

Asplenium nidus; The Bird's Nest Fern: Developmental Studies and Its Conservation

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ABSTRACT

Asplenium nidus L. commonly called as Bird's Nest Fern, is a threatened, ornamental fern, which is widely used as novel foliage ornamental plant and local people use it in worship. The taxon is threatened due to over exploitation, habitat destruction and genetic barriers. To understand the constraints in the regeneration, reproductive biology studies are made. It is observed that more sporophytes are produced in composite population (13.3%) in comparison to isolate population (10%). This pattern is suggestive of the fact that the parental sporophyte is heterozygous for recessive sporophytic lethal. On the basis of the results obtained *A. nidus* was initially adapted for outbreeding with the capacity for considerable amount of inbreeding. The low potential of sporophyte production in isolate gametophyte could be the constitution of the zygotic genotype.

Keywords: Reproductive Biology; *Asplenium nidus*

1. Introduction

Asplenium nidus (Bird's Nest fern) is a threatened ornamental plant belongs to family Aspleniaceae [1]. It is commonly used in horticulture trait as decorative plant, local people use it in worship, and in folk medicine (to treat asthma, sores and weakness) and hygienically to treat halitosis (bad breath) [2]. It occurs as epiphyte, as well as terrestrial plant and prefer warm, humid areas in partial to full shade and grows on rich organic matter.

The taxon is becoming rare and threatened day by day due to climatic factors, over exploitation for nursery trade and also by genetic barriers. Present study deals the reproductive biology of *Asplenium nidus*, which would give an insight for conservation strategies.

2. Material and Methods

Fertile fronds with ripen spores of *A. nidus* were collected from Calicut, Kerala. The fronds were kept in brown paper packets and stored in desiccator for the release of the spores at room temperature. The spores were surface sterilized with 2 percent sodium hypochlorite solution for two minutes and rinsed thoroughly with double distilled water before sowing in the petriplates in

sterilized Parker's macro—and Thompson's micronutrient culture medium (P & T) gelled with 1 per cent agar. The culture plates were kept in the culture room having light intensity ranges between $47.3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ - $56.8 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ at $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$ temperature for 16 hr light photo period followed by 8 hr dark photo period.

Periodically the spore germination and subsequent gametophyte growth, differentiation and sex ontogeny were observed under Nikon trinocular Eclipse 80i microscope and the photographs were taken using a camera Nikon DS Fi 1.

Periodic observations of gametophytes of stock culture were made and ratios of gametophytes bearing male, female or bisexual conditions were recorded (**Table 1**). Before initiation of gametangia in stock cultures, the gametophytes were isolated and placed in different petriplates containing P & T media in the following manner:

- 1) 25 petriplates with single gametophyte in each (isolate culture); and
- 2) 10 petriplates with 25 gametophytes in each petriplates (composite culture).

After the initiation of gametangia, all the isolates and composite populations were flooded from above with sterile distilled water twice in a week for bursting the antheridia and release of antherozoids, which fertilized the egg of archegonium. Percentage of sporophytes pro-

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duced at each level was recorded (Table 2).

Five plates of isolate and two of composite population were kept unwatered throughout the course of experiment to check the sexuality of the species. These populations did not produced sporophytes thus proved the sexual nature of the species. Five composite cultures were also maintained up to six months to observe the regeneration pattern of the gametophyte. Periodic sowing of spores was done to check their viability.

3. Observations

Spores of *A. nidus* are monolete, bilateral $22 \times 37 \mu$ (at X 240) in size, polar and *Vittaria*-type germination is found [3] (Figures 1(a) and (b)). About 47 percent spores were germinated after 47 days of spore sowing which became 100 percent after 60 days of spore sowing. The viability of spores was totally lost after six months when kept at room temperature.

The spore coat ruptures at the laesura region leading to formation of first hyaline rhizoid and chlorophyllous protonemal initial cell (Figure 1(c)). The protonemal initial cell divided transversely repeatedly and a filamentous, unidimensional protonema consisting of 5 - 6 cells was developed (Figure 1(d)). Further repeated transverse

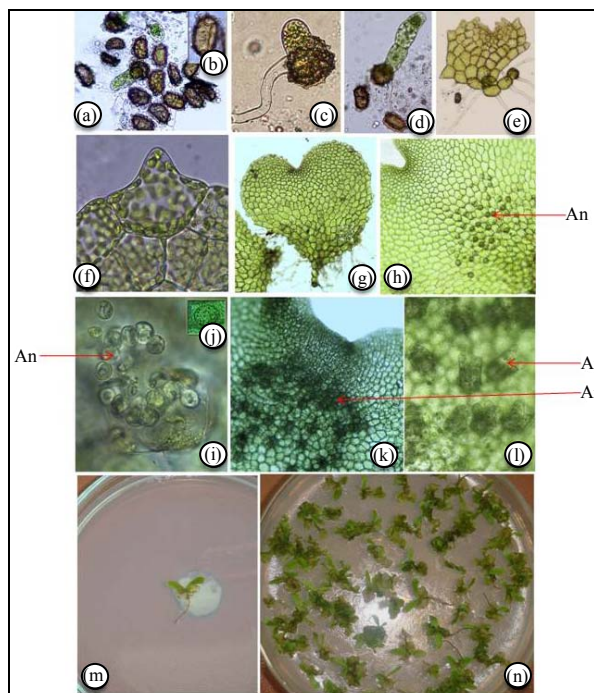


Figure 1. (a), (b) Monolete spores; (c) Emerging protonema; (d) Filamentous stage; (e) Two-dimensional stage; (f) Unicellular hair; (g) Cordate gametophyte; (h) Antheridia showing in between rhizoid of gametophyte; (i) Enlarged view of antheridia; (j) Single antheridium; (k) Archegonia below the apical notch; (l) Enlarge view of archegonia; (m) Sporophyte in isolate culture; (n) Sporophyte in composite culture.

Table 1. Chronological changes in sex ratio of a composite culture in *A. nidus*.

Sample size	Days after sowing	Neuter	Male	Female	Bisexual
20	60	20	0	0	0
20	65	15	5	0	0
20	70	12	8	0	0
20	75	10	10	0	0
20	80	8	12	0	0
20	85	8	10	2	0
20	90	7	8	4	1
20	95	7	6	5	2
20	100	6	4	5	5
20	105	7	4	6	3
20	110	8	3	7	2

Table 2. Breeding behaviour of different population in *A. nidus*.

Population type	Number of gametophyte studied	Number of sporophyte produced	Sporophyte produced (%)
Isolate (A)	20	2	10
Composite (A × A)	150	20	13.3

divisions in terminal and subterminal cells developed a two-dimensional protonema after 15 days of spore germination (Figure 1(e)). Subsequently repeated longitudinal and transverse divisions in the daughter cells of the two dimensional protonema formed a broad spatulate prothallus. In some case the anterior region of the germ filament may not take part in the formation of an expanded thallus and may be sluggish. In such cases a prothallial plate is formed from the intercalary cell. Prothallial development of *Asplenium* mainly of the *Aspidium*-type, with most of the germ filaments developing an obconical meristematic cell in one of the daughter cells of the terminal cell and the terminal cell developing a hair before initiating plate formation. In such cases the prothalli are hairy from the early stages of development onwards [3]. The adult prothallus was cordate, thalloid in nature (Figure 1(f)). The prothalli have papillate protrusion on the peripheral walls of the marginal cells. These are simple, unicellular hairs and are mammillate with a conspicuously swollen base and suddenly narrowed to short, subconical apex (Figure 1(g)) [3,4].

Gametangial development is of common leptosporangiate type. After 60 days of spore sowing, antheridia developed at the posterior zone of gametophyte between

rhizoids (**Figures 1(h)-(j)**) and after 80 days of spore sowing archegonia, were developed below the apical notch (**Figures 1(k) and (l)**). Within three months the gametophyte became bisexual and maximum bisexual condition were observed after 100 days of sowing. Later the antheridia degenerated and only archegoniate gametophytes were observed which ultimately became neuter. The details of sex ontogeny are appended in **Table 1**.

In composite populations sporophyte production was 13.3 percent (**Figures 1(n) and 2**) (**Table 2**) while in isolate population sporophyte production was 10 percent (**Figures 1(m) and 2**). No sporophyte was produced in unwatered population, proving the sexual nature of the taxon.

4. Discussion

Asplenium nidus is a threatened taxon due to over exploitation, habitat destruction and genetic barriers [5]. The reproductive biology studies are essential to understand the cause of rarity and success of reproduction.

Greater local adaptation was detected in outcrossers than in selfers when accounting for environmental differences [6], but the difference was not significant when species was included in their model. A significant difference would have indicated that population genetic processes that dominate selfing population or species, such as genetic drift, may limit local adaptation more often than gene flow, which should be more limiting in outcrossing species.

During studies it was observed that more sporophytes were produced in composite population in comparison to isolate population. e.g., in *A. nidus*, 13.3 percent sporophytes were produced in composite population (**Figure 2**) (**Table 2**).

Our observation indicates that *A. nidus* initially adapted for outbreeding with the capacity for considerable amount of inbreeding. The possible explanation for the lack of sporophyte production in isolate gametophyte could be the constitution of the zygotic genotype. This pattern is suggestive of the fact that the parental sporophyte were heterozygous for recessive sporophytic lethal [7-9].

For the colonization of open or barren habitat, if spores land sufficiently in distance only the chances of intragametophytic selfing are possible. It has been observed that

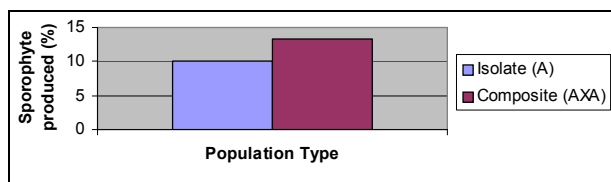


Figure 2. Percentage sporophyte produced in isolate and composite population of *Asplenium nidus*.

the majority of taxa having intragametophytic selfing and crossing were successful colonizers and rich in population density [10-18].

Since *A. nidus* have capacity to form sporophyte through intragametophytic selfing thus may colonise the barren land. Thus during the early stages of colonization of new habitat intragametophytic selfing predominates and at later stages mating occurs at the level of intergametophytic selfing. After a number of growing seasons the mode of sexuality will be transformed into almost obligatorily intergametophytic selfing.

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