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# A CLASSROOM EXERCISE FOR PROPAGATION OF BAMBOO ORCHID- *ARUNDINA GRAMINIFOLIA* (D.DON) HOCHR.

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**Abstract**

The present communication briefly outlines a simple and efficient class room exercise using the bamboo orchid- *Arundina graminifolia* to demonstrate hand pollination and horticultural seed germination. Hand pollination and subsequent seed capsule development was carefully monitored that provides an opportunity to discuss floral morphology and associated reproductive biology. Four to six wks old capsules were harvested and sown on (i) coarse dead bark collected from old mango tree + coconut coir (1:1), (ii) saw dust + coconut coir + loam soil (1:1:1) and (iii) loam soil. The seeds germinated only on coarse mango bark + coconut coir (1:1) and the seedlings grew nicely without supplying any external nutrients. The different sequential steps of embryo morphogenesis and protocorm formation were traced out. While thousands of scientific reports furnished information for *in vitro* seed germination, very few provided concise description of hand pollination and horticultural seed germination for use in field and classroom exercises. This paper provides a detailed and easy to follow protocol for hand pollination of orchid flowers and horticultural seed germination.

## Introduction

*ARUNDINA GRAMINIFOLIA* (D. Don) Hochr. (=*Arundina bambusifolia* Lindl.), commonly known as 'Bamboo Orchid' is a wild terricolous orchid found in India, Nepal, Bangladesh, Sri-Lanka, Thailand, Singapore, South China, Peninsular Malaysia, Japan, Borneo and Indonesia; and naturalized in Pacific Islands, Hawaii, Puerto Rico, Costa Rica, Panama through introduction. It is the only species in this genus. The plants are 1.5 – 2.5 m tall, resembling to bamboo plant. The long stems have leaves alternating along their length, similar to *Epidendrum* orchids. It produces pinkish flowers on 15-30 cm long terminal inflorescences may be branched, emerging from the top of the tall cane like stems that produce several flowers sequentially, so that there will be one at a time over an extended period (Singh and Duggal, 2009). The flowers are white with a purple-to-pink lip, and shaped similar to a *Cattleya* flower. They are 5-8 cm across and can appear twice in a year, winter and summer. David Don first described this species as *Bletia graminifolia* in Prodromus Florae Nepalensis in 1825, based upon a collection from Nepal. In 1910, Benedict Hochreutiner transferred it to *Arundina* in the Bulletin of the New York Botanic Garden. The generic name is derived from the Greek word '*arundo*' in reference to the reed-like stems of the plant and the Latin words '*gramineus*' (grass-like) and 'folius' (leaf). *Arundina* is considered to possess activities of detoxification, antiarthritis and abirritation and is used as antidote and demulcent (Hossain, 2009, 2011; Liu *et al.*, 2004; Roy *et al.* 2007). Stilbenoids are the major components in this plant as well as triterpenes. It contains a novel stilbenoid designated as arundinan possess the function of regulating immunity (Liu *et al.*, 2004). Unfortunately, ruthless collection by increasing orchid lovers, over-exploitation for medicinal purposes, deforestation for urbanization, destruction of habitats by reclamation, shifting cultivation and killing of pollinators has led to reduction in natural populations of this orchid. Therefore, conservation of this orchid is utmost important to meet up our future demand.

In nature, germination of orchid seeds yields a very few plants as compared to the number of seeds a plant can produce (Stoutamire, 1964), this feature had led to the concept of production of plants by *in vitro* asymbiotic germination in nutritive medium which was first developed by Knudson (1922). Although now-a-day orchids are predominantly propagated through *in vitro* aseptic culture of seeds, the need of horticultural germination is still important for understanding the biology and physiology of orchid seeds and role of mycorrhizal fungi in orchid seed germination. Moreover, without a well equipped laboratory, it is not possible to propagate orchid *in vitro*. Therefore, the aim of the present study was to develop an easy technique for horticultural germination of seeds of *Arundina graminifolia*. The paper presents a complete methodology incorporating artificial pollination, subsequent seed and capsule development and *in vivo* seed germination in horticultural condition. After completing this exercise, the students will be able to: (1) describe orchid flower morphology and associated reproductive biology; (2) recognize seed capsule development and maturation; (3) it will also help horticulturist and amateur grower for propagation of this orchid.

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## Materials and Methods

*Plant Material*

Field grown plants of *Arundina graminifolia* (D. Don) Hochr. were collected from the hilly forest of Khagrachori district of Bangladesh and maintained in the Orchidarium of the Botany Department of Chittagong University. The plants were properly nourished, subsequently clamps were divided and plants repotted on a regular basis. Flowering occurred in December-January were used for this exercise.

*Hand Pollination Procedure*

Firstly, column and anther cap on a fully opened flower was identified (Fig. 1a). The anther cap and pollinia were gently removed with a tooth pick (Fig. 1b). The pollinia and anther cap was taken out from the gynostemium by applying slight upward pressure to the bottom of the anther cap (Fig. 1c). The anther cap was removed carefully without dislodging the pollinia thus only pollinia were adhered to the tip of the toothpick (Fig. 1d). After removal of anther cap the pollinia was transferred to the same flower or another flower by gently placing the pollinia onto the stigmatic surface (Fig. 1e). The pollinia from another flower were removed before cross-pollination with the pollinia from the donor flower. During this step, gentle upward pressure was applied against the stigma while retracting the toothpick to maintain contact between the pollinia and stigma. After hand pollination, the flowers closely monitored for flower senescence, capsule development, and capsule dehiscence. Once capsule development time is estimated by allowing capsules to dehisce, repeated the hand pollination procedures. The capsules were harvested after four to six wks of pollination.

*Preparation of Potting Medium and Culture of Seeds*

Three potting mixture (i) coarse dead bark collected from old mango tree + coconut coir (1:1), (ii) saw dust + coconut coir + loam soil (1:1:1) and (iii) only loam soil were used for seed germination. These were taken in community culture pots and the seeds were sown in such a way that the seeds embedded 1-1.5 cm deep in the potting mixture. The pots were kept in the corridor covering with a metal net box and regular watering was done for maintaining proper moisture. The germinated seedlings transferred to the new potting medium when these attained at a height of 2-3 cm.

*Establishment of the Seedlings in Orchidarium*

The germinated seedlings were thinned by transferring to new potting medium every two months interval for their better growth and finally transferred to community plastic pots containing loam soil and humus (1 : 1) and kept in the Orchidarium (at 25-30°C and RH 60-70%).

## Results and Discussion

Within 3-4 days of pollination the gynostemium or column started swelling which is a visible sign of potential fruit setting. Almost all flowers set fruits after pollination irrespective to self or cross pollination (Fig. 1f). Pollinating young, fully open flowers is recommended since pollen is most receptive within 2-4 days after flowers are open. Likewise, using young flowers less than one wk from opening ensures that the stigmatic surface is receptive to pollen. After one wk, flowers close and pollen becomes brown and unreceptive.

Orchid flowers are trimerous, possessing three sepals and three petals. The third petal is modified into a labellum or lip typically found oriented toward the bottom of the flower. Potential pollinators use the labellum as a landing platform, directing them to the gynostemium (Kauth *et al.*, 2008). A distinguishing feature of the Orchidaceae is the gynostemium or column, which is the fusion of the style, stigma, and stamens (Dressler, 1981). The anther cap and pollinia are located at the front of the gynostemium, while the stigma is located directly behind the anther on the underside of the gynostemium which is the main barrier of natural self pollination. The pollen grains are joined together into masses called pollinia. During a pollination event, pollinators deposit pollinia onto the stigmatic surface. A successful pollination event depends on pollen and flower age. *Arundina* flowers remain open for several days, while inflorescences continually flower for several months.

Among the potting media used in this experiment the seeds germinated only on coarse mango bark + coconut coir (1:1). The seeds germinated and produced tiny seedlings within 80-120 days of sawing. Although the germination percentage was poor (0.001%) the seedlings grew nicely without supplying any specialized mineral nutrients. Reason behind the germination of seeds in this medium was possibly the presence of compatible mycorrhizal fungi in the mango bark.

David Cameron, the Curator of the Birmingham Botanical Garden reported self-sown seedlings of orchids in several pots and described it as an accidental case of orchid seed germination in the garden (Cameron, 1844, 1848). David Moore, Director of the Glasnevin Botanical Gardens in Ireland was the first man who became success in germination of orchid seeds in horticultural condition (Moore, 1849; Yam and

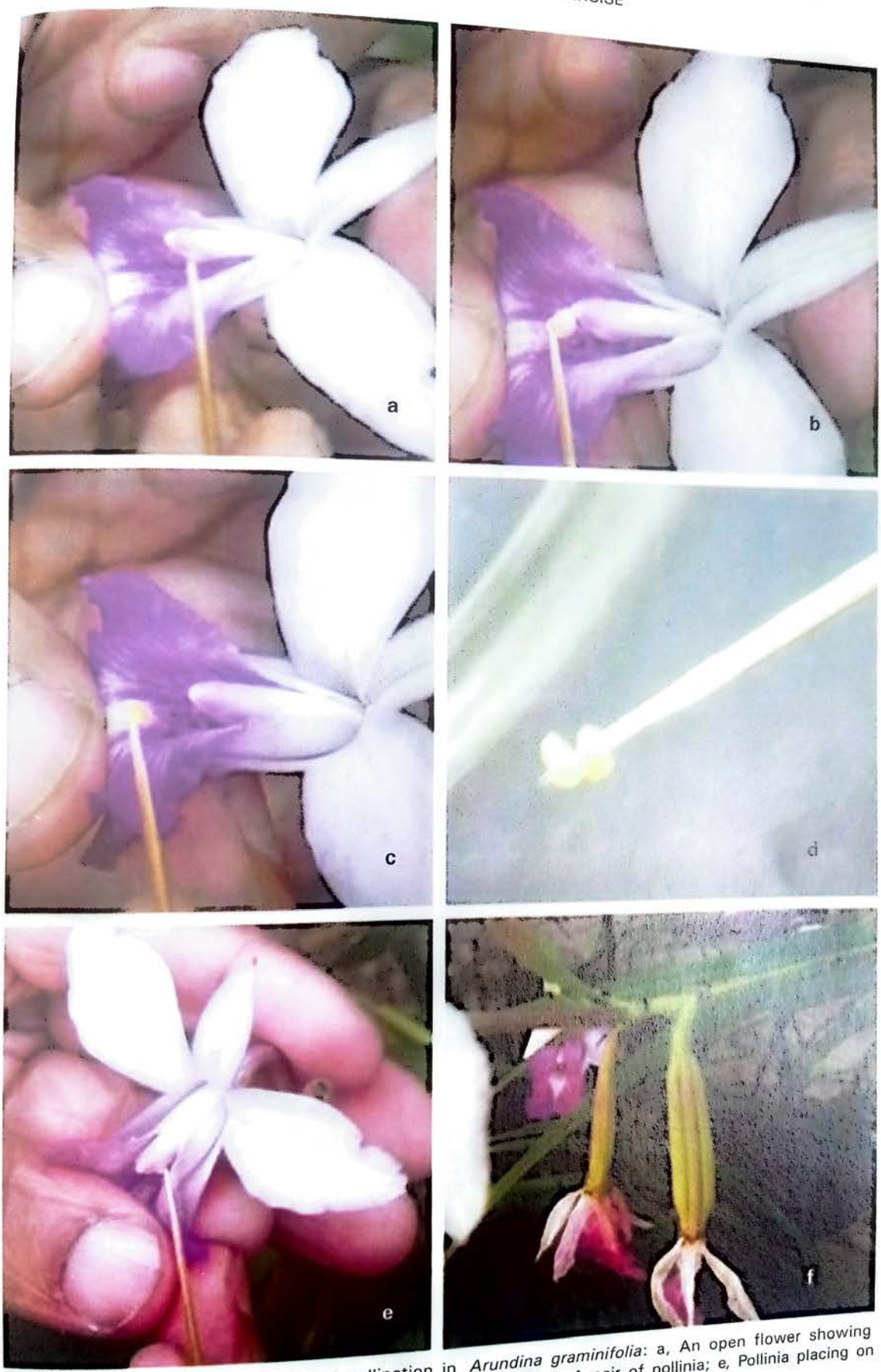


Fig. 1. a-f. Sequential steps of hand pollination in *Arundina graminifolia*: a, An open flower showing sigma and pollinia; b-c, Removal of pollinia with a tooth pick; d, A pair of pollinia; e, Pollinia placing on stigmatic surface; f, Capsules developed after hand pollination.


Fig. 2. a-i. Germination of seeds and embryo morphogenesis of *Arundina graminifolia*: a, Mature seeds; b, Stage-0: Swelling of embryo after four wks of culture; c, Stage-1: Parenchymatous cell mass/spherule with dense chlorophyll; d, Stage-2 Protocorm with appendicle indicating future shoot; e, Stage-3: Leaf development from the appendicle; f-g, Stage 4: Germinated seedlings; h, Individual seedlings growing in community pot; i, Plants established in the Orchidarium.

Arditti, 2009). He reported that orchid seeds can be germinated if scattered at the base of a mature plant. This approach was an innovative major horticultural and biological advance in Orchid Science. Other two contemporary British horticulturists, Richard Gallier and J. Cole also claimed about their success in germination of *Phaius* seeds sown in common soil and *Epidendrum elongatum* sown on blocks of wood covered with moss (Cole, 1849; Gallier, 1849). Half a century after Moore's discovery, Noe¨l Bernard made another quantum jump when he formulated a method for symbiotic germination of orchid seeds *in vitro* (Bernard, 1899, 1909). His method was a significant conceptual and technological innovation that opened a new avenue for the culture of these bizarre plants and foreshadowed modern biotechnology. Despite the fact that orchid seeds were being germinated under horticultural conditions their requirements especially role of mycorrhizal fungi were not known at that time.

Orchid seeds are unique due to presence of only a few

endosperm (Fig. 2a). The outer integument called testa is transparent, reticulate and persistent which consists of cells without protoplasts (Fig. 2a). Germination of seeds followed a peculiar metamorphogenetic pathway. Five distinct developmental stages were traced during seed germination. Stage 0: no visible germination occurred, only viable embryos swelled up by absorbing water and nutrition (Fig. 2b); Stage 1: Cell number increased through repetitive cell divisions and produced a round shape parenchymatous cell mass called spherule that came out by rupturing the testa; a few rhizoids were present at the posterior/basal part; this stage is supposed to be factual germination stage (Fig. 2c); Stage 2: the spherules enlarged in size, became compact and a growth appendicle appeared at the anterior/upper portion delimiting the meristimatic zone for development of foliar organs; critically this stage is called protocorm stage, an intermediate structure between seed and seedling (Fig. 2d); Stage 3: the first leaf primordium emerged out (Fig. 2e); Stage 4: roots emerged from the basal part of the protocorm and gradually a young seedling developed (Fig. 2F,G). Similar mode of embryo morphogenesis and protocorm formation was reported in a number of orchid species (Batygina *et al.*, 2003; Hossain, 2013; Hossain *et al.*, 2009, 2010, 2012; Leroux *et al.*, 1997; Pathak and Vij, 2007; Pathak *et al.*, 1992, 2001, 2011; Piri *et al.*, 2013; Vij and Pathak, 1988).

The individual seedlings presently obtained were established in community pots where they attained a height of 4-5 cm with 3-4 leaves and kept in the Orchidarium of the Botanical Garden of Chittagong University (Fig. 2h,i).

This exercise represents an excellent opportunity to incorporate both technical and horticultural aspects of orchid hybridization and seed germination in the amateur's garden as well as into the classroom exercise for academic purposes. *Arundina* has several attributes that allow for field exercise and classroom demonstrations. First, plants flower twice in a year under a natural photoperiod since no specific cultural requirements are necessary to induce flowering. Second, the column, anther cap, and pollinia are visible without a microscope. Third, seed capsule formation is nearly 100% after pollination. Fourth, this orchid is receptive to both self- and cross-pollination. Finally, capsules become mature within short time (30–60 days) as compared to many other orchids.

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