H1_224_PF: SOIL WATERING, FERTILIZER AND PLANT HORMONE ENHANCE EFFICIENCY OF PHOTOSYSTEM II OF THE TRANSPLANTED LICHEN *Parmotrema tinctorum* (DESPR. EX NYL.) HALE

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Abstract: This experiment examined the effect of fertilizer and plant hormone (FH) upon soil watering (W) on chlorophyll fluorescence of the lichen *Parmotrema tinctorum*. The experiment was conducted in the secondary forest at Khao Yai National Park by transplanting 640 fragmented thalli on polyethylene net oriented toward the East. They were exposed to four treatments: 1) control-dry soil (without soil watering, without FH), D0 2) dry soil with FH, DFH 3) soil watering without FH, W0 and 4) soil watering with FH, WFH. After a year, chlorophyll fluorescence parameters, Fv/Fm, Φ_{PSII} and ETR, indicated that lichen receiving WFH had the highest values of Φ_{PSII} measured 0.373 and ETR 55.1 µmol $e^{-}m^{-2}s^{-1}$; whilst, DFH had the highest values of Fv/Fm noted for 0.691. However, the lowest Fv/Fm, Φ_{PSII} and ETR were observed from lichen receiving W0 measured 0.656, 0.324 and 47.8 µmol $e^{-}m^{-2}s^{-1}$, respectively. Therefore, fertilizer and plant hormone enhanced the efficiency of photosystems II of lichen greater than soil watering alone. However, appropriate supply of moisture, nutrient, and other growth factors in order to maximize lichen production under natural habitat need further studies.

Introduction: The lichen *Parmotrema tinctorum* has been used successfully for silk dying and air quality monitoring in Thailand. In addition, natural products of this lichen have potential to be utilized for various purposes. Therefore, it is essential to enhance growth of this lichen for sustainable utilization. Naturally, lichens have extremely low growth rate comparing with vascular plants. This is because they depend on atmospheric moisture for carbon assimilation only in early morning. Their photosynthetic process terminates soon after thalli dry out before mid-morning when relative humidity is low. Previous studies reveal that growth of *P. tinctorum* increases upon receiving extra moisture evaporated from soil watering.[1] Fertilizer and hormone stimulate growth of lichens.[2,3,4,5] This study aims to enhance photosynthetic capacity of the lichen *P. tinctorum* through soil watering and application of fertilizer and growth hormone. Chlorophyll fluorescence parameters, Fv/Fm, Φ_{PSII} and ETR were used to investigate the efficiency of photochemistry or photosynthetic performance of the lichen.

Methodology: ***Transplantation site:*** Secondary forest, previously a tropical rain forest at Khao Yai National Park (KYPN), Thailand was used as an experiment ground.

Transplanted method and sample collection: Thalli of *P. tinctorum* grew at secondary forest were collected. Only healthy thalli were selected for transplantation. The transplanted lichen consisted of 3-4 cm^2 fragmented thalli, including 3-4 lobe margins. A thallus fragment was tied on 5x5 cm black polyethylene net mesh size 2x2 mm by 0.5 mm monofilament fishing line (Figure 1), thereafter referred to as transplanted thalli. Forty transplanted thalli were fixed on a transplanted frame making up of PVC pipe lining with black polyethylene net. They stand on the ground at 45-degree inclination facing the East under 50% black shading net (Figure 1).

Experimental design: 2x2 factorial design composed of 2 main factors: 1) Water; the treatment including no soil watering or dry soil (D) and soil watering or wet soil (W). Soil watering was practiced by spraying water on the ground under the transplanted frames during 9:00 hr. to prolong soil evaporative moisture. This application extend thallus moistening which enhance photosynthetic activity, and 2) Fertilizer and plant hormone (FH); composing of a mixture of 10% Hoagland solution and 2 µmol/l IBA. The application of FH was performed by spraying about 5 ml of the mixture directly over the transplanted thalli on both D and W soils every 15 days. Therefore, the treatments were as follow: 1) D0 (control)-dry soil without FH, 2) DFH-dry soil with FH, 3) W0-wet soil without FH and 4) WFH-wet soil with FH.

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These treatments were organized on sixteen transplanted frames, which divided into each 8 wet and dry soil treatments. A transplanted frame had 8x5 thalli (column x row), of which each column alternately received F0 and FH treatments, making up (8x2) x (4x5)2 = 640 thalli. In addition, two thalli were transplanted to each 16 transplanted frames for measuring of initial physiological parameters. They make up a total of 672 transplanted thalli altogether. A single thallus was collected from each transplanted frame for measuring chlorophyll fluorescence every two months from August 2015 to August 2016.

Chlorophyll fluorescence measurement: Eight thalli of each four treatments (D0, DFH, W0 and WFH) were measured for Fv/Fm, Φ_{PSII} and ETR under laboratory condition following Boongpeng *et al*. (2014)[6] by mini-PAM (Walz inc., Germany) at Ramkhamhaeng University. The parameter Fv/Fm indicates the maximum quantum efficiency of photosystem II (PSII) or photosynthetic capacity providing information of lichen health. The value Φ_{PSII} indicated the capacity of (PSII) during instant CO_2 fixation, and ETR reveals electron transport rate.[7]

Microclimate: Light intensity, air temperature, and relative humidity at transplantation field were recorded by CR10x datalogger (Campbell Scientific, Inc. USA) every months during August 2015 to August 2016.

Data analysis: Two-way ANOVA was used to analyze the differences of chlorophyll fluorescence parameters every two months from the four treatments (D0, DFH, W0 and WFH). The differences between the four treatments were examined by one-way ANOVA, and all pairwise multiple comparison verified by Tukey Test ($P<0.05$).

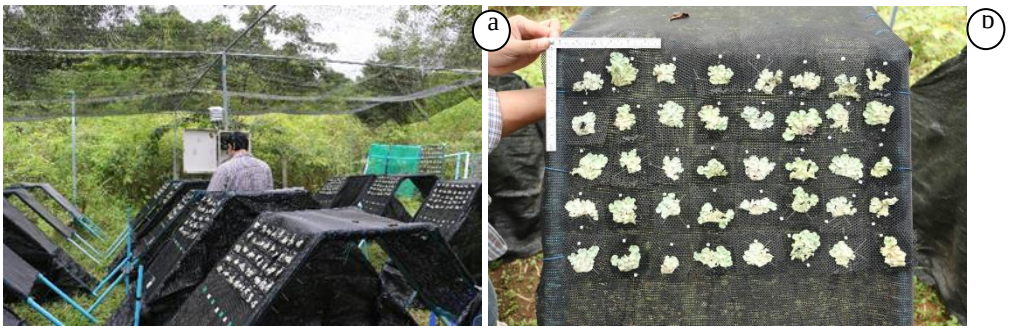

Figure 1. (a) Transplantation field of the lichen *P. tinctorum* at KYNP, (b) the transplanted thalli on black polyethylene nets fixed on transplanted frame.

Results and Discussion: All chlorophyll fluorescence parameters, Fv/Fm, Φ_{PSII} and ETR, exhibited annual variations. Illumination, temperature, and relative humidity fluctuated throughout the observation period, unparalleled with fluorescence parameters (Figure 2a-d).

It revealed multifaceted roles of microclimate on physiological performance of lichens through the interaction of all factors beyond an influence of a single factor alone as commonly perceive. And, this interaction need further intensive studies.

Influence of soil watering: The quantum efficiency, Fv/Fm, from two to four months after transplantation and annual average differed significantly ($p<0.05$). By contrast Φ_{PSII} and ETR of lichen from the dry soil was significantly higher than those of the wet soil (Table 1 and Figure 2a-c). However, these mean values from the dry soil were slightly higher than those of the wet soil treatment. It implied that soil watering alone during 9 hr. might exert negative effect on photosynthetic process of the lichen.

Influence of fertilizer and growth hormone: Table 1 indicated that Fv/Fm, Φ_{PSII} and ETR of the FH treatments were significantly higher than those without FH treatment ($p<0.001$) (Figure 2a-c). It indicated that an application of the mixture of fertilizer-growth hormone could enhance photosynthetic capacity of the lichen.

Interaction between soil watering and a mixture of fertilizer-growth hormone: The parameters Φ_{PSII} and ETR (Table 1) indicated that soil watering together with FH increased photochemical activity of the lichen. While, Fv/Fm were not affected by the combination of these factors, which revealed that water supply did not enhance quantum yield of PSII.

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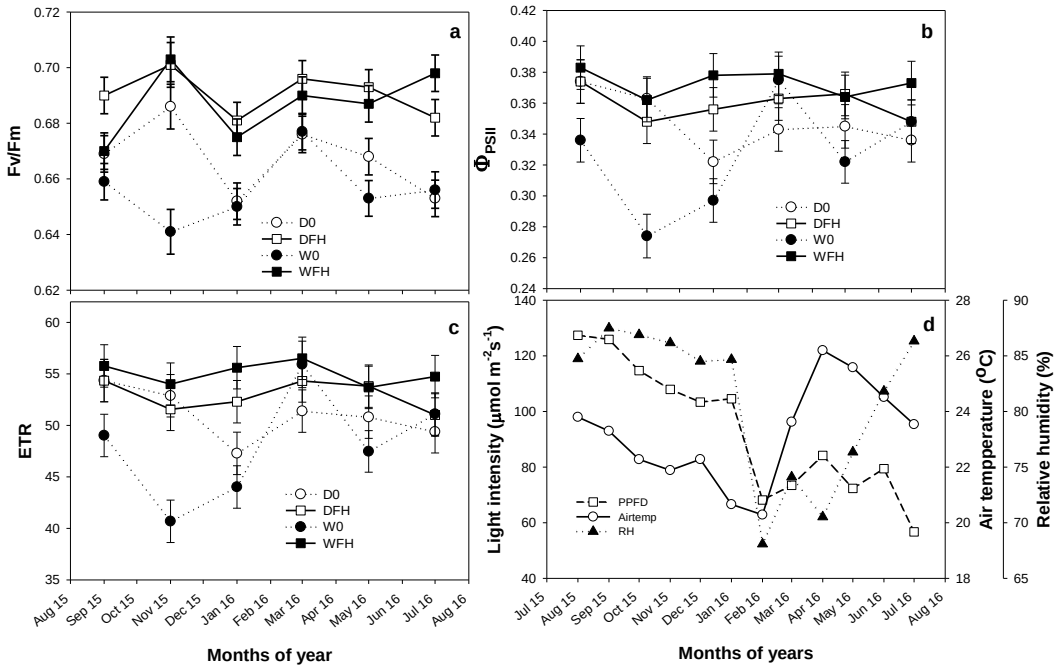

Figure 2. Chlorophyll fluorescence parameters of the transplanted lichen *P. tinctorum* at KYNP under soil watering and fertilizer-plant hormone treatments. Mean values (n=8) of Fv/Fm (a), Φ_{PSII} (b) and ETR (c) The four treatments were D0=control-dry soil without FH (*open circles*), DFH=dry soil with FH (*open squares*), W0=soil watering without FH (*closed circles*), and WFH=wet soil watering with FH (*closed squares*). (d) Microclimates including light intensity (PPFD; *open squares*), air temperature (Airtemp; *open circles*) and relative humidity (RH; *closed triangles*) recorded every month from August 2015 to August 2016.

Groups treatment analysis (D0, DFH, W0 and WFH): Table 1 revealed that DFH lichens had the highest average Fv/Fm value and subsequently lower in WFH, D0 and W0 thalli ($p<0.001$). Whilst the Φ_{PSII} and ETR had the highest average in WFH and consequently lower in DFH, D0 and W0 ($p<0.001$). Interestingly, Fv/Fm, Φ_{PSII} and ETR values between DFH and WFH were not different statistically, while D0 and W0 show significant difference between groups ($p<0.001$). This evident indicated that quantum yield of lichen were affected by the mixture of fertilizer-growth hormone greater than soil watering. However, effects on growth rate will be presented in the upcoming publication with in-depth information.

This results indicated that application of the mixture of fertilizer-plant hormone to transplanted lichen enhance the efficiency of photosystems II in *P. tinctorum*. This effect is supported by Welch *et al.* (2006)[2] and Wang *et al.* (2010)[4] who reported that photosynthesis and growth of lichen is induced by fertilizer and IBA. However, all chlorophyll fluorescence parameters under W0 had the lowest values, which suggested that other factors associated with soil watering could deteriorate PSII capacity. Nevertheless, an application of the mixture of fertilizer-plant hormone could remedy the damaging effects of wet soil alone.

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Table 1. Statistical analysis of chlorophyll fluorescence parameters, Fv/Fm, Φ_{PSII} and ETR, of the transplanted lichen *P. tinctorum* at KYNP under four treatments; D0=control-dry soil without FH, DFH=dry soil with FH, W0=soil watering without FH, and WFH=wet soil watering with FH. The group treatments were compared by one-way ANOVA followed by a Tukey's post hoc test, and different letters ($^{a, b, c}$) indicate statistical differences at p<0.05. Two-way ANOVA was used to check the overall differences of the two main effects; 1) soil watering and 2) Fertilizer and plant hormone.

Chlorophyll fluorescence Parameter	Month	Dry soil		Wet soil		group treatments (*F values*)	ANOVA effects; *F values*		
		D0	DFH	W0	WFH		Soil watering	FH treatment	Interaction (Water x FH)
Fv/Fm	1 Aug 15	0.712 (initial, n=16)							
	1 Oct 15	0.669^{ab}	**0.690^{a}**	0.659^{b}	0.670^{b}	4.730**	6.535*	6.937**	0.718
	1 Dec 15	0.686^{a}	0.701^{a}	0.641^{b}	**0.703^{a}**	16.086***	6.265*	20.364***	7.411*
	1 Feb 16	0.652^{bc}	**0.681^{a}**	0.650^{c}	0.675^{b}	5.317**	0.389	15.509***	0.055
	1 Apr 16	0.676^{b}	**0.696^{a}**	0.677^{b}	0.690^{a}	2.904*	0.272	8.009**	0.367
	1 Jun 16	0.668^{bc}	**0.693^{a}**	0.653^{c}	0.687^{b}	10.967***	3.638	28.010***	0.731
	1 Aug 16	0.653^{b}	0.682^{ab}	0.656^{b}	**0.698^{a}**	6.611***	1.370	17.836***	0.627
	Average	0.667^{b}	**0.691^{a}**	0.656^{b}	0.687^{a}	31.597***	5.608*	84.755***	1.066
Φ_{PSII}	1 Aug 15	0.348 (initial, n=16)							
	1 Oct 15	0.374	0.374	0.336	**0.383**	2.286	1.042	2.950	2.867
	1 Dec 15	**0.363^{a}**	0.348^{a}	0.274^{b}	0.362^{a}	7.778***	2.593	2.401	4.910*
	1 Feb 16	0.322^{b}	0.356^{a}	0.297^{b}	**0.378^{a}**	5.395**	0.010	13.945***	2.231
	1 Apr 16	0.343	0.365	0.375	**0.379**	1.568	3.316	0.847	0.421
	1 Jun 16	0.349	**0.366**	0.322	0.364	2.452	0.893	5.765*	0.596
	1 Aug 16	0.336	0.348	0.348	**0.373**	1.329	1.903	1.877	0.208
	Average	0.347^{b}	0.359^{ab}	0.324^{c}	**0.373^{a}**	12.448***	0.597	27.226***	9.757**
ETR	1 Aug 15	50.4 (initial, n=16)							
	1 Oct 15	54.4	54.3	49.0	**55.8**	2.243	0.966	2.867	2.896
	1 Dec 15	52.9^{a}	51.6^{a}	40.7^{b}	**54.0^{a}**	7.430***	1.916	2.902	4.321*
	1 Feb 16	47.3^{b}	52.3^{ab}	44.0^{b}	**55.6^{a}**	5.304**	0.001	13.754***	2.158
	1 Apr 16	51.4	54.3	55.9	**56.5**	1.450	3.040	0.829	0.364
	1 Jun 16	50.8	**53.8**	47.5	53.7	2.380	0.784	5.582*	0.675
	1 Aug 16	49.4	51.0	51.1	**54.7**	1.276	1.848	1.726	0.254
	Average	51.7^{b}	52.9^{ab}	47.8^{c}	**55.1^{a}**	12.664***	0.368	28.087***	9.763**

Significant level *p<0.05, **p<0.01, *** p<0.001. Bold texts are maximum and underline texts are minimum values.

Conclusion: Obviously, an application of the mixture of fertilizer-plant hormone to transplanted lichen could enhance the capacity of photosystems II in *P. tinctorum* through an increasing of Fv/Fm, Φ_{PSII} and ETR values. Despite the fact that these parameters declined by soil watering only. This information expressed the importance of factor interaction between moisture and nutrient under natural habitat that govern lichen production.

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