研究資料（Research record）

Effects of biochar on the early growth characteristics of teak seedlings planted in sandy soil in northeast Thailand

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Abstract

Teak (*Tectona grandis* L. f.) seedlings planted in sandy soil in northeastern Thailand often experience suppressed growth due to nutrient deficiency and drought stress. Based on a preliminary field pot experiment, it was observed that biochar increased the root growth of the seedlings. To verify the effects of biochar under field conditions, teak seedlings were planted in a sandy soil treated with 1 kg of biochar in July 2014 (2.5 Mg ha^{-1}), and were cultivated until November 2015. Biomass, photosynthetic rate, and the concentrations of elements in soil and plant materials were compared between biochar and control (no application) treatments.

The biochar used in this study contained nitrogen, calcium, and potassium, and thus acted as a fertilizer. Its application accelerated the growth of teak seedlings, increased their photosynthetic rates, and chlorophyll and calcium concentrations in plant tissues. It was concluded that biochar is a useful material to improve the quality of sandy soils and accelerate the growth of teak seedlings.

Key words : sandy soil, charcoal, photosynthesis, chlorophyll, nutrients

1. Introduction

Teak (*Tectona grandis* L. f) is an important timber species in tropical regions (Tewari 1992). In general, the soil of natural teak forests in Thailand is alkaline, with a pH between 6.8 and 7.8 (Kaosa-ard 1989). The soils in Asian tropical uplands are characterized by low clay content, low pH, low fertility, low water holding capacity, and low cation exchange capacity (CEC) (Suzuki et al. 2007, Kayama et al. 2016, 2017). Previous analyses demonstrated that teak planted in sandy soils showed reduced growth (Tangmitcharoen et al. 2012) and experienced drought stress (Kayama et al. 2017). Thus, soil improvement is essential to promote the growth of teak seedlings in this type of soils.

Biochar (charcoal) can play a role in increasing the water holding capacity and CEC of sandy soils (Kammann et al. 2011, Yuan and Xu 2011, Novak et al. 2012). The application of biochar increases the content of several nutrients in soil, such as N, Ca, and Mg (Yuan and Xu 2011, Jha et al. 2016, Kayama et al. 2016, Rezende et al. 2016), and can act as a fertilizer. In reforestation programs conducted in Laos, biochar was applied during the planting of woody species (Sovu et al. 2011). In the preliminary pot experiment in the present study, the effects of biochar were examined and the growth characteristics of teak seedlings were monitored (Kayama et al. 2016). Adding 4% of biochar increased soil water content and accelerated teak root growth. Moreover, Rezende et al. (2016) reported that applying activated biochar also accelerated the growth of teak seedlings, however verification tests in a field environment have not been conducted to this date.

The objective of this study was to verify the validity of biochar in a field environment. Teak seedlings were cultivated in a sandy soil with biochar, and it was hypothesized that using this material can improve soil quality, which should accelerate the growth of teak seedlings. To verify this hypothesis, the growth characteristics of teak seedlings were measured in the early growth period, and they were compared to those of seedlings in untreated sandy soil.

2. Materials and methods

The experiment was conducted in the Northeast Forest Seed Center in Khon Kaen Province, northeastern Thailand (16°16′ N, 102°47′ E, 191 m a.s.l.) and the meteorological data of this area were reported by Kayama et al. (2016). The sandy soil here was classified as Acrisol using the FAO/UNESCO soil

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taxonomy, and as Ultisol using the USDA taxonomy (Food and Agriculture Organization 1993, Kyuma 2003). An area covering 840 m^2 was selected as the experimental plot, and in April 2014 land preparation procedures were carried out at this site. A clone of teak seedlings was selected from Mae Hong Son Province (clone number 21), and it was planted in various locations (Royal Forest Department, unpublished data), then the treatment with biochar and the control (without applied biochar) were established. Three 12 m × 10 m blocks for each treatment were set up in the experimental site, and their positions were randomized, maintaining a 2 m distance between each block. In June 2014, before planting the seeds, 60 kg of biochar—made from *Acacia* wood bought from a market in Thailand—was prepared. The *Acacia* biochar was produced in a kiln at approximately 400°C, and the density was 0.68–0.72 g cm^{-3} (Forest products research division, Royal Forest Department, Ministry of Agriculture and Cooperatives 1984). Then, the biochar was crushed to fragments measuring less than 5 mm in diameter, and subsequently 1 kg batches of biochar were placed in separate plastic bags.

A total of 252 teak seedlings were planted in each block in July 2014: 42 seedlings were planted inside each block; 22 were planted outside and were considered as buffer trees; 20 were planted within the buffer trees and were set as the target trees. To plant the seedlings, a hole (30 cm × 30 cm, and 30 cm deep) was excavated, and the soil removed during the process was collected and mixed with 1 kg of biochar in order to prepare the treatment. After the mixing phase, a teak seedling was planted in the hole, which was then filled with the treated soil. The biochar application area measured 10 m × 8 m in each block, and the amount of biochar was 2.5 Mg ha^{-1}. All the above procedures were conducted also for the control treatment, except for the addition of biochar in the soil. Soil moisture sensors (SM150, Delta-T Devices Ltd., Cambridge, UK) were also inserted at the center of two blocks.

Soil texture and chemical properties were measured, including soil pH, CEC and concentrations of C, total N, available P, and base cations. Three seedlings located at the three corners of each block were selected, and surface soil samples (0–5 cm) were collected from beneath these seedlings in July 2014. The soil data of biochar treatment were included its particle. The chemical properties of biochar were also measured, and four samples of crushed biochar were collected for this purpose. The detailed methods of soil analysis are described in Kayama et al. (2021).

After the planting phase, the height and root collar diameter of all seedlings were measured, together with the photosynthetic rates at different light saturation (P_{sat}) and stomatal conductance (gs). Nine individuals with healthy teak leaves were selected from the second level from the top, and P_{sat} was measured five times throughout the experiment (October 2014, February 2015, May 2015, July 2015, and October 2015), in the mornings (09:00–11:00). The measurements were conducted using a portable gas analyzer (LI-6400, LiCor, Lincoln, NE, USA). The photosynthetic photon flux, temperature and CO_2 concentration were recorded at 1,800 µmol $m^{-2}s^{-1}$, 28°C, and 38.0 Pa, respectively. After the photosynthetic rate was measured, an area of 0.66 cm^2 of leaf disks was collected, and chlorophyll was extracted using dimethyl sulfoxide (DMSO). The concentration of chlorophyll was measured using a spectrophotometer (AE-VIS721, A and E Lab. Co., London, UK), and was calculated by the equation described in Barnes et al. (1992).

In order to determine the biomass of teak seedlings, the dry masses of leaves, stems, and roots were measured. At the end of November 2015, four teak seedlings were randomly collected from each of the three blocks. The other teak seedlings were left in place to determine their long-term effects on teak growth. The detailed methods used to determine the biomass are described in Kayama et al. (2021). The leaf area was also calculated from the data on length and width, using the equation from Tondjo et al. (2015). The concentrations of N, P, K, Ca, Mg, and Na in leaves and fine roots were analyzed using dried samples that were ground to a fine powder. N concentration was determined using an NC analyzer (Sumigraph NC-220F, Sumika Chemical Analysis Service, Tokyo, Japan). The remaining samples were digested using the HNO_3-HCl-H_2O_2 method (Goto 1990), and the concentrations of P, K, Ca, Mg, and Na were analyzed using an ICP analyzer (ICPE-9000, Shimadzu, Kyoto, Japan).

All parameters were statistically analyzed using Kyplot

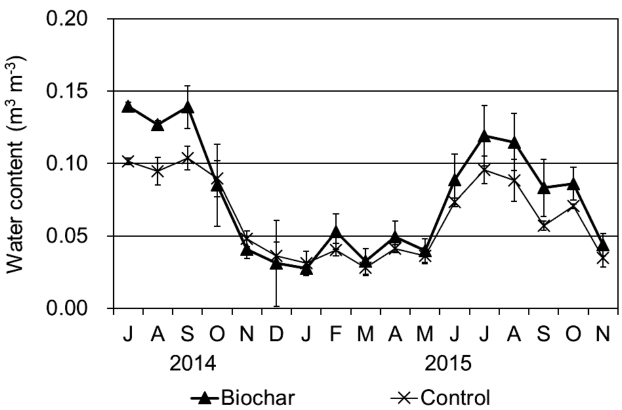

Fig. 1. Average soil water content in biochar and control treatments (July 2014–November 2015, n = 2).
The data for the control treatment were from Kayama et al. (2017). The data of the control treatment in Tables 1, 2, and 3, and Fig. 2 and 3 were also from Kayama et al. (2017).

5.0 (Kyens Lab, Inc.) The differences between parameter means were evaluated by analysis of variance (ANOVA), and compared between biochar and control treatments.

3. Results

The water content was low from November 2014 to May 2015, and high during the rainy season (June–September 2014 and June–September 2015, Fig. 1). When comparing the two treatments, soil water content was higher in the biochar treatment during the rainy season; however, there was little difference in soil water content between biochar and control treatments in the dry season. There were no significant differences between the two treatments in each month.

The biochar used in the experiment contained 47% of C (Table 1), its pH was 8.5, and high concentrations of N, Ca, and K were detected in it. In terms of soil texture, the sand content was approximately 80% in each treatment (Table 2), and there were no significant differences between sand, silt, and clay contents between the two treatments ($P > 0.05$). Soil pH, CEC and the concentrations of N, Ca, and K were significantly higher in the biochar-treated soil ($P < 0.01$). There were no significant differences in the content of other nutrients between the two treatments.

Between October 2014 and the end of the experiment, tree height and root collar diameter values were significantly higher in biochar-treated seedlings ($P < 0.001$, Fig. 2). At the end of the experiment, tree height and diameter in the biochar treatment increased by 67 cm and 13.9 mm, respectively. In contrast, the control treatment did not show any obvious effects on growth, and here the values increased only by 12 cm and 4.6 mm, respectively.

In the biochar treatment, P_{sat} showed significantly higher

Table 1. Chemical properties of the biochar (Mean ± SE, n = 4).

pH	CEC (cmol kg^{-1})	C (g kg^{-1})	N (mg kg^{-1})	P (mg kg^{-1})
8.51 ± 0.04	46.6 ± 6.0	424 ± 87	13,182 ± 1257	263 ± 30
Ca (mg kg^{-1})	Mg (mg kg^{-1})	K (mg kg^{-1})	Na (mg kg^{-1})	
6477 ± 488	502 ± 73	2782 ± 336	30.2 ± 3.1	

Table 2. Texture and chemical properties of soils in biochar and control treatments (Mean ± SE, n=9). The mean values of each parameter were analyzed using ANOVA. ** P < 0.01, *** P < 0.001, and n.s. = not significant.

Treatment	Texture (%)			pH
	Sand	Silt	Clay	
Biochar	80.2 ± 0.6	14.0 ± 0.3	5.8 ± 0.5	5.55 ± 0.13
Control	80.2 ± 0.5	14.1 ± 0.7	5.7 ± 0.9	4.53 ± 0.08
Statistical test	n.s.	n.s.	n.s.	***
	CEC (cmol kg^{-1})	C (g kg^{-1})	N (mg kg^{-1})	P (mg kg^{-1})
Biochar	1.99 ± 0.14	8.43 ± 1.62	267 ± 46	7.60 ± 2.20
Control	1.30 ± 0.10	7.00 ± 2.32	96 ± 16	8.62 ± 2.01
Statistical test	**	n.s.	**	n.s.
	Ca (mg kg^{-1})	Mg (mg kg^{-1})	K (mg kg^{-1})	Na (mg kg^{-1})
Biochar	221 ± 43	39.6 ± 9.7	73.9 ± 8.7	1.37 ± 0.35
Control	53 ± 8	22.9 ± 2.9	22.7 ± 2.9	2.67 ± 0.59
Statistical test	***	n.s.	***	n.s.

values in October 2014, May 2015, and October 2015 (P < 0.001, Fig. 3), and the same was observed for the gs values, except for May 2015 (P < 0.05). P_{sat} and gs showed the highest values in the biochar treatment in October 2014, but these were lower in February and May 2015. In July 2015, the values of P_{sat} and gs in the biochar treatment increased. In contrast, the control treatment showed low P_{sat} and gs values in each measurement period, and P_{sat} decreased in October 2015. The chlorophyll concentration showed significantly higher values in the biochar treatment in October 2014, February 2015, and October 2015 (P < 0.05). The highest chlorophyll concentration for both treatments was observed in October 2015.

Before the planting phase, the dry masses of leaves, stems, and roots of teak seedlings measured 1.6 g, 1.5 g, and 2.1 g, respectively. At the end of the experiment, the survival rate of seedlings was 55% in the control treatment and 92% in the biochar treatment, with only five dead seedlings during the experimental period. The leaf area in the biochar treatment was significantly larger than that in the control treatment (P < 0.001, Table 3). Additionally, the dry masses of leaves, stems, and roots showed significantly higher values in the biochar treatment (P < 0.001).

Ca concentration in the leaves and roots, and K concentration in the roots were significantly higher in the biochar treatment (P < 0.001, Table 4). There were no significant differences in the concentration of other nutrients between the two treatments.

4. Discussion

Based on the results of this study, various positive effects of biochar application were confirmed. Generally, studies have shown that biochar application can alter the physical and chemical properties of soil (Glaser et al. 2002) and can decrease its bulk density (Glaser et al. 2002). The bulk density of sandy soil was measured at 1.56 g cm^{-3} by calculating the soil dry mass (42 kg) and the volume of a single hole (27,000

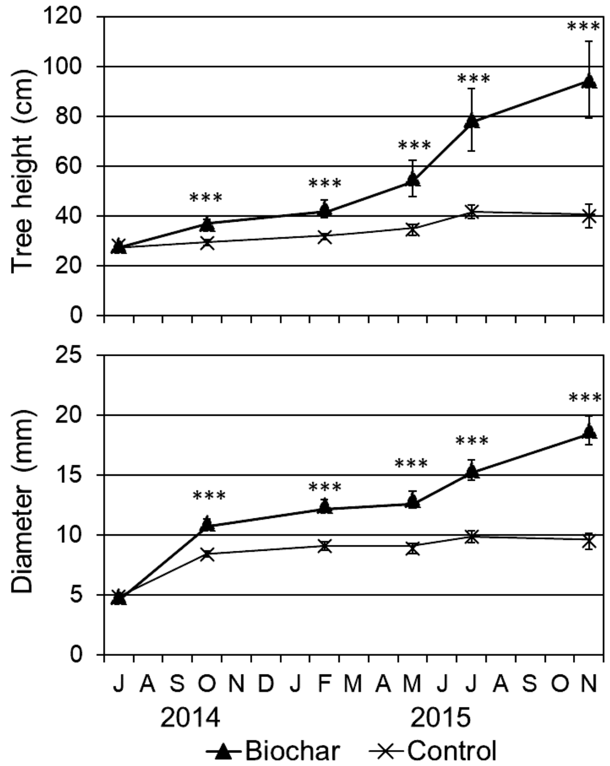

Fig. 2. Tree height and basal diameter of teak seedlings in biochar and control treatments (mean ± SE, n = 60). The mean values of each parameter were analyzed using ANOVA. *** P < 0.001.

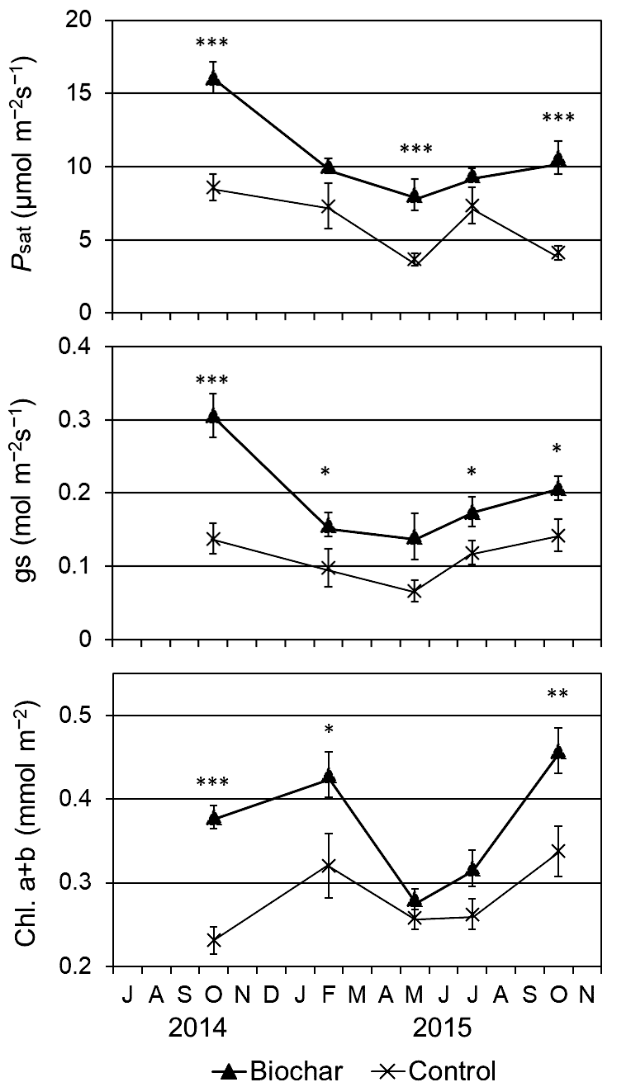

Fig. 3. Photosynthetic rate at light saturation (Psat), stomatal conductance (gs), and chlorophyll concentration (a + b) of teak seedlings in biochar and control treatments (9:00–11:00, mean ± SE, n = 9). The mean values of each parameter were analyzed using ANOVA. ** P < 0.01, *** P < 0.001.

cm^3), while the density of the biochar used in this experiment was 0.68–0.72 g cm^{-3} (Forest products research division, Royal Forest Department, Ministry of Agriculture and Cooperatives 1984). The density of sandy soil applied 1 kg of biochar was 1.51 g cm^{-3}, and the decrease in bulk density was 3.2% in the experiment. However, to accelerate the growth of seedlings, the bulk density should decrease by 8% (Zoghi et al. 2019). Thus, the 3.2% decrease in soil bulk density obtained through biochar application is not sufficient to accelerate the growth of teak seedlings. Biochar application also did not increase soil moisture content (Fig. 1), and therefore it did not alter the physical properties of the soil during the experiment.

Biochar has also the ability to alter the chemical properties of soil by increasing the retention of available nutrients in plants (Glaser et al. 2002). Biochar application increased the CEC (Table 2), and nutrient retention was probably altered. Biochar showed high pH values and high concentrations of N, Ca and K (Table 1), and the treatment also increased the pH and concentration of these elements in the soil (Table 2). Thus, the results of this study suggest that the application of biochar is important because it alters the chemical properties of soil. In addition, biochar also acts as a fertilizer and soil acidity corrector.

The survival rate of teak seedlings in the biochar treatment

Table 3. Leaf area and dry mass of each part of teak seedlings grown in biochar and control treatments (mean ± SE, n = 12). The mean values of each parameter were analyzed using ANOVA. *** P < 0.001.

Treatment	Leaf area (cm^2)	Leaf dry mass (g)	Stem dry mass (g)	Root dry mass (g)
Biochar	848 ± 121	64.0 ± 10.2	249 ± 38	226 ± 37
Control	92 ±34	5.1 ± 1.4	26 ± 4	36 ± 7
Statistical test	***	***	***	***

Table 4. Concentrations of elements (N, P, K, Ca, Mg, and Na) in leaves and roots of teak seedlings grown in biochar and control treatments (November 2015, mean ± SE, n = 12). The mean values of each parameter were analyzed using ANOVA. *** P < 0.001 and n.s.= not significant.

Treatment	N (mg g^{-1})		P (mg g^{-1})	
	Leaf	Root	Leaf	Root
Biochar	21.4 ± 1.0	8.4 ± 0.5	0.92 ± 0.03	0.32 ± 0.03
Control	18.9 ± 1.0	7.6 ± 0.3	0.76 ± 0.06	0.33 ± 0.03
Statistical test	n.s.	n.s.	n.s.	n.s.
	K (mg g^{-1})		Ca (mg g^{-1})	
	Leaf	Root	Leaf	Root
Biochar	1.99 ± 0.14	8.43 ± 1.62	267 ± 46	7.60 ± 2.20
Control	1.30 ± 0.10	7.00 ± 2.32	96 ± 16	8.62 ± 2.01
Statistical test	**	n.s.	**	n.s.
	Mg (mg g^{-1})		Na (μg g^{-1})	
	Leaf	Root	Leaf	Root
Biochar	221 ± 43	39.6 ± 9.7	73.9 ± 8.7	1.37 ± 0.35
Control	53 ± 8	22.9 ± 2.9	22.7 ± 2.9	2.67 ± 0.59
Statistical test	***	n.s.	***	n.s.

was higher (92%) compared to that in the control treatment (55%), therefore it was concluded that the use of biochar is likely to enhance the survival of teak seedlings. According to Wehr et al. (2017), lime has also the same effect and, as biochar also contains Ca (Table 1), the enhanced survival of seedlings may be similar to that observed when using lime.

The teak seedlings in the biochar treatment presented a significantly higher tree height, diameter, leaf area, and dry mass compared to those in the control treatment (Fig. 2, Table 3). Thus, it was inferred that the use of biochar resulted in an accelerated teak seedling growth in sandy soil. This trend was obvious when compared with the trend observed in the preliminary pot test (Kayama et al. 2016). Moreover, the teak seedlings in the biochar treatment had high Ca concentrations in their leaves and roots (Table 4), and this element originated from the biochar (Table 1). There have been cases in which Ca application enhanced teak growth (Barroso et al. 2005, Zhou et al. 2012, Wehr et al. 2017). In contrast, when teak seedlings were grown without any Ca addition to the culture medium, a drastic decrease in growth was observed, and Ca concentration in the roots was low (Barroso et al. 2005). Thus, it was concluded that the storage of Ca in teak plant tissues is important for growth, and that the application of biochar enhances Ca uptake. Furthermore, K concentration in the roots was also significantly higher in the biochar treatment (Table 4). In general, teak seedlings demand a greater amount of K in roots (Behling et al. 2014), and the K stored there restricts teak growth (Kayama et al. 2021). Therefore, the application of biochar increased the concentration of K in soil and, as a result, teak seedlings could accumulate K in roots, probably leading to an acceleration of growth.

Moreover, the teak seedlings in the biochar treatment showed high P_{sat} values in October 2014 and February 2015 (Fig. 3). In contrast, the N and P concentrations in the leaves, that showed a positive relationship with the photosynthetic rate (Evans 1989, Raaimakers et al. 1995), were low in November 2015 (Table 4) but chlorophyll concentration—which is closely associated with the photosynthetic rate (Enríquez et al. 1996)—was high at the same time (Fig. 3), suggesting that the photosynthetic rate was regulated by chlorophyll concentration in the leaves.

Compared with other biochar experiments conducted by Rezende et al. (2016), the accelerated growth of teak seedlings was not observed when normal biochar was used. Those experiments used a fertilizer with a pH greater than 6 (Rezende et al. 2016), while the soil pH in this experiment was 4.5, and no fertilizer was used. In addition, nutrient concentrations in the present experimental soil (Table 2) were considerably lower than those observed in Rezende et al. (2016). Poor soil conditions probably mean that even when using normal biochar, growth is accelerated.

In contrast, teak seedlings in the control treatment presented suppressed growth and showed low dry mass values (Fig. 2, Table 3). However, the only nutrient with low concentrations in teak seedling leaves in the control treatment was Ca (Table 4). According to Zech and Drechsel (1991), Ca deficiency levels in leaves border 5.5 mg g^{-1} (138 µmol g^{-1}) and, based on this value, the teak seedlings in the control treatment did show Ca deficiency. It was also observed that the leaves presented a striped yellow and green pattern, which may further indicate Ca deficiency (Barroso et al. 2005). Moreover, the control treatment showed low P_{sat} values and low chlorophyll concentrations in October 2015 (Fig. 3). Ca^{2+} plays an important role in maintaining chlorophyll, and stabilizing and aggregating light-harvesting chlorophyll-protein complexes (Han & Katoh 1993). Ca deficiency is known to decrease the functionality of photosynthetic apparatuses—such as chlorophyll and Rubisco (Lavon et al. 1999)—therefore it was inferred that such deficiency caused the decrease in chlorophyll in this experiment and, as a result, the photosynthetic rate was suppressed.

Finally, based on the findings of this study, it was concluded that biochar could alter the chemical properties of sandy soil in northeastern Thailand and could accelerate the growth of teak seedlings. Ca was the most important nutrient for teak growth, and biochar acted as Ca fertilizer. However, it was confirmed that the teak roots growing in the horizontal axis developed over the biochar application area (30 cm × 30 cm), and therefore biochar should be applied over a large area for it to be effective. Future studies following this experiment will examine the quantitative effects of biochar on the establishment of silviculture methods.

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タイ東北部の砂質土壌に植栽したチーク苗の初期成長における炭の効果

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要 旨

タイ東北部には砂質土壌が広範囲に分布し、砂質土壌に植栽されたチーク (*Tectona grandis* L. f.) 苗は、養分欠乏と乾燥ストレスによって成長が抑制される。ポットを用いた野外環境下での予備試験では、炭の添加によってチーク苗の根の成長は増加した。炭の効果を野外の圃場において検証するために、1 kg の炭 (2.5 Mg ha^{-1}) を添加して育成するチーク苗の植栽試験を実施した。チーク苗は 2014 年 7 月に植栽し、2015 年 11 月まで育成した。チーク苗のバイオマス、光合成速度および土壌と植物体中の元素濃度を炭処理区と炭を添加しない対照区で比較した。

炭には窒素･カルシウム･カリウムが含まれ、これらの元素は養分の役割を果たしていた。炭の添加は、光合成速度･クロロフィル濃度･カルシウム濃度の増加に貢献し、その結果チーク苗の成長は促進された。本試験結果から、炭は砂質土壌の改良に有用な資材であり、チーク苗の成長促進効果をもたらすことが明らかになった。

キーワード：砂質土壌、炭、光合成、クロロフィル、養分

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