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FLOWER BIOLOGY OF TWO DIOSPYROS SPECIES NEIGHBORLY LIVE AT CSC AREA: DO POLLEN VIABILITY AND TUBE GROWTH RATE SHIRE ENDEMIC DISTRIBUTION

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Abstracts

Aims of study were to compare flower structure, pollen viability and pollen tube growth rate of two Diospyros species neighborly live in Cibinong Science Centre (CSC) park, Diospyros blancoi and D. celebica, and pursue if pollen-tube growth rate shire reasons endemic distribution of D.celebica in native habitat. Floral structure was in situ or ex situ morphologically observed. Germination test and pollen tube growth incubation were done in Sarfatti medium and digitally captured and computerize analysis. The result showed that, both species have flower part resemblance each other. D. blancoi has bigger size in almost all flower parts. D. blancoi showed much more pollen viability (41% vs 7%) and tube growth rate than D. celebica1 (66 vs 0.57 µm per minute). Average pollen-tube length of both species at about 31/2 hours incubation was significantly different each other (342.679 ± 37.067 vs 128,673 ± 49,215µm). Most observed D. blancoi female trees set high number of fruit year to year and almost observed D. celebica female trees set very low number of fruit and some time without fruit in the year. It is associated with geographical distribution difference between the two species. Very significantly different in pollen-germination percentage and pollen-tube growth rate provide a tool of further study to know microenvironment for reproduction and propagation of the endemic species, D. celebica.

Key words: *D. blancoi*, *D. celebica, flower biology, pollen germination, po tube growth*

1. INTRODUCTION

1.2. Back Ground

D. blancoi A.DC.(butter fruit) and *D.celebica* Bakh (black ebony) is two members of the genus Diospyros of the family of Ebenaceae. Genus Diospyros consists of 400 – 500 plant species distributed through out pantropical region and economically is usable by mainly as wood or timber trees. Many Diospyros species have good quality of wood[1)]. In Indonesia *D. celebica* is often called as 'kayu hitam' (black wood) or 'eboni' generally because its dark and fancy wood color mainly on adult or old plant individual[2)]. Diospyros is usually unisex. Habitat *D. blancoi* (formerly called *D. philippensis* (Dsr) Gurke, according to[3)] is up to 800 m and very common and widespread in the Philippine, while those of *D. celebica* is up

to 540 m[4], above sea level and strictly endemic in Celebes Island. Two Diospyros species grow neighborly in Cibinong Science Center. *D. blancoi* set much more fruit set than *D. celebica* (ebony).

During the last decades many studies have been carried out on the relationship between pollen and progeny performance, but the evidence for pollen-tube growth rate as an indicator of progeny performance remains equivocal. The idea of a positive relationship between pollen and progeny performance based on an overlap in gene expression between gametophytic and sporophytic stage of lifecycle[5].

Incomplete of pollen tube formed in the pistil can lowering the tube growth rate but the growth rate is not depend on the genetic content of the pollen[6]. The pollen tube unablities to reach ovules in ovarium seems tobe claimed as incompatibility mechanism and then polination fails to set fruit. Therefore, fruiting success may depend on the vigor of the pollen-tube constructed. Some pollen can germinate on stigma an can develop pollen tubes but it is uncertanity that the pollen tube is not so strong or long to reach micropyle or ovules that physically cause fertlization fail to occure. Incompatibity may be caused by inhibition of pollen germination, growth retardation or disorientation of pollen, or failure in nulear fussion[7]. It is shown later that fertilization occurs in most self penetrated ovules, with ensuing initiation of endosperm cells, prior to pistil abscission[8].

1.2. Aim of Study

The aim of present study was to uncover generally the floral biology, if pollen viability and pollen tube growth rate significantly differ, of two species, *D. blancoi* and *D. celebica* grow neighborly in Cibinong Science Center, and do the pollen-tube growth rate shires endemic distribution of *D. celebica* ?

2. METODOLOGY

2.1 Study site

Present study was executed from September 2007 to March 2008 at Cibinong Science Center (CSC). Ten male individuals of *D. blancoi* and six male individual of *D. Celebica* growing as plant collection or shadowing plant area were observed as plant material. All observed plant were reproductively adult age. In nature *D. celebica* distribution is endemic in Celebes island. The both species is dioeciously plant and sex proportion in the area almost the same. Fresh male flowers of the species were synchronically picked and brought to P-MAC (Plant Morphology, Anatomy and Cytology) Laboratory of Biology Research Center, Indonesian Institute of Sciences (LIPI) nearby habitat of the plant, for pollen germination test.

2.2. Pollen germination test

In vitro pollen germination (G) test and pollen tube incubation were basically done in Sarfatti's pollen germination test medium[9]. Original medium composition was 100 mg H3BO3, 432 mg Ca (NO3)2 4 H2O, 300 mg KNO3, 408 mg Mg SO4 7H2O and 150 g Sucrose in 1 liter distilled water. We also modified the concentration by 2X and 4X dilution. We call solution without dilution as medium C 1000, 2 X dilutions as medium C 500 and 4X-dilution as medium C 250. The g-test and pollen tube growth incubation were carried out 10 – 15 replications of each medium with incubation time from 200 – 250 minutes, in optimal incubation temperature for pollen germination or pollen tube growth , 25 - 26°C[10].

2.3. Observation

Microscopic observation was done under 10X objective lens of Nikon Eclipse 80i Microscope with Nikon DS Camera Head DS-5M and then connected to DS-L1 DS Camera Control Unit for digitally capturing observed SFC-images. Pollen-tube length of

the SFC-images was measured with Motic Image plus 2.0 computer program and then measurement table was directly exported to XL statistical program to be further analysis.

3. RESULT AND DISCUSSION

3.1. Flower Structure, fruit set, tree canopy and native habitat

Though both species have flower precisely resemblance each other, *D. blancoi* has bigger size in almost all flower parts. Though *D. celebica* is dioecy, some rudiment stamens were still observable on young flower bud of female flower, attached at the basic of corolla. Female flower of *D. blancoi* had no rudiment stamen at all but according to[4]. 4–5 or 8–10 staminodes are present.

3.2. Pollen germination and pollen tube growth

D. blanchoi showed much more pollen germinability, 41% vs 7%, and tube growth rate, 1.66 vs 0.57 µm per minute, than *D. celebica* (Table 1). Generally, after more than 3.5 hours incubation the average of pollen-tube length of both species was significantly different each other, 342.679 ± 37.067 vs 128.673 ± 49.215.

Pollens of *D. blanchoi* showed good germinations which almost of its were followed by formatting vigor and straight pollen tubes (Fig. 1 A). Almost pollens of *D. celebica* tended to germinate in burst (abortive?) which were not followed formatting vigor and straight pollen tubes (Fig. 1 B). Tube growth in the majority of in vitro germinated pollens stop may be due to several reasons such as callose deposition at the tips, swelling, bifurcation and bursting[11]. Therefore, we excluded the burst-like pollen germination because it has no enough vigor tube growth to enter or make (?) style canal up to ovule so it can not fertilize them. It had been categorized as bad pollen tube[12]. In present study, bad pollen tubes seem to be as bursting only and *D. celebica* showed signicantly much more bad pollen tubes than *D. blancoi.*

Almost all *D. blancoi* individual observed set high number of fruit in all season of the year while *D. celebica* set fruit very rare at least in the CSC area. Our experience in other non natural planting location also showed approximately the same fruiting capacities[13]. In previos studies it has been found evidence for the positive relationship between pollen-tube growth rate and seed siring success of the pollen donors in self-incompatible, deciduos tree[5]. However,

Table 1 Comparative flower, fruit set, tree canopy and habitat aspects of *D. blancoi* and *D. celebica.D.blancoi* was higher for all observed item

Item	*D. blancoi* A.DC.	*D. celebica* Bakh	References
Male flower	in 3-7 flowered cymes	In 3-7 flowered cymes	Prosea book
width size (cm)	0.67 ± 0.07	0.41 ± 0.03	Present study
Merous	4-merous	4 merous	Prosea book
Stamen number	24 - 30	16	Prosea book
Female flower	solitarily	1-3 flowered cymes	Prosea book
width size (cm)	1.15 ± 0,13	0.765 ± 0.054	Present study
Style number	4-5 per ovary	Monostyle per ovary	Prosea book
ovulate	8 or 10 uni-ovulate locules	4-8 uni-ovulate locules	Prosea book
Rudiment anther	Present, 4-5 or 8 – 10 *)	Present, in young bud only	Present study
Fruit set (mature)	High number	rare	Present study
Tree canopy in CSC	Well developed	Well developed	Present study
Habitat and distribution status	Up to 800m, wide distri buted in Philippine	Up to 540m endemic, in Celebes, Indonesia	Prosea book

Prosea book =Suryanegara et al 1995 *) = cited from Suryanegara et al 1995, but absent in present study

clarifying if pollen-tube growth rate shire lower fruit setting of *D. celebica* need further study of sex ratio effects or pollination physiology and ecology. Environment favourabilty has been found to affect biomass allocation, number of inflorescence, cover and competitive ability of a plant species[14]. Characteristic syndromes of inhibition or malfunction of pollen tubes during at least seven different stages (only three stages we mention for this report) in the pollination process including: failure to germinate: abortion of tubes on stigma surface and abortion of tubes at various levels in the stylar canal[15].

Table 2. Comparative pollen germinability, tube length and tube growth rate of *D. blancoi* and *D. celebica. D.blancoi* was higher for all parameters.

Observed Item		*Diospyros blancoi*		*Diospyros celebica*	
		Value	**St dev**	**Value**	**St dev**
rata2(%)	germination	41,134	4,664	7,403	2,509
Average (µm)	tube length at C 1000	311.544	81.654	182.747	69.993
	at C 500	332.813	75.943	86.496	29.607
	at C 250	383.681	112.838	116.777	45.273
	Average all C	*342.679*	*37.067*	*128.673*	*49.215*
incubation	time (minute)	207		225	
average (µm)	per minute	1.66		0.57	

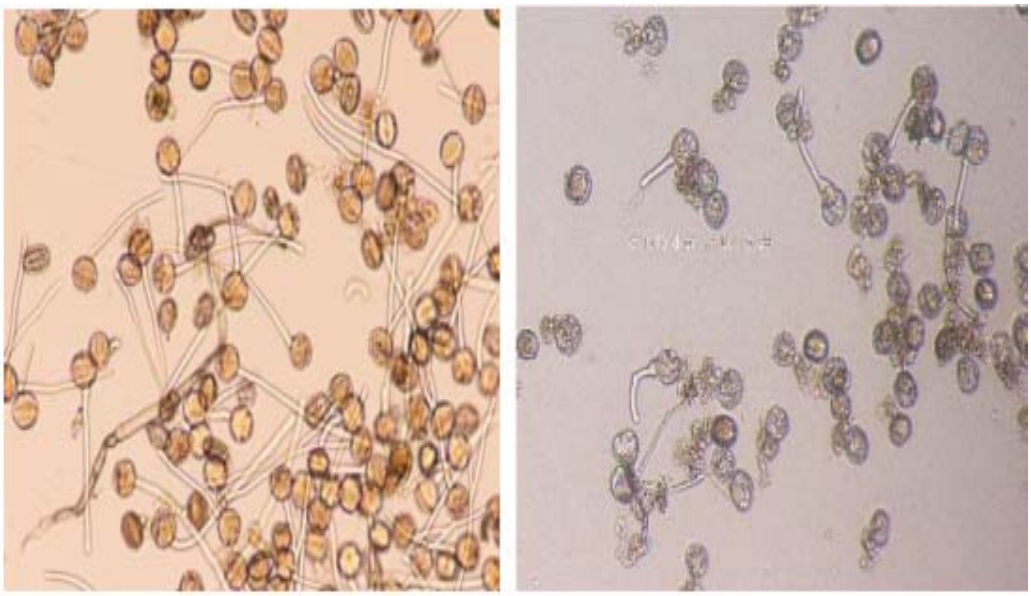
We modified the Sarfatti medium by diluting 2X and 4X. Therefore we germinated pollens for the pollen-tube growth rate analysis in three medium concentration termed as 1000 (without dilution), 500 (2Xdilution), and 250 (4X dilution). Respon of Pollen-tube development to the medium concentration was different between the both species. Increasing the medium concentration the higher pollen-tube growth rate of *D. celebica* is, but increasing the medium concentration generally lower pollen-tube growth rate of *D. blancoi* is (Fig.2).

3.3. Pollen viabilty an tube growth rate vs endemic distribution

Very significantly difference in viabilitas and pollen tube growth rate between the two Diospyros species confronted with fruit set successful seem to be one of interested indicators to further study uncovering required microenvironment or bioreproduction and propagation of endemic species, *D. celebica*. Present study is not able to answer if the pollen viability and pollen growth rate shire the endemic distribution because there is a (great number of ecophysiological and phytogeographical factors should be studied before. However, this is a preliminary study about relationship the functional pollen with endemic. Cultivations of *D. celebica* at non native-habitat in the CSC area show well development of its performance of vegetative canopy and in this case, were not different with *D. blancoi.* It is may implies macro environment (at the non native habitat) was not very important factor in studying why the species suffers endemic distribution. It was reported that the fast growing-tube pollens shared in good offspring formation[5]. Existence of a dioecy (may be also

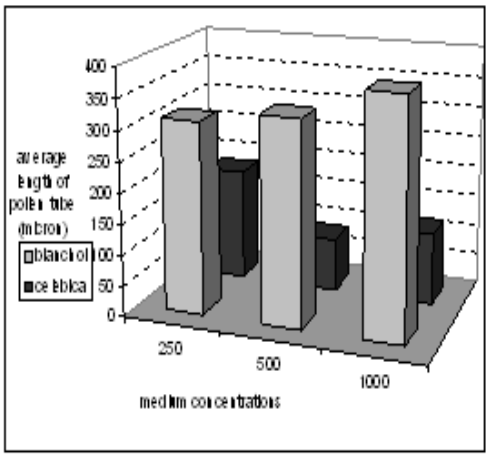
monoecy) endemic population on a given habitat from generation to generation in long time absolutely requires good offspring originated from pollen donor.

Fig.1. Pollen tubes of *Diospyros blancoi* (A): almost had high germination and good pollen tubes, and *D. celebica* (B) : almost pollen germinate in burst and with bad pollen tubes



A B

Fig. 2. Effects of medium concentration (see text for detail) to pollen tube growth.



4. CONCLUSION

It is concluded that pollen viability and pollen tube growth rate significantly differ, of two species, *D. blancoi* and *D. celebica* grow neighborly in Cibinong Science Center. *D. blanchoi* was higher for all observed item. Pollen tubes of *D. blancoi* tend to be longer in higher dilution factor, but pollen tubes of *D. celebica* tend to be longer in lower dilution factor. It is of great possibility that *D. celebica,* based on the pollen viability and pollen tube growth rate, reproductively need the special micro or macro environment condition for continuing its existence from generation to generation in one location, though the pollen-tube growth rate is surely

not the only one reason for its endemic distribution. Therefore, further study should be needed.

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