Breeding System of an Endemic orchid from Southern Thailand

Corybas ecarinatus Anker & Seidenfaden

รูปแบบการสืบพันธุ์ของกล้วยไม้เฉพาะถิ่นของภาคใต้ของไทย

Corybas ecarinatus Anker & Seidenfaden

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[1]นักศึกษาปริญญาโท คณะสิ่งแวดล้อมและทรัพยากรศาสตร์ มหาวิทยาลัยมหิดล [2]ผู้ช่วยศาสตราจารย์ คณะสิ่งแวดล้อมและทรัพยากรศาสตร์ มหาวิทยาลัยมหิดล [3]รองศาสตราจารย์ ภาควิชาพฤกษศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล [4]หัวหน้าส่วนวิจัย สำนักวิจัยและพัฒนา องค์การสวนพฤกษศาสตร์

Abstract

***Corybas ecarinatus* Anker & Seidenfaden is an endemic and rare terrestrial orchid originated from south Thailand. Since only insufficient information on its ecological requirement is available, relevant studies for conservation purpose is urgently needed. For the present study, thus, breeding system of *C. ecarinatus* was conducted. An area located in Muang district, Phang-Nga province was chosen as the study site. Possibility of five different types of breeding system, including spontaneous autogamy, self-pollination, cross-pollination (xenogamy), agamospermy and natural-pollination were then investigated *in situ* at the site. The results indicated that xenogamy or cross pollination was the most likely typical breeding system for *C. ecarinatus*. Relatively Low fruit set rate were observed from hand pollinated plants (30 %), as well as from natural population (23%). Seeds obtained from both treatments were found to have relatively low viability of 51 ± 8% and 55 ± 7%, respectively. As indicated from the experimental results, this orchid probably requires specific vectors for their pollination. Thus, any environmental change would have significant impacts on their survival. Micropropagation of their seeds obtaining from hand pollinated plants might be a suitable choice for *ex situ* conservation purpose. This would lead to mass production of seedlings for subsequent reintroduction program of this especially rare and beautiful orchid.**

Keywords: *Breeding system, pollination, seed viability*

บทคัดย่อ

กล้วยไม้ *Corybas ecarinatus* Anker & Seidenf. เป็นกล้วยไม้ดินถิ่นเดียว ที่พบเฉพาะทางภาคใต้ของไทย เนื่องจากข้อมูลทางด้านนิเวศต่างๆที่เกี่ยวข้องกับกล้วยไม้ชนิดนี้ ซึ่งจำเป็นต่อการใช้ในการวางแผนการอนุรักษ์กล้วยไม้ชนิดนี้ยังมีจำกัด จึงทำการศึกษารูปแบบการผสมเกสรของ *C. ecarinatus* ที่สถานีศึกษาบริเวณพื้นที่อำเภอเมือง จังหวัดพังงา โดยทำการศึกษาความเป็นไปได้ของรูปแบบการผสมเกสร 5 ลักษณะ ซึ่งได้แก่ การผสมตัวเองตามธรรมชาติ การผสมในต้นเดียวกัน การผสมข้ามต้น การผสมตัวเองโดยไม่ต้องอาศัยอวัยวะสืบพันธุ์ และ การผสมพันธุ์ตามธรรมชาติ ซึ่งจากผลการศึกษา แสดงให้เห็นว่า กล้วยไม้ *C. ecarinatus* มีรูปแบบการผสมเกสรแบบ xenogamy หรือการผสมข้ามต้น โดยมีอัตราการติดผลค่อนข้างต่ำเพียง จากการผสมด้วยมือ (ร้อยละ 30) และการติดผลในสภาพธรรมชาติ (ร้อยละ 23) ส่วนกลุ่มการทดลองอื่นๆไม่พบการติดผล โดยเมล็ดที่ได้มีระดับความมีชีวิตของเมล็ดต่ำเพียงร้อยละ 51 ± 8 และ 55 ± 7 ตามลำดับเท่านั้น จากข้อมูลที่ได้จากผลทดลองดังกล่าว ชี้ให้เห็นว่ากล้วยไม้ชนิดนี้มีความเฉพาะเจาะจงต่อรูปแบบการผสมเกสรในการขยายพันธุ์ ซึ่งต้องอาศัยผู้ผสมเกสรที่เหมาะสมเท่านั้น ดังนั้นการเปลี่ยนสภาพแวดล้อมใดๆก็อาจส่งผลอย่างรุนแรงต่อความสามารถในการดำรงอยู่ของกล้วยไม้ชนิดนี้ การขยายพันธุ์ด้วยการเพาะเลี้ยงเมล็ดที่ได้จากการผสมพันธุ์ด้วยมือในสภาพควบคุม น่าจะเป็นวิธีการหนึ่งที่เหมาะสม เพื่อจุดประสงค์ในการอนุรักษ์นอกพื้นที่แหล่งกำเนิดตามธรรมชาติ และนำไปสู่กระบวนการเพิ่มจำนวนของกล้วยไม้หายาก และสวยงามชนิดนี้ เพื่อใช้ในโครงการนำคืนสู่ถิ่นกำเนิดได้ต่อไป

คำสำคัญ: *รูปแบบการสืบพันธุ์, การผสมเกสร, ความมีชีวิตของเมล็ด*

Introduction

Genus *Corybas* is one of terrestrial orchids found from India, south China to New Zealand and Western Pacific islands. This single leaf and single underground tuber orchid genus comprises about 100 species (Van Steenis, 1972). Their natural habitats include tropical rain forests, upper mountain forest, as well as temperate lowland peat bog. Most of them grow as terrestrial in humus or soil but some species can grow as epiphyte on the mossy trunks, larger branches of trees, and in the fibrous trunks of tree fern (Pridgeon et. al., 2001). Some tropical species, however, grow in sphagnum moss in upper montane forest (Dransfield et al., 1986). In temperate lowland peat bog of New Zealand and Australia, several temperate *Corybas* species can also be observed (Clarkson et al., 1999).

From all orchids species discovered in Thailand, terrestrial orchids seem to be more vulnerable to environmental changes than epiphytic ones (Swarts & Dixon, 2009). Several of them, especially the endemic ones are, thus, threatened and endangered. Originally described from southern Thailand, an endemic terrestrial orchid *Corybas ecarinatus* Anker & Seidenf. (Figure 1), was found on limestone cliffs near Khao-Sok National Park in Suratthani province. (Anker and Seidenfaden, 2001). The second site was recently discovered in Muang District, Phang-Nga province (Pumicong, personal communication).

From preliminary observation, most of the primary forest around these sites was strongly cleared, which successively transformed natural forest areas into different land use purpose, especially for agricultural activities. Orchids in the area, thus, were evidently endangered. Only a small fragment of primary forests might still be

considered as safe from such activities. High anthropogenic input to the area, thus, led to very limited suitable habitats for this sensitive orchid. As a newly discovered orchid, basic information on their ecological status is quite limited. Due to their uniquely beautiful flowers and leaves, they are most likely to be under treat of illegal collecting for supplying both domestic and foreign markets. In order to gain better understanding about its ecological specification, breeding system of the orchid was then examined for this study.

Objectives

To examine breeding system of *C. ecarinatus* in its natural habitat

Material and Methods

Study site

The study site was located in Muang District, Phang-Nga Province. This was a highly disturbed tropical rainforest in a hilly region rich with limestone surrounded by rubber, palm and fruit plantations.

Plant materials

For experimental purpose, 80 similar sized *C. ecarinatus* plants showing young flower buds were randomly chosen from five separate populations. Each population was at least 10 meters from the others. This covered the area of about 0.2 km^2.

Breeding system

Based on Dafni's approach (1992), experiments on breeding system were done for the present study. Five different types of breeding system, including (a) sponataneous autogamy (b) self pollination (c) cross pollination (xenogamy) (d) agamospermy and (e) natural pollination were chosen for the purpose. Except cross and natural pollination, which was conducted on 20 and 30 of *C. ecarinatus* plants, respectively, 15 plants were applied. Prior to the experiments, young buds of the tested plants were covered with nylon mesh. Five days after opening flowers were observed, the first three different pollination types were conducted. For autogamy, non extra treatment was done on the tested plants, while opening flowers were self-pollinated for type (b). For type (c), however, selected plants from different colonies were hand crossed. Covering of flower buds was not required in case of types (d) since large flower buds of the selected plants were removed above their ovary before the experiment commenced. As an experimental control, natural pollination on selected plants was allowed to occur. For all the treatment, seed capsule setting and development were observed every three days and numbers of fruiting plants were recorded on day 50. The obtained fruit were subsequently used for determination of seed numbers and seed viability test.

Seed viability determination

For seed viability determination, seeds fleshly obtained from each capsule were stained with 1% (v/v) 2, 3, 5-triphenyl tetrazolium chloride (TTC) 24 hours under dark condition at 20 degrees Celsius. They were

subsequently observed using stereo microscope. Red color in the embryo indicated viable seeds (Figure 2). The total numbers of available seeds, as well as viable seeds from each capsule were recorded.

The similarity of data was then tested using a computer package SPSS one-way ANOVA to determine the different in seed production in each breeding type.

Results

There are none fruit set on spontaneous autogamy, self pollination and agamospermy. Only hand cross and natural pollination set seed capsule with fruit setting rate of 30 and 23%, respectively (Table 1). From the total number of about 4,200-4,400 seeds in each capsule**,** only about half of the orchid seeds in each seed capsule were viable (Table 2). High similarity of the results obtained from both types of breeding systems were observed and recorded.

Discussion

The low fruit set rate and similarity in number of fruit setting obtained from hand cross and natural pollination of *C. ecarinatus* indicated self-incompatibility of the orchid. It was consistent with the study of Neiland and Wilcock (1988), who studied fruit set, nectar reward, and rarity in the Orchidaceae. They founded that several orchids in tropical area showed low fruit set rate of less than 50% (Neiland and Wilcock, 1998), but this finding was not equivalent with results of Hacth (1952). It reported that an endemic *Corybas* species in New Zealand, *Corybas saprophyticus*, was almost certainly fertile and all the flowers set seeds (Hatch, 1952). From this finding, it showed the necessity of *ex situ* micropropagation of this particular orchid due to its slow natural propagation processes. This would lead to mass production of seedlings for subsequent reintroduction program of this especially rare and beautiful orchid.

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Table 1: Results of *Corybas ecarinatus* breeding system experiment

Breeding system	No. of flowers	No. of fruits	Fruit set (%)
Spontaneous autogamy	15	0	0
Self pollination	15	0	0
Cross pollination	20	6	30
Agamospermy	15	0	0
Natural pollination	30	7	23

Table 2: Number of seed capsules, seeds and percentage of viable seed obtaining from different pollination methods, i.e. hand cross and natural pollination*

Type of pollination	Number of seed capsule	Number of seed per capsule	Number of viable seed	Number of non-viable seed	% viable seed
Hand cross	6	4402±449[a]	2275±510[a]	2126±255[a]	51±8
Natural	7	4263±997[a]	2359±581[a]	1904±592[a]	55±7

Data was presented as means ± standard deviation

* For each parameter, means followed by similar letters were not significantly different at 95% confidence level

Figure 1: An inflorescence of *Corybas ecarinatus* Anker and Seidenfaden .

Figure 2: TTC positive red coloration of *C. ecarinatus* seed (right) and negative coloration seed (left).