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CHARACTERISTICS OF LAND SUITABILITY FOR *Didymoplexis pallens* Griff. (ORCHIDACEAE) AT BOGOR BOTANIC GARDENS

Raden Vitri GARVITA[1,*], Kartika Ning TYAS[1],
Rizmoon Nurul ZULKARNAEN[1], Hary WAWANGNINGRUM[1], Dian LATIFAH[1]

[1] Research Center for Plant Conservation, Botanic Gardens and Forestry - National Research and Innovation Agency Republic of Indonesia (BRIN) - Jl. Ir. H. Juanda No. 13. Bogor, 16122, Indonesia

Abstract

The orchid's present distribution faces daily threats including habitat loss and unsustainable exploitation. Didymoplexis pallens is a leafless rhizomatous terrestrial mycotroph. One of the factors that affect the orchid population is the abundance of orchid mycorrhizal fungi (OMF) in substrates, unfortunately, the information regarding the interaction between OMF and the presence of orchids is still not widely known. Whereas this information is required in the ex-situ conservation strategy of this terrestrial orchid in Bogor Botanic Gardens. The soil chemical analysis and mycorrhiza total number were taken by the Indonesian Soil Research Institute to determine levels of phosphorous, and nitrogen in the form of nitrate and ammonium, organic carbon, pH, texture, and mycorrhiza. The requirements for every orchid to live were different. This research could provide knowledge about soil properties that could aid in the relocation of this orchid's habitat. Litter decomposition can affect physical and chemical properties as well as the continuity of organic C as in soil. OMF is a kind of mycorrhiza symbiosis that arise among fungi and orchids, which is very required for the long-term viability of an orchid population. Numerous factors, or pairings of factors, could impact OMF distribution and orchid population distribution.

Keywords*: Orchidaceae; Didymoplexis pallens, Soils; Mycorrhiza*

Introduction

The species of *Didymoplexis* are small saprophytic orchids, achlorophyllous plants and emerge above ground only when flowering and fruiting, so they were known as 'Ghost Orchids'. Field investigators frequently neglected them. Because of their inconspicuous growth habits and restricted flowering seasons, it is exceptionally hard to found them [1]. According to [2] the highest species diversity of *Didymoplexis* is observed in Java with six species, one of them is *D. pallens* Griff, whereas Thailand and Borneo are occupied by five species each. *D. pallens* is native to Indonesia [3], a leafless terrestrial herb with a pale brown fleshy rhizome.

The existence of this orchid in nature is increasingly threatened, this is caused by partial degradation ecosystems such as selective logging, slash and burn agriculture, forest fragmentation, pollinator problems, keystone species change/losses, genetic erosion, weed introduction, animal introductions, fuelwood gathering, small fires/changing fire regimes, localized pollution [4, 5].

The existence of Orchidaceae is highly dependent on the interaction with orchid mycorrhizal fungi (OMF) for all nutrients, particularly in seed germination, and in some cases will continue to early in life and proceeds to varying degrees as adults [6–8]. The study about understanding the relationship between orchid diversity and OMF has received a lot of attention, but there are still

* Corresponding author: vitrigarvita@gmail.com

obstacles, namely the lack of information that explains the fundamental aspects of OMF diversity such as differences and abundance in OMF composition on the presence of orchids [6, 7, 9].

The interaction between orchid populations and distribution with abiotic and biotic variables, including climate, light conditions, substrate types, soil conditions and mycorrhiza, will give each other a reciprocal response [7, 8, 10], but the information of its processing is still lack. The previous studies suggest that habitat and soil conditions affect fungal composition in orchid populations. For example, *M. Waud et al.* [11] found that the existence of orchids with OMF density distribution is directly proportional, implying that as orchids become more distant from one another, the presence of OMF in the soil decreases dramatically; meanwhile the diversity of OMF affiliated with *Bipinnula* was well correlated with soil nutrients where N nutrient were lower [10].

The essential significance of orchid conservation is demonstrated by successfully propagated and restored orchids, as well as identification and introduction of the critically important mycorrhizal fungi symbiont. Unfortunately, orchid mycorrhizal fungi are still understudied. It makes difficult to carry out conservation efforts for this orchid. Therefore, in this study we propose a habitat of orchid propagation for conservation of *Didymoplexis pallens* Griff in order to establish self-sustaining populations in natural habitat. Understanding the biological and ecological requirements for orchid seedling survival is critical to the orchid's effective dispersion and survival in the ecosystem.

The object of this study was to determine the relations between habitat suitability factors, including soil condition and mycorrhiza with population and distribution of *D. pallens*, also to find out about flowering and fruiting stage of this orchid in Bogor Botanic Gardens. This study is part of acquire knowledge required for the successful conservation of *D. pallens* Griff.

Experimental

Materials

The research method was carried out using a survey method with a parallel land evaluation approach, namely conducting qualitative and quantitative evaluations simultaneously based on physical criteria [12]. The intensive field survey works were carried out from September-October 2019 during the wet season. The observation was conducted under the bamboo clumps at Bogor Botanic Gardens, Indonesia. Soil samples were taken in the field from eight distinct locations where *D. pallens* were observed in December 2019 by digging about 20 x 20cm away from shoots and then carefully approaching underground parts of the plants (Fig. 1). Approximately 1.0kg of soil samples was collected at each sample point, homogenized, and then used for soil analysis.

Methods

The soil chemical analysis and mycorrhiza total number were taken by the Indonesian Soil Research Institute, which is located in Bogor, Indonesia. ISRI soil laboratories employed standard chemical soil analysis to determine levels of phosphorous, potassium, nitrogen, organic carbon, pH, soil texture, and mycorrhiza. The results of the soil analysis were translated through a table of assessment criteria based on general empirical properties compiled by the Soil Research Institute of the Ministry of Agriculture of the Republic of Indonesia [13]. The influence of abiotic factors on the presence of *D. pallens* was analysed using SPSS.

Results and discussion

Didymoplexis pallens population, distribution, and habitat

Abiotic and biotic factors, such as the existence of adequate OMF in the soil, have a significant impact on the sustainability of orchid populations. The development of OMF hyphae can help absorb water and nutrients in the soil, especially phosphate. *D. pallens* can be found on shady grassland at Bogor Botanical Gardens (Fig. 1A-H). Most of these orchids reside under a bamboo clump collection with a litter of bamboo leaves, as seen in figure 1.G, so it gave high C soil content in soil analysis. The bamboo collections are *Gigantochloa verticillate, G. atroviolacea, G. atter, G. apus, Dendrocalamus giganteus, D. asper* and *Bambusa vulgaris*. The majority of this orchid population was found in areas with cover ranging from 60-80% using the HabitApp application [14].

The presence of this orchid demonstrates the significantly important roles of bamboo leaf litter in the existence of this species in Java. In other seasons, these plants are difficult to spot among the dry leaves of bamboo, but they are easy to spot in the wet season. The habitat and inflorescence of *D. pallens* can be seen in figure 2. According to [15] *D. pallens* orchid in India is also growing in bamboo forest and flowering around May until June.

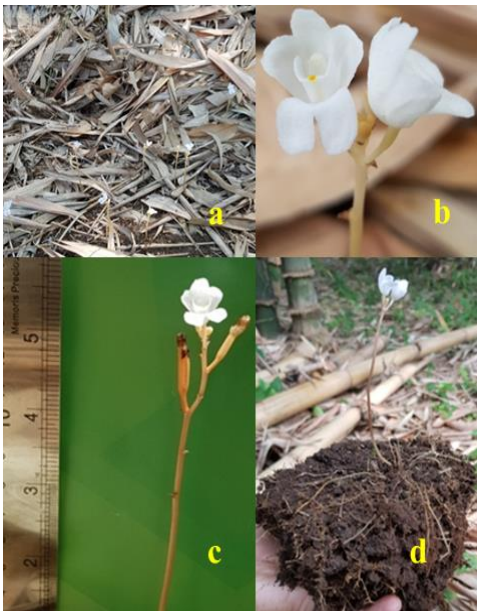

Fig. 2. *Didymoplexis pallens*: a. growth habitat of mature flowering plants amongst bamboo leaf's litter; b. an open flower from front view; c. an open flower and immature fruit's front view; d. habitus and soil samples

Cation Exchange Capacity (CEC) is a chemical property linked to soil fertility [16, 17]. A high soil CEC can greatly affect soil fertility and the availability of soil macronutrients such as C and N. According to the research of [18], soil fertility with a high C and N composition is sufficient to sustain plant biodiversity. Soil with high CEC absorbs and provides nutrients more effectively than soil with a low CEC. Table 1 shows that sites B and F have high CEC (24.99 and 24.64mmol/kg). Based on the Regulation of the Minister of Agriculture of the Republic of Indonesia number 70/permentan/SR.140/10/2011 concerning organic fertilizers, biological fertilizers, and soil enhancers, it is stated that the total propagules of mycorrhizal arbuscular are more than 50 spores/gram dry weight of the sample in biological fertilizers.

The samples of soil taken in the middle of the rainy season showed that the number of mycorrhizal spores was found 0-9 spores/gram soil samples. It is possible that the mycorrhizal spores in the location have germinated, grown and symbiotic with baboo roots or other plants in the location area, or were washed away by rainwater. The soil in Bogor Botanical Gardens has acid to slightly acid pH, low to moderate CEC, and very low to very high SC. The P nutrient content was very high in all locations where *D. pallens* grew. The C content is low to high. Meanwhile, the N content tends to be moderate, only locations A and E have low content. The K content is very low to moderate (Table 1).

Figure 3 shows that the variables, such as P_2O_5, K_2O, pH, and Soil Saturation (SC), influenced the abundance population of *D. pallens* by 51.7% (Component 1) and the other variables contributed 28.2% (Component 2). Thus, the presence of *D. pallens* is closely related to abiotic factors where the condition is also influenced by biotic factors such as the presence of bamboo.

The main source of carbon compounds available is the decomposition of organic materials present in the form of dead decaying such as leaves and bamboo litter. Organic matter is the primary

source of carbon (C), nitrogen (N), and phosphorus (P). Mycoheterotrophy orchid is highly dependent on OMF to absorb nutrients from their surroundings. OMF is present in soil to obtain nutrition from the organic matter [19]. The N present in leaf litter soil is in the form of inorganic N (nitrates and ammonium) and organic N (amides and amino acids). Fungi activity in decomposing litter is highly dependent on the litter moisture conditions and climate factors. The presence of leaf litter keeps soil moisture levels higher, indicating the presence of organic matter as a habitat for fungi.

Table 1. Population of *Didymoplexis pallens*, mycorrhizal fungus and its several soil parameters in the eight sites at Bogor Botanical Gardens

Parameter observed	A	B	C	D	E	F	G	H
Number of *D. pallens* flowering population	165.00	103.00	170.00	148.00	7.00	0.00	5.00	0.00
Number of mycorrhizal (spore/g)	2.00	9.00	3.00	5.00	1.00	0.00	0.00	0.00
pH	5.50	4.60	6.30	6.10	4.70	5.00	4.60	5.30
	acid	acid	Slightly acid	Slightly acid	acid	acid	acid	Acid
C (%)	1.83	4.86	3.29	2.93	3.32	4.34	3.12	2.40
	low	high	high	medium	high	high	high	medium
N (%)	0.17	0.46	0.35	0.30	0.18	0.49	0.29	0.25
	low	medium	medium	medium	low	medium	medium	medium
C/N	11	11	9	10	18	9	11	10
	medium	medium	low	low	high	low	medium	Low
P_2O_5 (mg/100g)	75.00	81.00	92.00	165.00	69.00	83.00	82.00	66.00
	very high	very high	very high	very high	very high	very high	very high	very high
K_2O (mg/100g)	7.00	11.00	33.00	34.00	21.00	12.00	20.00	23.00
	very low	low	medium	medium	medium	low	low	medium
CEC (cmol/kg)	16.70	24.99	19.45	18.78	19.66	24.64	19.05	23.89
	low	medium	medium	medium	medium	medium	medium	medium
SC (%)	53.00	17.00	71.00	65.00	18.00	33.00	24.00	53.00
	high	very low	very high	high	very low	low	low	High
Sand (%)	12.00	10.00	31.00	32.00	9.00	9.00	25.00	54.00
Clay (%)	33.00	25.00	28.00	25.00	37.00	34.00	40.00	12.00
Dust (%)	65.00	65.00	41.00	43.00	54.00	57.00	35.00	34.00

Note: soil test conducted in December 2019. Assessment criteria based on general empirical properties [12]

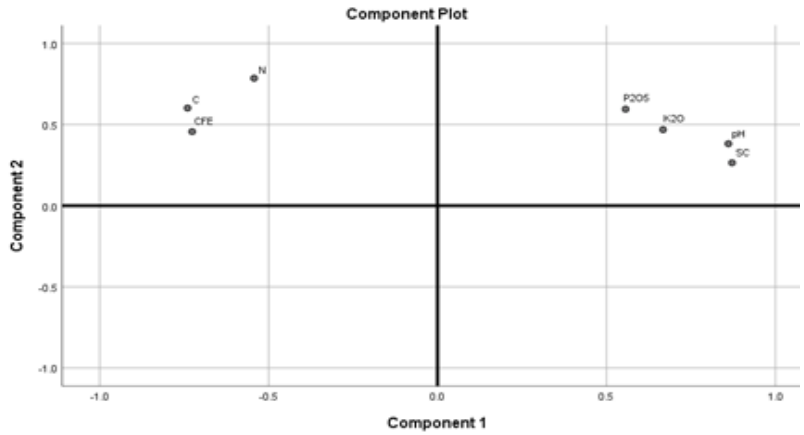

Fig. 3. Abiotic factor components affecting *Didymoplexis pallens* populations in Bogor Botanic Gardens

The presence of *D. pallens* is poorly understood because it appears only during reproductive phase, at wet season, and spends most of their life cycle underground. Development from seed to flower in several MPHs was successfully conducted by researcher in symbiotic or a symbiotic culture i.e. *Gastrodia pubilabiata* required 4 months, 6 months in *E. roseum* and 4-6 months in *D. pallens* [20].

The orchid mycorrhizal fungi are able to obtain carbohydrate from outside the orchid, although there is some diversity of source. Both of fungus and orchid are using organic C from the photosynthetic mycorrhizal species. Orchid fungi have unspecialized requirements for nutrients other than C sources. Most can use a wide range of N compounds. It seems likely that organic sources of N, and possibly also P, are important for these fungi growing in soil [21].

Mycorrhiza

Orchidaceae life is entirely or partially dependent on fungi or carbon, as well as other essential resources such as abiotic and biotic factors. As a result, the distribution of OMF affects orchid populations [6–11]. Our results indicated that the number of flowering *D. pallens* was not proportional to the abundance of OMF (Table 1). It could imply that not all OMF discovered were equally effective at supporting *D. pallens* life history stage [22]. With only two of OMF at site A and a population of 165 flowering orchids, we predicted that two of the OMF are effective supporting *D. pallens* life.

Mycorrhizal fungi distribution can influence the orchid distribution, which the orchid associated with an appropriate mycorrhizal fungus. Mycorrhizal fungi are more abundant at B site 9, but the orchid population is 103. This population fewer than C site, 170, with number of mycorrhizas is only 3. It can be means that type of mycorrhizal fungi at B site is partially appropriate fungi for *D. pallens*.

Knowledge of the ecological specificity of orchids and the mycorrhizal fungi is likely to be the key to device successful orchid trans-location programs to an ex-situ conservation site. The distribution of mycorrhizal fungi can influence orchid distribution and it may be critical for orchids, which cannot persist unless they are associated with an appropriate mycorrhizal fungus [23]. Therefore, knowledge of orchid mycorrhizal fungi within soil or other substrates is important to understand the distribution of *D. pallens*.

The seed germination of *Caladenia arenicola* is related with present of several soil factors [24]. Water holding capacity of the soil may be affected by potassium which relates to the presence of clay. Leaf litter presence is to maintain higher soil moisture levels and also indicates the presence of organic matter on which soil fungi depend. To maintain higher soil moisture levels and also indicate the presence of organic matter that supports the presence of fungi are indicated by the presence of leaf litter. The potassium and organic carbon were related to soil structure and texture properties, and thus are indirectly rather than directly related to seed germination. There are many factors which may affect the distribution of mycorrhizal, which in turn may affect distribution of orchid in the field.

Many studies have been done to understand of orchid-fungus interaction, i.e., investigating the distribution and role of mycorrhizal fungi in the soil by using seed packets to ‘bait’ for appropriate fungi. The location of fungi and environmental condition for seed germination can be detected by seed packets [23]. Based on [25] study, the seed packets can detect fungi in orchid roots but not found in soil samples. [10] showed that the distribution of OMF in the soil may not be evenly distributed and the variety of OMF is different so that it has an impact on the distribution of orchids. *W.D. Bunch et al*. [26] showed that soil pH and percentage of organic materials C and N in populations of *Cypripedium acaule* habitat have a variety of mycorrhiza composition.

Conclusions

The prerequisites for each orchid to grow were varied. This study may give information about the soil properties that can support to translocation of the habitat of this orchid. However, a more detailed study into the characterization of mycorrhizal, the interaction between bamboo leaf litter and

mycorrhiza, and their significance in the orchid development stage is required. Litter decomposition can have an impact on soil characteristics as well as the stability of organic C in the soil. One type of mycorrhiza symbiosis that occurs between fungi and orchids is OMF.

Many factors, or combinations of factors, may influence OMF distribution, which in turn may influence orchid population distribution. The presence of appropriate suitable OMF in the soil is certainly necessary for an orchid population's long-term viability.

Abbreviations

OMF : Orchid mycorrhiza fungi	P : Phosphorus	K_2O : Potassium oxide
C : Carbon	K : Potassium	CEC : Cation exchange capacity
N : Nitrogen	P_2O_5 : Phosphorus pentoxide	SC : Soil saturation

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