Original Research Article

Effect of Canopy Gaps on Growth and Water Use Efficiency of Seedling of Vulnerable Species, *Hopea sangal* Korth

.

ABSTRACT

Aims: *Hopea sangal* Korth. is listed as vulnerable species and recently its remnant habitat was rediscovered in the degraded forest near the springs area in East Java. In the forest, the regeneration of the seedlings and saplings understory is affected by the heterogeneity in environmental factors especially various light levels due to the canopy gaps. *H. sangal* is considered a shade-tolerant species, hence the establishment of the seedling in its natural habitat occurs under the closed canopy. This study aimed to understand the Water Use Efficiency (WUE) and Relative Growth Rate (RGR) of *Hopea sangal* Korth seedlings grown in two different levels of tree canopy shades.
Study design This research was using a completely randomized design, with 9 replications.
Place and Duration of Study: This study was conducted in the Purwodadi Botanic Garden, East Java, between March – July.
Methodology: We used seedlings of *H. sangal* collected from the field in Pasuruan which were planted in plastic pots and acclimatized to obtain seedlings at similar size. The microclimate was measured weekly using solarimetri and sling psychrometer at 08.00; 10:00; 12:00; 14:00 and 16:00. RGR was measured by harvesting the seedling and whole plant WUE was measured using the gravimetric method.
Result: We observed the light level during the periodic opening of the canopy gap was significantly different at a specific time (8-12 am), with the highest light intensity was at 10.00 of 333.57 µmol photon $m^{-2}s^{-1}$. The canopy shades differences did not affect the WUE of seedlings (P = 0.333), meanwhile, there was a significant difference in the seedling RGR (P = 0.025). The seedlings that were grown under a higher gap and received higher light intensity periodically during a day had higher RGR than those under a closed canopy. The WUE has a positive relationship with the RGR of seedlings (R2 = 0.5; P < 0.05).
Conclusions: This study suggested that the *H. sangal* is one of the shade-tolerant species capable of responding to gap opening sunlight. The study also showed RGR of the seedling positively correlated with the WUE of plants, indicating that the seedling could use the water supply efficiently to grow rapidly.

Keywords: seedling, canopy gaps, Hopea sangal, Water use efficiency, Relative growth rate

1. INTRODUCTION

One of the plant species collected around the water spring in the district of Pasuruan, East Java by the exploration team of Purwodadi Botanic Garden was the seedlings of a rare plant species, *Hopea sangal*. It belongs to the Dipterocarpaceae family. The name *H. Sangal* was obtained from the identification process based on literature and discussion. The identification results have been verified by experts on Dipterocarpaceae, Prof. Dr Peter S. Ashton, of the Royal Botanic Gardens Kew, Richmond, England. The conservation status of *H. sangal* based on the IUCN (the International Union for Conservation of Nature) is vulnerable A2cd ver 3.1 and current population trend is decreasing [1]. The remaining habitat of this plant species was also declared as a new habitat in the region of Pasuruan (rediscovery) [2]. Based on information obtained from the Herbarium Bogoriense, Biology Center-LIPI, *H. sangal* was also found in the Banyuwangi district in 1898, Malang district in 1934 and Blitar regency in

1935 [3]. The remaining habitat *H. sangal* was newly discovered in the district of Pasuruan, located close to the northern coast of Java, while the three locations where it was first discovered were located closer to the southern coast of the island of Java [2]. In terms of ecology, this information is likely to give us new knowledge about the habitat of *H. sangal* in East Java.

Figure 1. The tree and canopy of *H.sangal* in its natural habitat
Left: The tree stand; Right: The tallest tree among others, in Pasuruan, East Java

Dipterocarpaceae in Indonesia is not evenly distributed on each island with most found west of the Wallace Line and northwest of the Indonesian archipelago [4]. The Dipterocarpaceae family consists of more than 500 species in 14 genera and mostly grows well in Asia, especially in Sumatra and Kalimantan [4,5]. Generally, In Java, there are 10 species of Dipterocarpaceae, two of them are from the genus Hopea: *H. celebica* and *H. sangal*. However, the population of *H. sangal* has been declared as a rare species [6,7]. The next editions of the publication by Ashton Wong in 1982 and 1994 also did not mention the existence of *H. Sangal* in East Java.

The new habitat where *H. sangal* was rediscovered is a degraded forest near the spring area in East Java. This remaining habitat becomes a great concern because only one individual tree was found in that area [2]. Moreover, the flowering timing and masting periods of Dipetrocarpaceae is seasonal and does not occur throughout the year. Previous studies proposed that the mass flowering of Dipterocarps is associated with the El Nino phases and it evolved as a response to the seasonal tropic dry climate. This reproductive strategy of Dipterocarps is important for seedling survival in tropical forest [8,9].

The tropical forest has heterogeneous canopy structures that affect environmental factors, especially light availability and microclimate which would affect the forest regeneration. The canopy gap has an important role in providing the habitat that is suitable for the seedling and sapling to grow, hence it affects the species richness and diversity in the forest [10]. The light availability is also different in the type of forest. For example, the primary forest consists of larger trees and higher stratification of canopy layers than the regeneration forest. Therefore the light availability in regenerating forests higher than in the primary forest. The disturbed and degraded forest has a larger canopy gap and lower stratification layer hence this type of forest would allow the higher radiation of sunlight to the forest floor [11].

The functional adaptation of the plant varies among species. Several plants species could tolerate the low intensity of light and are able to compete with another plants species to survive in the forest and some species only can grow under high light intensity [12,13]. The response of Dipterocarpaceae seedlings to the light intensity is usually different depending on the species. For instance, the responses of two species of Dipterocarpaceae seedlings: *H. helferi* . and *H. odorata* were different to various light conditions[14]. A previous study of *Shorea selanica* also stated that the high percentage of shading would reduce the intensity of sunlight received by plants and it would inhibit growth processes [15]. *H. sangal* is considered a shade-tolerant species, hence the establishment of the seedling in their natural habitat occur under the closed canopy. An earlier study suggested that *H. sangal* has the highest rank in size-specific height, diameter, and aboveground biomass growth rates performance under low light conditions than 21 species of Dipterocarpaceae, but it had lower growth performance under medium and high light treatment [16].

Earlier studies measuring the growth performance of the *H. sangal* seedling were using the shade house, hence there is still a lack of information about its growth performance under variable or non-constant light intensity. In this study, we used two different levels of canopy shades to replace the shade house. We also measured the microclimate differences between two levels of canopy shades and investigated the relative growth rate (RGR) of the seedlings. The RGR has relation to plants' ability to adapt and compete to survive and it would give information on how plants respond to differences in abiotic factors [17].

Besides investigating the RGR we also observed the water use efficiency of the seedling. The plant's distribution in the natural habitat is related to their various responses to their environment [18]. The response of the plants to water availability is related to their functional strategies. The extreme drought, for instance, changes the plant biomass allocation to reproductive organs [19]. The water deficit would disrupt the balance of chemicals in the plant which results in reduced photosynthesis results or all physiological processes running abnormally. Water crucially impacts the dynamics of plant growth, productivity and reproduction [20–22]. The Hopea is distributed mostly in lowland forests that have dry climates or in the dry season of less than 4 months [4,5,23]. This suggested that plants cope with the seasonal water limitation. The new remnant habitat of *H. sangal* was found close to the spring area, in which the presence of the water is abundant. Hence it is important to understand the water use efficiency of this species. Moreover, the previous study proposed that the water use of each species of the Dipterocarpaceae family varied. For instance, *Shorea contorta, Parashorea malaanonan, Hopea maliboto* and *Hopea plagata,* the species with the lowest water use *was H. plagata* with a transpiration rate *was* 3.3mm day^{-1} and its water use was 4 kg day^{-1} with the trunk's diameter of 6.6 cm [24]. Besides genetic factors, the water use efficiency for plant growth is also influenced by ecological factors such as temperature, humidity, wind speed, weather, and sunlight. Water use efficiency is used as a link between timber production and management of water use by plants.

Our objective in this study was to understand the physiological performance of *H. sangal* seedlings in two different levels of canopy gaps and microclimate. We tested these hypotheses: (1) there will be significant differences in the RGR and WUE of the seedlings under different canopy gaps and, (2) does the WUE has a relationship with the seedling RGR?

2. MATERIALS AND METHODS

2.1. Materials and Location

The research materials were seedlings of *H. sangal* collected from the field in Pasuruan which were planted in plastic pots. Before the research began, the seedlings were acclimatized to obtain seedlings of similar size. Due to seedlings of this vulnerable species was limited, we used 18 seedlings in this study. The diameter of the plastic pots used was 29x20x19 cm in the same media which consisted of sediment and compost mixture with a ratio mixture of 3:1.

2.2. Experiment set-up

The selected seedlings were established beneath closed-canopy trees. The canopy gaps were divided into two groups. One group was placed under a closed canopy tree with 94% of shade and the other group was placed at a location slightly more open in a shade of 88%. The percentage of shades (94% and 88%) was obtained from the comparison of the average measurement results with the open or without shade results . Each group consisted of 9 replications.

The research activities started with weighing all the pots and seedlings. The soil from each pot was taken to measure the water content of the soil by the gravimetric method, then all the pots were covered with transparent plastic and tied with a rubber band so that the unintended water will not enter the pot and there is no evaporation from the soil. The pots were weighed every five days and then re-watered to field capacity.

2.3. Microclimate Measurement

To know the differences in microclimate under the two shades canopy gaps, we collected data on energy light using solarimetri, temperature, and humidity using a sling psychrometer. The observations were performed weekly with the frequency of observation five times at 08.00; 10:00; 12:00; 14:00 and 16:00.

2.4. Relative Growth Rate

The method used was a completely randomized design with two treatments: 94% shades and 88% shades treatments. The observed parameters were relative growth rate/RGR (g g^{-1} $week^{-1}$), water use (kg $month^{-1}$), and Water Use Efficiency (WUE) (g DM kg^{-1} H_2O). The RGR obtained by harvesting method and the rate of relative growth was calculated using the following equation [25]

RGR = (ln ln W2-W1) / (t2-t1)

Where:

ln= natural logarithm
t1 = time one
t2 = time two
W1 = dry weight of plant in time 1
W2 = dry weight of plant in time 2

2.5. Transpiration and Water Use Efficiency

The WUE was quantified at whole plant level by using gravimetric technique. The analysis of plants' water use efficiency was calculated for each plant as the ratio of biomass production during a definite growth period to water transpired during the same period. The average value increment of biomass was obtained by comparing the measurement results with the amount of the sample's biomass. Meanwhile, the amount of water that transpired during the experiment was calculated by measuring evaporative water loss from the pots experiment.

2.6. Statistical Analysis

The experiment was analyzed as a completely randomized design with two different shades of conditions and 9 replicate seedlings per treatment. The following model was applied in this study:

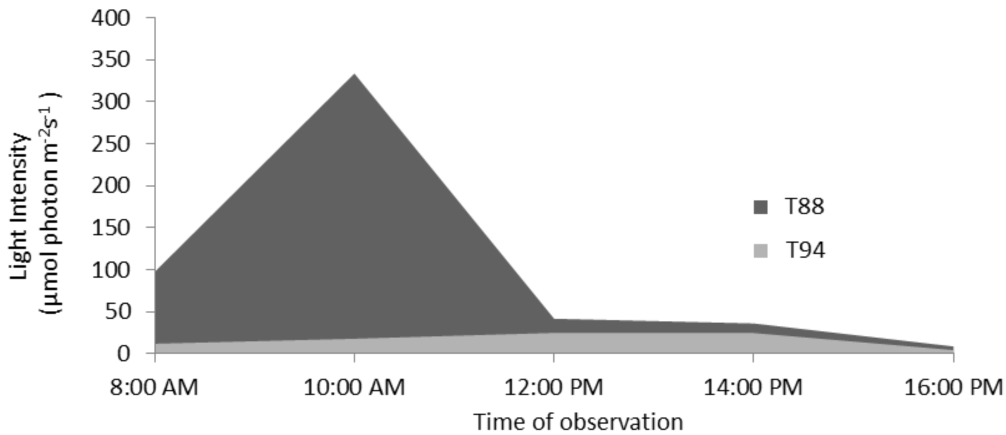
$$Y_{ij} = \mu + a_i + e_{ij}$$

where Y_{ij} is the measured values for seedling j under the treatment i; μ = constant; a_i = effect of shades treatment and e_{ij} = error term. The normality and randomness of data were tested using Kolmogorov-Smirnov and Run tests. The normal data were analyzed using variance analysis (ANOVA) with a confidence level of 95%. The relationships between RGR and WUE was analyzed with regressions using Statistica 64 software version 13.3.

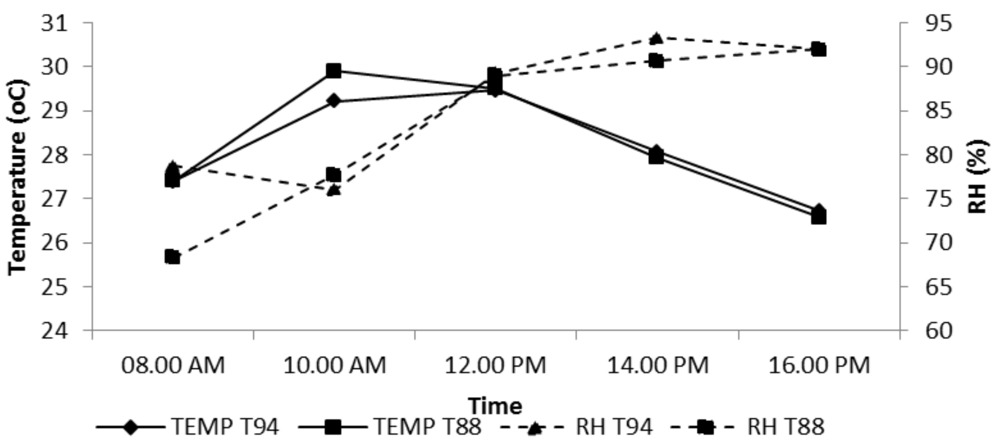
3. RESULTS AND DISCUSSION

3.1. Microclimate under different canopy gaps

The light intensity, temperature, and relative humidity of both canopy-shaded areas were measured from 08.00 until 16:00. Even though the level of shades under the tree canopy did not have large differences of 88% and 94%, interestingly the light level during the periodic opening of the gap was significantly different. It occurred at a specific time (08:00 – 12.00). Under the 88% shaded area, the light intensity increased from 08.00 and reached its highest point at 10.00 and then decreased until 16.00, while in 94% canopy shade, the light increased from 08.00 and reached its highest point at 14.00 and then decreased until 16.00 (Fig 2a). The highest light intensity was at 10.00 of 333.57 µmol photon $m^{-2}s^{-1}$ while at same time light intensity that entered 94% shade was 17.67 µmol photon $m^{-2}s^{-1}$. The differences in gap size at canopy shades increase in the duration of periods of full sunlight in the understory. Hence, at 8 – 12 am the seedling under the 88% shades received maximum solar energy.



(a)



(b)

Fig 2. (a) Mean value of light intensity between 08.00 -16.00, while (b) mean value of temperature and relative humidity between 08.00-16.00

Temperature and relative humidity have a negative correlation. If the temperature increases, the humidity will decrease and vice versa. Temperature, humidity, and light intensity affect seedling growth and transpiration affects seedling water use. In both different canopy shade conditions between 88% shade and 94% shade, temperature and humidity did not show a significant difference. The temperature at 08.00 would gradually increase and reached the highest value by 10:00 in both shaded areas. Temperatures descended from 12:00 until 16:00. The highest humidity occurred at noon in the 94% shade area (Fig 2b).

3.2.The seedling growth, Water Use, and Water Use Efficiency

The Relative Growth Rate (RGR) is the plant's ability to produce biomass as the result of assimilation per unit of dry weight per time expressed in the g g^{-1} $week^{-1}$. The results showed that *H. sangal* seedlings placed in two different amounts of shade have significantly different RGRs with P = 0.025. The RGR of *H. sangal* seedlings that were placed in 88% shade were higher than those placed in 94% shade (Fig 3).

The seedling grown under 88% shade had RGR 0.02 g g^{-1} $week^{-1}$, while those under 94% shades was 0.01 g g^{-1} $week^{-1}$.

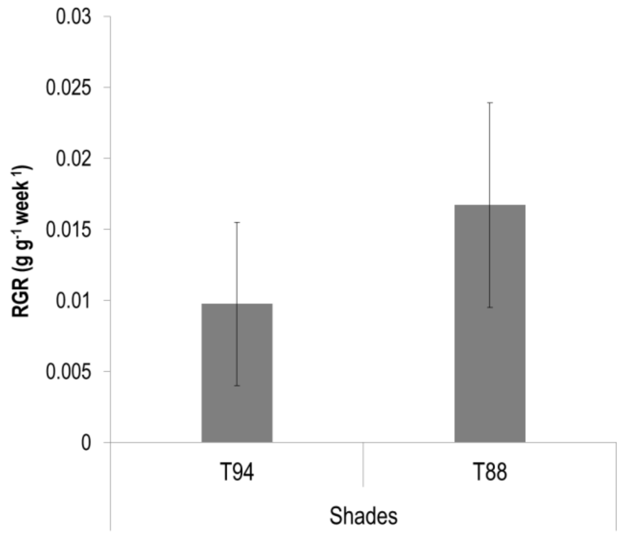
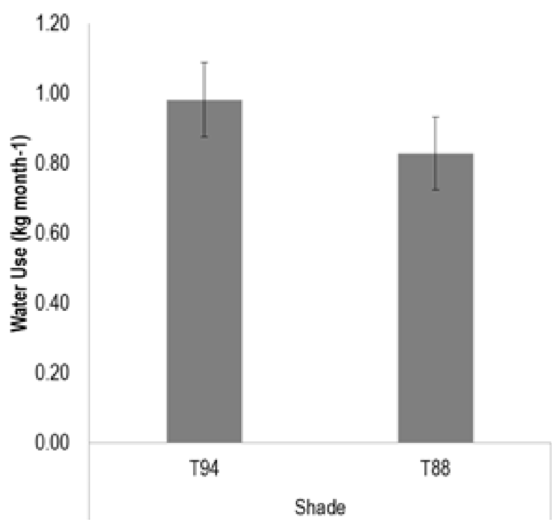
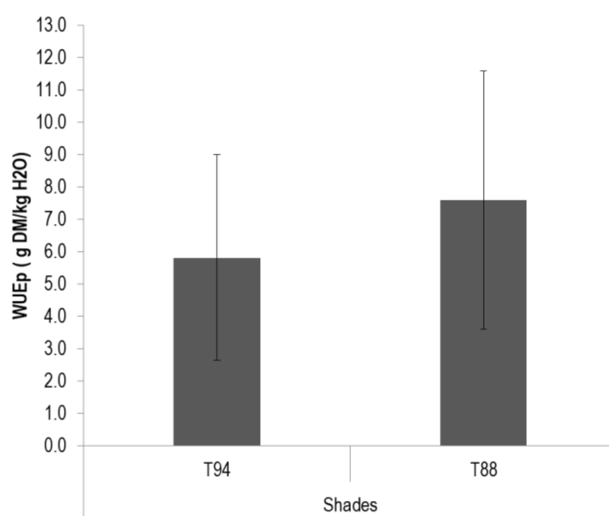


Fig 3. Mean value of RGR of seedling of *H. sangal* growth in two shaded areas T94= 94% shades and T88 = 88% shades, *P* < 0.05

The significant differences in RGR were due to a difference in the light intensity received by seedlings. Our result showed that seedling RGR was higher under 88% shades canopy than under 94%. A higher RGR value indicates that the seedling grows faster at the given size. Under the canopy gap in the forest, the responses of shade-tolerant species toward the light level were different. Some shade-tolerant species could not tolerate the higher light intensity during the periodic openings [13]. In the right amount of light intensity plants grow properly and the growth will increase concurrently as much as sunlight received because assimilates produced during the process of photosynthesis are greatly influenced by the intensity of the sun [16]. Based on our result, *H. sangal* is one of the shade-tolerant species that had a great response toward the higher light levels during the periodic opening of the canopy gap. It has the capability to respond to the higher sunlight with the higher RGR under 88% of the canopy gaps area.





(a) (b)

Fig 4. (a) Water use of *H. sangal* seedling under two different treatments at P > 0.05, while (b) Water Use Efficiency of *H. sangal* seedlings at P > 0.05

The amount of water required by the plants is different for each individual, depending on the plant

Figure 4 : Water Use Efficiency of ***H. sangal*** seedlings

species and external factors. The data showed that the amount of water use of *H. sangal* seedlings in both areas were not significantly different at P = 0.333. The seedlings under 88% shades treatment used a lower amount of water (0.83 kg month^{-1}) compare to the 94% shade treatment which used 0.93 kg month^{-1} (Fig 4a). Water use efficiency (WUE) is expressed by the number of grams of the dry weight of plants per kg of water transpired. The seedling with the highest WUE was those grown under 88% shades about 7.6 g DM kg^{-1} H_2O and 94% was 5.8 g DM kg^{-1} H_2O (fig 4b). Based on our statistical analysis the WUE of seedlings that were grown under different shades was not significantly different at *P* > 0.05.

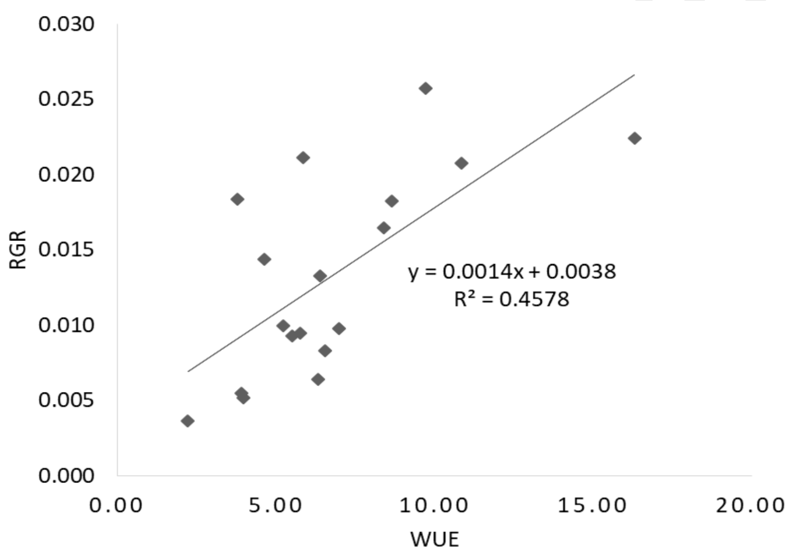


Fig 5. Relationship between RGR and WUE of *H. sangal* seedlings, the model was significant at P <0.05 the slope is indicated a positive relationship

The models show that WUE has a positive relationship with the RGR of seedlings (R2 = 0.5; P < 0.05). The seedling that has higher WUE grew faster showed by the RGR value (see fig 5). Water use efficiency is the effectiveness of water use by plants in the transpiration process for their growth or biomass addition, in comparison to the amount of water that is absorbed. Hence it will affect plant growth or biomass. Based on this study, the seedling could use the water supply efficiently to grow rapidly.

Water in the plant plays a large role in physiological processes. Water composes protoplasm of 85-90% of the plant's weight acts as a reagent in the process of photosynthesis and also has a function as a salt and substance solvent for cell growth. Water in the plant's system will be released into the air by the transpiration process. Under the 88% canopy shade area, the *H. sangal* seedlings produced higher biomass than those that were placed in the 94% shade area. Interestingly, this study also found that at the higher RGR, the water use of the seedling was lower compared to 94% shade. Thereby the seedlings of this species could optimize carbon assimilation and reduce water losses. The earlier study also proposed that the amount of Dipterocarpaceae water use is lower than 7 other families of plants with the same diameter and in the same climate [26].

The seedling *of H. sangal* had faster biomass increment under the higher level of light during a day illustrating that the seedling requires the higher light intensity to maximize carbon assimilation. This study also suggested that the seedling may be able to compete to grow under lower stratification layer forest or the larger canopy gaps. The high WUE of *H.sangal* was contributed to its growth and it would benefit the seedling for surviving the dry month season. Understanding the light requirement and also the WUE of the seedling will give information on the environmental suitability for the seedling to grow properly. This information would be needed for further conservation action of this vulnerable species.

4. CONCLUSION

The differences in light intensity during periodic opening under the canopies significantly affect the seedling growth but not the water use and water use efficiency in *H. sangal* seedlings. Based on our study *H. sangal* is considered one of the shade-tolerant species that had a great response toward the higher light levels during the periodic opening of the canopy gap. High WUE of seedlings positively affected plant growth. It suggested that the seedlings of *H. sangal* are able to optimize the carbon assimilation and reduce water losses, hence seedlings could use the water supply efficiently to grow rapidly.

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