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Gametophyte development of *In Vitro* cultured *Diplazium esculentum* (Retz.) and *Asplenium nidus* L.

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ABSTRACT

This study was conducted to investigate the gametophyte development of *In Vitro* spore cultured *Diplazium esculentum* (Retz.) Dryopteridaceae commonly known as Vegetable fern, between the months of March and May 2015 at Natural Science Research Center, Central Mindanao University Musuan, Bukidnon. *D.esculentum* (Retz.) have bean-shaped monolete spore enclosed with a multicellular, rounded, and short stalked sporangia arranged around the side of the veins or veinlets of mature frond having 2-3 pinnane. Its gametophyte development from spore to heart-shaped ptothallus formation is relatively short that only takes approximately 3 weeks considerably faster compared to other fern species such as *Asplenium nidus* L. *D.esculentum* (Retz.) is very easy to propagate when appropriate medium and physical condition is provided. *Asplenium nidus L.* which is locally known as bird's nest, pasdak or pakpak lawin is an economically important fern that best serves as an ornamental plant and an edible fern. The study was conducted to investigate the gametophyte development of *Asplenium nidus L.*in a Cyathea sp. medium. Swelling took place seven days after the spores were sown, 12 days for its rhizoid and germ filament formation and 23 days for the cordate shaped prothallus to appear. Spore germination follows that of the *Vittaria* type while it followed the *Aspidium* type for the prothallus development.

KEYWORDS. Spore culture, *D. esculentum* (Retz.), gametophyte development.

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INTRODUCTION

The Philippines is composed of over 14,000 species of plants which represents the 5% of flora in the world (Heaney & Regalado, 1998; Catibog-Sinha & Heaney, 2006). All in all, about 8,050 species are angiosperms, 33 species gymnosperms, approximately 1100 species Pteridophytes (Amoroso, 1997; Delos Angeles & Buot, 2012), 1,271 species bryophytes, 3,555 species of fungi and molds, about 1355species algae, and 790 species are lichens (Catibog-Sinha & Heaney, 2006). However, there is still a handful of plant groups poorly studied in the Philippines (Madulid, 1996).

Vegetable fern (*Diplazium esculentum*) is a fern species found and used in East to South Asia and Oceania. Distribution is widespread and even invasive in regions where there is plentiful heat, water, and light shade. This fern plant prefers acidic soil and thrives in hot conditions. Most of the fern's habitat is lower story forestry but it is also found in irrigation ditches and roadside gulleys (Chiou et al. 2006). *Asplenium nidus L.* is an economically important fern that belongs to the family Asplineaceae. It is locally known as bird's nest, pakpak lawin and pasdak. This epiphytic fern is native to the tropical and temperate forest of the Philippines and other Southeast Asian countries, East Africa, Eastern Australia, Papua New Guinea and Hawaiiand prefers a habitat that is warm, humid, shaded and soil or bark with rich organic matter. Aside from clinging in the trunks of trees, it is also commonly found as a terrestrial ornamental houseplant. The use of this fern varies from country to country and includes ornamental, tool in worship, medicinal and food.

In the Philippines, this fern is primarily used for ornamental purposes. Recently the book of Dr. Amoroso and Dr. Villalobos (2012) published this fern as one of the edible ferns in the country providing a wider dissemination of the fern's economic use. Although it is not still considered threatened in the Philippines, it is already categorized as threatened in India due to overharvesting (Srivastava and Uniyal, 2013). In the country it is also being harvested and sold in ornamental plant exhibits and malls and is becoming a favourite ornamental fern that is often displayed in homes, resorts and hotels.

Similar to all pteridophytes, *A. nidus* L. has an alternating life cycle of a diploid sporophyte and haploid gametophyte. Studying the ontogenic development of this fern species is important in understanding it developmental biology and for propagation purposes. This study will investigate the gametophye development of this ornamental fern using in vitro culture with the use of tree fern medium.

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Commonly known as "Paku" in the Philippines, the frond of *D. esculentum* and *A. nidus* are found to contain high antioxidants and) and considered as one of the most famous edible fern in the country (Copeland, 1942; AVRDC, 2009; Amoroso, 1990). Since it is widely distributed (Chiou et al. 2006) no significant threats has been noted for this plant (IUCN Red list, 2014). Several studies have already been conducted on its morphology and ecology (Amoroso, 1990, 1993, 1997; Amoroso et al. 2010, 1995) but at present no local data on its gametophyte development. Hence this study was conducted to observe the gametophyte development of *D. esculentum* (Retz.) and *A. nidus* L. under In-Vitro culture.

MATERIALS AND METHODS

Place and duration

D. esculentum (Retz.) and *A.nidus* samples were collected from the fernery of Central Mindanao University. In-Vitro observation was conducted at the National Science Research Center, CMU between the months of April and August 2015.

In Vitro observation

The following methods are followed during in-vitro culture: 1. Detach the sporangia/spores from the mature frond and place them in a paper envelope. Carefully close the paper envelope and gently crush it to expose the spores and settle at the bottom of the paper. Remove the unnecessary plant materials and transfer the spores to a clean paper. 2. Mix the spores with distilled water. Using a dropper, sow the spores into the prepared media made of sterilized crushed tree fern trunks. 3. Place the culture medium into the growth room with sufficient amount of lighting and temperature between 24-25^{o}C. 4. Using a stereomicroscope, monitor the spore germination by taking some samples from the culture media. Due to a very limited time, observation was made up to the formation of prothallus. 5. Photo-documentation was employed for further analysis of its gametophyte development.

RESULTS

D.esculentum (Retz.) have bean-shaped monolete spore enclosed with a multicellular, rounded, and short stalked sporangia arranged around the side of the veins or veinlets of mature frond having 2-3 pinnane. Gametophyte

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development of *In Vitro* cultured *D.esculentum* (Retz.) was observed in a very short period. Compared to other fern species such as Asplenium nidus that takes 4-5 weeks of prothalli formation (Khan, et al. 2008), *D. esculentum* (Retz.) is relatively faster with about 3 weeks from spore to heart-shaped prothallus. This further means that *D. esculentum* (Retz.) is relatively easier to grow/propagate as long as appropriate medium is provided.

A. Habit of *D. esculentum*

Fig. 1 (Left) Dorsal view of the frond; (Middle) Circinnate venation of young *D. esculentum* (Retz.) frond; (Right) Ventral view of a fertile frond of *D. esculentum* (Retz.) showing the sporangia.

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B. Gametophyte development

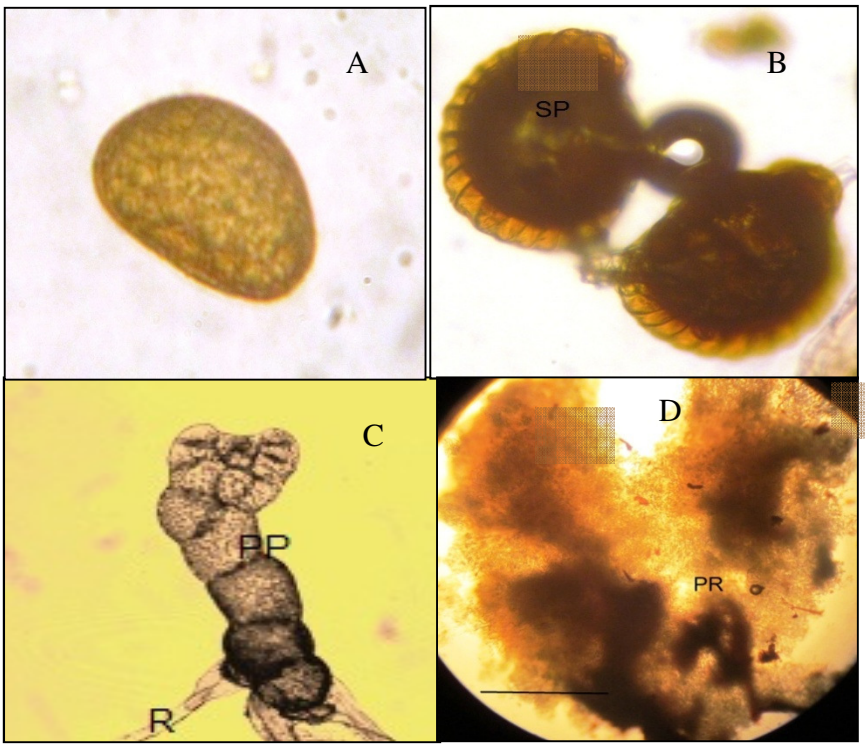

Fig. 2 Sporangia (SP) of *D. esculentum* (Retz.) with spores, day 1 (A); Close-up view of a monolete bean-shaped spore of *D. esculentum* (Retz.). 400x (B); Development of Prothalial plate (PP) with rhizoids (R) at the posterior end.400x after 5-6 days of inoculation (C); Heart-shaped prothallus (PR) of *D. esculentum* (Retz.) after 15-16 days (Approx. 3 weeks) of inoculation at 100x (D).

The spores of *Asplenium nidus* L. is arranged linearly on the underside of its fronds (Figure 2). The spore is monolete, bilateral, and polar anditsspore germination follows that of a *Vittaria*-type (Figures 3) where the spore cell grows and ruptures at the lesure. The spore began swelling few days after it was sown and after 12 days the spore cell ruptured at the lesure region leading to the formation of the first hyaline rhizoid and chlorophyllous protonemal initial cell

(Figure 4). The first rhizoid developed from the basal cells. A transverse and repeated cell division resulted to a filamentous and unidimensional protonema with 5- 6 cells (Fig. 3a).After 10 days from the spore germination, a bidimentional protonema was observed (Fig. 3b). Further cell division of the bidimentional protonema led to the formation of the broad and spatullate prothallus (Figure 3c).

The development of the prothallus of the *Asplenium nidus* took place 23 days after spore germination following the *Aspidium* type of development which is characterized by the a germ filaments developing a meristematic cell in one of the daughter cells of the terminal cell and the terminal cell developing hairs before the beginning of the plate formation thus making the prothalli hairy from the beginning up to the end of its development. The adult prothallus is shaped-cordate, and thalloid in nature (Fig. 3c). It also has a protrusion on the peripheral walls of the marginal cells. They are simple and unicellular hairs which are tapered towards the apex and characterized by swollen base.

Swelling of the spore was observed on the seventh day after they were sown in the Cyathea sp. medium. And after 12 days, a hyaline rhizoid and chlorophyllous protonemal initial cell began to develop while it took another11 days for its protonema to develop into a cordate shaped prothallus which agrees with is similar with the gametophyte development of *Asplenium foreziense* (Herrero et.al, 2002). Thus it took 22 days for the prothallus to appear after the spores were sown. The *Vittaria*-type of germination is observed in the *A. nidus* gametophyte development while it follows the *Aspidium* types in its prothallial development. A high percentage of emerging prothallus became observable using the naked eye 30 days after it was sown which shows a positive result of its high germination rate which agrees with the findings of Amoroso C.B. & Amoroso V.B. that *A. nidus* L. has one of the highest percentage of spore germination among several species of ferns they examined thirty days after sowing.

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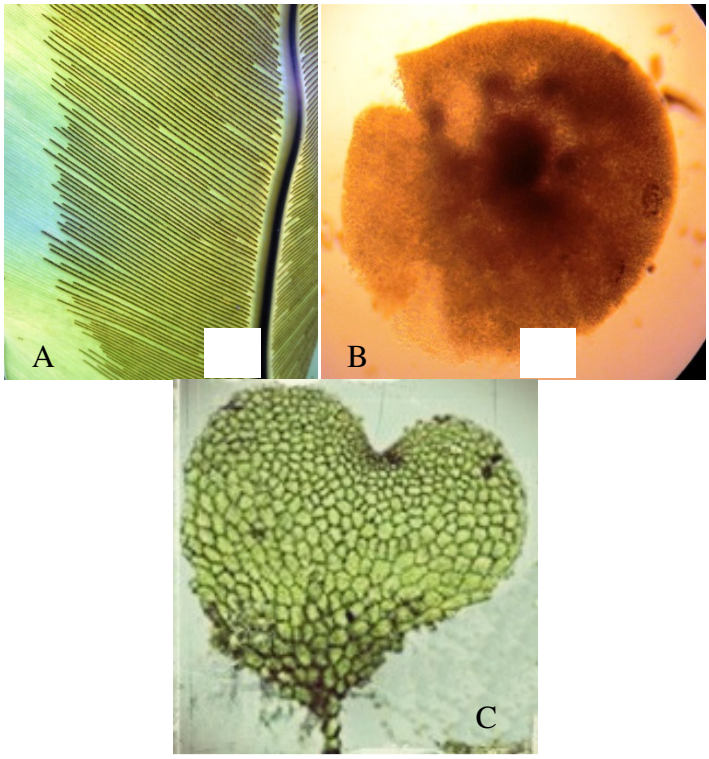

Fig 3. Sori arrangement of *A. nidus* (A); monolete spore (B); and cordate prothallus (C).

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