

Physiological Character of *Merremia peltata* (L.) Merrill from Bukit Barisan Selatan National Park Lampung

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ABSTRACT

Merremia peltata invaded the vegetation of Bukit Barisan Selatan National Park (BBSNP) in Lampung Province, Indonesia. In this site, the invasion has reached more than 7000 hectares. Therefore, it is necessary to assess the physiological characters of *M. peltata* as an invasive plant species under different shading level in BBSNP. The experiment design of this study was split block design with factorial. The first factor (main plot) was shading treatment that consisted of 0, 55, and 90% shades. The second factor (sub plot) was 3 species of plant that consisted of *M. peltata*, *Mikania micrantha*, and *Ipomoea batatas*. Each experimental unit was replicated 3 times. The result showed that the physiological characters such as carbon fixation rate (CFR), relative growth rate (RGR), leaf area ratio (LAR), photosynthetic nitrogen use efficiency (PNUE) of *M. peltata* higher than the other two species and interconnected. Physiological characters associated with RGR can be supporting the invasiveness of *M. peltata*, especially in BBSNP. Supporting invasiveness with resources allocation to leaves biosynthesis and relatively efficient nitrogen were used for photosynthesis.

1. Introduction

Merremia peltata (Convolvulaceae) locally known as *mantangan* invaded Bukit Barisan Selatan National Park (BBSNP), Lampung Province, Indonesia covering an area more than 7000 ha. This vast invasion impacted BBSNP considerably hindering natural regeneration (Master *et al.* 2013) and driving elephants to migrate northward creating conflict of interest with human settlement (Irianto and Tjitrosoedirdjo 2010). Some physiological characters of *M. peltata* that may support its invasiveness compared with *Mikania micrantha*, a species with known physiological characters admitted as being invasive (Deng *et al.* 2004) and a non invasive crop, *Ipomoea batatas*. Knowledge and understanding of plant invasion mechanism are an important information for managing invasive plant species. In most cases of plant invasions were usually associated with lack of natural enemies of the invading species, although *M. peltata* is implicated as a native species to Indonesia, so far no natural enemies being reported attacking the plant. Irianto and Tjitrosoedirdjo (2010) speculated that the invasiveness of *M. peltata* may be attributed to changes in its environment into a more open one,

facilitating a greater availability of light and therefore supporting its fast growth. The domination of invasive plants is usually indicated by fast growing, expansive dispersion, and efficient resources use (Matzek 2011). The invasive nature of *M. peltata* is hypothesized due to some physiological characteristics. In order to identify the *M. peltata*'s physiological characteristics some of its physiological characters were compared with *M. micrantha* which was known as an invasive plants (Deng *et al.* 2004) and *I. batatas* which belongs to non-invasive cultivated plants (Tjitrosoedirdjo 2010). The area was known as a degraded forest leaving only a meager trees (Master *et al.* 2013).

The hypothesis of this study was the *M. peltata* growth which was thought as an invasive species affected by shade. Therefore, the objective of the research was physiological characters of *M. peltata* from BBSNP as an invasive plant species under different shading level which support them to invade a certain ecosystem.

2. Materials and Methods

2.1. Materials

M. peltata (from BBSNP in Pemerihan Village, West Lampung Regency), *M. micrantha*, and *I. batatas* were used in this research. Soil and compost manures fertilizer with 3:2 (w/w) were used as planting media.

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2.2. Experimental Design

This research was carried out at the green house and field experiment. Experiment design was a split block design with factorials. The first factor (main plot) was shading treatment by using shade net of 0, 55, and 90% shades. The second factor (sub plot) was 3 species of plant i.e. *M. peltata*, *M. micrantha*, and *I. batatas*). Each experimental unit was replicated 3 times.

Observed variables were carbon fixation rate (CFR), relative growth rate (RGR), leaf weight ratio (LWR), specific leaf area (SLA), leaf area ratio (LAR), net assimilation rate (NAR), leaf nitrogen, and photosynthetic nitrogen use efficiency (PNUE).

2.3. Plant Propagation

M. peltata was tried to be grown using stem cutting, but failed to grow and subsequently *M. peltata* was grown from seeds. Seeds were separated from the fruit capsules (brown, dry, chapped). The good quality of seeds (not rotten) were germinated in soil from BBSNP. Seeds were planted in the planting medium and covered with soil. Two weeks old seedlings were transferred into transparent plastic that containing soil from BBSNP. Seedlings of *M. peltata* were transferred to polybag planting medium. The thirty centimeters height seedlings were planted in planting media. The experiment was conducted using polybag (black colour and in size 25 cm x 25 cm), with 25 cm diameter and 20 cm height of planting medium with Cikabayan soil (were dug to 20 cm depth).

M. micrantha were propagated by using stem cuttings. The stem cuttings were used in 15 cm length and consisting of 3 leaves. Then, seedlings was adapted until 3 weeks period in the planting medium.

I. batatas were propagated by using stem cutting. The stem cuttings were used in 30 cm of length. These seedlings were planted by immersing 3-4 segments to the planting medium (3:2 w/w). Then, the seedlings was adapted until 3 weeks period in planting medium.

2.4. Measuring of CFR

The CFR was measured by using portable photosynthesis system (Li-qor 6400 xt). Measurement was used Photosynthetic Active Radiation (PAR) of 2000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. Measurement was performed on the full width of the leaf (in the position of fifth to seventh leaves from the tip of the stem).

2.5. Plant Growth Analysis

Plants were harvested on 0 days after treatment and 90 days after treatment. Dry biomass of leaves, roots, stems and tubers were oven dried at 80°C until dry weight constant. Leaf area was determined by gravimetric method. Parameters of growth analysis observed were: $\text{RGR} = \ln W_1 - \ln W_0 / (T_1 - T_0)$; $\text{LWR} =$

LW / DM ; $\text{SLA} = \text{LA} / \text{LW}$; $\text{LAR} = \text{LWR} \times \text{SLA}$; and $\text{NAR} = \text{RGR} / \text{LAR}$ in this research. Where, W is the dry weight of plants, T is time, LW is leaf dry weight, DM is dry weight of plant, and LA is the leaf area (Rodosevich *et al.* 1997). Nitrogen content was analyzed by using Kjeldahl method. Nitrogen was measured as nitrogen content per leaf area unit. PNUE was ratio between CFR with leaf nitrogen content.

2.6. Data Analysis

Data were analysed using analysis of Variance (ANOVA) and followed by Duncan's Multiple Range Test (DMRT) with confidence test level of 95%. The relationship among variables was tested by using correlation (Pearson correlation = r). The grouping (based on the degree of similarity) of *M. peltata* with *M. micrantha* and *I. batatas* was tested by cluster analysis. Data analysis was processed by using SPSS v 16.0 program.

3. Results

3.1. Carbon Fixation Rate

Shading intracts with plant species significantly in affecting Carbon Fixation Rates (CFR). CFR of *M. peltata* (19.0 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) lower than CFR of *M. micrantha* (25.2 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), however, it was still the higher than CFR of *I. batatas* (Table 1). The reduction CFR of *M. micrantha* was about 14.7% under 55% shade, and down to 55.5% under 90% shade, while that of *M. peltata* was 23.8% and 43.2% respectively. That of *I. batatas* was 19.0% and 81.0% respectively. Shading of 90% intensity created low light condition leading to plant death.

3.2. Plant Growth

All plant growth variables were showed significantly correlation (Table 2). The correlation between RGR with NAR ($r = 0.711$, $P < 0.05$), RGR with LAR ($r = -0.229$, $P > 0.05$), LAR with LWR ($r = 0.701$, $P < 0.05$), LAR with SLA ($r = 0.674$, $P < 0.05$), RGR with leaf nitrogen content ($r = 0.733$, $P < 0.05$), and RGR with PNUE ($r = 0.946$, $P < 0.05$) were significantly different. These results significantly affected to plant growth.

Relative growth rate of *M. peltata* is slightly lower than that of *M. micrantha* but higher than that of *I. batatas* (Table 3). $\text{RGR}_{M.peltata} = 12 = 9.0 \times 1.4 < \text{RGR}_{M.micrantha} = 13 = 6.4 \times 2.1$. $\text{LAR}_{M.peltata}$ (under 0% shade, 9 cm^2g^{-1}) contributed more to the RGR of *M. peltata* if compare to $\text{LAR}_{M.micrantha}$ (under 0% shade, 6.4 cm^2g^{-1}). On the other side, *M. micrantha* was contributed more by $\text{NAR}_{M.micrantha}$ (2.1 $\text{mg cm}^{-2} \text{ day}^{-1}$) if compare to $\text{NAR}_{M.peltata}$ (Table 3).

M. micrantha reached high content of leaf nitrogen and PNUE especially in shade net of 0% and 55% (Table 4). Leaf nitrogen content of *M. micrantha* was the highest in 90% shade net (118.0 g m^{-2}), but PNUE

Table 1. CFR of *M. peltata*, *M. micrantha*, and *I. batatas* in shade net level treatments

Shade Net Treatment	Plant Species	CFR ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$)
Shade net 0%	<i>M. peltata</i>	19.0 ^c
	<i>M. micrantha</i>	25.2 ^a
	<i>I. batatas</i>	14.7 ^d
Shade net 55%	<i>M. peltata</i>	14.5 ^d
	<i>M. micrantha</i>	21.5 ^b
	<i>I. batatas</i>	11.9 ^e
Shade net 55%	<i>M. peltata</i>	10.8 ^e
	<i>M. micrantha</i>	11.2 ^e
	<i>I. batatas</i>	2.8 ^f

Data with the same letter in the same column is not differ significantly at $\alpha=0.05$ (DMRT test)

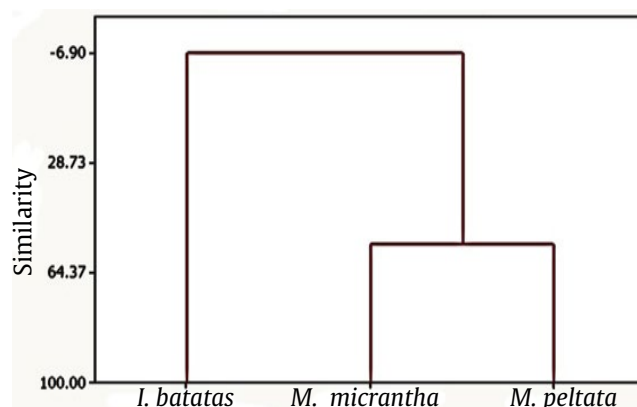


Figure 1. Dendrogram of the degree of similarity between *M. peltata*, *M. micrantha*, and *I. batatas*

Table 2. Correlation of carbon fixation rate and plant growth variables

	CFR	RGR	LWR	SLA	LAR	NAR	Leaves nitrogen	PNUE
CFR	1							
RGR	0.856**	1						
LWR	0.211	0.388*	1					
SLA	-0.823**	-0.725**	-0.033	1				
LAR	-0.442*	-0.229	0.701**	0.674*	1			
NAR	0.763*	0.711**	-0.322	-0.776**	-0.779**	1		
Leaf nitrogen	0.954**	0.733**	0.192	-0.869**	-0.489**	0.698**	1	
PNUE	0.875**	0.946**	0.298	-0.775**	-0.310	0.712**	0.744**	1

Note : * = significant at $\alpha=0.05$, ** = significant at $\alpha=0.01$

Table 3. Plant growth analysis in shade net level and plant species treatments

Shade Net Treatment	Plant Species	RGR ($\text{mg g}^{-1} \text{ day}^{-1}$)	LWR (mg g^{-1})	SLA ($\text{cm}^2 \text{ g}^{-1}$)	LAR ($\text{cm}^2 \text{ g}^{-1}$)	NAR ($\text{mg cm}^{-2} \text{ day}^{-1}$)
Shade net 0%	<i>M. peltata</i>	12 ^b	300 ^c	30.1 ^f	9.0 ^d	1.4 ^d
	<i>M. micrantha</i>	13 ^a	240 ^d	26.4 ^g	6.4 ^{ef}	2.1 ^a
	<i>I. batatas</i>	11 ^c	350 ^b	42.0 ^c	14.7 ^b	0.8 ^f
Shade net 55%	<i>M. peltata</i>	11 ^c	290 ^c	41.4 ^c	12.0 ^c	0.9 ^e
	<i>M. micrantha</i>	12 ^b	220 ^d	36.1 ^{de}	8.1 ^d	1.5 ^c
	<i>I. batatas</i>	10 ^c	370 ^a	52.8 ^b	19.6 ^a	0.5 ^g
Shade net 90%	<i>M. peltata</i>	10 ^c	150 ^f	38.8 ^{cd}	5.7 ^f	1.8 ^b
	<i>M. micrantha</i>	5 ^d	200 ^e	34.8 ^e	6.8 ^e	0.8 ^f
	<i>I. batatas</i>	3 ^e	200 ^e	76.4 ^a	14.8 ^b	0.2 ^h

Data with the same letter in the same column is not differ significantly at $\alpha=0.05$ (DMRT test)

Table 4. Leaves nitrogen in shade net level and species of plant treatments

Shade Net Treatment	Plant Species	Leaf Nitrogen (g m^{-2})	PNUE ($\mu\text{mol CO}_2 \text{ g}^{-1}\text{N s}^{-1}$)
Shade net 0%	<i>M. peltata</i>	137.5 ^b	0.14 ^{bc}
	<i>M. micrantha</i>	171.1 ^a	0.15 ^{ab}
	<i>I. batatas</i>	119.2 ^c	0.12 ^d
Shade net 55%	<i>M. peltata</i>	112.0 ^c	0.13 ^{cd}
	<i>M. micrantha</i>	138.8 ^b	0.16 ^a
	<i>I. batatas</i>	95.6 ^d	0.12 ^d
Shade net 90%	<i>M. peltata</i>	89.4 ^d	0.12 ^d
	<i>M. micrantha</i>	118.0 ^c	0.09 ^e
	<i>I. batatas</i>	53.0 ^e	0.05 ^f

Data with the same letter in the same column is not differ significantly at $\alpha=0.05$ (DMRT test)

PNUE_{*M. micrantha*} (0.09 $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ N s}^{-1}$) was lower than of PNUE_{*M. peltata*} (0.12 $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ N s}^{-1}$). PNUE_{*M. peltata*} was relative stable in all level shade net, that were 0.14 $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ N s}^{-1}$ (in shade net of 0%), 0.13 $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ N s}^{-1}$ (in shade net of 55%), and 0.12 $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ N s}^{-1}$ (in shade net of 90%), respectively.

Based on all parameters of physiological, the degree of similarity between *M. peltata* with *M. micrantha* was 55%, whereas, the degree of similarity between *M. peltata* with *I. batatas* was -6.90% (Figure 1).

4. Discussion

Carbon fixation rate (CFR) of *M. peltata* under full sunlight (0% shade) was slightly lower than that of *M. micrantha*. When under 55% shade, the reduction of CFR on *M. peltata* was higher than *M. micrantha*, however the reduction was somehow greater on *M. micrantha* under 90% coverage. The condition actually reduced the growth considerably and tended to kill the plants (Taiz and Zeiger 2010).

CFR of *M. peltata* in shade of 0% were higher than in other shading level. *M. peltata* still showed the ability to bind CO_2 high enough, although grown in conditions of light exposure. In this case, *M. peltata* classified as strong enough plant in the high-light condition. CFR of *M. peltata* was not significantly different with CFR of *M. micrantha* in the shade of 90% and CFR of *I. batatas* in 55% shading. But overall, CFR of *M. peltata* was between *M. micrantha* and *I. batatas*.

Relative growth rate of *M. micrantha* reached the highest value, followed by *M. peltata* and *I. batatas* gained the lowest. The RGR of *M. micrantha* was high at the 0% shading, which was influenced by NAR. Leaf weight ratio is the proportion of biomass allocated to the leaves. *M. micrantha* allocated a smaller proportion of biomass for leaves synthesis, but had very high NAR indicating a very high physiological contribution to RGR (Long *et al.* 2004). This was supported by high content of nitrogen and high PNUE, so physiological *M. micrantha* was superior to *M. peltata*. The high RGR enables the plants to grow rapidly and invasively to occupy space and gain resources (James and Drenovsky 2007). NAR of *M. micrantha* reached a high value when compared with the other two species of plants. The high of NAR also indicate rate increase of plant's biomass per leaf area unit per time unit. The high of NAR are followed by the high of RGR. Therefore, NAR is a physiological index that contributes to the high values of RGR. NAR variations determine the variations of RGR (Shipley 2006).

High nitrogen content of the leaf per leaf area unit will affect the increase rate of biomass per time unit, the amount of CO_2 tied up and used for photosynthesis, as well as determine the efficiency of nitrogen use for photosynthesis. The increasing supply of nitrogen causes the increase of chlorophyll content, the rate increase of photosynthesis, and biomass (Feijuan and Cheng 2012).

The photosynthetic nitrogen use efficiency determined how efficient the availability of nitrogen used for photosynthesis. At the shade of 0%, the full light exposure which means pretty high light stress. Therefore, plants must be able to utilize its resources to maintain photosynthesis, their growth rate, and their nitrogen allocation. The high values of PNUE indicate that the small amount of nitrogen is able to influence the process of photosynthesis, and is also able to form the plant's biomass, especially the leaves (McDowell 2002).

Specific leaf area (SLA) of *I. batatas* in the 55% shade reached the highest values when compared to SLA of *M. peltata* and *M. micrantha*. However despite this high value of SLA, it gave a very low RGR, most likely because this plant accumulated food reserve as tubers, preventing it from productive utilisation and infact increase the rate of respiration. The change of SLA results in changes of the leaves' ability to generate the leaves' photosynthate (Durand and Goldstein 2001; Salehian and Eshagi 2012).

Three species of plants showed the lowest values (such as CFR, RGR, LWR, LAR, NAR, and PNUE parameters) in the shade net of 90%. The condition was so bad for most species and tended to kill species used in this experiment. Infact only *M. peltata* that still survive.

In the preliminary of work it was planned to utilise plant cutting of *M. peltata* to carry out the experiment. Despite a great effort to grow *M. peltata* stem cuttings failed to grow. Stem cuttings with different length, grown in the green house (high humidity), put in horizontal fashion, different physiological age, the use of chemicals to stimulate root initiation all of them failed to grow. The cuttings turned brownish, later on blacken and finally dead and rotted. Some cuttings grew 1.2% when wrapped in plastic, 0.7% when laid horizontally, and about 0.8% when kept under green house condition. It seemed require a high humidity, but not from the preliminary study, we decided to use the seeds for plant propagation which was easily germinate. Therefore, in this experiment we used the seedlings from the seeds.

Propagations of *M. peltata* by seeds has been successfully carried out. *M. peltata* seeds were separated from the fruit capsules of *M. peltata* were selected. The successful percentage of the propagation of *M. peltata* seed is much better (the viability percentage is 100%) compared with stem cutting. *M. peltata* successfully grown from seeds was due to the supporting structures of the seeds. The structure of *M. peltata* seeds was hairy and strong, and it was very suitable to be distributed through water.

The mechanism of *M. peltata* invasion seems due to its ability to response to a new open environment, with plenty of light, by changing LAR, through a greater resource allocation for leaf synthesis. Although the CFR was lower than that *M. micrantha* (infact the values were relatively high). It showed a very low RGR for all species, but displayed an interesting differences, i.e. *M. micrantha* showed high the NAR, but low the

LAR, meaning that *M. micrantha* has a high contribution to its the RGR, while *M. peltata* has a high the LAR and low the NAR, meaning that while the physiological index was lower than that of *M. micrantha*, but it has higher the LAR, i.e. allocating more photosynthate for leaves synthesis.

Physiological character of invasive alien species (IAS) is known to have the growth rate, the ability to capture resources, and the high efficiency (Feng *et al.* 2007). Some variables of *M. peltata* was not significantly different from *M. micrantha* and *I. batatas*. Although, there was a tendency of *M. peltata* to produce a response between *M. micrantha* and *I. batatas*. The degree of similarity was almost 55% which showed that *M. peltata* and *M. micrantha* had high similar characters.

The carbon fixation rate, RGR, NAR, and PNUE in the shade of 90% were variables to support *M. peltata* as a typical IAS. *M. peltata* demonstrates its ability to maintain its several physiological processes in a state of less energy. CFR of *M. peltata* showed high. The RGR, NAR, and PNUE of *M. peltata* reached the highest in this shade compared to *M. micrantha* and *I. batatas*. The high of CFR mean the rate of CO₂ binding of photosynthesis is also high. RGR was growth rate that seems to be influenced by net assimilation rate. It means that the RGR was influenced by the amount of CO₂ used for photosynthesis purposes. The impacts of high RGR and NAR were also followed by high of PNUE. The high used of nitrogen in photosynthesis makes *M. peltata* more efficient. It was efficient due to the high shade condition, *M. peltata* can still take advantage of its little resources to proceed photosynthesis. The CFR, RGR, NAR, and PNUE are all factors associated with the physiological processes. The physiological factor of *M. peltata* showed the highest in low light stress conditions indicates that *M. peltata* belongs to the plant species that was resistant to low-light stress. Plant species that was resistant to high shade stress (low light) indicates that the life range of *M. peltata* is very wide living. The ability to live on a wide range (from high, moderate to low shades) was included in IAS group (Nagel and Griffin 2004; Poorter 2010).

The physiological characters such as CFR, RGR, LAR, and PNUE of *M. peltata* showed higher values. These results indicated that physiological characters that are associated with RGR and supporting the invasiveness of *M. peltata* were related to resource allocation to leaf biosynthesis and relatively efficient nitrogen use for photosynthesis. It was rather different from *M. micrantha* with higher value of NAR indicating a high physiological index, with relatively high CFR and RGR. The NAR indicates a physiological index, a greater value indicates a greater physiological contribution to the growth of the plant. This is in line with finding reported by Deng *et al.* (2004), who indicated the important physiological contribution to its invasiveness. The correlation between LAR with LWR and LAR with SLA showed that leaf biomass

allocation and leaf area per weight leaf to contribute more to the high ratio of leaf area.

M. peltata had a comprehensive range of life, starting from the low to high shades (the shades of 0%, 55%, and 90%). This capability was another factor contributing to the success of *M. peltata* to survive in various light conditions.

M. peltata had similar physiological characters with *M. micrantha* based on the overall observed variables. These were indicated that *M. peltata* had some characters of IAS like *M. micrantha* with degree of similarity was 55%.

It can be concluded that *M. peltata* acclimatized to the low light intensity (shade 90%) were able to survive and when they grown in the shade of 55% and 0% able to improve the quality of leaf in the form of PNUE like *M. micrantha* and also able to use a greater leaf area to support RGR. These conditions provide opportunities *M. peltata* to become invasive.

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