

Growth and Development of Apogamous *Adiantum lunulatum* Burm. f. Gametophyte from Dry and Humid Areas in Java Island

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ABSTRACT

Adiantum lunulatum Burm. f. has been widely used in aesthetic needs and medical fields. Detail information about the life cycle of *A. lunulatum* is still unknown, particularly about its gametophyte generation. Present work aimed to study and compare the growth and development of the gametophytes of *A. lunulatum* from dry and humid areas. Research method consists of two stages: (1) Spores were collected from two locations, Pasuruan and Bogor and (2) Spores were planted and observed the growth and development of gametophyte. The result showed that natural habitat of the plant in various dry and humid areas, affecting the rate of growth and development rates of *A. lunulatum* gametophyte. The gametophyte from the dry area showed faster rate of growth and development than that of humid areas. The spores collected from dry area need 9 weeks for germination, growth, and development while the spores of humid area took 22 weeks.

Keywords: Bogor, gametophyte generation, Pasuruan, spore

INTRODUCTION

Adiantum lunulatum Burm. f. belongs to the family Pteridaceae [1]. This fern is characterized by short suberect rhizome and covered with dark brown scales (3 mm long); stipes grooved, black, 8-18 cm long; lamina pinnate, usually bearing 8-15 alternate pinnae with an abnormal terminal leaflet or with prolonged, leafless rachis may start rooting at tip when contact with soil; leaflets crescent-shaped, thin, and glabrous. Sori along the edges, abaxial leaflets reflexed, elongate soral flaps [2, 3]. The species is growing in dry areas sometimes found on the muddy ground and crack rocks and is a tropical species distributed from lowlands to an altitude of 1,000 m [3].

Some *Adiantum* species have been widely known for their high economic value, including *A. lunulatum* which is often used for aesthetics and medical importance.

Some features of *Adiantum* sp. are unique and is widely cultivated as an ornamental plant [2]. This species has also long been used as medicine ingredients to

cure various diseases for its antioxidant properties [4].

As a fern species, *A. lunulatum* has two independent generations in its life cycle, i.e., gametophyte and sporophyte. The gametophyte generation is started with the formation of spores, and continued with the growth and development of spores until prothallus formation to form reproductive organs, archegonium and antheridium. Archegonium produces female gametes, while antheridium produces male gametes [5].

A. lunulatum is the apogamous type and produces 32 spores in each sporangium. This type is distributed mostly in dry areas. Previous studies reported that gametophyte of apogamous ferns grows faster than that of sexual reproduction type. The mature gametophyte of apogamous fern is relatively smaller compared with the sexual one [6]. Apogamous fern is also characterized by the fast growth of reproductive organs which occurred at the young gametophytic stage, nevertheless, the reproductive organs producing either antheridium or archegonium, allowing the propagation of this type cannot be through fertilization. During sporophytic generation,

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an apogamous fern produces leaves rather before root formation [7].

A. lunulatum produces homosporous and the gametophytes of homosporous ferns usually form green heart-shaped (cordate) prothallus. The other has ribbon or filament shape [6]. Information about gametophyte generation is very important to be used for distinguishing among fern species and understanding the reproduction system, distribution, ecology and evolution [8, 9, 10]. For example, some morphological features of gametophyte stage including the spore germination type, early development, mature form, trichomes, and gametangia are quite useful tool for taxonomic delimitation [11, 6, 12].

Many studies on the sporophyte generation of the species have been done, but the information about the growth and development of the gametophyte is not available. This study was conducted in order to assess the growth and development of a gametophytic stage of apogamous *A. lunulatum* originating from dry and humid areas under laboratory conditions.

MATERIALS AND METHODS

Research materials

Mature spores from fertile leaves of *A. lunulatum* were collected from dry and humid areas. One fertile leaves was from one individual per location. Every individual consisted of 3 to 4 frond containing mature spores, and 20 to 30 leaflets in each frond.

Research method

The research began with field sampling, and followed by spores seedling and gametophyte observations. This research was conducted at the Laboratory of Ecology and Plant Resources, Department of Biology, Faculty of Mathematics and Natural Sciences - Bogor Agricultural University.

Sampling

Sampling locations for dry and humid areas were determined based on herbarium studies that had done previously in Herbarium of Bogoriense Bogor. Pasuruan and Bogor represent dry and humid areas respectively. Field sampling was based on an exploration or cruising method [13]. Fertile fronds containing mature spores of each individual were taken from two different areas. The fertile fronds containing mature spores were rinsed under running water to avoid contamination with other species spores, and then the fronds were preserved in Samson envelope papers. Each sample from each area

was preserved in different plastic bags and labeled.

Gametophyte growth and development observation

Spores were planted in medium containing vermiculite, Sphagnum moss and perlite with ratio 2: 2: 1. The medium was put in a plastic box, covered with a paper filter, and sterilized using boiling water and cool it down for one night, then as many as 0.003 g spores for each treatment were sown on media [19]. Growth and development of gametophytes were observed using stereo microscope once a week for and repeatedly under 10x for each treatment. Detailed photographed using light microscope were taken regularly.

Observed parameters of gametophyte development were shape alteration of protalus in every stage, spore germination, filaments, spatula, mature gametophyte and reproductive organs either archegonium or antheridium. Growth measured parameter was the number of cells in each stage [14].

Data analysis

The experiments were performed using Complete random design and three replications, with a total of 6 experimental units. The data were analyzed using *Ms.Excel*.

RESULTS AND DISCUSSION

Gametophyte development

The development of *A. lunulatum* gametophyte under laboratory conditions were observed for 22 weeks. The results showed that the gametophyte development consists of four stages, spore germination, filaments formation, spatula, and mature gametophyte. The mature gametophyte is marked by the emergence of antheridium. Each phase has a specific protalus form (Table 1).

Spore germination

Early growth of spores after seedling is marked by broken spore wall, followed by the first cell formation to form rhizoid (Figure 1A). This stage is called spore germination stage. It was observed that the spore germination

Tabel 1. Developmental stage of *A. lunulatum* gametophyte

No.	Habitat types	Developments stage			
		Spores germination (DAP)	Filaments (WAP)	Spatula (WAP)	Mature gametophyte (WAP)
1.	Dry area	7	3	5	9
2.	Humid area	4	2	4	22

Note: DAP: Day after planting; WAP: Week after planting

ation between dry and humid areas showed different velocity. Spores from humid areas began to germinate in the fourth day, while the spores from dry areas were started to sprout on the 7th day after sowing. This is presumably due to the environmental influence of the natural habitat of the spores. Spores from humid areas germinated faster because the environmental conditions of the media resemble the environmental conditions where the spores originated.

Spores observation under the microscope showed that the first cell observed from both locations were green. This result is consistent with the previous study in *Adiantum* that showed spore germination of *Adiantum capillus-veneris* began at the third or fourth day after planting, and rhizoid elongation from new spores occurred in the next two days [15]. The first green cells in gametophyte formation played a role in photosynthesis [16]. It was also reported that the growth pattern of the first gametophyte ferns is strongly influenced by the climate where they grow [17]. Light is one of the crucial environmental factors that has to play a significant role in spore germination. Therefore, spore germination will not occur without light [18].

In this study, germination type of the studied fern corresponds to the Vittaria type. This type is the most common type of spore germination in homosporous ferns [6].

Filaments and spatula

The green cells of the germinated spores continue to divide and multiply in each week to enter the filament stage (Figure 1B). *A. lunulatum* gametophytes originating from humid areas began entering to filament stage at the second week, while the filaments of gametophyte from dry area occurred at the 3rd week. Both of the filamentous prothalli from dry and humid areas had not undergone a significant difference in size and shape which was seen from the number of cells of each prothallus (Table 2). At the fourth week, the amount of cell increased, and the size of prothallus got bigger and started to shape a spatula-like (Figure 1C).

At the stage, the total number of cells differ between dry and humid prothalli (Table 2), although the shape of prothallus was not changed. The total cells in prothallus from the dry area began to increase significantly in spatula stage compared to the two previously stages. At the end of spatula stage, the prothallus formed a notch, which marks the initiation of mature gametophyte stage.

Mature gametophyte

The mature gametophyte is the last stage of fern gametophyte development before entering to sporophyte generation. In this stage, the observed gametophyte formed a heart-shaped sheet with a real notch at the end of its protalus, as well as rhizoid and reproductive organs. The reproductive organs of this type had already

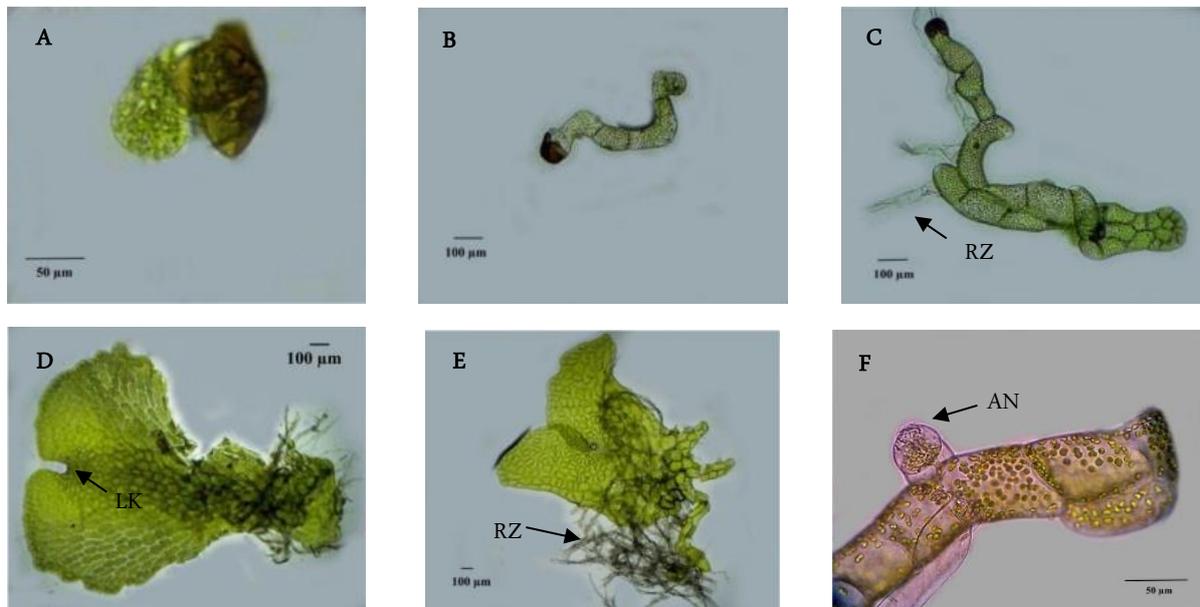


Figure 1. Development stage of *A. lunulatum* gametophyte. A: Germinated spores; B: Filament; C: Spatula; D: mature gametophyte (humid ferns); E: Mature gametophyte (dry ferns); F: Antheridium (reproductive organ); LK: Indentation (notch); AN= Antheridium; RZ=Rhizoid.

been formed in the filaments and spatula stages of young gametophyte, and the number increased when entering mature gametophyte stage. Reproductive organ, i.e., antheridium develops on gametophyte is which is located close near to rhizoid (Figure 1F).

Prothallus from dry area reached to mature gametophyte stage at the 9th week. While the prothallus from humid area initially grew slower, the development rate decreased when it entered in mature gametophyte stage, and it took 22 weeks to complete the stage (Table 1). In this stage, a curve has been formed completely, and form a symmetrical heart-shaped gametophyte (Figure 1D and 1E). The gametophyte development of *A. lunulatum* follows the *Adiantum*-type as described before [6]. In this type, the location of the meristematic tissue turned into a notch on the apex of the thallus.

The crucial environment factors that affected the mature gametophyte stage are moisture and light intensity. The characteristics of fern distribution also have an important role, because each species tends to develop its sporophyte stage in optimal condition. It can be concluded that apogamous *A. lunulatum* from dry habitat develops gametophytic stage more rapidly.

The total number of gametophyte cells from the first week to the third week were not significantly varied. At the fifth week, the cells originating from dry area began to increase significantly, three times higher compared to that of humid areas. The cells proceed to multiply until

the final stage reached (adult gametophyte), and at the tenth week, the number of gametophyte cells originating from dry areas reached five times higher than that of humid area (Table 2 and Figure 2).

The number of cells increased differently on each stage, and the cell division rate from dry area is faster than that from the humid area. This result is in accordance to the previous study [19] that showed the gametophyte cells would continue to proliferate along with the age of the gametophyte and the total number of the proliferating cell was varied among species.

Thus, gametophyte development of observed in apogamous *A. lunulatum* in each stage is closely related to the number of proliferating cells. Gametophytes of individual originated from dry area grew and developed more rapidly compared with that from the humid area. Although the media showed of both gametophytes has the same component and the gametophytes grew at the same time, both of them are originated from a different environmental condition which affected the rate of growth and development of each gametophyte. The dry area is the most suitable habitat for supporting the growth and development of gametophyte [7]. Previous study [20] reported that fern grown in dry areas tend to have an apogamic reproduction. The dry condition is thought to be the cause of an individual gametophyte originating from dry area grows and develops faster when is planted in a new condition far from its natural habitat. It is a fern adaptation to avoid water deficiency before entering to sporophyte phase.

Our result is in accordance with the previous study [21] that reported the migration of spores from a great distance would produce short-lived gametophyte. The factor assumed to be the cause of the gametophyte growth and development of the individual from Pasuruan was more rapid compared with the gametophyte originating from the humid area, Bogor.

Table 2. Average cell number in gametophyte of *A. lunulatum*

No.	Habitat types	W1	W3	W5	W7	W10
1.	Humid area	1	4	13.33	25.33	35
2.	Dry area	1	3.66	43.33	108.33	143.33

Notes: W1, W3, etc : Week (Time).

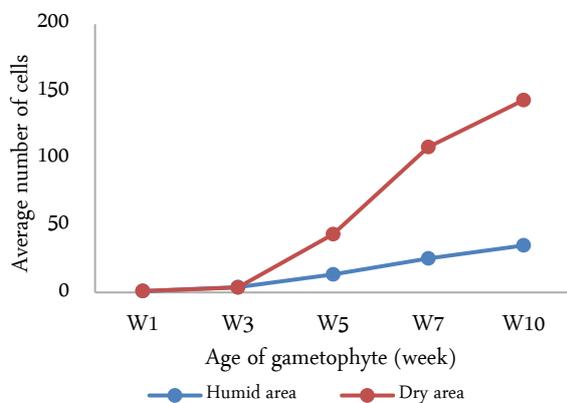


Figure 2. The average number of cells on *A. lunulatum* gametophyte

CONCLUSION

The development of *A. lunulatum* gametophyte consists of four stages like, spore germination, filament, spatula and mature gametophyte. Different origin habitat of the observed apogamous *A. lunulatum* caused different growth and development rate of their gametophytes. Gametophyte originated from dry area grew and developed faster compared with the gametophyte from the humid area.

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