

การเพิ่มการเติบโตของไลเคนที่ย้ายปลูกโดยการให้น้ำเพิ่ม และทิศทางของแทลลัส

Enhancing growth of transplanted lichens by water treatments and thallus orientation

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บทคัดย่อ: ไลเคนเป็นสิ่งมีชีวิตที่โตช้า กระบวนการสังเคราะห์ด้วยแสงและการเติบโตของไลเคนใช้แสงและน้ำจากบรรยากาศในสภาพของ หมอก น้ำค้าง และน้ำฝน วัตถุประสงค์ของการทดลองในครั้งนี้เพื่อทราบถึงสภาพแวดล้อม ที่ส่งเสริมการเพิ่มผลผลิตไลเคน *Parmotrema tinctorum* ที่ย้ายปลูกบนวัสดุเทียม โดยการให้น้ำด้วยวิธีต่างๆ ในป่าฝนเขตร้อนอุทยานแห่งชาติเขาใหญ่ โดยย้ายปลูกไลเคน *P. tinctorum* จำนวน 960 แทลลัสบนตาข่ายไนลอนที่เอียงทำมุมประมาณ 40 องศากับพื้นดินซึ่งหันไปทางทิศตะวันออก ทิศตะวันตก ทิศเหนือ ทิศใต้ และในแนวราบ โดยมีการให้น้ำ 3 วิธีคือ กลุ่มควบคุมซึ่งไม่ให้น้ำ กลุ่มที่ให้น้ำโดยตรงบนไลเคน และกลุ่มที่ให้น้ำบนผิวดิน พร้อมทั้งบันทึกสภาพภูมิอากาศจุลภาค และวัดอัตราการเติบโต หลังการย้ายปลูก 16 เดือนไลเคนที่ให้น้ำบนดินมีอัตราการเติบโตเฉลี่ยรวมของทุกทิศ สูงกว่าไลเคนที่ไม่ให้น้ำคือ 0.32 และ 0.29 มม./เดือน ตามลำดับ โดยแทลลัสที่หันไปทางทิศต่างๆและได้รับน้ำจากดินมีอัตราการเติบโตสูงกว่าแทลลัสที่ไม่ได้รับน้ำ ยกเว้นแทลลัสที่หันไปทางทิศตะวันออกและไม่ได้น้ำ มีอัตราการเติบโตสูงสุด วัดได้ 0.39 มม./เดือน และสูงกว่าพวกที่หันไปทางทิศเดียวกันและได้รับน้ำจากดินซึ่งวัดได้ 0.37 มม./เดือน ส่วนไลเคนที่ให้น้ำโดยตรงตายหลังจากย้ายปลูก 4 เดือน นอกจากนี้พบว่า ไลเคนที่ไม่ให้น้ำ และให้น้ำบนดิน มีการสร้างโลบใหม่ร้อยละ 42 และ 43 ตามลำดับ ส่วนไลเคนที่ย้ายปลูกในแนวราบร้อยละ 50 ไม่สร้างโลบใหม่ ในช่วงเช้าไลเคนได้รับแสง 180 -1,500 ไมโคร โมลต่อตารางเมตรต่อวินาที ความชื้นสัมพัทธ์ในเวลากลางคืนสูงกว่าร้อยละ 80 อุณหภูมิตลอดทั้งปีวัดได้ 8-33 องศาเซลเซียส ฤดูฝนส่งผลให้การเติบโตของไลเคนย้ายปลูกและการสร้างโลบใหม่ลดลง โดยเฉพาะไลเคนที่ย้ายปลูกในแนวราบ เนื่องจากมีน้ำขังน้ำฝนจึงเป็นปัจจัยหลักที่ทำให้ไลเคนที่ย้ายปลูกมีการเติบโตลดลง แต่สร้างไอซีเดียเพิ่มขึ้นซึ่งต่างจากไลเคนที่เติบโตตามธรรมชาติซึ่งมีการเติบโตสูงสุดในฤดูฝน

Abstract: Lichens have slow growth rates. Their photosynthesis and growth depend on resources from the atmosphere i.e. fog, dew and rain water. The objective of this study was to find the best environmental man made condition, in semi natural habitat, that enhance production of the lichen *Parmotrema tinctorum* on artificial substrate by different water treatment. The experiment was performed at the open site in the abandon tropical rain forest at Khao Yai National Park. Nine hundred and sixty thallus fragments of *P. tinctorum* were attached on 9x9 cm² nylon meshes. These artificial substrates stand over the ground at about 40° inclination facing the East, the South, the West, the North and horizontal. Three water treatments were applied; without extra water (control), wetting the soil to increased evaporation and spray with water directly over lichen thalli. Growth of the thalli and microclimate of the habitats were measured. Sixteen months after transplantation, the

transplanted lichens which received soil evaporative moisture had higher growth rate than those without watering (control) accounting for 0.32 and 0.29 mm/month respectively. Thalli that received soil moisture in all aspects had higher growth rate than those received no water, except the thalli that facing the East. The east facing thalli that had no treatment of water had higher growth rate than those received soil water accounting for 0.39 mm/month, which was the highest growth rate among all transplants. Whilst, thalli that face the same direction and got soil water had growth rate of 0.37 mm/month. The transplanted lichen which received water directly over thalli died completely in four month. In addition, transplanted thalli without water and those received soil water generated small new lobes accounting for 42% and 43% respectively. The horizontally transplanted thalli produced new lobes 50%. The transplanted lichen had illumination $180 - 1,500 \mu\text{molm}^{-2}\text{s}^{-1}$, relative humidity at night was higher than 80% and temperature ranged from 8-33 °C. The transplanted thalli had the lowest growth rate, and generate less new lones in rainy season, especially those that were transplanted horizontally. Therefore, rain was the important factor that caused reduced growth of the transplanted lichens, but numerous isidia were produce.

Introduction: Lichen *Parmotrema tinctorum* (common name in Thai Phaggardnotang) is a foliose macrolichen which widely distributed in all types of forests in Thailand¹. This lichen had high growth rate comparing with other species² and has potential uses in pharmacy, agriculture and environment¹. *P. tinctorum* has been used as bioindicator for air pollution index^{3,4}. This lichen would be in great demand in the future because of its potential uses. Therefore, increase natural production of this lichen is necessary for sustainable utilization and conservation. However, lichen requires specific habitat and environmental condition such as illumination, moisture and temperature for growth and regeneration. The objective of this study was to find the best environmental man made condition, in semi natural habitat, that enhance production of the lichen *P. tinctorum*. It was hypothesize that 1) increasing water availability would enhance growth and production 2) different orientation of lichen thallus to the sun causes different growth.

Methodology

Transplantation: The transplantation started in October 2008 and last until February 2010. Nine hundred and sixty thallus fragments of *P. tinctorum* (Despr. ex Nayl.) Hale collected from the bark of trees and rocks in secondary forests were attached on 9x9 cm² nylon meshes by nylon thread. These artificial substrates stand over the ground at about 40° inclination facing the East, the South, the West, the North and horizontal. Sixteen thalli were placed on each aspect (Figure 1). Three water treatments were applied; without extra water (control), spray with water directly over lichen thalli, and wetting soil, by which lichens received extra water from soil evaporation. Water used in the experiment came from reservoir in the park and store in a tank for general uses. This water was retreated before used in the experiment by passing through several layer of filtering materials. The transplantation area was located in the abundant tropical rain forest near the training center at KYNP. The transplanted thalli were adhered on nylon board which stand over the ground at 40 degree inclination and orientation to the N, S, E, W aspects and horizontal (parallel to the ground)

Figure 1. Lichen transplantation A) transplanted lichens on artificial substrates B) Sixteen thalli on each aspect and C) transplanted lichen on 9x9 cm² nylon meshes



Microclimate: Microclimate of each aspect, illumination, temperature and air humidity, was measured by Licor-1400 (Licor Inc., Lincoln, Ne, USA) in every mid season during October 2551 to February 2553

Growth measurement: Photographs of the thalli were taken at the end of season, and growth was calculated from digital images by using AxioVision LE. Rel. 4.1 software.⁵

Spaying water of experiment Water treatment: Except the control group, the transplanted lichen thalli received additional water by directly spaying over thalli, and by wetting the ground under the stands which increase soil evaporation. Thus, lichen in the latter treatment got extra, pure, water from the ground. Water was applied three times a day; 8.00-8.30 am., 12.00-12.30 pm. and 17.00-17.30 pm. by using automatic timer.

Results, Discussion and Conclusion

Effects of different water treatment and aspect orientation on germination and growth of the transplanted lichens

Growths of the transplanted lichens in various treatments were shown in Table 1. After sixteen months of transplantation, lichens in the control group facing the east had the highest growth rate accounting for 0.39 mm/mo, whereas those received extra water evaporated from the ground had average growth rates of 0.32 and 0.30 mm/month respectively. By contrast, lichens which received water by directly sprayed on thalli died almost completely in 4 months (Table 1). The highest growth rate was found on the east-facing side of control group accounting for 0.39 mm/month. The lowest average growth rates of 0.24 mm/month was found on the North and Horizontal facing sides without water treatment. Growths during rainy season of every treatment were low comparing with other seasons.

Table 1. Growth rate of *P. tinctorum* in different season after sixteen months of transplantation on each different aspect orientation and water treatment; control (C), wetting the ground (SI) and spray directly over thalli at KYNP during October 2551 to February 2553

Aspects	Treatments	Growth rate (mm/month, 16 month)				
		cold season	summer	rainy	cold season	16 month
East	C	0.61±0.37	0.59±0.44	0.14±0.64	0.20±0.30	0.39±0.31
	SI	0.52±0.32	0.52±0.46	0.15±0.45	0.31±0.25	0.37±0.25

		Tha					
South	C	0.52±0.27	0.35±0.35	0.18±0.54	0.15±0.22	0.29±0.26	
	Sl	0.46±0.27	0.43±0.32	0.32±0.40	0.3±0.27	0.35±0.23	
	Tha						
West	C	0.44±0.28	0.38±0.31	0.33±0.54	0.24±0.23	0.31±0.21	
	Sl	0.36±0.22	0.31±0.28	0.32±0.53	0.42±0.35	0.32±0.26	
	Tha						
Horizontal	C	0.44±0.26	0.43±0.30	-0.12±0.36	0.02±0.30	0.24±0.23	
	Sl	0.40±0.29	0.38±0.25	-0.07±0.47	0.25±0.25	0.27±0.19	
	Tha						
North	C	0.48±0.31	0.21±0.22	0.14±0.38	0.15±0.23	0.24±0.16	
	Sl	0.33±0.28	0.21±0.39	0.20±0.42	0.27±0.28	0.27±0.24	
	Tha						

The generation of small new lobes of the transplanted lichens

Generation of tiny new lobes was observed after 8, 12 and 16 months of transplantation accounting for 51%, 82% and 85% respectively (Table 2). Most of the new lobes were generated after the first observation, which was eight months after transplanted and passed through the rainy season.

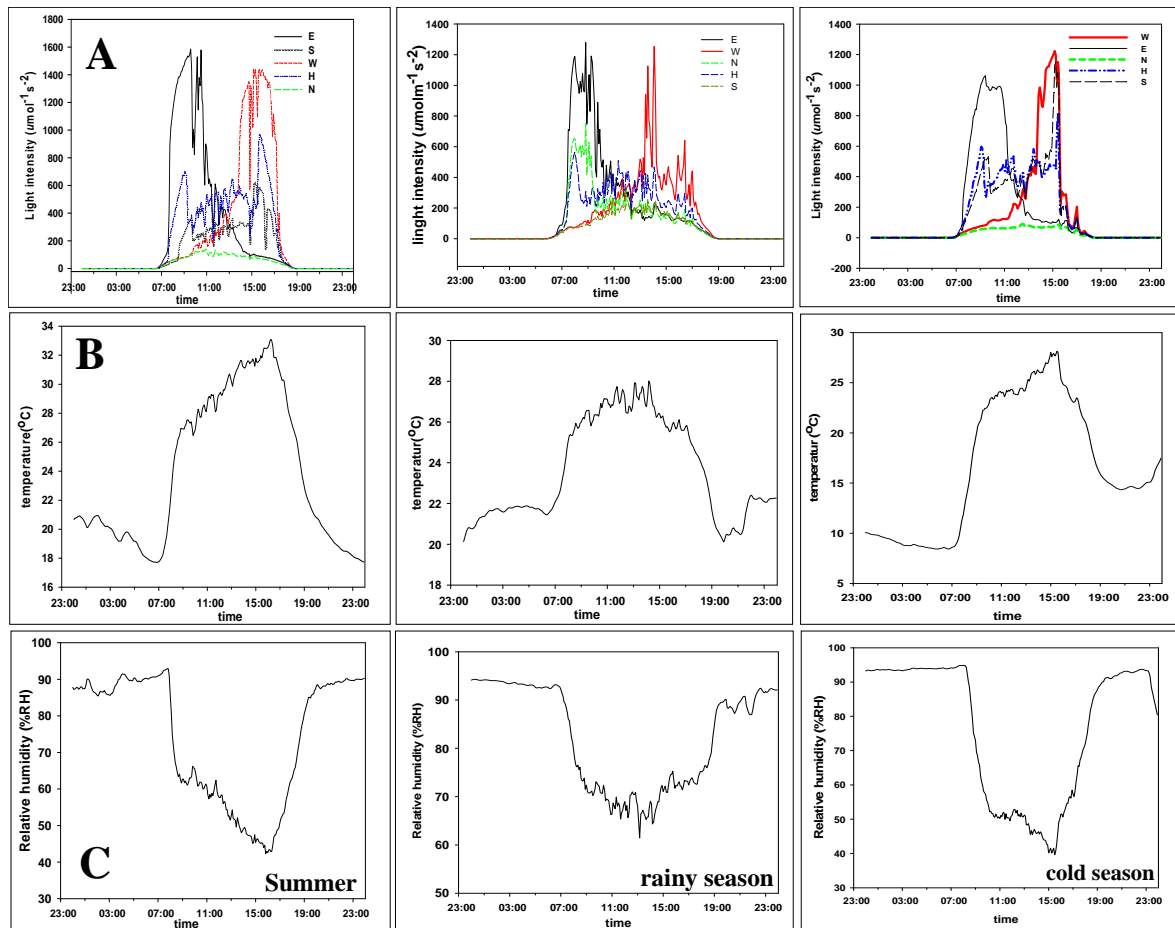
Table 2. Proportion of small new lichen lobes generated after eight, twelve and sixteen, months of transplantation on artificial substrates with different water treatment; control (C) without extra water, wetting the ground (Sl) and spray directly over thallus (Th) and different aspect orientation

Aspects	Treatments	8 months	12 months	16 months
East	C	7	8	9 (%)
	Sl	5	8	8
	Tha			
South	C	5	9	9
	Sl	4	9	10
	Tha			
West	C	7	9	9
	Sl	7	9	9
	Tha			
Horizontal	C	2	6	6
	Sl	3	6	7
	Tha			
North	C	5	9	9
	Sl	6	9	9
	Tha			
Total C		26	41	42
Total Sl		25	41	43

Microclimate of the transplanted lichens

Lichen transplanted thalli received illumination of 180 - 1,500 $\mu\text{molm}^{-2}\text{s}^{-1}$ (Fig. 2.), relative humidity than higher 80% for about 13 hours and temperature ranged 8-33 °C.

Figure 2. Microclimate of the transplanted lichen, *P. tinctorum*, measured during May 2008 to January 2018 at KYNP. A) Illumination at different aspects of orientation B) Temperature and C) Relative humidity (1, 2 and 3 mean summer, rainy season, and cold season)



This experiment revealed that transplanted lichens oriented to the East aspect both control and soil water treatment had the highest growth rate. This was because this side had bright illumination in early morning while thalli were moist with atmospheric humidity during the night. Growth rate declined during rainy season, which was different from natural grow lichens that have the highest growth rate during this season². On the other hand, the transplanted lichen produced numerous isidia, and small new lobes during rainy season. They allocated carbohydrate to make these vegetative propagules instead of growing. The reason for this needs further investigation. However, Kon (2003) reported similar evidence from his experiment with the same species of lichen in Japan⁶. In addition, transplantation of thalli over nylon substrate with small mesh size caused stagnant of water in thalli over longer period during rainy season, and consequently numerous isidia were produced⁷. After rainy

season, growth rates increased with higher rate observed from the thalli treated with soil moisture (Figure 3). This was probably due to longer moistening of thalli, lower temperature which enhanced CO₂ assimilation.

Figure 3. Average growth rates of *P. tinctorum* after sixteen months transplantation on artificial substrate with different water treatments and aspect orientation at KYNP

Aspects	Control		SI	
	Average	SD	Average	SD
E	0.39	±0.31	0.37	±0.25
S	0.29	±0.26	0.35	±0.23
N	0.24	±0.16	0.27	±0.24
W	0.31	±0.21	0.32	±0.26
H	0.24	±0.23	0.27 </td <td>±0.19</td>	±0.19
Average	0.29		0.32	

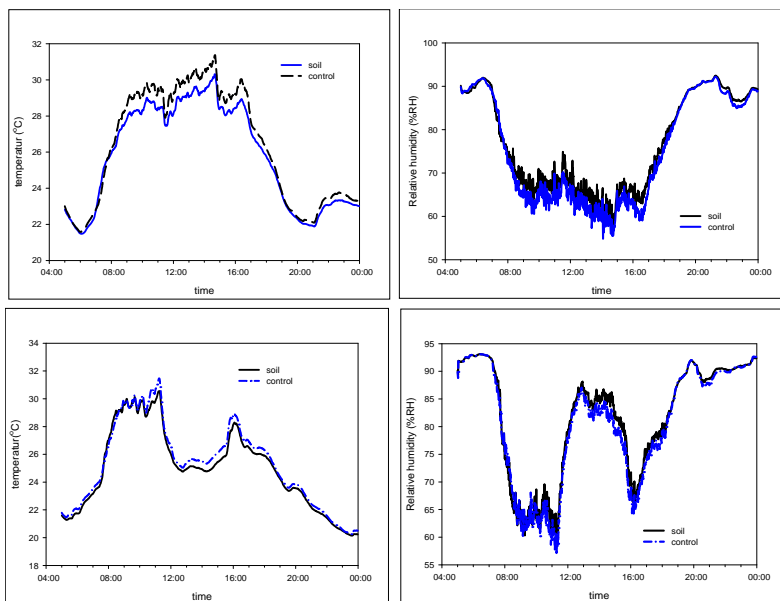


Figure 3 show that average growth rate of the transplanted thalli with received evaporated water from soil was higher than those without additional water (control). Although the highest growth rate was observed from the East facing thalli without water treatment, nevertheless the other aspects (S, N, W and H) with soil water treatment had higher growth rates than those without water treatment. This was probably due to the E received high illumination in early morning, which caused high evaporation and the thalli dried faster than the other aspects causing termination of photosynthesis sooner than the others. In order to enhance production of lichen by transplantation, a few factor need to be considered, which are aspects and methods of water treatments. In conclusion this experiment showed that increasing lichen growth and biomass production can be achieved by applying clean water to prolong thallus moisture. However, magnitudes of increases need longer investigation

References

1. Lichen Research Unit. *OEPP Biodiversity series*, 2004.
2. Osathanon N., *MSc thesis*, 2004.

3. Ohmura Y., Kawachi M., Kasai F., Sugiura H., Ohtara K., Kon Y, Hamada N., *Bull. Natl. Mus. Nat. Sci., Ser. B*, 2009, 35(2) 91–98
4. Boonpeng C., Thanomjitt R., Boonpragob K., *The 35th congress on science and technology of Thailand*, 2009, 62.
5. Hookert. N., Brownd. H. *Lichenologist*, 1977, 65-75.
6. Kon Y., Mineta M., Kashiwadani H. *J. Jpn. Bots.* 2003, 208-213.
7. Santanoo S., Boonpragob K., *The 35th congress on science and technology of Thailand*, 2009.

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Keywords: lichen transplantation, growth rate, water treatments, thallus orientation