ความสามารถในการขยายพันธุ์และเติบโตของไลเคนโดยใช้โครงสร้างสืบพันธุ์แบบไม่อาศัยเพศ

ESTABIISHMENT AND GROWTH CAPABILITY OF LICHENS USING ASEXUAL REPRODUCTIVE STRUCTURES

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บทคัดย่อ : ไอซิเดีย (Isidia) และซอริเดีย (soredia) เป็นหน่อ สืบพันธุ์ แบบไม่อาศัยเพศ (หรือ vegetative propogule) ของไลเคนหลายชนิด สามารถแพร่กระจาย ได้ไกล และเติบโตได้ในสภาพแวดล้อมที่เหมาะสม การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบความสามารถในการ ขยายพันธุ์และเติบโตของไอซิเดียของ *Parmotrema tinctorum* และซอริเดียของ *P. santi-anglelii* ที่พบมากในป่ารุ่นสองในอุทยานแห่งชาติเขาใหญ่ โดยการย้ายปลูกไอซิเดียและซอรีเดียบนกระดาษกาวสองหน้าซึ่งเป็นที่อยู่อาศัยเทียม โดยให้กระดาษกาวเอียงทำมุมกับทิศตะวันออก 135 องศา ผลการทดลองพบว่า 10 เดือนหลังการย้ายปลูก ซอรีเดียและไอซิเดียมีสัดส่วนการงอกร้อยละ 84.5 และ 42.2 ตามลำดับ และหลังย้ายปลูกเป็นเวลา 23 เดือน มีการงอกเพิ่มขึ้นเป็นร้อยละ 92 และ 76 ตามลำดับ ส่วนการเติบโตจำแนกเป็น 6 กลุ่มตามขนาดของพื้นที่แทลัสที่เติบโตคลุมพื้นผิวกระดาษก าวสองหน้าจากน้อย-มาก คือ 0 1 2 3 4 และ 5 ซึ่งพบว่าซอรีเดียเติบโตได้ดีกว่า ไอซิเดียอย่างมีนัยสำคัญทางสถิติ และอิทธิพลของสภาพแวดล้อมรอบแปลงปลูก ไม่มีผลต่อการเติบโตของหน่อ สืบพันธุ์ทั้งสองชนิด มุมที่ใช้ย้ายปลูกและภูมิอากาศจุลภาคมี ความเหมาะสม ต่อการเติบโตของหน่อ สืบพันธุ์แบบไม่อาศัยเพศ เทคนิกการย้ายปลูกด้วยไอซิเดีย และซอริเดีย นี้ สามารถนำไปประยุกต์ใช้ ได้ต่อไปในการเพิ่มมวลชีวภาพของไลเคน โดยใช้ทรัพยากรไลเคนน้อยที่สุด

Abstract: Vegetative diaspores were produced by lichens such as soredia and isidia and they were able for dispersal and growth in suitable condition. The objective of this study is to compare growth and dispersal ability of isidia of *Parmotrema tinctorum* and soredia of *P. santi-anglelii*. These two lichens commonly found in the Secondary Forest (SF) at Khao Yai National Park. Soredia and isidia were adhered on double-faced glue tape (DFGT), and they were attached facing East with 135^{o} angle of inclination. Ten months after transplantation, the percent germination of soredia was higher than isidia accounting for 84.5 and 42.2 % respectively. These propagules were able to grow into small thalli. After twenty three months of transplantation germination of soredia and isidia increased more than those observed after 10 months, accounting for 92 and 76 % respectively. Thalli that germinated from diaspores and covered surface of the DFGT were categorized into 6 classes (0, 1, 2, 3, 4 and 5) for statistically analysis. The growth capacity of soredia was significantly higher than isidia. Environment factor do not have important effect on the growth of lichen diaspores. The angle of inclination and microclimate of the transplantation materials favored establishment and growth of the vegetative diaspores.

These transplantation technique can be later applied for increasing biomass production of lichens by using the least resources of lichen stock.

Introduction: Lichens produce many novel natural products that can be potentially utilized as lead compounds in drug industries and other medicinal usages. However, they are constantly threatened by human activities, i.e. air pollution and habitat encroachment. Therefore, cultivation of lichens for biomass production is an essential for sustainable utilization and conservation. Apart from sexual spores, lichens also reproduce by asexual means by using vegetative propagules, e.g. thallus fragments and diaspores. Thallus fragments, if used as a means in cultivation, also result in depletion of stock lichens, particularly when the natural population of that species is small and close to extinction. Cultivation by means of diaspores is hence an interesting and effective reproduction method considering its minimal usage of lichen biomass resource. Isidia and soredia are the most common forms of lichen diaspores which are dispersed by wind and water [1]. Soredia and isidia have different structures. The photobiont in isidia are bound by an outer cortex layer of fungal mycelium whilst the photobiont in soredia are loosely interspersed with hyphae of the mycobiont [2]. The dispersal and growth capability of such diaspores in the nature are still poorly known. Transplantation studies suggested that substrates and microclimate have influence on the growth and survival of soredia [3] and isidia [4]. This study aimed to compare the growth of different lichen diaspores—isidia and soredia—by transplanting them into their original forest conditions to minimize the effects of changes in habitat microclimate.

Methodology: Isidia of *Parmotrema tinctorum* (Despr. ex Nyl.) Hale and soredia of *Parmotrema sancti-angelii* (Lynge) Hale were collected in April 2007 from the Secondary Forest (SF) in Khao Yai National Park (KYNP), Thailand. They were common lichen species in SF and produced different types of diaspores. The diaspores were spread separately on the surface of 100 pieces of double-faced glue tape (DFGT). The tapes were adhered to square pieces of plastic net. The net were attached in place by two parallel nylon ropes, making an angle of 135^{o} toward the East. The ropes were 1.5 m above the ground. The transplantation field was in the same forest where the specimens were collected. Green shading nets were installed 0.5 m above the transplanting nets. Growth data was recorded separately for 4 different areas of the transplanting site, e.g. Northeast (NE), Southeast (SE), Northwest (NW) and Southwest (SW), to investigate the effects of environmental conditions around the transplanting area. Microclimate (illumination, temperature and moisture) were measured every 5 minutes for 24 hour, using Data logger Licor-1400 (Licor Inc., Lincoln, Ne, USA). Development of isidia and soredia into lichen lobes was examined with the aid of hand magnifiers and photographs taken. Statistical analysis of diaspore types and directions were evaluated using Sigmaplot 11.0 and Sigmastat 3.5 software.

Result, Discussion and Conclusion: Six months after transplantation, isidia of *P. tinctorum* and soredia of *P. sancti-angelii* developed into small thalli (0.5 mm) on DFGT. Ten months after transplantation, the percentages of isidia and soredia germination were 84.5 and 42.2% respectively. Twenty-three months after transplantation, diaspores developed into larger thalli of 0.5 cm (Figure 1). The percentages of isidia and soredia germination increased as shown in Table 1. Germination percentages of soredia were higher than those of isidia, particularly in the NE area in which all of the soredia samples developed into thalli (100% germination). Interestingly, the lowest germination percentage (85.7%) of soredia was found in SE area in which the highest germination percentage (84.6%) of isidia was also found. Germination percentages of isidia were lowest (66.7%) in NW.

Table 1 Numbers of diaspores developed into thalli and their germination percentages. The transplantation site was separated into 4 areas according to their spatial directions.

Areas in the transplantation site	Vegetative diaspore types			
	No. of Germination		% Germination	
	Isidia (n)	Soredia (n)	Isidia (%)	Soredia (%)
NE	11 (15)	15 (15)	73.3	100
NW	6 (9)	13 (15)	66.7	86.7
SE	11 (13)	6 (7)	84.6	85.7
SW	10 (13)	12 (13)	76.9	92.3
SUM	38 (50)	46 (50)	-	-
Average	-	-	76.0	92.0

Growth of diaspores was classified into six classes according to their percentage of coverage on DFGT (Figure 1). Two-way ANOVAs were used to compare the effects of areas in the transplantation site (data not shown). Soredia was shown to have higher percentage coverage than isidia ($P<0.001$). Five soredia samples were classified into class 5 (figure 1F) while only one isidia sample had enough coverage to be categorized into the class. This indicated a higher growth and dispersal capacity of soredia in SF. The different areas in the transplantation site had no influence ($P=0.88$) on the growth of both types of diaspores ($P=0.24$). This indicated that the environmental conditions in the similar transplantation areas had no or negligible effect on diaspore germination and growth.

It can be concluded that the microclimate conditions in the transplantation area in SF (Figure 2) were suitable for *P. tinctorum* and *P. sancti-angelii* diaspores to germinate and develop into thalli. Isidia transplanted on barks from the tree bases up to the canopy level of four ecosystems (Tropical Rain Forest, Dry Evergreen Forest, Lower Montane Forest and SF) in KYPN had more growth than soredia transplanted in the same experiment. This is particularly true in SF. However, the survival percentages of the diaspores were extremely low at the mid-trunk to the canopy levels, suggesting unfavorable microclimate conditions [2].

The angles of transplantation were shown to have significant roles in diaspore germination. In a previous study (unpublished), *P. sulphuratum* isidia were transplanted on DFGT and attached to three different angles of 90^o, 135^o and 180^o toward the East. Isidia samples with 135^o attachment had the highest survival percentages (50%), with one sample having more than 100% coverage on DFGT. On the contrary, all of the samples with 90^o and 180^o attachment failed to grow. The successful germination and growth percentages in the current study, in which 135^o installation was used, confirmed the previous findings.

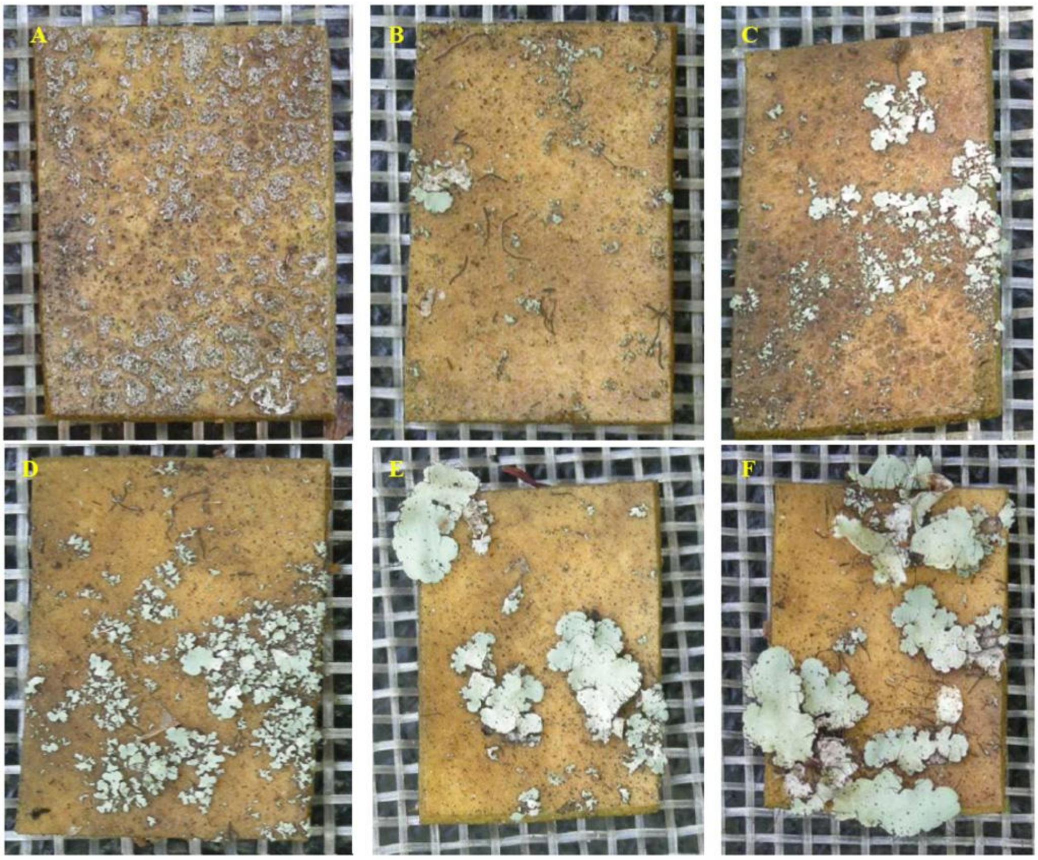

Figure 1 Growth of isidia and soredia on double-face glue tape (DFGT) after being transplanted for 23 months. The results were classified into 6 classes according to their percentages of coverage of developing thalli on the pieces of glue tape. Class 0, no growth of isidia and soredia (A); class 1, 1-10% coverage (B); class 2, 10-20% coverage (C); class 3, 20-40% coverage with small lobes (D) ; class 4, 20-40% coverage with larger lobes (E); class 5, >40% coverage (F) (net holes = 1mm).

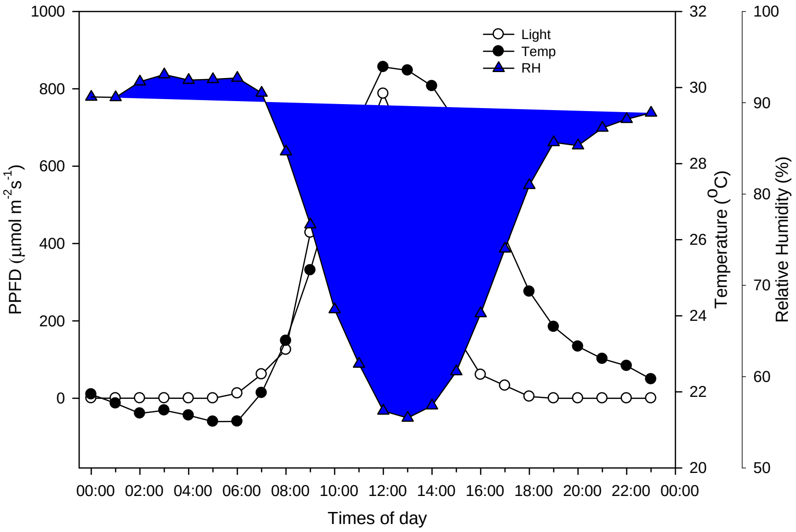

Figure 2 Microclimate conditions (light intensity —○— , temperature —●— and relative humidity —▲—) of isidia and soredia in transplantation site, measured in July 19th, 2008. Each value was an average of 1 hr. measurement.

In conclusion, vegetative diaspores are able to grow into small thalli after 23 months of transplantation (Figure 1). Soredia had higher percentage and growth capacity than isidia, indicating that soredia of *P. sancti-angelii* had higher distribution capacity than isidia of *P. tinctorum* in SF. The inference of microclimate in SF played a very importance role on the growth and germination of diaspores.

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Keywords: diaspore, dispersal, isidia, microclimate, soredia, transplantation, vegetative propogule

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