GROWING CAPACITY OF DIFFERENT THALLUS STRUCTURE AND SIZE OF *Parmotrema tinctorum* LICHEN ON ARTIFICIAL SUBSTRATES

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Abstract: Lichen regenerates by spore discharging and germination of broken thallus. The latter is more efficient because both the algae and fungi are together at symbiosis stage, whereas the former needs to wait for compatible algae. However, different part of thallus has dissimilar capacity to grow. It was hypothesized that large lichen thallus has higher growth rate than the small ones, and complete lobe tip has the highest growth rate. The objectives of this study were to observe 1) effect of thallus size on growth and 2) growing capacity of different parts of thallus. The study was performed by transplantation thallus fragments of *P. tinctorum* on nylon meshes in secondary forest at KYNP. Twenty fragments of small thalli with diameter less than 3.8 cm and ten larger thalli were attached on nylon meshes. In addition, 30 different parts of thalli consisted of complete lobe tip, without lobe tip and isidia lobe were transplanted. Twelve months after transplantation, the small thalli had higher growth rate than the large ones accounting for 0.93 and 0.76 mm/month, respectively. Thallus fragment with complete lobe tip had the highest growth rate of 1.04 mm/month, which was significantly higher than those without lobe tip and with isidia, which accounting for 0.64 and 0.42 mm/month, respectively, after transplantation for 6 months. The complete thalli with lobe tips consisted of active growing zone of lichen, which enhance thallus growth. However we anticipated that the transplanted thalli without lobe tip and those with isidia would grow better and have larger biomass production in the future because some new lobules germinate from these thalli. However, time frame required for this establishment is not known, and it needs a long term investigation.

Introduction: Thailand is situated in tropical region, where high biodiversity and luxurious lichen flora could be found. Record in 2009 showed that the country hosted 1,692 species consisting of 450 species of new records and 150 taxa. Lichens have high potential to be utilized for dyes and several applications because of their unique chemical products.[1] The lichen *Parmotrema tinctorum* (Despr. ex Nayl.) Hale is a foliose macrolichen which is widely distributed in all types of forests in Thailand. This lichen had high growth rate compared with other species[2] and has a potential to be used in pharmacy, agriculture and environment. It has been used as a bioindicator for air pollution[3,4] This lichen would be in great demand in the future because of its prospective uses. Therefore, the increased biomass production of this lichen is necessary for sustainable utilization and conservation. The objectives of this study were to observe 1) effect of thallus size on growth and 2) growing capacity of different parts of thallus. It was hypothesized that large lichen thallus has higher growth rate than the small ones, and complete lobe tip has the highest growth rate.

Methodology:

1. **Study site**

The transplantation area was located in the tropical rain forest near the training center at KYNP. It was situated between 14° 05′ to 14° 35′ north latitude and 101° 05′ to 101° 50′

east latitude on the western most part of Dongrak mountain range. Climate is under the influence of Asian monsoon, which is characterized by extreme wet and dry season cycle.[2]

2. Transplantation technique

2.1 Transplantation of different sizes of lichen thalli to observe growth capacity:

Thirty thallus fragments with complete lobe tips of *P. tinctorum* collected from the bark of trees and rocks in secondary forests were attached on nylon meshes by nylon thread (Figure 1). These artificial substrates stood over the ground at about 40^{o} inclination facing the east. Diameters of the transplanted thalli consisted of 20 small thalli with diameters less than 3.8 cm and 10 large ones with diameters greater than 3.8 cm. The transplantation started in February 2010 and lasted until February 2011(12 months).

(A) (B) (C) (D)

Figure 1. Transplantation site of lichen at KYNP A) overview of transplanted area B) the lichen *P. tinctorum* C) lichen thalli on artificial substrates in the east and D) small and large lichen thalli on nylon meshes.

2.2 Transplantation of different parts of thalli to observe growing capacity:

Sixty thallus fragments of *P. tinctorum* consisting of 20 of each thallus with complete lobe tip, without lobe tip and with isidia were attached on nylon meshes stand facing the west (Figure 2). Thallus growth was measured at the end of each season. The transplantation lasted for 6 months from October 2010 to February 2011.

(A) (B) (C) (D)

Figure 2. Transplantation of lichen by using different parts of thallus fragments A) transplantation substrates stand on ground B) thallus with normal lobe tip C) thallus without lobe tip and D) thallus with isidia.

3. Growth measurement:

Photographs of the thallus were taken at the end of each season, and growth was calculated from digital images by using AxioVision LE. Rel. 4.1 software.

Results, Discussion and Conclusion

Growing capacity of small and large thalli after transplantation:

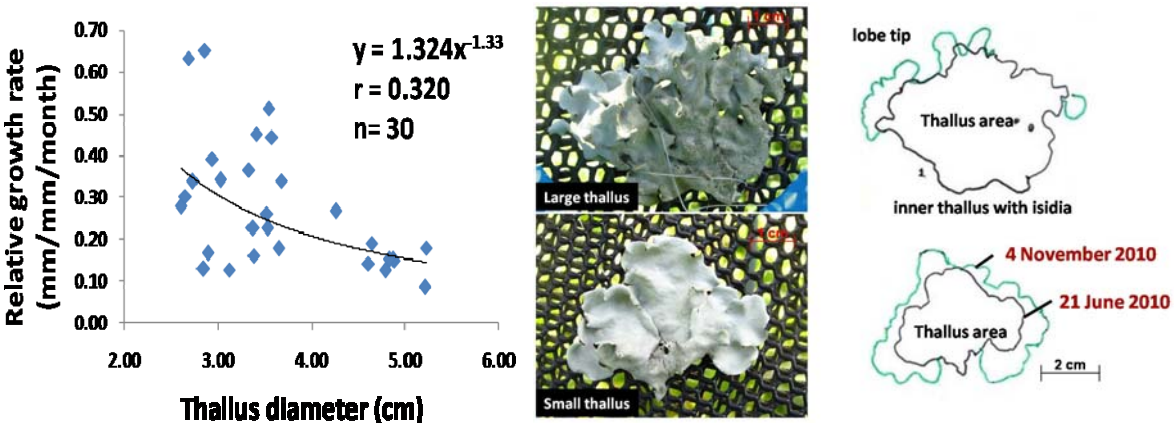
After 12 months of transplantation, both small and large thalli had the highest growth rate during the rainy season, and small thalli grew faster than the large ones accounting for 0.93 mm/month and 0.76 mm/month (Table 1). However, the growth rate of the small thalli was not significantly different from that of the large ones.

Table 1. Average growth rate of small and large thalli of *P. tinctorum* after transplanted on artificial substrates (diameters: small thalli-less than 3.8 cm, and large thalli-greater than 3.8 cm).

	Growth rate (mm/month, 12 months)			
Thallus size	Summer	Rainy season	Cold season	12 months
Large thallus (n=10)	0.8±0.46	0.96±0.38	0.53±0.34	0.76±0.2 a
Small thallus (n=20)	1.06±0.46	1.09±0.55	0.65±0.34	0.93±0.38 a

*Average growth rate 12 months values in vertical column followed by the same letter do not differ significantly at $P < 0.05$ by Student t-test comparisons.

Relative growth rate of different thallus sizes was shown in Figure 3. Small thalli had higher relative growth rate than large thalli.



(A) (B)

Figure 3. Growth and relative growth rate of various sizes of the transplanted thalli of *P. tinctorum* A) relative growth rate of 32 individual thalli B) direction of thallus expansion from lobe with isidia and lobe with complete lobe tip from 21 June 2010 (black line) to 4 November 2010 (green line) of large and small thalli.

The small thalli had relatively smooth surface which had more area than the wrinkle large thalli. Growth rates varied depending on smooth area at the tip of the lobe and this variability obscured the differences in growth rate between thalli caused by age of the thallus.[5]

In addition, radial growth depended largely on processes within a region approximately 2 mm behind the lobe tip.[8] Small thalli of *P. tinctorum* had high relative growth rate, which declined as thalli gained larger size.

Growth rate of different parts of thallus after transplantation

Growth of different parts of thallus of *P. tinctorum* varied considerably (Table 2). Thallus fragments with complete lobe tips had the highest growth rate of 1.04 mm/month, which was significantly higher than those from the other parts of thallus ($P \leq 0.001$). Growth of the thalli without lobe tips and the thalli with isidia were 0.64 and 0.42 mm/month, respectively, after transplantation for 6 months.

Table 2. Average growth rate of different parts of thalli of *P. tinctorum* after transplantation on artificial substrates by facing the west for 3 months and 6 months at KYNP.

Thallus (n=60)	Average growth rate (mm/month)		
	after 3 months	after 6 months	average 6 months
Complete lobe tip	0.95 ± 0.27	1.13 ± 0.62	1.04 ± 0.3 a
Without lobe tip	0.36 ± 0.27	0.72 ± 0.56	0.64 ± 0.27 b
Isidia lobe	0 .00 ± 0.36	1.14 ± 0.54	0.42 ± 0.24 b

*Average values in vertical column followed by the same letter do not differ significantly at $P < 0.05$ by Turkey's pairwise comparisons.

The complete thalli had lobe tips which are growing zone of lichen,[6] which enhanced growth of this area. However, we anticipated that the transplanted thalli without lobe tip would grow better in the future because some new lobules germinated from these thalli. Nevertheless, the time frame required for this establishment is not known, and it needs a long term investigation.

Growth appears to be dependent on carbohydrate production in a small area of the lobe tip.[7] In addition the concentration of ribitol, arabitol and mannitol was greatest at the lobe tip.[7] Despite the transplanted thalli with isidia had the lowest growth rate, they developed many new lobules from the isidia. This type of thallus allocated carbohydrate photosyntate to produce new lobes rather than to expand area of the original thallus. This stage was recognized as the establishment phase, of which growth was slow. This type of thallus was supposed to have the largest biomass production in the future, if all new lobe survive and grow. A long term investigation was also needed for this aspect.

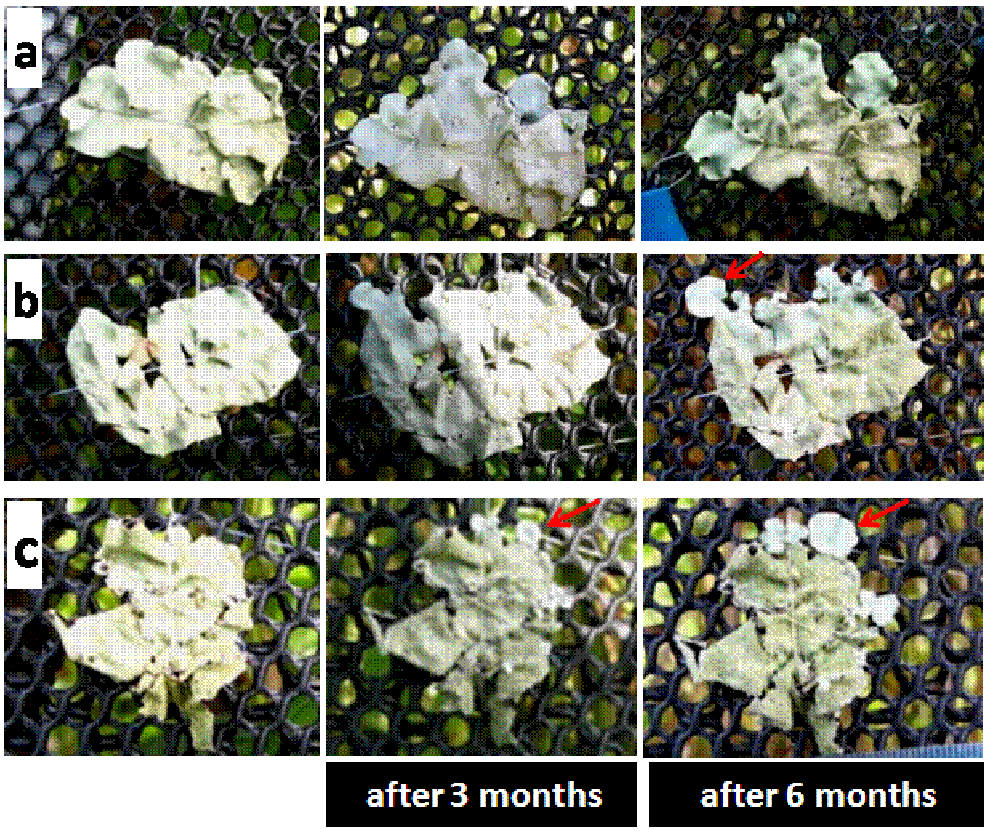


Figure 4. Transplantation of lichen using different parts of thallus. Complete lobe tip (row a), Without lobe tip (row b) and isidia thallus (row c) after transplanted for 3 months and 6 months.

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Keywords: lichen transplantation, *P. tinctorum*, thallus size, growing capacity