การใช้พลังงานแสงในกระบวนการโฟโตเคมีของไลเคน *Parmotrema tinctorum* จากแหล่งอาศัยจุลภาคสามแบบ ณ อุทยานแห่งชาติเขาใหญ่

PHOTOCHEMICAL ENERGY UTILIZATION OF THE LICHEN *PARMOTREMA TINCTORUM* FROM THREE MICROHABITATS AT KHAO YAI NATIONAL PARK

มงคล แผงเพ็ชร และ กัณฑรีย์ บุญประกอบ

Mongkol Pangpet and Kansri Boonpragob
Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkapi, Bangkok 10240, Thailand; Fax: (662)310-8395, e-mail address: golkol@hotmail.com

บทคัดย่อ: ไลเคนใช้น้ำจากบรรยากาศในการเติบโต และไม่สามารถรักษาน้ำไว้ภายในได้ เมื่อแทลลัสได้น้ำพอเพียงกระบวนการเมตาบอลิสซึมของไลเคนจึงเกิดขึ้น ไลเคนในป่าเขตร้อน มีอัตราการเติบโตต่างกัน โดยอาจขึ้นอยู่กับระยะเวลาที่ได้รับความชื้นสูง และความสามารถในการใช้พลังงานแสง การศึกษานี้ใช้ตัวแปรของ chlorophyll fluorescence ได้แก่ Maximum quantum yield of PSII (Fv/Fm), Quantum yield of PSII (ΔF/Fm'), Electron transport rate (ETR), Photochemical quenching (qP) และ Non-photochemical quenching (NPQ) เพื่อศึกษากิจกรรมโฟโตเคมีของไลเคน *P. tinctorum* ที่อาศัยอยู่บนหินกลางแจ้ง บนหินในร่ม และบนเปลือกไม้ในที่ร่ม ผลการตรวจสอบพบว่ากิจกรรมโฟโตเคมีของไลเคนบนหินกลางแจ้ง และเปลือกไม้ในร่ม สูงสุดในช่วงเวลา 7:30-8:30 น. ขณะที่ไดรับความเข้มแสงต่ำประมาณ 200 μmol $m^{-2}s^{-1}$ ในทางตรงกันข้ามไลเคนบนหินในที่ร่ม มีกิจกรรมดังกล่าวสูงสุดเมื่อเวลา 11:00 น. ภายใต้ความเข้มแสงประมาณ 460 μmol $m^{-2}s^{-1}$ แต่ไลเคนบนหินกลางแจ้งมีกิจกรรมนี้สิ้นสุดลงเร็วกว่าพวกอยู่ในที่ร่ม ความชื้นสัมพัทธ์ในอากาศมีผลต่อกิจกรรมของไลเคนบนหินกลางแจ้งและเปลือกไม้มากกว่าหินในที่ร่ม อาจเนื่องจากแทลลัสได้รับจากน้ำที่ระเหยออกมาจากหินและดินภายใต้ร่มเงา การศึกษานี้ช่วยให้เข้าใจความสำคัญของปัจจัยต่างๆที่มีอิทธิพลต่อการเติบโตและการเพิ่มมวลชีวภาพของไลเคนชนิดนี้ซึ่งสามารถประยุกต์ใช้กับไลเคนชนิดอื่นในเขตร้อน อันจะนำไปสู่การอนุรักษ์และการเพิ่มผลผลิตของไลเคน *P. tinctorum* เพื่อการใช้ประโยชน์ยั่งยืนในอนาคต

Abstract: Lichens are poikilohydric, by which thallus water contents depend on atmospheric moisture. They are metabolically active only when thalli are moist with atmospheric water. Different growth rates of lichens in the tropical rain forests probably determined by duration of exposure to high humidity and the ability to utilize limited light energy in the forest. This study use chlorophyll fluorescence parameters, Fv/Fm, ΔF/Fm, ETR, qP and NPQ to observed photochemical activity of the lichens *Parmotrema tinctorum* inhabited exposed rock, shaded rock and shaded bark. The investigation revealed that lichens from sun rock and shaded bark maximized their photochemical activities during 7:30-8:30 hours while PAR was as low as 200 μmol $m^{-2}s^{-1}$, whereas those of the shade rock achieved such activity at about 11 hours under 460 μmol $m^{-2}s^{-1}$ illuminations. *P. tinctorum* on the sun rock had heir photochemical activity terminated earlier than the other shade bark and shaded rock (9, 11:30 and beyond 12 hours) respectively. Relative humidity effected photochemical activities of the lichens on the sun rock and the shade bark more than those on the shaded rock. The latter probably got evaporative moisture from porous rock and soil. This study enhance an understanding of the underlining factors that influence growth and biomass production of the

lichen *P. tinctorun*, which may applicable to other lichens in the tropic. It leads to future management to increase production of the lichen *P. tinctorum* for conservation and sustainable utilization of the novel products from this lichen.

Introduction: Lichens have very slow growth rate, one of the reason is because they are pokilohydric which cannot store water in their thalli. Water content of thalli fluctuate with atmospheric water. They are metabolically active when RH is high over 80%, which normally occurs during the night and early morning [1]. During this period thalli are moist with atmospheric water (fog and dew), and lichens perform photosynthetic activities when illuminate with early morning sun. As thallus dry out, which usually occur fast, before 9 hours, photosynthesis terminate, and lichen become inactive [2]. Lichens have short period to assimilate carbon for growth, which results in slow growth rate (mm/year). Growth of lichens also depended on environmental factor e.g. relative humidity, light intensity, temperature and etc., which vary among microhabitats. Natural conditions of microhabitat affect lichen photosynthetic performances. A long photosynthetic period benefits lichen growth. Chlorophyll fluorescence technique is an efficient indirect measurement of photosynthesis that has been widely used to assess carbon assimilation under stress [3]. The hypothesis of this study is lichens on shade habitat has longer photochemical activity than those on exposed site. Chlorophyll fluorescence analysis allows nondestructive, near instantaneous measurement of key aspects of photosynthetic light capture and electron transport [4]. Thus, it has been used extensively to study photosynthetic efficiency of lichens growing in natural habitats. The parameter Fv/Fm indicates maximum quantum yield of PSII and can be used to indicate lichen photosynthetic efficiency in environmental stress [3]. *Parmotrema tinctorum* (Despr. ex Nyl.) Hale is a foliose lichen with green algae, *Trebouxia sp,* photobiont. This lichen has very high growth rate and is common in Khao Yai National Park (KYNP) [2]. It grows on various substrates including rock, tree trunk and branch at the forest canopy. These microhabitats provide different meteorological factors. The objective of this study is to investigate photochemistry of *P. tinctorum* on three nature habitats by using chlorophyll fluorescence technique. The results of this study will lead to an understanding of underlining factors that determine growth rates of lichens, which can be used in transplantation to enhance biomass production for utilization of lichen substances.

Methodology: The lichen *Parmotrema tinctorum* were collected from 3 types of substrates: 1) the shaded bark of Ficus sp. and Cassia grandis L.f. near Khao Yai Training Center 2, 2) sun and 3) shaded sandstone rocks near Pratumnak (the Royal lodge) at Khao Yai National Park. Chlorophyll fluorescence were measured on 2 samples of lichens on shade rock, 3 shade bark and 2 on sun rock. The measurements were performed during 8-24 June 2007 by using MINI-PAM (Walz, Effeltrich, Germany) [3, 5]. A fiber optic probe was securely fixed at 7 mm distance and 60^{o} from lichen thallus, using a distance clip and a 2 mm spacer ring. The potential quantum yield of photosystem II (PSII) was examined through the parameter Fv/Fm, which represented maximum chlorophyll fluorescence. Predawn measurements were performed to assess maximum yield of fluorescence or maximum photosynthetic capacity. Relative humidity were recorded using LI-1400 (LI-COR), and temperature and light intensity during fluorescence measurements were recorded with thermocouple and micro-quantum sensor attached to Mini-PAM. Data analysis was conducted with the help of Sigmaplot software (Jandel Scientific, San Rafael, Calif.).

Results, Discussion and Conclusion: The differences in chlorophyll fluorescence parameters, Fv/Fm, ΔF/Fm' and ETR, measured from lichens at three different microhabitats in this study indicated clearly periods of their photosynthetic carbon assimilation and growth conditions. Lichens growing in both shaded habitats had Fv/Fm as high as 0.62, whereas those from fully-illuminated conditions had 0.37 of this value. The difference was mainly due to light condition. Lichens on exposed rock had maximum fluorescence values (Fm) much lower than those growing in the shade because the amounts of chlorophyll in the sun lichens are generally lower than the shaded ones [6], this result in lower yield of fluorescence from chlorophyll resulting in lower Fv/Fm (Table 1).

Table 1. Fv/Fm (mean +SD) of lichens on three different habitats. Lichens on exposed rocks had the lowest average Fv/Fm comparing with those on shaded rock and bark. The measurements were performed during 8-24 June 2007. (n= 3, 3 and 2 on shade bark, shade rock and sun rock)

Substrate types	Fo	Fm	Fv/Fm
Shade bark	803±140	2332±305	0.656±0.028
Sun rock	552±53	838±203	0.323±0.083
Shade rock	862±43	2217±98	0.606±0.002

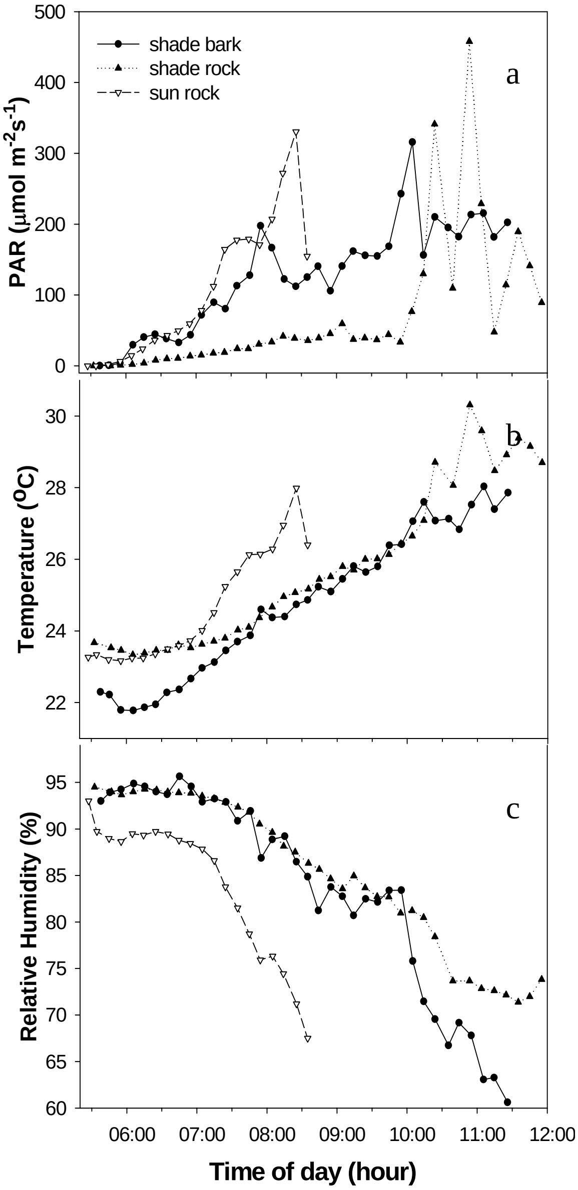

Fig 1. (on the right) Microclimate on three different habitats, showing ten-minute average values of light intensity (a), temperature (b) and relative humidity (c) of lichens on three substrates during 8-24 June 2007.

Relative humidity fluctuated follow illumination and temperature (Fig1), which related to time of day. Fig. 2a shows that fluorescence parameter ΔF/Fm' declined noticeably fast from dawn to midday. Values of ΔF/Fm' as low as 0.15 were observed at 8:15, 10:30 and after 12:00 hours from lichens on shade bark, sun rock, and shade rock, respectively. These values immediately decline followed the reduction of relative humidity. Fig 2b show correlation of ΔF/Fm' and atmospheric water vapor. The decline of this fluorescence parameter to zero indicated termination of photochemical activity due to rapid loss of water from thallus on the three habitats, which occurred approximately 2-4 hours after sunrise. The values, reported in lichens in extreme environments, indicated photosynthetic active period [3, 4, 5, and 8]. This evidence suggested that water content in the thalli was far more important to lichen photosynthetic performances than high light intensity at such condition.

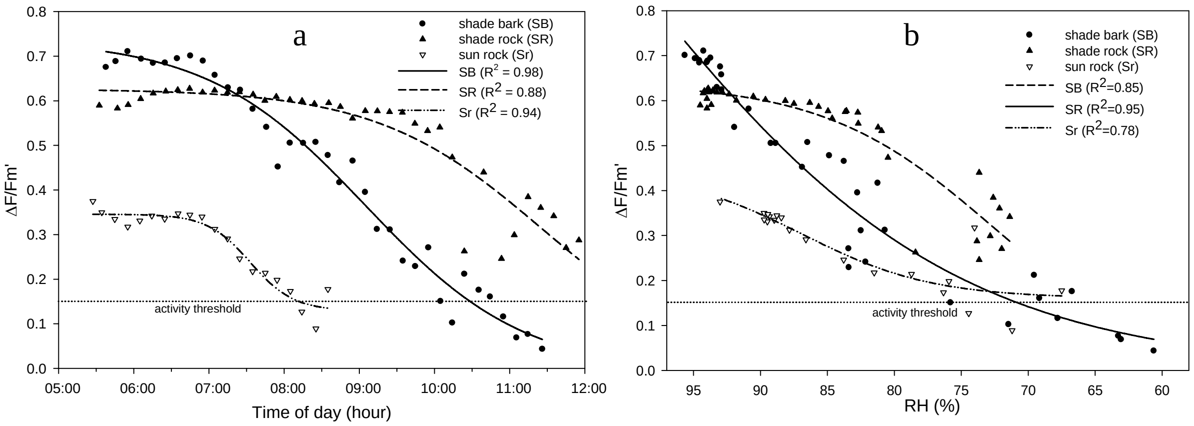

Fig. 2 a, b. a) Chlorophyll fluorescence parameter, Fv/Fm' of lichens growing naturally on exposed rock, shaded rock and bark during 5-12 hours at KYNP. b) ΔF/Fm' of lichens from the three different habitats as a function of relative humidity. (8-24 June 2007)

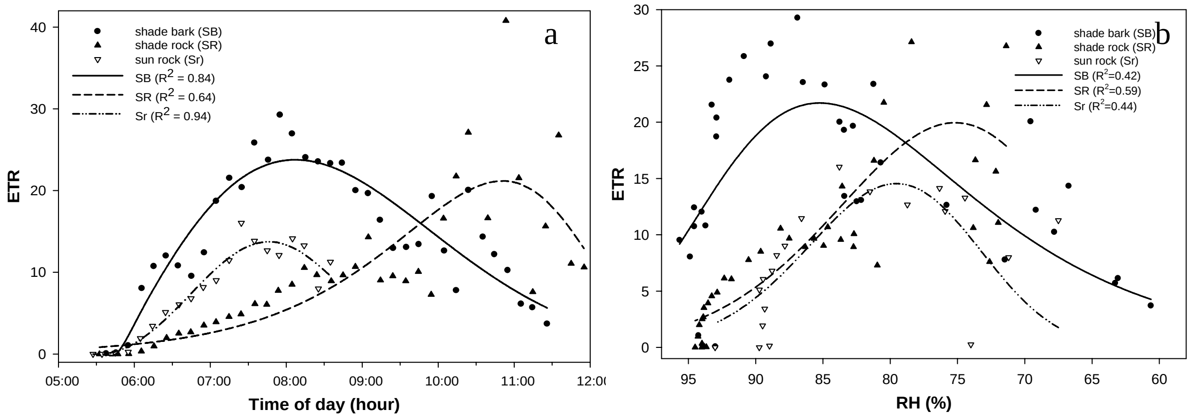

Fig.3 a, b. a) ETR of lichens growing naturally on shade bark, shade rock and sun rock and during 5-12 hours at KYNP. b) ETR of lichens growing at KYNP as a function of RH. (8-24 June 2007)

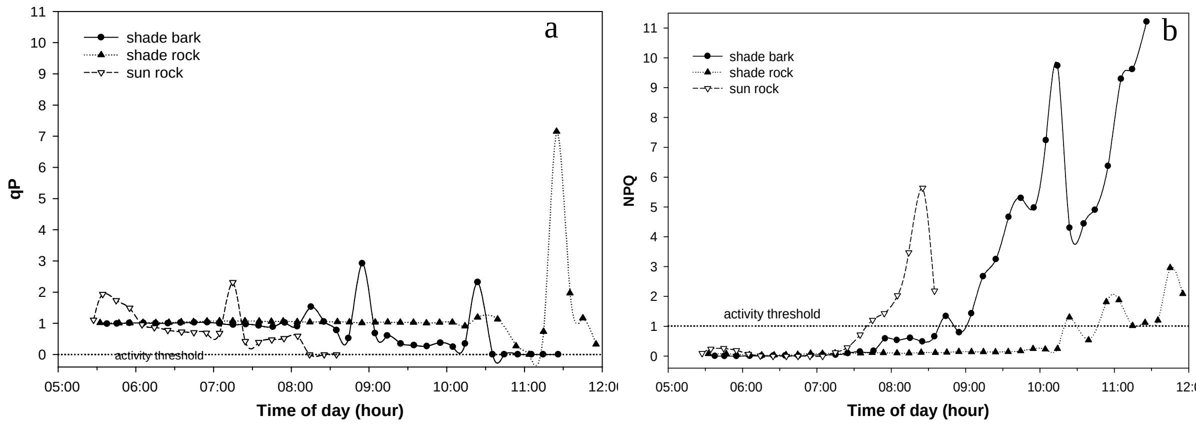

Fig. 4 a, b. a) fluorescence parameter, qP, of lichens growing on shade bark, shade rock and sun rock during 5-12 hours at KYNP. b) NPQ of lichens growing on threes substrate conditions. (8-24 June 2007)

Electron transport rates (ETR) indicated that utilization of light energy for photochemical activity varied among lichens on the three substrates (Fig. 3a). This parameter agrees with values of ΔF/Fm' and qP (Fig. 2a and 4a), which demonstrated that lichens on the shaded bark and sun rock achieved the highest photochemical activity during 7:30-8:30 hours, whereas the shade rock inhabitant continue such activity in longer period, and maximized at 11 hours. Orientations of the microhabitat were the primary cause of this variation. The dependent of ETRs on atmospheric humidity varied considerably among lichens on the three habitats (Fig. 3b). Lichen on shaded bark had the highest ETR when RH was approximately 85% and PAR was only 200 µmol $m^{-2}s^{-1}$. Those on shaded rock and sun rock achieved this peak at RH 85% and 75%, which correspond to PAR 160 and 460 µmol $m^{-2}s^{-1}$ respectively. Surprisingly, the shaded-rock lichen seems to continue electron transport at RH as low as 70 or 65 %, which rarely occur in the temperate and desert regions, where lichens suspend their metabolic activities when RH lower than 80 % [9]. Lichen on shaded rock in this study probably got evaporative water from porous rock substrate and moist soil. Therefore, they were less dependent on atmospheric humidity. Moreover, low ETR when RH were high (Fig. 3b) revealed that insufficient illumination during early morning play role in inefficient photochemistry of the lichens under such condition.

Photochemical quenching (qP) and non photochemical quenching (NPQ) (Fig. 4a, b) supported the above interpretation. The parameter qP of the lichens inhabit exposed rock, shaded bark and shade rock had qP reduced to zero indicating that all PS II centers were closed [4, 5] because lichen thalli lost almost all water. NPQ indicated higher heat loss in sun rock and shade bark than the shade rock during 8-12 hours (Fig. 3b) because energy received by chlorophyll of the previous two lichens dissipated as heat due to termination of photochemical energy conversion of PSII as thalli dried out.

In conclusion, bark and rock have different physical property; lichens growing on shade barks and sun rocks utilize low illumination during earlier morning, while RH was still high (Fig. 1). However, the bark has higher moisture content and dried out slower than the rock in the similar shaded situation. In addition the shaded rocks were protected against intense illumination by tree canopy. By contrast, sun rocks were exposed to direct solar radiation and higher temperature, which enhanced evaporation (Fig.1). These factors affect period of photochemical energy utilization, and as consequence growth of lichens. Lichens on shade rock had the potential to grow fast. A maximum growth rate of 1.5 mm/month from *P. tinctorum* grow on rock was reported [2]. While lichens transplanted thalli at KYNP on bark had the maximum growth rate of approximately 0.95 mm/month [10].

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