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Research Article

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Stomata characteristics and chlorophyll content in two plant species regenerating with sprout and seeds after burning at Peat Swamp Forest in Batang Alin-Indonesia

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ABSTRACT

Regeneration capability of plants with sprouting and seeding are two important characters in forest recovery after burning. Characteristics of stomata and chlorophyill contents of Anthocephalus cadamba Miq and Mallotus leucodermis Hook regenerating with sprout and seeds after burning at peat swamp forest in Batang Alin were studied and compared with the same unburned plant species. The two plant species studied with different regeneration mechanisms, sprouting and seeding, showed different response on environmental changes after burning. In Anthocephalus cadamba Miq., characteristics of stomata and chlorophyll content were not factors determining adaptation capability of seeding regeneration in burned location, while adaptation capability of sprouting regeneration was determined by characteristics of stomata. In Mallotus leucodermis Hook f., characteristics of stomata and chlorophyll content determined adaptation capability on seeding regeneration, meanwhile on sprouting regeneration it was determined by characteristics of stomata only.

Keywords: Stomata, chlorophyll, regeneration, sprout, seed, burned location

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INTRODUCTION

Peat swamp forest is a unique ecosystem with its diverse flora and fauna. It plays an important role in maintaining environmental balance, prevents flood in wet season and releases moisture back to air during dry season. However, peat swamp forest is so fragile (1), that it is so prone to disturbance and difficult to come back to initial condition. One of disruptions in peat swamp forest is vegetation burning.

In Indonesia vegetation burning is a phenomenon that usually happens in dry season(2). In 1997/1998 Indonesia faced the worst forest fire in the world and this was noted as one of the worst environmental disasters in that century. It was estimated 2.124.000 ha peat swamp was burned in 1997/1998, in which 624.000 ha existed in Sumatera(3). Forest burning has been a problem until now in Indonesia.

Forest burning has negative impact on biodiversity, public health, transportation and forest industry(4). Forest burning is a disrupting factor in ecosystems in the world and affects plant species reproduction(5). The burning peatland will causes irreversible drying, thus it forms material which can not absorb water. The main impact of forest burning is shown in vegetation. Generally plants die directly due to worst forest burning(1). Burning can cause vegetation to die at various stages of growth and development.

Vegetation burning affects physical environmental changes, thus influences regeneration in the forest. Plants have 2 basic mechanisms to regenerate after burning, resprouting and seedlings(6). For some species of plants fire can be a trigger for seeds to grow as seedlings and finally become new individuals(5). The recovery capacity of forest vegetation after burning and other disturbances involves four main processes; tree survival, the resprouting of damaged trees, germination of seeds in the seed bank, and seed rain(7).

Plant response on environmental changes is shown at plant performance. After burning there are changes on plants anatomy, physiological and biochemical acclimation of leaves. The adaptation ability for stressing conditions is crucial for plants establishment after burning under natural conditions. Different plant may show different responses to stress conditions, depending on their different genetic origin and different morpho-anatomical potentials to show plasticity(8). Different environment can change individual behavior, morphology and physiology(9).

In the last several decades there has been intensive conversion from peatland to agricultural, rubber and oil palm plantations resulting in the disturbance of ecosystem(10). The fact in field has proven that the conversion of forest into plantations has caused problem on environment like degradation of biodiversity and extinction of indigenous species. Batang Alin-Pasaman Barat is one of areas in West Sumatera Province, Indonesia in which peatland has been disturbed because of conversion to oil palm plantations. The process of land clearing was done by cutting the plants followed by burning. Based on a survey done, not all entire burned land is planted by oil palm, but some parts are grown by regenerated plants dominated by *Anthocephalus cadamba* Miq., and *Mallotus leucodermis* Hook f. after burning. Both plants show regeneration by resprouting and seeding. Capability to resprout and recruiting new individuals from seeds after burning are two very important characters to survive in an ecosystem that is prone to burning(5; 11).

Vegetation should have certain characters which can help to overcome problem dealing with environment changes after burning. Up till now there has not been an information on characteristics of stomata and chlorophyll content on plants regenerated by sprouting and seeding after peatland burning. Characteristic of stomata and chlorophyll content relate to plant physiological response which show plants adaptation to environment changes due to burning. Ecophysiological response of plant species due to burning may play an important role in forest recovery(12).

EXPERIMENTAL SECTION

Plant materials were collected in May 2014 in Batang Alin area–Pasaman Barat, West Sumatera Province, Indonesia. Before taking plant samples vegetation analysis was done. Plant samples were collected from vegetation 3 years after burning and from unburned vegetation as a control. Two dominant species of plants, *Anthocephalus cadamba* Miq and *Mallotus leucodermis* Hook f, were collected to determine their characteristics of stomata and chlorophyll content.

Ten individuals of each plant species and type of regeneration (sprouting and seeding) were collected in each location. Leaves samples were cut by using plant scissors and labeled. The samples were then put into plastic bags and then were placed in jetfresh to keep them fresh. Samples were brought to Laboratory of Biology Faculty of Mathematics and Natural Science, Padang State University and kept in freezer. Observation of stomata characteristics was done using SEM (Scanning Electron Microscope). Parameters observed were density, length, and width of stomata.

The measures of chlorophyll content was done using spectrophotometer on 649 and 665 nm wavelength. Then chlorophyll content was measured by formula:

$C = (20{,}2 \times D649) + (8{,}02 \times D665) \times 50/1000 \times 100/5 \times 1/\text{leaf sample mass}.$

where

C	= Chlorophyll Content (mg chlorophyll/gr leaf)
20,2 dan 80,2	= Coeficient of Chlorophyll Absorbance
D649 dan D665	= Value of Extract Absorbance
50/1000 x 100/5	= Dillution Factor

Data of stomata characteristics and chlorophyll content were analysed using t test with 5 % confident level.

RESULTS AND DISCUSSION

Stomata

Results of t test (5 % confident level) showed different density, length, and width of stomata of *Anthocephalus cadamba* Miq. regenerated with sprouting in burned location vs unburned location. Density of stomata in unburned location was higher than in burned location. Length and width of stomata regenerated with sprouting in burned location was higher than in unburned location as shown in Table 1. Further, the result showed that density, and length of stomata of individuals regenerated with seeding were not different between burned and unburned location, while width of stomata in burned location was higher than in unburned location.

Table 1. Density, length, and width of stomata of *Anthocephalus Cadamba* Miq. and *Mallotus leucodermis Hook.f.* on regeneration with sprouting and seeding in burned vs unburned locations

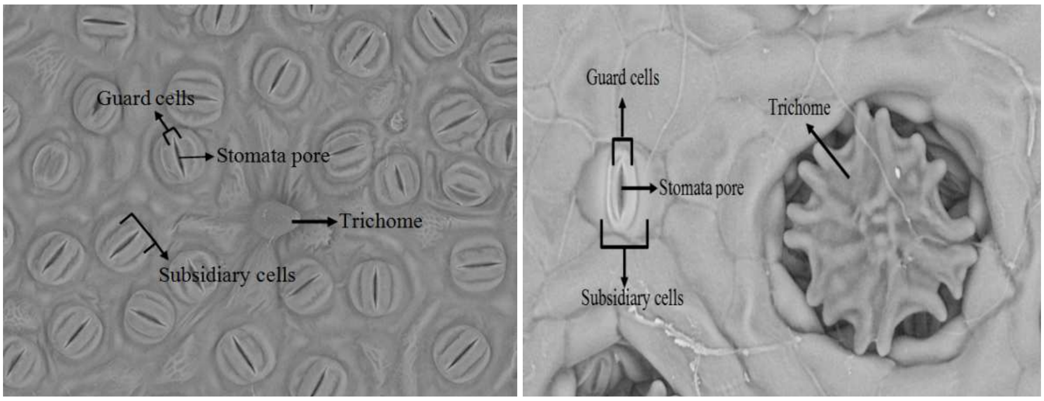
Species	Regeneration	Location	Density (/mm²)	Length (µm)	Width (µm)
A. cadamba	By Sprouting	Burned	403.76 a	27.67 a	25.77 a
		Unburned	478.95 b	25.41 b	23.67 b
	By Seedling	Burned	466.42 a	25.73 a	24.74 a
		Unburned	394.02 a	25.04 a	22.79 b
M. leucodermis	By Sprouting	Burned	68.17 a	21.83 a	10.31 a
		Unburned	48.56 a	20.46 b	9.56 b
	By Seedling	Burned	34.97 a	21.40 a	9.78 a
		Unburned	25.84 a	20.30 b	11.48 b

Study on *Mallotus leucodermis* Hook.f. showed no difference on stomata density between burned and unburned location. Length and width of stomata in burned location were higher than in unburned one like in *Anthocephalus Cadamba*. Regeneration with seeding of *Mallotus leucodermis* showed that stomata density was not different between two locations, but length and width of stomata were different between two locations. Length of stomata in burned location was higher than in unburned location. On the other hand, width of stomata in burned location was lower than in unburned location.

Result of t test on *Anthocephalus cadamba* showed no difference on density and width of stomata on regeneration by sprout versus by seeding in burned location, as shown in Table 2. Regeneration by sprouting had higher width of stomata than regeneration by seeding. In unburned location density, length, and width of stomata were not different in two types of regenerations.

Table 2. Density, length, and width of stomata of *Anthocephalus cadamba* Miq. and *Mallotus leucodermis* Hook f. on sprouting vs seeding regeneration in burned and unburned locations

Species	Location	Regeneration	Density (/mm²)	Length (µm)	Width (µm)
A. cadamba	Burned	By Sprouting	403.76 a	27.67 a	25.77 a
		By Seedling	466.42 a	25.73 b	24.74 a
	Unburned	By Sprouting	478.95 a	25.41 a	23.67 a
		By Seedling	394.02 a	25.04 a	22.79 a
M. leucodermis	Burned	By Sprouting	68.17 a	21.83 a	10.31 a
		By Seedling	34.97 b	21.40 a	9.78 b
	Unburned	By Sprouting	48.56 a	20.46 a	9.56 a
		By Seedling	25.84 b	20.30 a	11.48 b



a b

Figure 1. SEM image of a Leaf stomata of *Anthocephalus cadamba* Miq. with 1000 times magnification b. Leaf stomata of *Mallotus leucodermis* Hook.f with 1000 times magnification

Study on *Mallotus leucodermis* in burned location showed that density and width of stomata from sprouting regeneration were higher compared to seeding regeneration, but stomata length was not different between two regenerations. In unburned location sprouting regeneration showed higher density of stomata than in seeding regeneration. Stomata length of *Mallotus leucodermis* in unburned location was not different between two regenerations but stomata width in seeding regeneration was higher than sprouting regeneration as shown in Table 2.

Chlorophyll content

Result of t test (5 % confident level) showed that chlorophyll content of both type of regenerations of *Anthocephalus cadamba* was not different between two locations.

Study on *Mallotus leucodermis* Hook.f. showed no difference on chlorophyll content of sprouting regeneration between two locations, as shown in Table 3. In seeding regeneration chlorophyll content in burned location was higher compared to unburned location.

Table 3. Chlorophyll content of *Anthocephalus Cadamba* Miq. and *Mallotus leucodermis Hook.f.* on regeneration with sprouting and seeding in burned vs unburned locations

Species	Regeneration	Location	Chlorophyll Content (mg/g plant)
A. cadamba	By Sprouting	Burned	6.51 a
		Unburned	4 .62 a
	By Seedling	Burned	8.34 a
		Unburned	7.73 a
M. leucodermis	By Sprouting	Burned	17.67 a
		Unburned	15.13 a
	By Seedling	Burned	29.44 a
		Unburned	19.81 b

Result of t test on chlorophyll content in *Anthocephalus cadamba* in both burned and unburned locations showed that there were significant differences between two types of regeneration. Chlorophyll content on seeding regeneration was higher compared to sprouting regeneration(as shown in Table 4).

Study on *Mallotus leucodermis* Hook f. in burned location showed that chloropyll content on seeding regeneration was higher compared to sprouting regeneration (as shown in Table 4), while in unburned location no difference in chlorophyll content between two types of regenerations

Table 4. Chlorophyll content of *Anthocephalus cadamba* and *Mallotus leucodermis* on sprouting vs seeding regeneration in burned and unburned locations

Species	Location	Regeneration	Chlorophyll Content (mg/g plant)
A. cadamba	Burned	By Sprouting	6.51 a
		By Seedling	8.34 b
	Unburned	By Sprouting	4.62 a
		By Seedling	7.73 b
M. leucodermis	Burned	By Sprouting	17.67 a
		By Seedling	29.44 b
	Unburned	BySprouting	15.13 a
		By Seedling	19.80 a

Anthocephalus cadamba Miq. and *Mallotus leucodermis* Hook.f. were two dominant species of plants found in peatland forest after being burned in Batang Alin, West Sumatera, Indonesia. Both species showed ability to regenerate by sprouting and seeding after burned. An understanding about are very crucial in an effort for forest recovery which damage due to burning. Stomata characteristics and chlorophyll content relate to plant physiological response showing adaptation to environmental stress.

Changes of stomata characteristics on *A. cadamba* as a response to environment after burning was the most dominant showed by sprouting regeneration, where density, length and width of stomata on sprouting were different between burned and unburned location. In the other side, on seeding the changes of stomata characteristics was found only in width which was different between two locations observed (as shown in Table 1). Stomata is a main gate between plants and atmosphere which play a central role in vegetation as their response environmental condition.

Density of stomata of sprouting regeneration tended to decrease after burning, while on seeding regeneration density and length of stomata were not different in two locations. It indicated that there was different physiology between the two types of regenerations in response to environment changes. Plants have specific characters which differentiate one type with another. The specific characters was believed as an adaptation to certain environment. The decrease of stomata density in sprouting regeneration of *A. cadamba* after burned is one of its adaptation to environment stress after burned. Burned location causes the vegetation to open, increases light intensity which in turn makes land dry. One of physical characters of peatland when it was burned, the effect is irreversible drying(13). Some plants was adapted by drying and reducing a number of stomata. There is a relationship between the resist drying plants with the lower number of stomata. Although number of stomata in sprouting regeneration decreased after burning, but its length and width increased. Stomata density and length had negative correlation, which could be seen several million years ago(14).

This data showed that seeding regeneration had physiological characters which were suitable to environment condition after burning, thus the plants did not need to reduce a number of stomata when facing environmental stress due to burning. Regeneration by seeding was suitable to water limited condition and very various for instance higher resistance to xylem cavitation, closing of stomata before drying and high tolerance of leaves for drying(15). Regeneration by seeding needed to grow rapidly and matured(16).

For these reasons the plants should increase photosynthesis activity and this had strong correlation with number of stomata which function in carbon fixation. Density of stomata related to an important process in plants, Carbon fixation, photosynthesis and transpiration. Non sprouting species had gas exchange rate higher than sprouting(16). *Anthocephalus cadamba* is a plant which is tolerant to light. Light is an important factor for plant growth(17). Stomata was needed for carbon fixation in photosinthesis thus gives advantages for growth. Then, it will making *Anthocephalus cadamba* seedling to grow rapidly and found dominantly after burning.

When *Anthocephalus cadamba* from seeding regeneration was compared to sprouting regeneration, both showed stomata characters which almost the same when they existed in the same location (burned and unburned location), except length of stomata from seeding regeneration was different from sprouting regeneration in burning location (as shown in Table 2). It was indicated that basically stomata from sprouting and seeding regeneration had characteristic of stomata which almost the same. But, when each type of regeneration existed in different location, changes could happen, mainly on sprouting regeneration (as shown in Table 1). It showed that environment factor caused changes on stomata characteristics mainly in sprouting regeneration. Sprouting and non-sprouting species coexist and represent the two main types of post-fire regeneration strategies(11; 18).

Stomata density was not different among individuals of *Mallotus leucodermis* either on sprouting or seeding regeneration in two locations. But there was differences on length and width of stomata on two types of regenerations in burned location. It indicated that both types of regeneration showed almost the same response to environmental changes by changing the size of stomata after burned. Number and size of stomata depended on species and environmental factor at the time leaves were developing.

When compared stomata characteristics from sprouting and seeding regeneration of *M. Leucodermis* they showed the same length of stomata when they existed in the same location (burned and unburned locations), but they had different number and width of stomata between two types of regeneration both locations. It indicated that basically characteristics of stomata of two types of regenerations tended to be different.

This result showed that on *Anthocephalus cadamba* environmental changes after burned did not change the chlorophyll content either on sprouting or seeding regenerations. Chlorophyll content in seeding and sprouting regeneration was not different between two locations. (as shown in Table 3). There was difference in chlorophyll content between sprouting and seeding regenerations (as shown in Table 4).

In burned location chlorophyll content of seeding regeneration (seeders) was higher compared to sprouting regeneration. In unburned locations the differences was also shown between two types of regeneration where seeding regeneration had higher chlorophyll content than sprouting one. It was indicated that there was different physiologically between two type of regenerations. Seeding regeneration had a better physiological performance on leaves because it had higher photosynthesis than sprouting one(16), thus its chlorophyll content was also higher. Seeding regeneration had shallow root and to maximize their survival they had specific characteristic of root which had higher efficience in acquisition soil sources compared to sprouting one. Root structure of seedlings made possible to be more efficient to explore the upper soil layers, while sprouting might store carbon and penetrate deeper soil layer(19).

In *Mallotus leucodermis* burning did not influence chlorophyll content on sprouting regeneration but it did on seeding regeneration. In seeding regeneration chlorophyll content was higher in burned location compared to unburned location. This condition was contrary to what happened to *Anthocephalus cadamba* where chlorophyll content of seeding regeneration was not different between burned and unburned location. Burning of vegetation has caused the area to open, thus sun light comes straight to forest ground. In an open environment light intensity increases thus increase photosynthesis. This condition is needed by pioneer plants as *Mallotus leucodermis* to grow rapidly. Regeneration by seeding needs to grow rapidly and mature. For these reasons the plants have leaves characteristics which relate to increase efficiency in growth and acquisition of sources compared to sprout(16).

It is predicted that this is the cause of the increase of chlorophyll content in seeding regeneration in burned location compared to unburned location. In burned location, chlorophyll content of seeding regeneration of *Mallotus leucodermis* was higher than sprouting regeneration. This is an indication that changes of environmental condition after burned increase photosinthetic activity in seeding compared to sprouting regeneration. In unburned location there was no different in chlorophyll content between sprouting regeneration and seeding regeneration. Plant biological response on condition after burned varied and depended on many factors such as ; (a) plant Species ; (b) Microclimate (soil temperature, light irradiance, etc); (c) density of population growth; (d) competition among plants; (e) limited nutrient resources; (f) fire intensities, and (g) season(8). Condition of vegetation after burning was different and significantly depended on burning level and development stages of vegetation(20).

CONCLUSION

Different mechanism of regenerations, sprouting and seedings, showed different response on environmental changes after burning. In *Anthocephalus cadamba*, characteristics of stomata and chlorophyll content were not factors determining adaptation capability of seeding regeneration in burned location, while adaptation capabilty of sprouting regeneration was determined by characteristics of stomata. In *Mallotus leucodermis*, characteristics of stomata and chlorophyll content determined adaptation capability on seeding regeneration, while on sprouting regeneration it was determined by characteristics of stomata only.

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