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Ecology of *Zanthoxylum acanthopodium*: Specific leaf area and habitat characteristics

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Abstract. *Junaedi DI, Nurlaeni Y. 2019. Ecology of* Zanthoxylum acanthopodium*: Specific leaf area and habitat characteristics. Biodiversitas 20: 732-737.* Andaliman (*Zanthoxylum acanthopodium*) is an important species in North Sumatra, Indonesia either from conservation, economy, or socio-cultural point of views. *Z. acanthopodium* is known as plant species difficult to cultivate and its natural distribution in Indonesia is restricted to Aceh and North Sumatra, Indonesia. This study aims to identify the modest characteristics of *Z. acanthopodium*'s habitat in natural and cultivated areas and to assess the importance of light in these two habitat types in North Sumatra. We surveyed four districts in North Sumatra and used specific leaf area (SLA) as proxy for the importance of light in *Z. acanthopodium* habitat. We found that the species grow naturally in high slope areas and not in a plain area. Similarly, in cultivated areas, this species grows well in sloped area with minimum inundated surface run-off water. *Z. acanthopodium* with higher SLA values occurs in natural habitat and high slope areas while those with smaller SLA are found in cultivated areas and low slope although the difference is statistically not significant. This study indicates the importance of light and surface run-off for suitable habitat of *Z. acanthopodium*. Further studies are needed to examine the quality, intensity, and frequency of light to support *Z. acanthopodium* growth; and to examine the role of landscape inclination and their position relative to sun exposure (i.e. aspect) for its survival and growth rate.

Keywords: Batak, leaf trait, North Sumatra, Rutaceae, traits-based ecology, *Zanthoxylum acanthopodium*

INTRODUCTION

Andaliman (*Zanthoxylum acanthopodium*) is a locally endemic species for Indonesia which naturally occur in the Provinces of North Sumatera and Aceh. In North Sumatera, *Z. acanthopodium* is distributed particularly in areas adjacent to Toba Lake such as Karo, Brastagi, and Asahan (Hartley 1966). *Z. acanthopodium* is also a culturally-important species for local people in North Sumatra. This species is used as spice with very unique taste that characterizes Batak traditional cuisine flavor. The species is economically important commodity because it has a relatively expensive price in local markets (Sibarani et al. 2013). Furthermore, it also contains bioactive compounds with anti-bacterial, anti-fungal, anti-oxidant, and medicinal activities (Majumder et al. 2014). *Z. acanthopodium* is relatively difficult to grow in cultivation, and most local farmers rely on naturally-grown seedlings from prescribed burning lands than sowing its seeds by themselves. Many natural populations exist in high slope habitat (Siregar 2010). The species also naturally occurs in rainforests and thickets at low and mid-altitude (Hartley 1966).

Despite the abundant studies about *Z. acanthopodium*'s bio-prospecting (such as potential anti-fungal, anti-oxidant and other important uses), there are only few ecological studies of Andaliman that have been conducted. Eco-physiological studies are important to support agriculture development of this species. Moreover, habitat ecology studies will help us to better understand *Z. acanthopodium* habitat to support its conservation and cultivation. Habitat ecology and eco-physiological studies can be analyzed using traits-based approach. Trait is defined as "morphological, anatomical, biochemical, physiological or phenological feature measurable at individual level" (Violle et al. 2007). Traits can be used as proxies in ecophysiology and habitat ecology studies because traits indicate physiological and ecological preferences of a species (van Kleunen et al. 2010). As such, traits are important tools for ecological studies of this species.

Specific leaf area (SLA) is functional trait that commonly used in traits-based ecology because it is a useful proxy to explain many eco-physiological parameters. SLA correlates with plant responses to abiotic conditions such as nutrient availability, shading, and water consumption (Martin et al. 2009; Poorter et al. 2009; Van Kleunen et al. 2010; Cornwell and Ackerly 2010; Kaupenjohann and Kowarik 2013; Gibert et al. 2016). SLA is a potential indicator to explain abiotic role in a plant habitat Thus, from practical perspective, SLA is an easy-to-measure parameter which contains informative data for decision makers or stakeholders.

By collecting detailed information of *Z. acanthopodium* ecology and habitat, we may support particular development for agriculture and conservation purposes of the species. First, eco-physiological characteristic of the species (such as shade-tolerance affinity, water consumption rate, etc.) may determine the propagation technique and agriculture development of *Z. acanthopodium*. Second, comprehensive habitat data can also assist stakeholders working on plant conservation to predict the natural occurrence, estimate the population, and prioritize the conservation area of this species. Lastly, information on seed germination characteristics and processes may support farmers to conduct more efficient and practical seed germination for *Z. acanthopodium* propagation through seeds.

Based on existing literature, seed propagation technique for *Z. acanthopodium* is relatively difficult. Farmers rely on prescribed burning to stimulate the seeds to germinate from soil seed bank stocks (Siregar 2013). Moreover, natural populations of this species also mainly occur in particular areas with hilly slope (Hartley 1966). In cultivation, *Z. acanthopodium* is also planted in non-plain areas with slope (personal observation). Due to previous information about habitat of the species (Hartley 1966), light factor may play important role for *Z. acanthopodium* survival in natural habitat. The response of this species to light intensity may be inferred from SLA as the proxy of plant eco-physiological response to abiotic factor such as light.

This study aims to identify the modest characteristics of *Z. acanthopodium* habitat in natural and cultivated areas and to assess the importance of light in these two habitat types in North Sumatra using SLA as the eco-physiological indicator. By testing the correlation between SLA and different habitat ranges of the species, we may be able to develop our understanding about its habitat requirements. This information is useful to support *Z. acanthopodium* conservation and its sustainable use as cultivated plant.

MATERIALS AND METHODS

Study area

The study was conducted in four sampling locations in four regencies around Toba Lake area, North Sumatra, Indonesia. These locations are: Tanjungan (Samosir District), Eden (Tobasa Distrik), Batumardinding, (Simalungun District), and Tanjung Beringin (Dairi District) (Figure 1). Samplings were conducted in March 2018 using purposive method. We selected these four locations based on records from previous studies, either for natural population (Hartley 1966) or cultivated plants (Lumban Raja and Hartana 2017). These four locations have tropical rainforest climate (Af based on Koppen-Geiger climate type) with rainfall of more than 2500 mm per year and mid to low air temperature (17-27°C) (www.climate-data.org). Most of cultivated *Z. acanthopodium* in our study areas were planted by farmers as supplementary plants in their coffee plantation, except for one location in Eden (exclusive *Z. acanthopodium* plantation as part of ecotourism area).

In general, the maximum slope at a district level in the studied areas is 40%. All the sampling locations are located in surrounding Lake Toba Lake with slope facing the lake. Detailed information of the topography of studied areas is presented in Table 1.

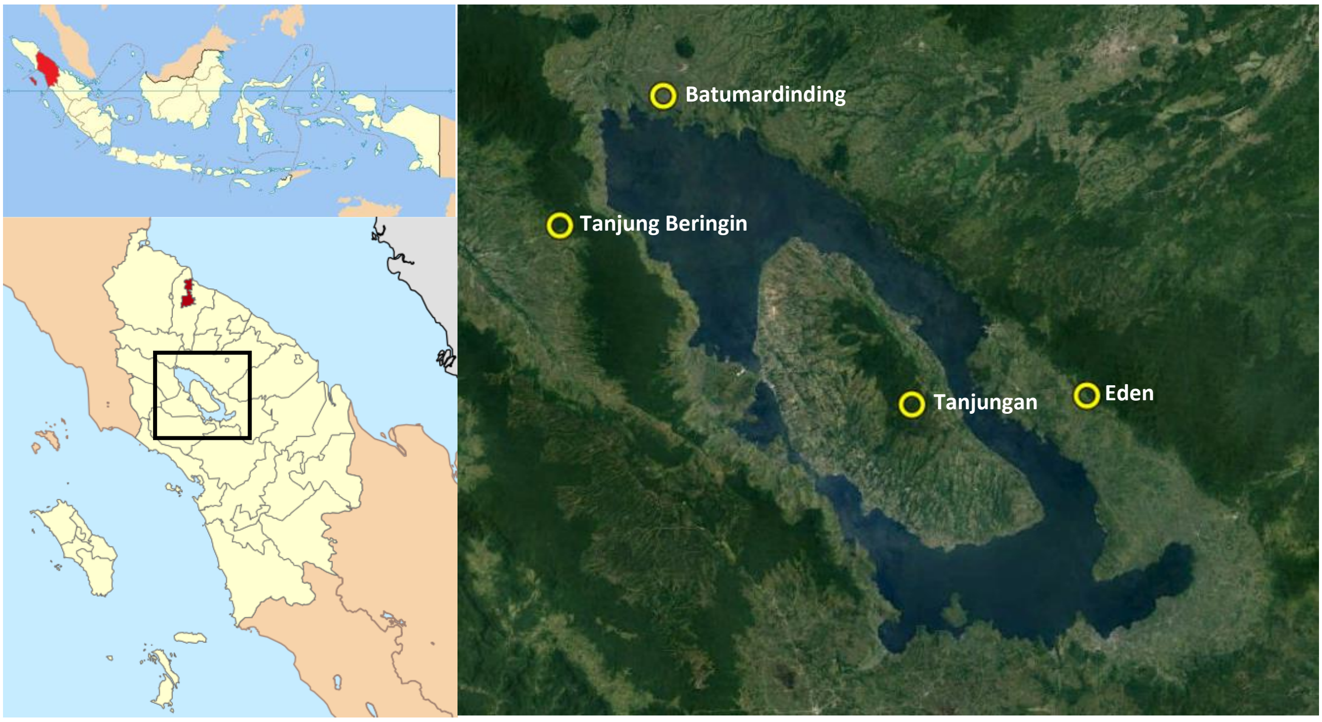


Figure 1. Sampling locations around Lake Toba area in four districts, North Sumatra (presented by yellow circle). Map is acquired from GoogleMap (google.com/maps/) and Wikipedia (wikipedia.org)

Table 1. General topographic conditions of four districts of studied areas

District	Topography detail	Sources
Samosir	Hilly and mostly not plane (0-2° = 10% area, 2-15° = 20% area; 15-40° = 55% area, and 40° = 15% area)	http://tpsamosir.limnologi.lipi.go.id/samosir.html
Tobasa	Most of the area dominated by mountainous area and lowland area (15-40° = 43% area; 2-15° = 29% area; 0-2° = 28% area)	http://samosirkab.go.id/web/geografis
Simalungun	Varied topographic condition with slope ranges from 0 to 35^0 slope (0-35° = 100% area)	Pokja Sanitasi Kabupaten Simalungun 2016
Dairi	Specific topographic profile with many hilly areas (up to 40° slope). Geographically, area of Dairi District is buffer zone that supports Toba Lake ecosystem (0-40° = 100% area)	https://samosirkab.bps.go.id/statictable/2016/08/04/4/letak-dan-geografi-kabupaten-samosir-tahun-2015.html

Procedures

Field survey and sampling location

The selection of sampling locations four districts is referred to previous study on natural distribution and cultivation area of *Z. acanthopodium* (Hartley 1966) and morphological variation of the species (Lumban Raja and Hartana 2017). In every sampling location, we collected dataset for every recorded individual (Table 2). The dataset consists of: leaf photo, leaf thickness, land slope, and land use type. Leaf photo was collected to measure specific leaf area (SLA). Leaf thickness measured using digital caliper Freder (0-150 mm). We simplified land slope into two categories: low (< 30° inclination) and high (> 30° inclination). Land use type is divided into cultivation area and natural area (Figure 2).

Table 2. Sampling location details (geographic coordinates, altitude, average temperature, slope inclination and slope aspect) of four locations

Samples	Geographic coordinates	Altitude (m asl.)	Average temperature (°C)	Slope inclination (%)	Slope aspect
Tanjungan	2.555792 N, 98.902174 E	1250	18.3	40%	North-face
Eden	2.590526 N, 99.038629 E	1100	21.2	35%	South-face
Batumardinding	2.702754 N, 98.928999 E	1150	21.6	5-10%	West-face
Tanjung Beringin	2.764007 N, 98.480617 E	800	22.1	< 5%	South-face

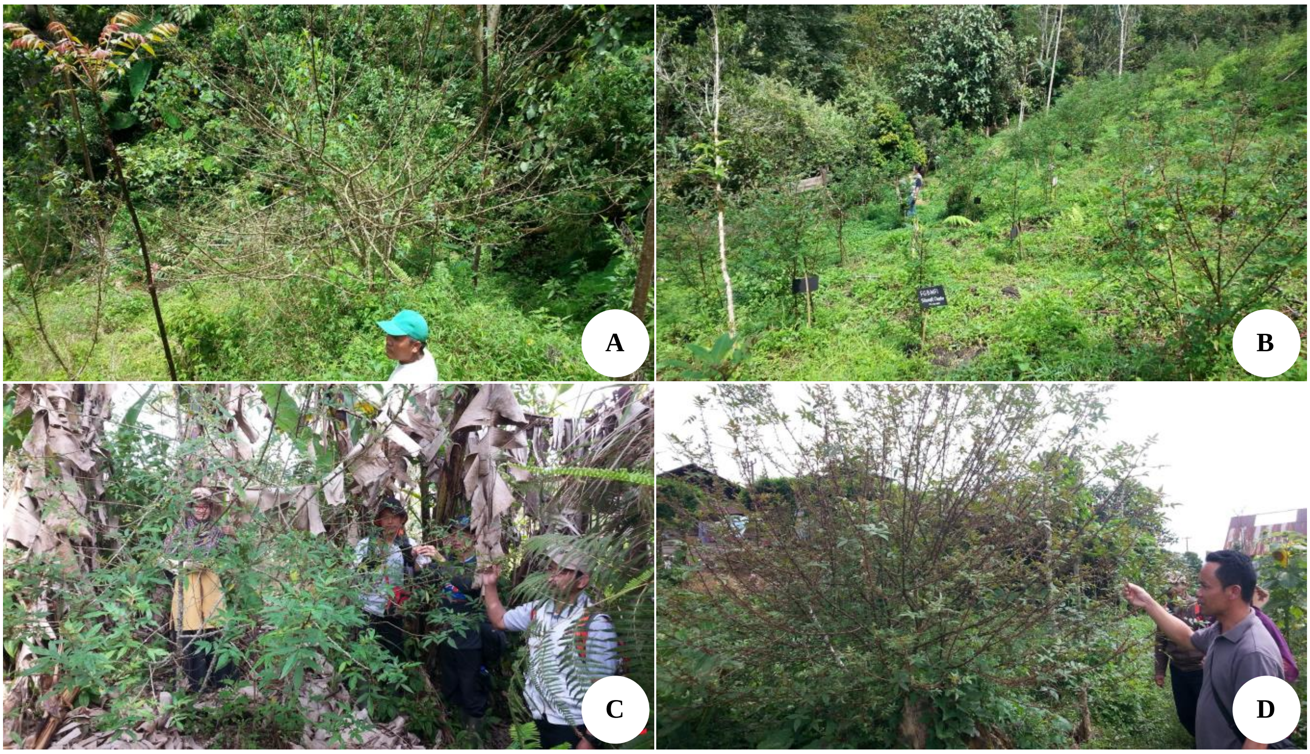


Figure 2. Four different habitats of *Z. acanthopodium* in different sampling locations. A. High inclination in natural area; B. High inclination in cultivated area; C. Low inclination in natural area; D. Low inclination in cultivated area

SLA data collection

SLA (in mm^2/mg) is formulated as the ratio between leaf area and leaf dry mass (Perez-Harguindeguy et al 2013). Leaf area was calculated by analyzing leaf photo using ImageJ with unit measurement of mm^2 (Schneider et al 2012).. Leaf dry mass data was obtained by weighing leaf specimen collected from the field after being dried in the oven. The leaf was collected from individual tree then it was stored into sealed plastic bag before it was put in MEMMERT-IN55 oven under constant temperature of 70^0 C for 72 hours. Then, the oven-dried leaf was weighed using OHAUS digital balance with unit measurement of mg.

Data analysis

Difference of SLA based on natural versus cultivated habitat

The difference of SLA between cultivated *Z. acanthopodium* occurring in cultivation land and wild *Z. acanthopodium* growing naturally in natural forest was tested using independent samples t-test (Welch 1947). A difference in SLA value in these two different habitat types might indicate different light factor effect to *Z. acanthopodium* in its habitat.

Habitat preference based on natural versus cultivated and high versus low slope

Bayesian logistic regression analysis was conducted to examine the proportion of habitat type of *Z. acanthopodium* (natural versus cultivated) based on the SLA values and formulated as:

$$p_i = e^{(gi)/(1+e\ (gi))} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ e1)}$$

Where: pi is the probability of an individual *Z. acanthopodium* grow in habitat and $g_i = a_0 + ß_1SLA_i + \varepsilon_i$. Dependent variable was coded as 1 for natural habitat and 0 for cultivated land.

Similar statistical analysis was also conducted to model the proportion of habitat in term of land slope preferences based on SLA value and equated as:

$$q_i = e^{(hi)/(1+e\ (hi))} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ e2)}$$

Where: qi is the occurrence probability of an individual *Z. acanthopodium* in two different slope categories (low and high) and $h_i = a_0 + \gamma_1SLA_i + \varepsilon_i$. High slope habitat was coded as 1 and low slope habitat was coded as 0. Bayesian statistical analysis for e1 and e2 were conducted in R Statistical Software (R Team 2015) using rjags (Plummer 2003) to call Just Another Gibbs Sampler (JAGS).

RESULTS AND DISCUSSION

Total samples and SLA data

We sampled 19 individual trees of *Z. acanthopodium* from four locations around Toba Lake in which 4 trees were from Tanjungan (Samosir), 5 trees were from Eden (Tobasa), 6 trees were from Batumardinding (Simalungun), and 4 trees were from Tanjung Beringin (Dairi). In total, 115 leaf samples were collected for SLA measurements. Measurements of SLA of *Z. acanthopodium* show that values vary from 29.38 to 88.55 mm^2/mg with mean SLA value of 54.05 mm^2/mg (Figure 3).

Statistical analysis to compare SLA data of *Z. acanthopodium* differentiated based on habitat land use (i.e., natural versus cultivated area) and habitat slope (high slope versus low slope) show they are statistically not different. This is indicated by the p-value of t-test statistic result which is 0.073 for different land use habitat and 0.075 for different habitat slope. Although, in general, the SLA values of *Z. acanthopodium* in natural habitat and higher slope are higher than those in cultivated area and low slope (Figure 4.A-B).

Logistic regression results for land use and land slope

Logistic regression results indicate clear SLA gradation between different land slope categories and modest differentiation for different land use habitats (Figure 5). The value ranges of credible interval of the logistic regression results do not have zero value (Table 3), meaning that there is statistically significant effect of using SLA value as indicator or proxy of the occurrence probability of *Z. acanthopodium* in different land use and land slope. In other words, SLA value of *Z. acanthopodium* reflect different habitat condition.

Table 3. Logistic regression analysis results for land use preferences (e1) and land slope preferences (e2). CI = credible interval

Model	Regression equation	2.5% CI	mean	97.5 CI
Land use (natural vs cultivated area)	Log (pi) = -2.577 + 0.087*SLA	0.003	0.082	0.198
Land slope (high vs low slope)	Log (qi) = -0.259 + 0.065*SLA	0.003	0.063	0.141

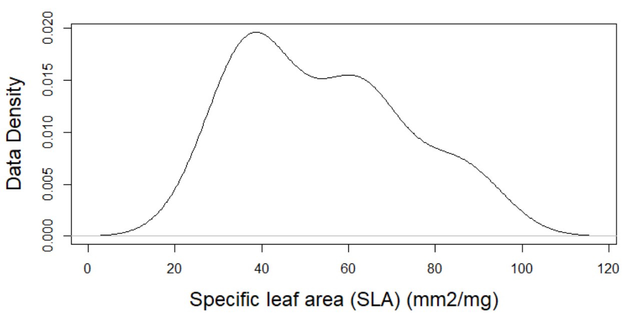
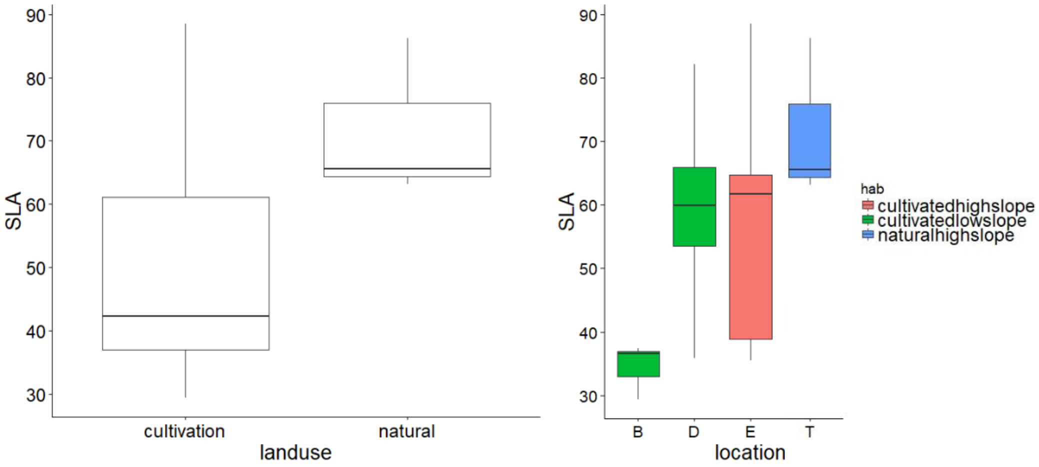
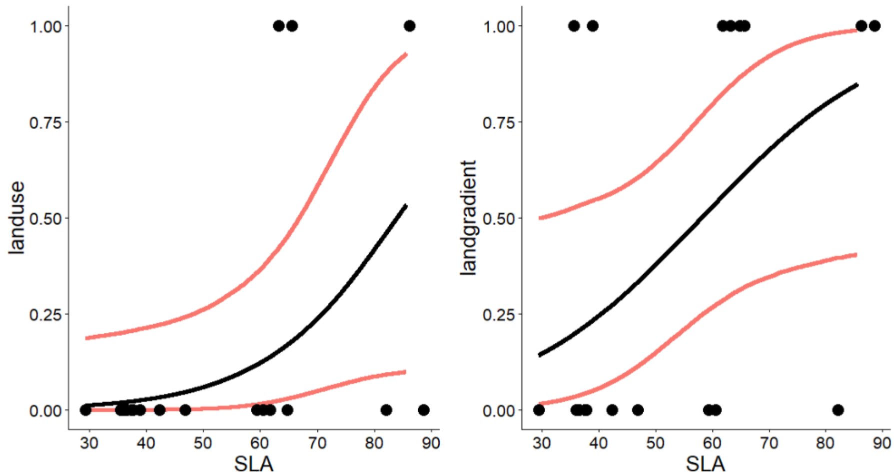


Figure 3. Data distribution of specific leaf area (SLA) of *Z. acanthopodium*. SLA data were acquired from 115 leaf samples from 19 individual trees



A B

Figure 4. A. Specific leaf area (SLA) value for *Z. acanthopodium* in natural habitat and cultivation area; B. SLA value for *Z. acanthopodium* based on the slope of habitat and sampling locations; note: B = Batumardinding (Simalungun), D = Tanjung Beringin (Dairi), E = Eden (Tobasa), and T = Tanjungan (Samosir)



A B

Figure 5. A. Predicted probability of *Z. acanthopodium* habitat preferences on different land use type (natural = 1, cultivation = 0) based on SLA value. B. Predicted probability of *Z. acanthopodium* habitat preferences on different land slope (> 30^0 slope = 1, < 30^0 = 0) based on SLA value. Black line is the predicted probability, red lines are the 2.5% and 97.5% credible interval that describe the uncertainty of the prediction in the model.

Discussion

This study shows that SLA may capture the difference in habitat conditions of *Z. acanthopodium*. The fact that *Z. acanthopodium* with larger SLA occurs in high slope habitat may reflect the importance of light factor for this species. Unlike low slope area (plain), high slope area may cause *Z. acanthopodium* to receive incomplete sunlight during daytime. The affinity of high slope area may also explain why natural populations mostly occur on foothills (Hartley 1966). High SLA value in this study reflects an adaptation to shading because SLA value in this study does not correlate with leaf thickness but correlates with leaf area (unpublished data). Larger leaf area is needed for *Z. acanthopodium* to maximize light irradiance acceptance by leaf in order to adapt with shading (Jackson 1967, 2009; Poorter and Bongers 2006). During the survey, we did not find any naturally grow *Z. acanthopodium* in plain and opened natural habitats. Thus, naturally grow *Z. acanthopodium* may need shading to support their life. The results of this study may also indicate that fully opened habitat with full sun exposure during daytime may not be suitable for the species. For agricultural application, shading treatment to some extent is needed to cultivate *Z. acanthopodium* in farmland. Thus, the species could be planted intercropped with higher trees that can provide shading in cultivation area.

Naturally grow *Z. acanthopodium* recorded in this study also occur in habitats with high slope. High slope habitat facilitates faster surface run-off water and minimizes water infiltration (Dodds 1997). *Z. acanthopodium* might be sensitive to inundated water or soil habitat with high water content or humidity. This less water preference may be indicated by relatively modest SLA value ranges (between 29.38 and 88.55 mm^2/mg) (Gibert et al. 2016). Further study is needed to examine the effect of soil water holding capacity (WHC) and ideal slope for *Z. acanthopodium* habitat to support their maximum growth. This study also suggests the importance of light preferences and surface run-off for *Z. acanthopodium* habitat suitability. Further studies are needed to examine the quality, intensity, and frequency of light to support *Z. acanthopodium* growth; and to examine the role of land inclination and their position relative to sun exposure to maximize the survival and growth rate of this species.

We are fully aware of the limitations of this study. First, SLA data in this study is not a time series data and may experience from phenotypic plasticity. Phenotypic plasticity may cause traits data (e.g., SLA) to have a wide range of values and is strongly influenced by fluctuation of abiotic habitat condition (Catoni et al. 2015; Turner et al. 2015; Vonesh et al. 2016). Second, we over-simplified the habitat slope into two categories and did not consider the position relative to sun exposure (i.e., aspect). We cannot quantify the level and duration of shading during daytime that experienced by *Z. acanthopodium* in this study. Therefore, we suggest further study to examine these factors and other eco-physiological parameters in order to define ideal habitat conditions for *Z. acanthopodium* growth. We also suggest to conduct analysis on soil physical and chemical properties of their natural habitat and conduct soil seed bank test to examine the diversity of species. Lastly, we also did not consider the cultivar differences of *Z. acanthopodium* in this study. Previous study showed that *Z. acanthopodium* populations in North Sumatra consist of several different cultivars that differences in leaf morphology (Lumban Raja and Hartana 2017). We suggest to conduct trait-based ecology study to test whether traits data varied among different cultivars.

In conclusion, this study shows that light and land slope are important factors for *Z. acanthopodium* abiotic habitat preferences. The SLA values of cultivated *Z. acanthopodium* and natural population are not significantly different, while SLA values between *Z. acanthopodium* in low slope and high slope are relatively different. Further studies are needed to examine the quality, intensity, and frequency of light to support this species growth; and to examine the role of land inclination and their position relative to sun exposure for *Z. acanthopodium* growth rate and growth success.

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