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The relationship between photosynthetic processes of the lichen *Parmotrema tinctorum* and rainfall at Khao Yai National Park

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Abstract. Water is one of the most essential factors for survival and growth of lichens. Their metabolisms depend on thallus water content, which rely on atmospheric humidity, rain, dew and fog. The aim of this study was to observe variations of net photosynthesis (NP), efficiency of PSII (Fv/Fm, ΦPSII and ETR) and photosynthetic pigments (Chl. a, b, a+b, a/b) of lichen *Parmotrema tinctorum* (Despr. ex Nyl.) Hale influenced by annual rainfall at Khao Yai National Park. The study was performed during September 2010-August 2011. The results showed that all physiological parameters likewise responded to rainfall. Those values were higher during rainy period (late February-mid October) and lower during dry period (late October-mid February), particularly in late drought all parameters were extremely low. However, after rain began, the values of those parameters raised even in the hot season. In addition, statistical testing illustrated that some physiological parameters had high positive correlation with rainfall and wet days. Therefore, physiological performance of this lichen closely related with rainfall regardless of seasons. Furthermore, obvious decline of NP after exposure to long dry period indicated that carbon assimilation was the most sensitive parameter to drought. Moreover, this work also demonstrated that photosynthetic related parameters of this species distinctly showed statistical significant positive correlations among each other.

Keywords: lichen, photosynthesis, chlorophyll, rainfall, Khao Yai National Park

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Introduction

Lichens depend on atmospheric water in the form of rain, dew and fog for survival and growth. Thallus water content fluctuates with air humidity, which affect their metabolism. Suboptimal thallus water content, both high and low, distinctly depressed CO_2 exchange rates in any thalli especially chlorolichens (Lange *et al*., 1993, 2007; Lange & Green, 1996). It is well-known that chlorolichens which have green algae as primary photobionts use both water vapour and liquid water to activate their metabolisms and produce positive net photosynthesis (NP). By contrast cyanolichens with cyanobacteria photobionts need only liquid water to achieve positive NP (Green *et al*., 1993, 2002; Schlensog *et al*., 2000).

On dry day, without rain, chlorolichens absorb humidity during the night and photosynthesis starts at near dawn until late morning then decline and terminate because of thallus desiccation. However, if there are rain events during the day, thallus is rewet and photosynthesis begin again (Lange *et al*., 2004). Many photosynthetic parameters from several lichens were measured to understand lichens' performances and water relation both in the field and in laboratory (Zotz *et al*., 2003; Lange *et al*., 2006; Lange & Green, 2008). However, study on seasonal rainfall and physiological responses particularly in tropical lichens are lacking. In addition, Thailand has high diversity of lichens, over 2,000 species was estimated by Lichen Herbarium (RAMK) of Ramkhamhaeng University, but their physiological processes is less known.

The epiphytic chlorolichen *Parmotrema tinctorum* (Despr. ex Nyl.) Hale is a common species in Thailand. It has high growth rate and grow luxuriantly in Khao Yai National Park (Lichen Research Unit, 2004; Wannalux *et al*., 2009). This species has been utilized to assess air quality including accumulation of air pollutants (Ohmura *et al*., 2009; Boonpeng, 2011), and to develop lichen dye (Poajaroen, 2007) and etc. In order to understand nature of this species and to increase its productivity, physiological measurements were performed throughout past few years (Boonpeng *et al*., 2011; Phaengphech & Boonpragob, 2013; Poajaroen & Boonpragob, 2013; Santanoo & Boonpragob, 2013). Notwithstanding, its physiological response to seasonal rainfall is not yet realized; hence, this study aimed to observe variations of net photosynthesis (NP), efficiency of PSII (Fv/Fm, ΦPSII and ETR) and photosynthetic pigments (Chlorophyll a, b, a+b, a/b) of the lichen *Parmotrema tinctorum* influenced by annual rainfall at Khao Yai National Park.

Materials and Methods

Study material

Thalli of epiphytic chlorolichen *Parmotrema tinctorum* with *Trebouxia* sp. photobiont, were carefully collected on barks in the UNESCO world heritage area, Khao Yai National Park located at 14˚ 25' N, 101˚ 21' E, elevation about 780 m asl. Each physiological measurement, five thalli were deliberately picked when they were dry in order to reduce physiological stresses during 6 hours of transferring to laboratory at Ramkhamhaeng University, Bangkok. Thereafter thalli were kept in controlled environment in light-dark condition of 12 hours, at room temperature of 24-28˚C, which closely represented temperature range at their natural habitat (Chayamarit, 2006). The physiological measurements were carried out on 19 September, 2 November, 5 February, 20 April and 18 August during 2010 to 2011.

CO_2 exchange measurement

Net photosynthesis (NP) of the lichen thalli was measured using open system of an Infrared Gas Analyzer (IRGA), LI-6400 (LiCor Inc., Lincoln, NE, USA), and attached to a transparent conifer chamber. Prior to the measurement, air dry thalli were weighted then placed on wet fabric and immediately sprayed with deionized water until saturated, which thallus water contents were 150-200% dry weight (DW). The samples were incubated and photosynthesis activated under PPFD (Photosynthetic Photon Flux Density) about 80 μmol $m^{-2}s^{-1}$, 24-28˚C. The thalli were sprayed with deionized water to prevent desiccation every 15 minutes for 2 hours. After that, each thallus with moisture content of about 60% DW was carefully enclosed with the conifer chamber, and exposed under LED lamp having PPFD 350 μmol $m^{-2}s^{-1}$, which is light saturation intensity of this species, and temperature 24-28˚C (Boonpeng *et al*., 2011). Air flow was adjusted to 100 μmol s^{-1} and NP was recorded after the fifth minute of measuring processes.

Chlorophyll a fluorescence measurement

Chlorophyll fluorescence parameters were accomplished by a portable modulated fluorometer (MINI-PAM, Heinz, Walz, Germany). In light-adapted, each thallus was incubated and photosynthesis activated under the same condition as gas exchange experiment. Then, actual efficiency of PSII (ΦPSII; (Fm’-Ft)/Fm’) and relative electron transport rate (ETR; ΦPSII x PAR x 0.5 x 0.84) of all thalli were measured under 350 μmol $m^{-2}s^{-1}$ of PAR (Photosynthetically Active Radiation), 24-28˚C and at optimal thallus water content of 80-120% DW (Boonpeng *et al*., 2011). Consequently, the same thalli were similarly treated in the dark for one hour. Finally, maximal efficiency of PSII (Fv/Fm; (Fm-Fo)/Fm) were measured under similar temperature and thallus water content.

Photosynthetic pigment analysis

Chlorophyll content from a total of five lichen samples from each sampling period was analysed. Each dry thallus weight 50 mg was cautiously rinsed with $CaCO_3$ saturated acetone to eliminate lichen acids for six times. Five millimeters of DMSO (dimethyl sulfoxide) containing 2.5 mg/ml of polyvinylpolypyrrolidone was then added into all sample tubes and incubated at 65˚C for 45 minutes. After that, the samples were cool in ambient temperature, subsequently 5 ml of DMSO was added (Ronen & Galun, 1984; Boonpragob, 2002). The aliquot was determined absorbance at OD 665 and

648 nm by spectrophotometer (Shimadzu UV-VIS, UV-160A). Lastly, pigment contents included chlorophyll a (Chl. a), chlorophyll b (Chl. b), total chlorophyll (Chl. a+b) and ratio of chlorophyll a/b (Chl. a/b) were calculated according to Barnes *et al*. (1992) and Boonpragob (2002). Importantly, the extraction process was performed in dark condition to prevent chlorophyll degradation in light condition.

Meteorological data

Daily rainfall during September 2010 to August 2011 in the study area obtained from Khao Yai National Park Meteorological Station (Code Station: 431031, Department of Meteorology of Thailand) was used. This station is located only 3 km from the sampling point. Unfortunately, this station did not provide reasonably variable daily relative humidity and temperature. However, representative seasonal temperature and relative humidity could be retrieved from long term study on microclimate at different forest types at Khao Yai National Park monitored by Lichen Research Unit, Ramkhamhaeng University.

Statistical analysis

All statistical analyses and graphs were performed using SigmaStat V. 3.5 and SigmaPlot V. 10.0 computer software. Pearson Product Moment Correlation or Pearson's Correlation (*r*) was used to test correlation among the physiological parameters and daily rainfall, and Linear Regression (r^2) was also employed to find the relationships of those parameters.

RESULTS

Rainfall

Annual rainfall and wet days or rainy days during the experimental period (September 2010-August 2011) in the study site at Khao Yai National Park was 2,580 mm/year, occurred in 145 days.

Rainy event could be distinctly separated into two periods. 1) Wet period, started from early September to mid October 2010 (1 September-20 October) and late February 2011 to late August 2011 (21 February-31 August), covering 242 days. It sometimes rained during early hot season (late February to early April) and predominant rain came later. There were 139 wet days (57%) and 2,531.8 mm for rainfall. 2) Dry period, lasted from late October 2010 (21 October) to mid February 2011 (20 February), covering 123 days. This period almost devoid of rain. Light rain occurred only in 6 days (5%) with 48.2 mm of rainfall. As shows in Fig. 1a.

Physiological responses

All physiological parameters of the lichen *P. tinctorum* responded to rainfall. The values were higher during wet period and lower during dry period, as show in Fig. 1b, c.

Net photosynthesis (NP) varied year round between 5.8 to 27.8 nmol CO_2 $g_{dw}^{-1}s^{-1}$. The highest value was measured in 20 April, the forth sampling period, after rain started, while the lowest value was observed during late dry period on 5 February. NP of the lichen at the beginning of the experiment, September 2010, was 21.5 nmol CO_2 $g_{dw}^{-1}s^{-1}$. It was almost the end of wet period in 2010. The NP values were subsequently declined to extremely low during the next sampling periods when dry season prevail. Nevertheless, NP substantially increased again after the first rain in early hot season, and resumed to normal at the last measurement which was wet period in 2011 (Fig. 1b).

Chlorophyll a fluorescence parameters included maximal efficiency of PSII (Fv/Fm), actual efficiency of PSII (ΦPSII) and relative electron transport rate (ETR) responded to rain in similar pattern as NP. Values of Fv/Fm, ΦPSII and ETR were 0.52-0.69, 0.25-0.37 and 37-55, respectively (Fig. 1b).

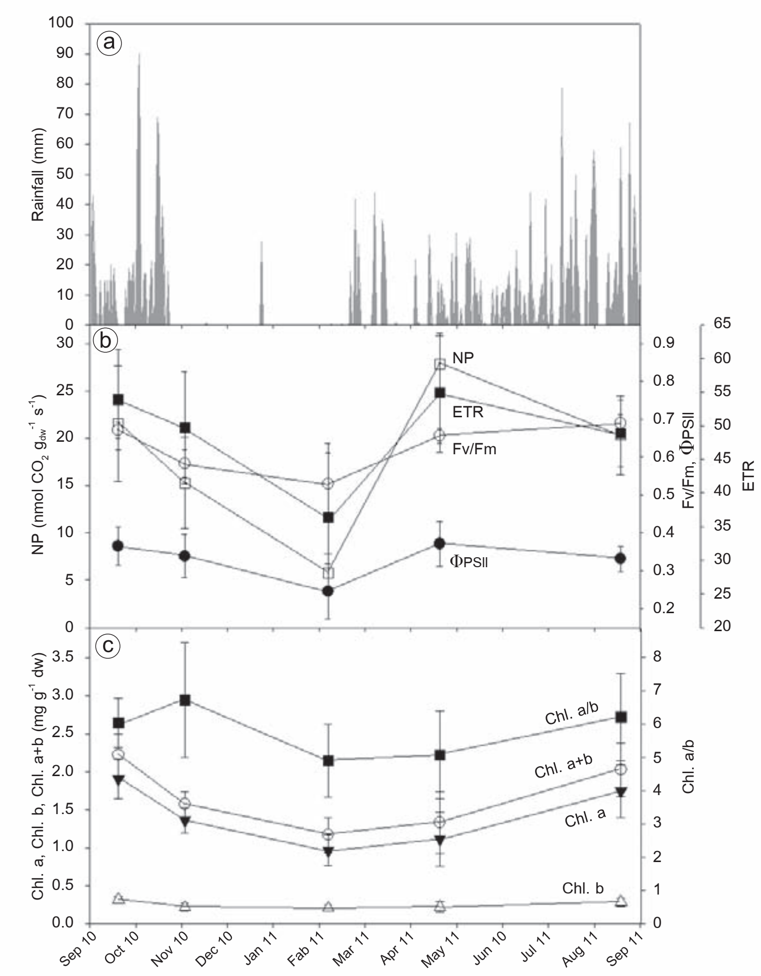

FIGURE 1. Variations of means and standard deviations (n=5) of photosynthetic related parameters of *Parmotrema tinctorum* against rainfall at Khao Yai National Park from September 2010 to August 2011. a) daily rainfall (mm); b) net photosynthesis (NP, open square), relative electron transport rate (ETR, close square), maximal efficiency of PSII (Fv/Fm, open circle) and actual efficiency of PSII (ΦPSII, close circle); c) photosynthetic pigments (mg g^{-1} dw), Chl. a (close triangle), Chl. b (open triangle), Chl. a+b (open circle) and Chl. a/b (close square).

Photosynthetic pigments consisted of chlorophyll a (Chl. a), chlorophyll b (Chl. b), total chlorophyll, of which Chl. a+b indicate vitality of light harvesting complex (LHC), had identical variations as NP. However, ratio of chlorophyll a/b, which indicates vitality of reaction centre (RC) was slightly differed. Unlike other parameters, it did not decline in the second sampling period, but higher value was detected. The amount of Chl. a, Chl. b and total Chl. were 0.97-1.9, 0.20-0.32 and 1.2-2.2 mg g^{-1} dw, respectively, while Chl. a/b was 4.9-6.7 (Fig. 1c).

DISCUSSION AND CONCLUSION

Exposure to extremely low water availability during long drought from late October to mid February caused severe decline of all photosynthetic related parameters of *P. tinctorum.* NP was the most affected, it tremendously declined about 79% comparing with the highest value. Similar effect was reported by Poajaroen & Boonpragob (2013) which found that this lichen from several forest types at Khao Yai National Park had the lowest NP during cool dry season. Ra *et al*. (2005) discovered that two chlorolichens *Parmelia sulcata* and *Platismatia glauca* from HJ Andrews (U.S. Pacific Northwest) obviously demonstrated lower net carbon uptake during summer, which had relatively lower precipitation than winter. Drought or drier condition also damages photosynthetic apparatus, but the impact seemed to be lesser than NP. In addition, assimilation pigments, Chl. a, Chl. b, total Chl. and Chl. a/b decreased about 49, 38, 45 and 27%, respectively during dry period, while efficiency of PSII, Fv/Fm, ΦPSII and ETR dropped about 25, 32 and 33%, respectively. Accordingly, Pirintsos *et al*. (2011) reported that lower Chl. a, Chl. b, Chl. a+b and Fv/Fm were found in chlorolichens *Evernia prunastri* and *Pseudevernia furfuracea* during drier period. Furthermore, similar result was found in *E. prunastri*, *Flavoparmelia caperata*, *Xanthoria parietina*, *Punctelia subrudecta* by Paoli *et al*. (2010) and Baruffo & Tretiach (2007).

Notably, the photosynthetic parameters markedly increased after rain resumed in early hot season particularly NP, and then gradually approached to their normal ranges in rainy season which came later (Fig. 1b, c). This relationship was significant according to Pearson's correlation statistical analysis in Table 1. Some parameters included Fv/Fm (r=0.99, p<0.01), Chl. a (r=0.92, p<0.05) and total Chl. (r=0.91, p<0.05) had high positive correlation with rainfall and wet days. Although, many parameters did not show statistical significant correlations with rainfall and wet days, nevertheless they had relatively high correlation coefficient (r) according to Table 1. In the future, increasing sample size (n) is required to elucidate this issue. The NP magnificently raised after received the first rainfall in hot season. Similar pattern is observed in *Usnea undulata*, but different from *Cladonia submultiformis*, *Relicina abstrusa* and *Relicinopsis intertexta* which have the peak NP in rainy season at the same study location as this one (Poajaroen & Boonpragob, 2013). This implied that photosynthetic mechanism of *P. tinctorum* could be recovered from dormant state during long dry period faster than other common species, *C. submultiformis*, *R. abstrusa* and *R. intertexta* at Khao Yai National Park. As such, it is reasonable to assume that rapid recovery in photosynthetic capacity of the lichen *P. tinctorum* after prolong drought enable this species to thrive and distribute abundantly at Khao Yai National Park and other ecosystems throughout the country. Other studies in this aspect are less known, nevertheless studies of Baruffo & Tretiach (2007), Paoli *et al*. (2010) and Pirintsos *et al*. (2011) strongly support our results.

Interestingly, ratio of Chl. a/b which usually employs to indicate vitalities of reaction centres (RCs) clearly increased on the second sampling period (2 November), different from other parameters (Fig. 1c). This caused by substantial decreasing of Chl. b at this time. It was possibly the combination of high temperature (~22.6˚C) and early drought. This evidence was supported by Pisani *et al*. (2007) who reported that Chl. b was more sensitive to temperature than Chl. a. Similar result was also found in epiphytic lichens grew in the Mediterranean area (Pirintsos *et al*., 2011).

Moreover, most photosynthetic parameters had high statistical significant positive correlation among each other (Table 1). The NP value noticeably related to all parameters except Chl. b (Table 1, Fig. 2a, b). Notwithstanding, a recent study clearly demonstrates strong positive correlation with NP (Boonpeng, 2011). However, more experiment on Chl. b sensitivity in the tropic was really required to verify this result.

In conclusion, the lichen *P. tinctorum* grew in the dry deciduous forest at Khao Yai National Park revealed clear trends of photosynthetic pattern. Its physiological performance, especially photosynthetic related parameters, closely related to rainfall. They declined during dry period especially at the end of drought, and resumed during wet period regardless of seasons. More

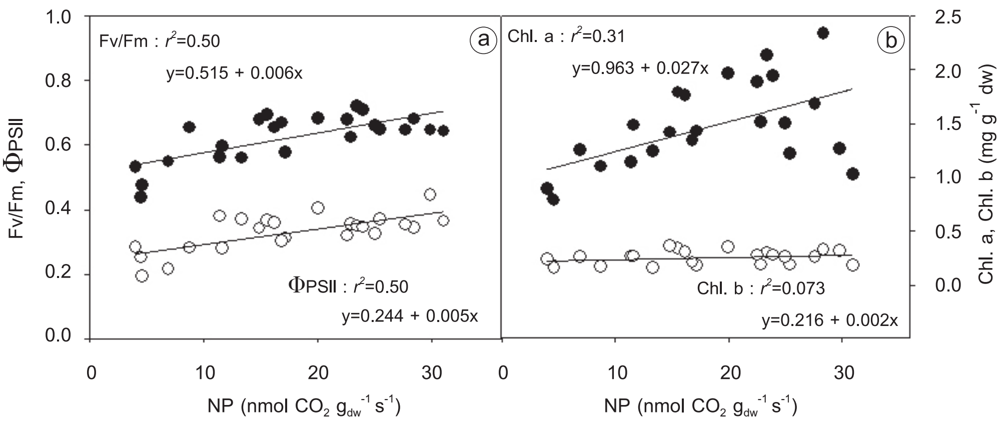

FIGURE 2. Linear relationships (r^2) among photosynthetic processes of *Parmotrema tinctorum*. a) net photosynthesis (NP) versus maximal efficiency of PSII (Fv/Fm, close circle) and actual efficiency of PSII (ΦPSII, open circle); b) NP against Chl. a (close circle) and Chl. b (open circle).

importantly, NP was the most sensitive photosynthetic parameter to drought followed by photosynthetic pigments and efficiency of PSII. Lastly and more importantly, all photosynthetic related parameters markedly illustrated statistical significant correlations amongst each other.

TABLE 1. Pearson's correlations (*r*) amongst photosynthetic parameters (n=24) of *Parmotrema tinctorum*, rainfall and wet days (n=5, amounts of rainfall and wet days were calculated during 15 days before each physiological measurement).

	Fv/Fm	ΦPSII	ETR	Chl. a	Chl. b	Chl. a+b	Chl. a/b	Rainfall	Wet Days
NP	0.71***	0.70***	0.70***	0.56**	0.27	0.54**	0.41*	0.63	0.79
Fv/Fm		0.60**	0.60**	0.77***	0.56**	0.77***	0.35	0.82	0.99**
ΦPSII			1.00***	0.43*	0.44*	0.45*	0.13	0.78	0.75
ETR				0.42*	0.44*	0.44*	0.12	0.77	0.75
Chl. a					0.66***	0.99***	0.46*	0.92*	0.82
Chl. b						0.74***	-0.33	0.80	0.84
Chl. a+b							0.37	0.91*	0.82
Chl. a/b								0.77	0.38
Rainfall									0.86

* Statistical significance of correlation at $p<0.05$, ** Statistical significance of correlation at $p<0.01$, *** Statistical significance of correlation at $p<0.001$

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