioned by Turner and Veldkamp (2009) that *Cananga odorata* var. *fruticosa* never sets seed. However, Some *Cananga odorata* var. *fruticosa* × *odorata* were cultivated in Thailand by pollinating *C. odorata* var. *fruticosa* using *Cananga odorata* var. *odorata* pollen (Dr Piya Chalermglin, pers. comm.), proving that *C. odorata* var. *fruticosa* carpels contain functional ovules. This project aims to investigate whether pollen tube formation and fruiting occur in *Cananga odorata* var. *fruticosa*, and to elucidate the reasons for the failure of fruit formation.

Materials and Methods

Cananga odorata var. *fruticosa* individuals were sampled, with three individuals from Hong Kong Zoological and Botanical Gardens and four from Tai Po Waterfront Park.

Observations were initially conducted before pollination. The morphologies of pistillate and staminate phase flowers were recorded.

Controlled pollination

- Aimed to observe if fruitset and pollen tube germination occur

In-vitro pollen tube germination under different sugar concentrations

- Done on Petri dishes, sucrose solutions used were in concentration ranged from 0% to 50%
- Referencing from previous researches (Lora et al., 2011), 24h was set for the pollen tubes to grow
- Pollen tubes stained by methylene blue stain → Examination of pollen tubes under a microscope → Calculation of pollen germination percentage: [(number of pollen tubes germinated / total number of pollen grains) × 100%]
- Aimed to test the viability of pollen

Microtome sectioning

- Sample dehydration → Clearing → Vacuum infiltration → Embedding → Sectioning using rotary microtome → Dewaxing → Staining using safranin O and fast green → Mounting using Neo-mount
- Aimed to observe stamen structure in the flower bud, pistillate and staminate phases

Results and Discussion

Flower in pistillate phase (some petals removed)

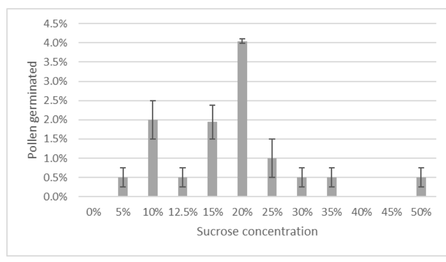
Flower in staminate phase (petals removed)

Morphology of flowers in pistillate phase: petals yellow, curved; stigmas moist, with brown tips; apex of stamen yellow; fragrant.
Morphology of flowers in staminate phase: petals yellowish-brown, soften and shrunken; stigmas moist, mostly brown; apex of stamen brown; stamens easily loosened from receptacle; intensely fragrant

Controlled pollination

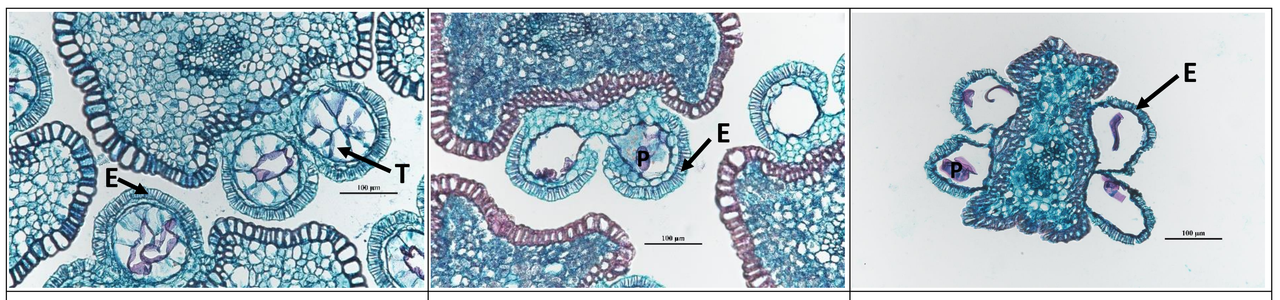
No fruiting and pollen tube germination was observed.

In-vitro pollen tube germination under different sugar concentrations



The germination percentages calculated were relatively low comparing with other Annonaceae species e.g. *Uvaria macrophylla* (Annonaceae) with 8.7% in average (Lau et al., 2017), the pollens were considered not viable.

Microtome sectioning (Showing transverse sections of stamen. Figure abbreviations: E = endothecium, P = pollen, T = tapetum.)



Bud | Female | Male

Transitioning from bud to pistillate phase then staminate phase, it was observed that although the endothecium shrank, it did not rupture even at the end of the staminate phase, indicating that the failure of endothecium breakage prevents pollen release.

It was hypothesized that *Cananga odorata* var. *fruticosa* contains only functionally female flowers. If *C. odorata* var. *fruticosa* is confirmed to be sterile and there are no natural populations, it suggests that it might be an artificial cultivar and should accordingly be renamed instead of being treated as a natural variety of *C. odorata*.

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