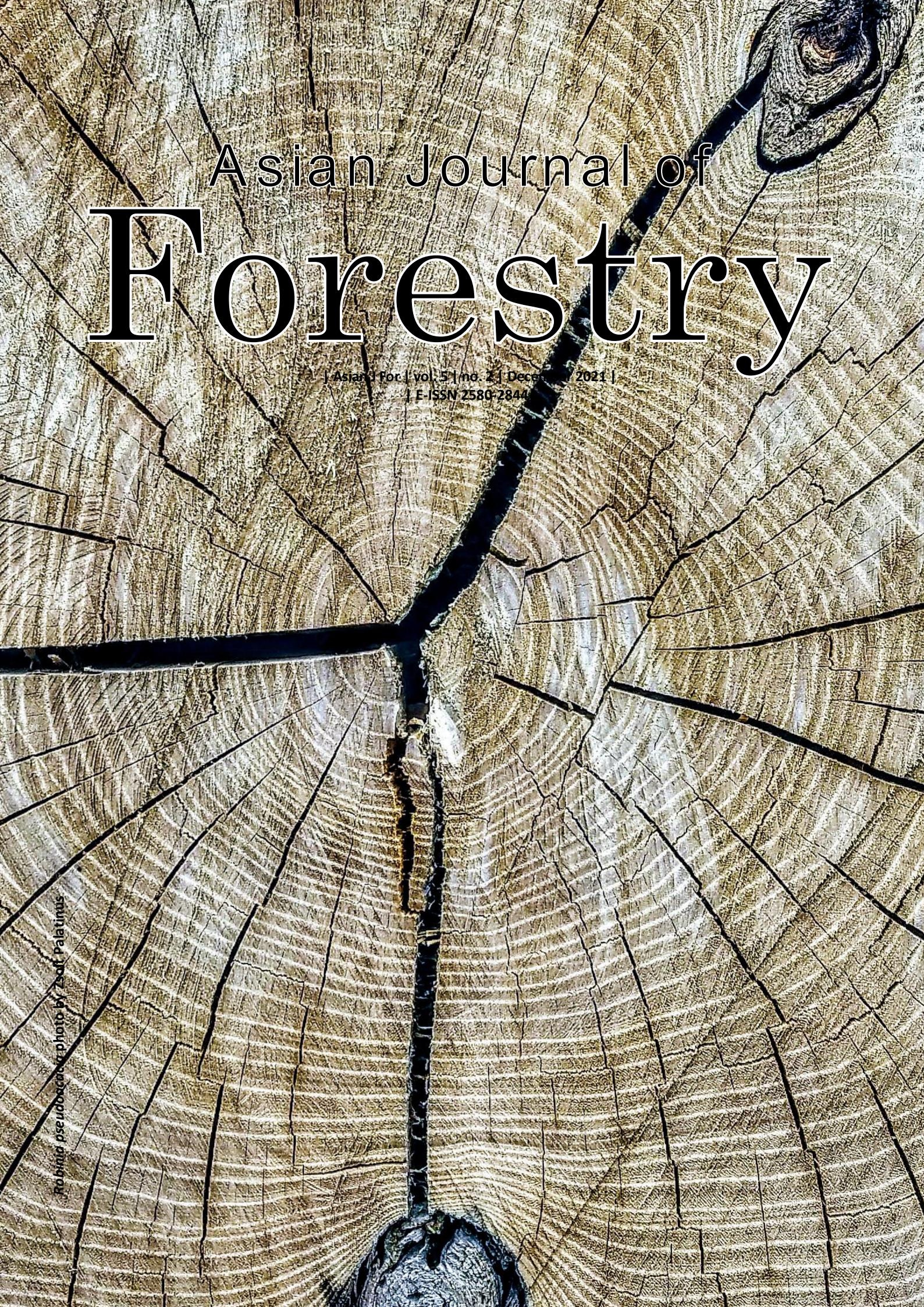
Asian Journal of Forestry

| Asian J For | vol. 5 | no. 2 | Dece[illegible] 2021 |
| E-ISSN 2580-2844



Robinia pseudoacacia photo by Zsolt Palatinus

Asian Journal of Forestry

| Asian J For | vol. 5 | no. 2 | December 2021| | E-ISSN 2580-2844|

Published semianually **PRINTED IN INDONESIA**

E-ISSN: 2580-2844

Asian Journal of Forestry

| Asian J For | vol. 5 | no. 2 | December 2021 |

ONLINE
http://smujo.id/ajf

e-ISSN
2580-2844

PUBLISHER
Society for Indonesian Biodiversity

CO-PUBLISHER
Universitas Sumatera Utara, Medan, Indonesia

OFFICE ADDRESS
Faculty of Forestry, Universitas Sumatera Utara. Jl. Tri Darma Ujung No. 1, Kampus USU Padang Bulan, Medan 20155, Sumatera Utara, Indonesia. Tel.: +62-61-8211633, email: editors@smujo.id

PERIOD OF ISSUANCE
June, December

Society for Indonesian Biodiversity

Universitas Sumatera Utara Medan, Indonesia

Published by Smujo International for The Society for Indonesian Biodiversity and Universitas Sumatera Utara, Medan, Indonesia

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Journal:

Saharjo BH, Nurhayati AD. 2006. Domination and composition structure change at hemic peat natural regeneration following burning; a case study in Pelalawan, Riau Province. Biodiversitas 7: 154-158. DOI: 10.13057/biodiv/d070213.

The usage of "et al." in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al. 1999. Future of health insurance. N Engl J Med 965: 325-329. DOI: 10.10007/s002149800025.

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in the book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds.). Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.). Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from the internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. DOI: 10.1038/msb.2008.24. www.molecularsystembiology.com.

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ASIAN JOURNAL OF FORESTRY
Volume 5, Number 2, December 2021 E-ISSN: 2580-2844
Pages: 51-59 DOI: 10.13057/asianjfor/r050201

Effect of low light condition on the growth and carbon use of legume seedlings

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Manuscript received: 2 March 2021. Revision accepted: 1 June 2021.

Abstract. *Qur'ani CG, Yoshimura K. 2020. Effect of low light condition on the growth and carbon use of legume seedlings. Asian J For 5: 51-59.* Plants exhibit flexible changes on their morphological and physiological traits to adapt with low light environments. However, the interactions between growth, functional traits among plant organs, and non-structural carbohydrates (NSCs) concentration that determine the adaptation strategies remain unclear. Three legume species recognized as shade-intolerant plants, i.e., *Robinia pseudoacacia* (L.), *Falcataria moluccana* (Miq.), and *Acacia mangium* (Willd.), were grown under full light (open) and 15% of light availability (shaded). Changes in shoot length, diameter, organ respiration rates, and NSCs concentration were observed throughout 6 months of the growing period. Only *R. pseudoacacia* developed longer (13.67%) and bigger (26.61%) shoots in the open site than in the shaded site. Specific Leaf Area (SLA) and Specific Root Area (SRA) were bigger in the shaded site than in the open site for all species (SLA=±2 times; SRA=±1-4 times). Dry mass, respiration rates, and NSCs in *R. pseudoacacia* were allocated more to the roots, but were more to the leaves in *F. moluccana* and *A. mangium.* Based on the interactions among morphological and physiological parameters, our results showed that different legume species develop specific growth, morphological traits and carbon use (NSCs) under shade conditions which exhibit flexibility changes as part of adaptation strategy to the low-light environment.

Keywords: Growth, legume, non-structural carbohydrates, respiration rates, shade-intolerant.

INTRODUCTION

Woody legume plants are known as fast-growing and pioneer species with high survival rates in various environmental conditions, particularly after disturbances (Hughes et al. 2011; Masaka 2016; Osunkoya and Othman 2005). As a result of their ability to colonize and dominate new habitats, some leguminous woody plants are often considered as invasive species (Akamatsu and Makishima 2014). The high rate of growth and subsequent secondary succession of leguminous wood species is attributed to their adaptability to high light intensity (Bush and Van Auken 1986), although a decrease in growth is observed under shade conditions (Sprent 1999).

Generally, plants show changes in morphological and physiological traits as the light intensity changes. The changes in leaf and root morphology to adapt with light conditions are common to establish relative growth rate (RGR) variation among light environments (Reich et al, 1998). Shade-intolerant species showed a higher specific leaf area (SLA) with less investment in carbon storage as part of adaptation to survive under shaded conditions. For example, the seedlings of *Acacia koa* exhibited no differences in relative allocation of carbon to the roots under varying light intensities (Craven et al. 2010). Meanwhile, in the case of *Acacia implexa*, seedlings in higher light conditions exhibited higher relative allocation to roots (Forster and Bonser 2009). These cases show that legume species have various flexibility changes, which work as strategies to survive under the shade

Seedlings in open sites have better chance in capturing light for photosynthesis than seedlings in shaded sites, resulting in higher carbon gain and carbon stock for metabolism (Pattison et al. 1998). High carbon gain corresponds to the needs for plant organ growth and correlates with higher carbon use in high light seedlings. Carbon balance can be achieved when each organ potentially uses its roles to support the whole-plant metabolic activity level, such as leaf for photosynthesis and roots for carbon stock. Although recognizing carbon balance within an individual plant is important for survival under the shade, carbon consumption within an individual is not associated with survival under the shade. The changes in functional traits of each organ represent the potential of using carbon stock (Bush and Van Auken, 1986), which are considered as the parameters that support and affect the whole-plant activity level. Respiration and carbon investment for each organ is not considered simultaneously as a compartment of whole-plant carbon balance.

The marked products of plant high or low rates in photosynthesis can be recognized through the availabilities of non-structural carbohydrates (NSCs). NSCs are used as resources for metabolism among the stored carbon, which explains the breakdown of the metabolism process when the deficiency of NSCs occurs. Generally, the photosynthesis rates decrease as light decreases and reduce the NSCs (Xie et al. 2018). A positive correlation was

found between biomass, NSCs concentration, and survival rates with light availability in shade-intolerant species (Zhou et al. 2020). These variations are due to the internal regulation between soluble sugars and starch depending on the adaptive strategies under different environmental scenarios (Dirk et al. 1999; Kami et al.2011; Xie et al. 2018). The amount of carbon stock corresponds to the stored carbohydrates that proportionally allocate for the needed organs under the shaded condition.

The interaction between morphological changes, organ potential on using the carbon stock, and the availability of NSCs have not been explored in shade-intolerant legume species, especially their response and characteristic changes under low light. We hypothesize that changes in carbon allocation, i.e., low carbon gain in shade conditions, result in the shift of whole-plant metabolic activity and biomass production in legume species. In this study, we focused on the effect of low light environments on the growth of legume species, and the connection on the ability of plant organs to use carbon stocks as well as the availability of NSCs concentration. From the results, we expect to understand the survival strategy of each species on their adaptation to the low light condition.

MATERIALS AND METHODS

Seedling origin

We used three legume tree species of *Robinia pseudoacacia* (L.), *Falcataria moluccana* (Miq.), and *Acacia mangium* (Willd.). *R. pseudoacacia* is a deciduous legume tree native to North America and grows in a temperature range of 2 - 13ºC for budburst (Cierjacks et al. 2013). *F. moluccana* is deciduous legume trees native to parts of Indonesia and Papua New Guinea, grown in the natural habitat of wet climate with optimal temperature of 22 - 29ºC (Hughes et al. 2011). And *A. mangium* (Willd.), an evergreen leguminous tree native to Australia, Papua New Guinea, Indonesia and Mollucas islands, grown in a wet climate with average annual temperature of 18 - 28ºC (Atipanumpai, 1989).

Planting

The seeds of *R. pseudoacacia* were collected in Yamagata Prefecture, Japan (38° 43' 2" N, 139° 51' 19" E), while *F. moluccana* and *A. mangium* seeds were collected from the provinces of East Java (-7° 50' 21" N, 112° 13' 24" E) and Central Java (-7° 47' 35" N, 110° 55' 42" E), Indonesia. Seeds were soaked in boiled water at 100ºC for 24 hours during which the water temperature decreased gradually up to room temperature, to remove the seed coats after water absorption. The seeds were then sowed in granite soil on May 18, 2018 (139 days of the year) and left under full sunlight. Before sowing, the soil was heated for 1.5 hour in 550ºC in an oven to remove organic matter/contents. Seeds were germinated within 7 - 10 days and immediately transplanted to pots (9 cm × 9 cm × 9 cm) in the nursery of Faculty of Agriculture, Yamagata University, northern part of Japan (38° 43′ 59″ N, 139° 49′ 28″ E). The area of the experiment location has an annual temperature of 13.3 °C, and annual precipitation is 1620 mm (AMeDAS, Japan Meteorological Agency).

Shading treatment and measurement of growth rate

Two types of light regimes were used after the transplanting on 194 days of year (DOY): (i) open site under the full sunlight; and (ii) shade under a black shading net (90 cm × 60 cm × 120 cm) with 15% of full sunlight maintained by measuring light intensity with the LI-190SA (Li-cor, Lincoln, NE) connected to a data logger (LI-1400, Li-cor, Lincoln, NE). The Relative Photosynthetically active Photon Flux Density (rPPFD) was automatically recorded every 5 minutes. We measured shoot diameter on the base of the aboveground part, shoot length between the basal position on the above-ground part, and the tip of the shoots of 30 seedlings for each species and light regime using a digital caliper and a ruler. These measurements were conducted once a week, from 195 to 295 DOY.

Organ respiration rates

Five or six seedlings for each species of each treatment were selected and separated into leaves, stems and roots on 289 DOY. We harvested the seedlings at the end of the growing season (6 months) when it is understood that seedlings growth is maximum as cell expansion had slowed or ceased (Ryan et al, 1995). We proved the theory by finding a slow increment of shoot length and diameter by the end of summer. However, root growth can still proceed well during fall (Lyr and Hoffmann, 1967). To investigate the metabolic activity for each treatment, each separated sample was put in a closed-air circulation chamber with the volume of 1.94×10^{-4} m^3 for small samples and 5.47×10^{-3} m^3 for bigger samples fitted with an Infrared Gas Analyzer (IRGA) (GMP 343, Vaisala, Helsinki, Finland) to measure the respiration rates. We carefully checked the air circulation, air temperature and no air leakage inside the chamber during the measurements through the observation of CO_2 concentration per second. Organ respiration rates (OR) were calculated using the following equation (Ideal Gas Law).

$$OR = \frac{1}{R} \times \frac{\Delta CO_2}{\Delta t} \times \frac{P_{atm} \times V_{cham}}{T_{cham}} \times 1 \quad [1]$$

where OR is the organ respiration rate (nmol s^{-1}), R is the gas constant (8.31 Pa m^3 K^{-1} mol^{-1}), CO_2/t is the change of CO_2 concentration inside the chamber for one second (ppm s^{-1}), P_{atm} is atmospheric air pressure (101.3 kPa), V_{cham} is the chamber volume (m^3), and T_{cham} is the temperature inside the chamber (K).

Morphological measurements

After measuring respiration rates, leaf surface area was analyzed using the flat-head image scanner (GT-600, Epson, Tokyo, Japan) and image analysis software (ImageJ, NIH, Bethesda, Maryland, USA). While the root surface area was analyzed using flat-head image scanner (V800, Epson, Tokyo, Japan) and image analysis software (WinRHIZO, Regent instrument, Quebec, Canada). All harvested materials, including leaves, stem and roots, were

dried in an oven at 65°C for 48 hours and the respective dry mass (DM) was measured. Specific Leaf Area (SLA) and Specific Root Area (SRA) were calculated by total leaf area/total leaf DM and total root area/total root DM, respectively.

Non-structural carbohydrates (NSCs)

Samples were ground to very fine powder for the analysis of non-structural carbohydrates (NSCs). The powder was extracted with 80% ethanol and the extract was once desiccated with heat. We analyzed soluble-sugar content by adding water to the desiccated materials followed by phenol-sulfuric acid assay, which induces a color-producing reaction that can be measured spectrophotometrically at 490 nm. After adding potassium hydroxide and acetic acid on the sediment of 80% ethanol extraction, starch was decomposed to glucose by the mixture of α-amylase and amyloglucosidase, we then estimated the starch content by analyzing the glucose content using a Glucose test kit (Fujifilm/Wako Chemical, Japan) and measured the colorimetric at 550nm.

Total NSCs concentrations were calculated as the sum of soluble sugar and starch concentration from the assays. Total NSCs pools were calculated as NSCs concentration multiplied by the total dry mass of the organ

Data analysis

Independent t-tests were used to examine the statistically significant differences between the two treatments within each species. All statistical analyses were performed with SPSS (version 25, *SPSS Inc*, Chicago, Illinois, USA). We also performed a Detrended Correspondence Analysis (DCA) to summarize the traits of carbon used for each species and each treatment. Dry mass, starch concentration per dry mass, soluble sugar concentration per dry mass, respiration rate per dry mass for each organ were used for this analysis, and multicollinearity was not seen among these traits.

RESULTS AND DISCUSSION

Growth rates and morphological traits in shoots and roots

All species showed a high increment of shoot length and diameter until mid-summer (from 231 DOY) in both the open and shaded sites, but the patterns varied among all species (Fig.1 and Fig. 2). In *R. pseudoacacia*, shoot length did not differ between open and shaded sites throughout the measurement periods (t-test p=0.248), but shoot diameter became smaller in the shaded site in the mid-summer (t-test p=0.029). In *F. moluccana*, shoot length increment in the shaded site was higher than in the open site in early summer (t-test $p \leq 0.001$) but caught up with the shaded site seedlings by mid-summer. The shoot diameter in *F. moluccana* in the open site was higher than the shaded site in mid-summer (t-test $p \leq 0.001$). In *A. mangium*, shoot length increment in the shaded site was higher than the open site treatment in mid-summer (t-test $p \leq 0.05$) while shoot diameter showed a similar pattern as *F. moluccana* (t-test p=0.006). Regardless of the species, the ratio of shoot length to diameter was significantly higher in the shaded site than in the open site (Fig.3).

In all species, SLA in the shaded site was twice as high as those in the open site (t-test *p_R. pseudoacacia*=0.005; *p_F. moluccana*=0.001; *p_A. mangium*=≤0.001) (Table 1). *R. pseudoacacia* had the lowest SRA in the open site, while *F. moluccana* had the lowest SRA in the shaded site. The highest SRA among all species in open and shade sites was observed *A. mangium*. SRA in *R. pseudoacacia* under the shade was five times higher than in open site (t-test p=0.028), while *F. moluccana* showed no differences between the two treatments (t-test p=0.184). SRA in *A. mangium* was 1.5 times higher in the shade than open site, but this was not statistically significant (t-test p=0.095).

Biomass production and allocation

At the overall organ level, dry mass (DM) in the open site was higher than the shade site in all species (Table 2). Leaf DM in open site was higher in *R. pseudoacacia* (t-test p=0.004) and *F. moluccana (*t-test p=0.032), and not significant but marginally higher in *A. mangium* (t-test p=0.065). Stem DM in open site was significantly higher in *R. pseudoacacia* (t-test p=0.009) and *F. moluccana* (t-test p=0.056), and marginally higher in *A. mangium* (t-test p=0.394). Root DM in the open site was higher than the shaded site in all species.

Regardless of light condition, root and leaf DM were higher than the other two organs DM in *R. pseudoacacia* and *F. moluccana*. But, in *A. mangium*, root DM was higher in the open site while leaf DM was higher in the shaded site

Organ level carbon properties

Among all species, a significantly higher leaf organ respiration (OR) in the open site than the shaded site was noted in *F. moluccana* only (t-test p=0.032) (Table 2). While, in *R. pseudoacacia* a significantly higher stem OR in the open site than the shaded site was observed (t-test p=0.03). All species showed significantly higher root OR in the open site than in the shade. Regardless of the light condition, OR in *A. mangium* was lower than the other two species for all organs. OR was not different among species for all organs in the shaded site. Roots in *R. pseudoacacia* and *A. mangium* had higher OR than other organs, whereas, leaves in *F. moluccana* had higher OR than other organs in open sites.

In all species, the non-structural carbohydrate (NSC) organ in the open site was higher than the shaded site (Table 2). Leaf and stem NSCs in *R. pseudoacacia* and *F. moluccana* were significantly higher in the open site than in the shade, but not in *A. mangium*. Leaf and root NSCs in the shaded site showed different amounts among species, but stem NSC in the shaded site showed no differences among species. Root NSCs of all species were significantly higher in the open site than the shaded site. In *R. pseudoacacia*, roots NSCs had the highest amount than the other two organs, but leaves in *F. moluccana* and *A. mangium* had higher NSCs than the other organs (stem and roots), regardless of light conditions.

Combination of carbon use properties among species and organs

Overall, leaf mass showed different direction from root mass and stem mass in the biplot (Fig. 4). Root starch content showed different direction from leaf and stem mass. Leaf sugar content showed different directions from root and stem mass. DCA 1 decreased on the sequence of respiration rates, sugar contents, starch contents and dry mass. DCA 2 decreased on the sequence of root, stem, and leaf traits, but showed opposite patterns in respiration rates. In all species, DCA 1 in the open site was lower than in the shaded site. DCA 2 was higher in *R. pseudoacacia* than other two species.

Discussion

Light regimes significantly correspond to the changes of morphology and carbon-use performances of current-year legume saplings (Chazdon and Pearcy 1986; Valladares et al. 1997). According to Madsen (1994), shade stress constrains shoot growth, eventually limiting the whole plant performance. In our study, none of the leguminous species under the shade conditions died throughout the experimental period. This agreed with our hypothesis that legumes adapt survival strategies under shade stress. Craven et al. (2010) observed significant positive growth response of legume *Acacia koa* to increasing irradiance (Photosynthetically Active Radiation, PAR, 21.28±13.06 µmol m^{-2} s^{-1}, 80.33±55.15 µmol m^{-2} s^{-1}, and 255.25±176.78µmol m^{-2} s^{-1}). Similarly, for our study, all three tree species had growth traits of shoot length and diameter (Fig. 1 and Fig. 2) as morphological adaptation to light and is consistent with that other light-demanding pioneer species (Walters and Bartholomew 1984, Sanford et al. 2003, Markesteijn et al. 2007, Smith and Shackleton 2000). Seedlings in the shaded site showed higher shoot length than in the open site in *F. moluccana* and *A. mangium* (Figure 1).

Table 1. Specific Leaf Area (SLA) and Specific Root Area (SRA) of each species in open and shade sites. Statistical analysis using independent t-test of n = 5 for each treatment; p-value ≤0.05 is significantly different. Asterisk symbols are significant differences of open and shade sites using independent t-test (p<0.05)

Species	Treatments	SLA(cm^2g^{-1})		SRA(cm^2g^{-1})	
		mean±SE	*p*	mean±SE	*p*
R. pseudoacacia	Open	130.08 ± 3.12	0.005*	180.01 ± 54.12	0.028*
	Shade	257.55 ± 22.70		829.18 ± 198.64	
F. moluccana	Open	108.01 ± 8.91	0.001*	544.16 ± 100.30	0.184ns
	Shade	252.02 ± 26.51		708.94 ± 52.77	
A. mangium	Open	115.82 ± 12.18	<0.001*	970.16 ± 57.39	0.095ns
	Shade	231.87 ± 17.19		1307.08 ± 161.65	

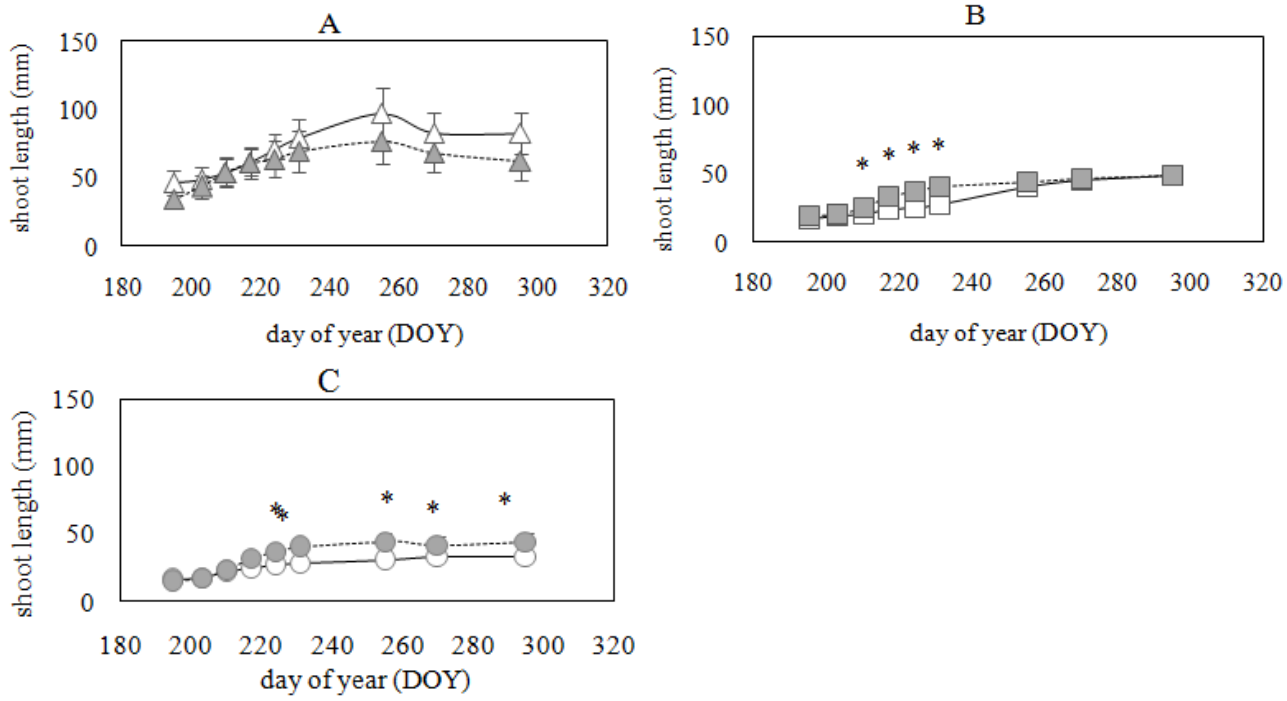

Figure 1. Changes in shoot length before and after the shading were applied in seedlings of *R. pseudoacacia* (A), *F. moluccana* (B), and *A. mangium* (C). Open symbols are seedlings in open sites (n=30) and close symbols are seedlings in shade sites (n=30). Error bars are standard errors. Asterisk symbols are significant differences of open and shade sites using independent t-test (p<0.05)

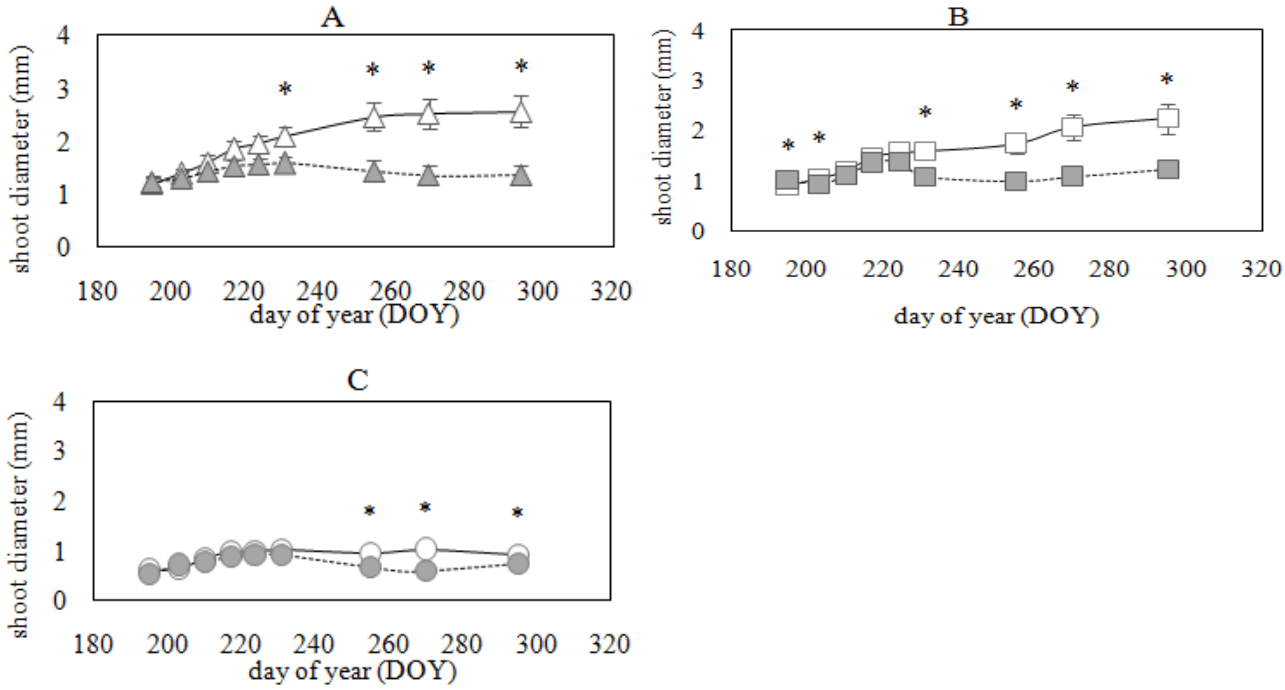

Figure 2. Changes in shoot diameter before and after the shading were applied in seedlings of *R. pseudoacacia* (A), *F. moluccana* (B), and *A. mangium* (C). Open symbols are seedlings in open sites (n=30) and close symbols are seedlings in shade sites (n=30). Error bars are standard errors. Asterisk symbols are significant differences of open and shade sites using independent t-test ($p<0.05$).

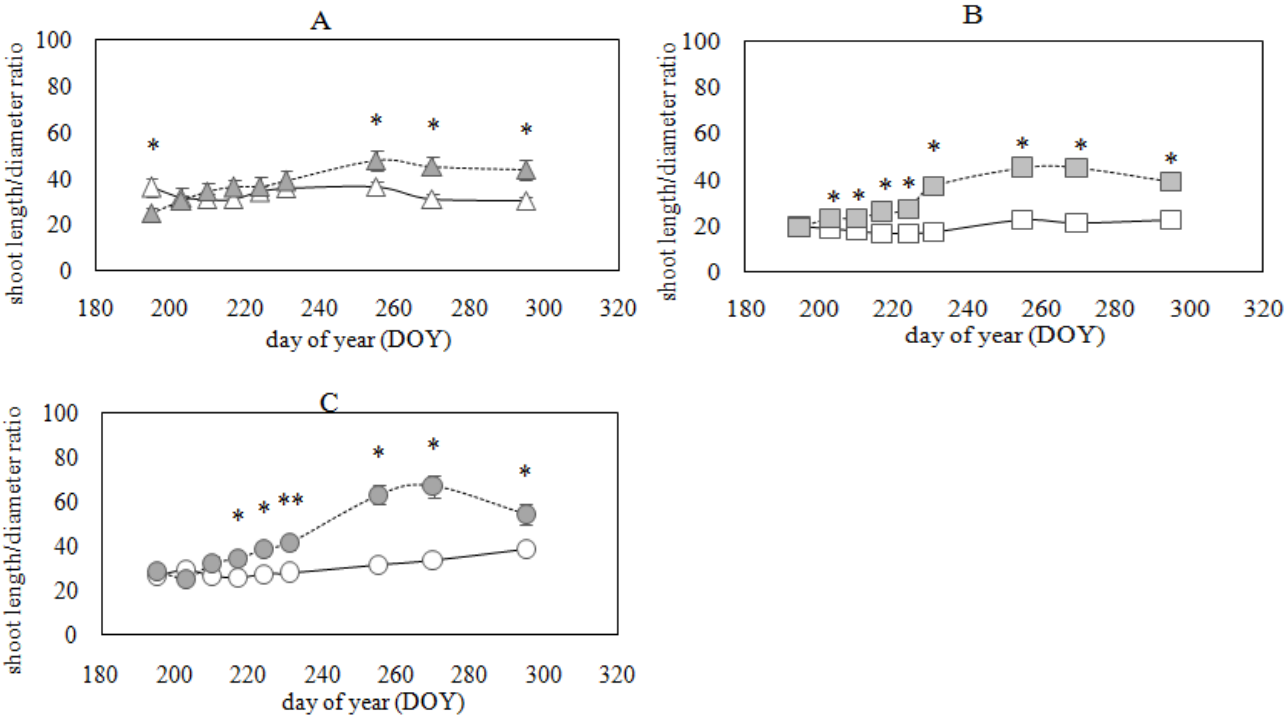

Figure 3. The ratio of length to diameter of shoot before and after the shading was applied in seedlings of *R. pseudoacacia* (A), *F. moluccana* (B), and *A. mangium* (C). Open symbols are seedlings in open sites (n=30) and close symbols are seedlings in shade sites (n=30). Error bars are standard errors. Asterisk symbols are significant differences of open and shade sites using independent t-test ($p<0.05$))

Table 2. Dry mass (DM), respiration rates and the amount of non-structural carbohydrate (NSC) in three organs (leaf, stem, and root) and whole-plant of seedlings of three legume species (*R. pseudoacacia*, *F. moluccana*, *A. mangium*) grown in open and shade sites. Mean values and p-values after the comparison between open and shade sites using Mann-Whitney U-tests are shown, and bold means significantly higher values ($p<0.05$) between open and shade sites. Different characters mean significant differences ($p<0.05$) among species after Holm's multiple comparisons of Kruskal-Wallis tests, and no characters mean no significant differences after the tests.

	Leaf			**Stem**			**Root**			**Whole-plant**		
	Open	**Shade**	**p**	**Open**	**Shade**	**p**	**Open**	**Shade**	**p**	**Open**	**Shade**	**p**
DM (g)												
R. pseudoacacia	**0.634** a	0.064	0.004	**0.503** a	0.104	0.009	**1.544** a	0.147	0.004	**2.681** a	0.314	0.004
F. moluccana	**1.030** a	0.152	0.032	0.321 a	0.064	0.056	**0.647** a	0.064	0.008	**1.998** a	0.281	0.016
A. mangium	0.078 b	0.035	0.065	0.027 b	0.018	0.394	**0.087** b	0.018	0.002	**0.193** b	0.071	0.009
Respiration rates (nmol s^{-1})												
R. pseudoacacia	3.343 a	0.924	0.052	**1.866** a	0.584	0.030	**6.268** a	1.211	0.004	**11.477** a	2.719	0.009
F. moluccana	**7.470** a	1.453	0.032	1.474 a	0.322	0.056	**4.313** a	1.700	0.032	**13.256** a	3.475	0.032
A. mangium	0.278 b	0.205	0.394	0.105 b	0.102	0.818	**1.255** b	0.360	0.026	**1.638** b	0.668	0.026
NSC (mg)												
R. pseudoacacia	**72.09** a	2.15 A	0.004	**88.20** a	8.94	0.004	**437.51** a	12.84 A	0.004	**597.80** a	23.93	0.004
F. moluccana	**156.94** a	15.72 B	0.008	**33.33** a	5.38	0.032	**24.75** b	4.29 A	0.032	**215.02** a	25.39	0.008
A. mangium	6.80 b	4.59 AB	0.329	2.02 b	1.16	0.177	**1.88** c	0.27 B	0.002	10.70 b	6.07	0.082

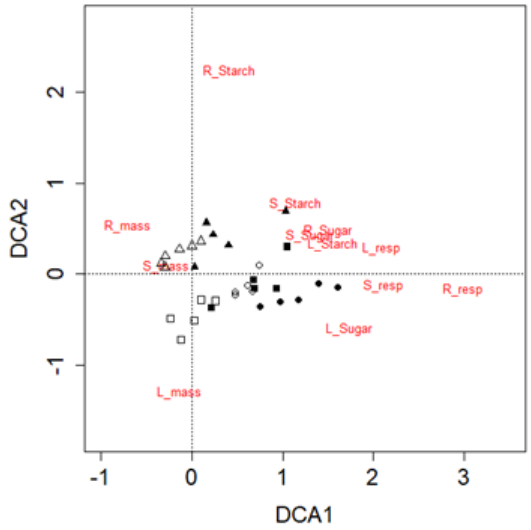

Figure 4. Detrended Correspondence Analysis (DCA) ordination of the entire dataset parameters of all species materials in open (open symbols) and shade site (close symbols). The seedlings of *R. pseudoacacia* are in triangle symbols, *F. moluccana* are in square symbols, and *A. mangium* are in circle symbols. Note: L: leaf; S: stem; R: root; mass: dry mass; resp: respiration rates

Under low-light conditions, shade-intolerant species prioritize shoot length growth over diameter increase (Evans and Poorter 2001; Gommers et al. 2013; Liu et al. 2016; Noguchi et al. 2001). This enhances light absorption and reduces carbon use. We also found in our study that seedlings in the shaded site had higher SLA than that in the open site (Table 1). Plants respond against light stress by allocating larger surface areas to capture light (Catoni et al. 2015; Xu et al. 2009). From these findings, leaf is the most preferential organ for capturing light as also stated by Hanba et al. (2002). Legner et al. (2014) showed that shade-tolerant species have higher plasticity on SLA than shade-intolerant species and this may be useful in species-specific N allocation patterns for supporting photosynthesis under the shaded conditions.

Seedlings in the open site have a better chance of capturing light for photosynthesis than seedlings in the shaded site, resulting in higher carbon gain and carbon stock for metabolism (Pattison et al. 1998). The NSC was highly allocated to the roots in *R. pseudoacacia,* and to the leaf in *F. moluccana* and *A. mangium* (Table 2). *R. pseudoacacia* can resprout from stump and horizontal roots after aboveground perturbations (Hoshino et al. 2021). It is a species with characteristic of high carbon storage in the roots because this species exhibits high maintenance respiration (Barbaroux and Breda 2002), high basal respiration rates in sapwood tissues, need access for the new leaf flush before leaf expansion in spring (Breda and Granier 1996; Landhäusser 2011), and resprout after experiencing aboveground damage (Cierjacks et al. 2013). These highlight that *R. pseudoacacia* species have higher function on carbon storage than the other two species. Meanwhile, *F. moluccana* and *A. mangium* showed better assimilation of carbohydrates by maximizing the carbon use for photosynthesis. Some invasive species such as *A. koa, F. moluccana,* and *P. cattleianum* contain high protein and amino acid represented by N storage in leaves, allowing rapid growth in heterogenous light environments (Funk et al. 2013). Also, recent study supports the idea that *Acacia* has high phenotypic plasticity in response to light availability that correlates with stored N that is useful for adaptation under changing light conditions (Funk 2008). On the aspect of carbon use, leaves have a function to assimilate carbohydrates, so higher leaf mass achieves higher carbon gain and higher sugar contents necessary for or from the photosynthesis process (Jurik et al. 1979).

Most of dry mass of each organ consists of structural carbohydrates. Soluble sugar can be used for metabolism while starch cannot. Starch is, however, used for the stock of carbohydrates (DCA axis 1 means the activity of each organ). Categorized by DCA axis 2, root is the most important organ for carbon storage, and the amount of sugar does not contribute to the function of carbon storage, compared to the amount of starch. Along the gradient of light conditions, shaded seedlings invest their carbon resources into metabolism higher. On the other hand, seedlings in the open site allocate their carbon resources into structure or carbon stock. The results on the respiration rate, carbon stock, carbon use, and NSCs suggested that seedlings in shade sites had smaller biomass production due to low ability of light capturing resulting in low carbon gain and carbon stock.

Throughout our findings, we understood that the strategy for legume trees for survival under shade conditions varies to ensure balanced carbon gain, carbon use, and carbon stock. In our results, we found the interactions between shoot growth (length and diameter), biomass, and NSCs concentration correlate positively with light availability (Figure 1, Figure 2, Table1, Table 2). The optimum use of carbon stock by each organ supports the survival strategy in low light environment as we recognized in shade site seedlings. *R. pseudoacacia* allocated higher storage to the roots and a low elongation to aboveground shoots relates to the ability of the species to recover after the death or damage of aboveground parts following disturbances, such as flood and clear-cut. The *R. pseudoacacia* are able to produce new sprouts from the roots when the aboveground parts are under environmental disturbances, which cause the decrease of carbon gain (Masaka 2016, Nicolescu et al. 2020). On the other hand, *F. moluccana* and *A. mangium* invest high NSC to the leaves, which ensures a greater carbon use efficiency during photosynthesis production and respiration. The two species development mechanisms for higher light capture by large shoot elongation and high biomass deposition to leaves under the shaded conditions.

The basic silvics information on the legume species is important for restoration activities. The comprehensive information on morphological and physiological changes due to light intensity in our study will be useful in identifying light tolerant species. Our study found that morphological and physiological changes are part of adaptation to light conditions, including tolerance to low light. However, at the end of our investigation, the

seedlings under shade conditions showed a low increment of biomass which we determined was due to low light intolerance. Many studies determined *R. pseudoacacia*, *F. moluccana*, and *A. mangium* as shade-intolerant species following observations on various light regimes (Hughes et al. 2011; Osunkoya and Othman 2005). This study examined the growth through the carbon allocation among organs to understand the survival behavior and adaptation strategies of legume species in low light conditions. Contrary to studies that stated that some legume species could not survive under the shade due to shade intolerance (Izaguirremayoral et al. 1995; Sprent 1999), we observed in our study that legume species perform an adaptation strategy through exhibit plasticity changes.

ACKNOWLEDGEMENTS

The authors would like to thank the members of Forest Creation Laboratory of Yamagata University for their insightful supports to gain the results and new knowledge through this study. We also thank Dr. Ashitani Tatsuya for use of his laboratory's equipment on NSC analyses. This work was supported by the research funding from The United of Graduate on Agricultural Science of Iwate University and scholarship from Japan Ministry of Education, Culture, Sports, Science and Technology.

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ASIAN JOURNAL OF FORESTRY
Volume 5, Number 2, December 2021 E-ISSN: 2580-2844
Pages: 60-70 DOI: 10.13057/asianjfor/r050202

A pilot study of seed ecology of *Allocasuarina robusta* to define strategies for its recovery program

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Manuscript received: 9 March 2021. Revision accepted: 1 June 2021.

Abstract. *Pearson MW. 2021. A pilot study of seed ecology of Allocasuarina robusta to define strategies for its recovery program. Asian J For 5: 60-70.* The *Allocasuarina robusta* is a threatened species of national and local significance. Population recovery is therefore important for the persistence of the species which can be conducted through community-based restoration activity. This pilot study investigated the process involved to facilitate seed recruitment of *A. robusta* as part of a threatened species project. The investigation aimed to improve seed recruitment in *A. robusta* occurring under natural conditions. Several experiments occurred, each examining a specific attribute in the seed recruitment process. The research design would help land managers and communities to conserve *A. robusta.* The results highlighted several experimental design flaws and identified opportunities in the recovery program of *A. robusta*.

Keywords: Experimentation, germination, Hindmarsh Tiers, Mount Lofty Ranges, pilot study, seed recruitment

INTRODUCTION

Community involvement is a fundamental aspect in environmental restoration. McGregor and McGregor (2020) described the need for environmental restoration and its intrinsic value to the overall community. Communities can drive a restoration project and contribute to restoration activities, for example by providing labour and time. However, communities cannot perform their role without adequate knowledge and technical information, for instance when restoration areas contain threatened or rare species (Gollan et al. 2012; Roger et al. 2020). Therefore, community requires direct research through citizen science programs or other community activities by providing detailed local knowledge (Gollan et al. 2012; Roger et al. 2020).

The purpose of revegetation in restoration goes beyond tree planting or direct seeding (Bischoff et al. 2008; Breed et al. 2012; Navarro-Cano et al. 2019). Revegetation should aim to protect biodiversity with adequate population by increasing genetic diversity or stimulating the natural recruitment process without the threat from introduced species (Breed et al. 2012; Padilla & Pugnaire 2006). Revegetation has proven useful in protecting natural communities against further degradation (Breed et al. 2012) and this should be viewed as environmental protection through a big-picture perspective (Hobbs 2017). As such, revegetation could all be undone by not understanding what occurs at an individual species level (Breed et al. 2012).

Identifying what environmental factors affecting the effective restoration of threatened species, such as *Allocasuarina robusta* (Macklin) L.A.S. Johnson [Casuarinaceae] requires understanding and testing the hypothesis and significance factors (Newman 2008), for example the factors affecting seed recruitment of *A. robusta*. Newman (2008) described that hypothesis testing and significance testing have a relationship to each other. The relationship can provide misinformation or incorrect inferences when these are not analysed (Newman 2008). A cornerstone of science is the generation of questions; this may involve creating or reaffirming knowledge already known or applying an experimental design differently, none of which will lead to misinformation. Misinformation from experimental design occurs at the execution stage, affecting the results and data analysis (Newman 2008; Symes et al. 2015).

The reporting of experimental design errors or the generalisation of results should not occur in a scientific research (Pennock, 2004). As such, undertaking trials is vital for developing appropriate scientific inquiry skills, reducing the possibility of reporting misinformation originating from incorrect experimental design (Symes et al. 2015). A new experimental method or application creates a tendency to focus on procedural components of the experimental design rather than the unexpected outcomes (Chen 2010; Symes et al. 2015). In this regard, designing a pilot study contains rigour that can test the original research question. As Chen (2010) explains, the concept of an experimental design identifies and engages with the theoretical aspect of testing.

A fundamental aspect of understanding restoration ecology is knowing what observations are required and why (Pennock 2004). The current investigation occurs in a simulated environment instead of a field study where Pennock (2004) used second criteria concerning the degree of control exerted in the experimental design. Reporting the experimental design needs

contextualisation towards the outcome's size (Newman 2008; Pennock 2004). A pilot study experimental design involves a degree of scalability where procedures and the testing rigour can be measured (Pennock 2004). For example, the results from Navarro-Cano et al. (2019) field plot investigation involved changing the scale to maintain genetic diversity in a species. Pennock (2004) extended the concept of scale to measure the time taken for recording the manipulative experimental data and the experiment's duration.

This pilot investigation aims to demonstrate the importance of experimental design in the restoration ecology. This investigation uses the species *A. robusta*, a threatened species (Minister for the Environment and Heritage, 2006), to test what environmental cues would simulate the populations to regenerate following the death of parent plants.

MATERIALS AND METHODS

Studied species

Allocasuarina robusta (Macklin) L.A.S. Johnson [Casuarinaceae], a threatened species of the Mount Lofty Ranges, is described as a monoecious shrub with smooth bark (Jessop & Toelken 1986; Wilson & Johnson 1989). The branchlets and the scale leaves of *A. robusta* are glabrous, with the immature scale leaves overlapping (Wilson & Johnson 1989). The female inflorescences produce aggregate fruit from a 3mm long peduncle; these may be sparsely pubescent or sessile on the peduncle (Jessop & Toelken 1986). *A. robusta* seed description is a samara with seed ranging from 5.5 to 6.0 mm in size (Wilson & Johnson 1989).

Pollination in Casuarinaceae occurs by the wind; the bracteoles develop into a fruit that contains a single winged samara seed (Swamy 1948). The female inflorescence develops a woody cylindrical infructescence consisting of whorls of tightly appressed hairs of enlarged floral bracteoles (Pannell & Myerscough 1993). Growth of the floral bracteoles becomes part of the formation of aggregate fruit in *Allocasuarina*. The aggregate fruit is initially hairy and then develops two woody valves with the seed filling the cavity (Swamy 1948). *A. robusta* stores the seed above ground and then releases seed through an environmental trigger (Jessop & Toelken 1986; Quarmby 2011).

Study area

The climatic conditions favouring *A. robusta* are in the Fleurieu Peninsula's (South Australia) wettest parts (Department for Environment and Heritage, 2007). The Fleurieu Peninsula has a temperate climate with moderately wet winters and hot, dry summers (Armstrong et al. 2003). Rainfall in the Fleurieu Peninsula can range from 400 to 1000 mm depending on altitude and aspect (Armstrong et al. 2003). *A. robusta* grows on soils described by the Department for Environment and Heritage (2007) as infertile acidic soils associated with peat bogs. The soil types range from mottled yellow ironstone soils, gravelly duplex soils and sandy glacial outwash soils (Department for Environment and Heritage, 2007). Hindmarsh Tiers, where *A. robusta* are mainly distributed, has sandy glacial outwash soils (Bickford et al. 2008).

A. robusta occurs in the Kanmantoo bioregion, including the southern Mount Lofty Ranges, Fleurieu Peninsula and Kangaroo Island (Department for Environment and Heritage, 2007). The vegetation is predominantly *Eucalyptus* open forests and woodlands. The habitat for *A. robusta* is the peripheries of wetlands where the mesophytes and hydrophytes meet. The critically endangered Fleurieu Peninsula wetlands communities have legislative protection from the Commonwealth and South Australian State governments (Department for Environment and Heritage, 2007).

Seed collection

A. robusta seeds were collected during September 2017 to February 2018 from 11 sites in the southern Mount Lofty Ranges in South Australia from Hindmarsh Tiers (Figure 1). The collection technique involved collecting aggregate fruit by hand from branches near the base close to the main stem and branches that had not hardened but were still flexible. These sites had additional tube stock planted from other populations within the *A. robusta* range. Sites were selected along an east to the west gradient, and two outlying populations on the north and south. The seeds were collected twice; first a mixture from all 11 sites to form a composite seed collection to test the different simulation techniques and secondly seeds were collected to investigate the populations at an individual level.

Seed examination

Different age of *A. robusta* seeds were collected and examined. Aggregate fruit from different positions on the *A. robusta* represents a cross-section of age to avoid environmental variability. In each population, sampling occurred on 10% of the population. The 10% sampling relates to the conditions stipulated in the permit from the Department of Environment and Water in South Australia. Each population had a variable number of *A. robusta* from 10 to 112 individuals.

Examination of seed from various populations was done under a dissecting microscope. The initial visual assessment enabled the development of a seed characteristic list. Several characteristics initially included were removed due to being reflective of seasonal variation in the seed. Seasonal variations, as defined by Cochrane et al. (2015), is a cause that affects the seed size (width and length) and the seed's plumpness.

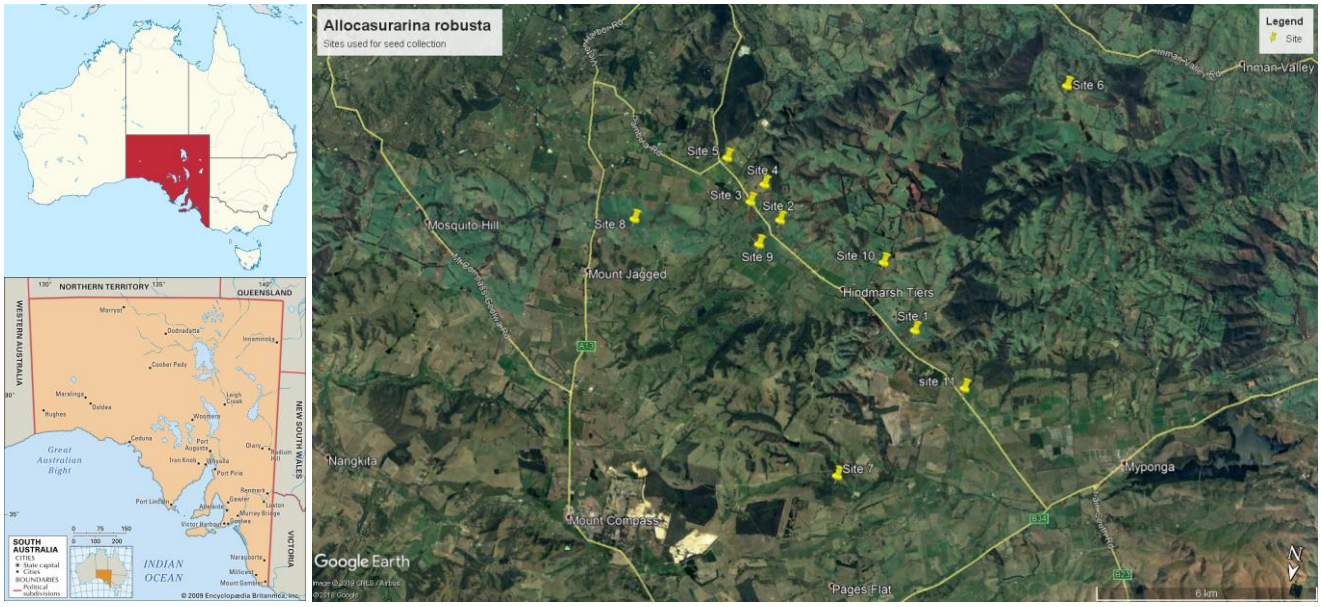

Figure 1. Seed collection sites of *Allocasuarina robusta* in the southern Mount Lofty Ranges in South Australia

The names assigned to the *A. robusta* metapopulations were the road names or nearby local features, i.e., Hindmarsh Tiers Road had two metapopulations with one sample allocated the road name Hindmarsh Tiers and the second sample named after a nearby local feature Hindmarsh Falls.

The *A. robusta* characteristics list used are as follows:

#1. The geometry of the seed and samara is/
1. seed and the samara are symmetric/
2. seed and the samara are asymmetric/

#2. Midrib in the samara/
1. shows no signs of tapering/
2. shows tapering away from the seed/

#3. Seed surface characterised by being/
1. entire with surface pitting/
2. entire without surface pitting/

#4. Samara colour is/
1. brown/
2. clear/

#5. Seed colour is/
1. brown/
2. black/

#6. The colour pattern between the samara and seed is/
1. ragged without any fading towards the end of the samara/
2. entire and fades towards the end of the samara/

#7. The surface texture of the seed is/
1. rough/
2. smooth/

#8. The placenta connection between seed and fruit/
1. is retuse with smooth edges/
2. is entire with smooth edges/

#9. The shape of the seed is/
1. generally square/
2. generally oval to round/

#10. The shape of the end of the seed is/
1. obtuse to acute in shape/
2. rounded to ovate is shape/

#11. The seed has a colour marking which gives it an appearance of/
1. a single colour without striations (stripes)/
2. striations (stripes)/

#12. Number of teeth/
1. 9 or more/
2. 8 or less/

#13. Number of protuberances on the Aggregate fruit are/
1. single on the back of the bracteole/
2. several on the back of the bracteole/

#14. The shape of branchlets are/
1. rounded or subangular/
2. angular/

#15. The phyllichinia has/
1. a shape that is rounded or subangular/
2. a central groove/

#16. Aggregate fruit are on/
1. pedicels less than 2 mm long or sessile/
2. peduncles 3 – 12 mm long/

#17. Length of teeth/
1. 0.6 – 1.5 mm long/
2. 0.3 – 0.5 mm long/

#18. Teeth bases are/
1. not overlapping/
2. overlapping/

#19. Bracteoles of fruiting cone/
1. thick and convex, often with separate angular or divided protuberances/
2. relatively thin and without any dorsal protuberances/

Competition

To determine if the presence of either the Burr medic (*Medicago polymorpha* L. [Fabaceae]) and Cocksfoot (*Dactylis glomerata* L. [Poaceae]) contributed towards acting as a surrogate nursery to allow for the establishment of *A. robusta*, a total of 30 punnets sown with the introduced species, i.e., 30 punnets of *D. glomerata* and 30 punnets of the *M. polymorpha*. Fifteen punnets were randomly selected and sown with *A. robusta*, which gave a combination of 15 *A. robusta* and *M. polymorpha* punnets and 15 *M. polymorpha* only punnets. In the repeated method *M. polymorpha* was replaced with *D. glomerata*. The experiment design contained fifteen punnets to act as control with *A. robusta* without any form of competition. Punnets remained in growth boxes for 100 days. The sowing of *A. robusta* did not occur until day 20. The 20-day lag time was to allow the *D. glomerata* and *M. polymorpha* to establish. Response variables were the time of germination and survivorship over 100 days.

Surface litter

As per the observations of Quarmby (2011), dying of *A. robusta* resulted in release of seeds from the canopy. The experiment designed was to mimic conditions of natural seed recruitment in the absence of fire. The experiment used a square squat pot to ensure variability of litter depth while maintaining a proper soil depth to allow a seedlings to establish. Square squats (470ml) procured from Garden City Plastics were filled with 100ml of growing media and seeds were sown at a density of 30 seed per pot to represent a natural seed rain. The first treatment had a layer of seed placed on the surface litter to replicate natural seed rain. The second treatment placed the seed on the interface between the surface litter and the growing media to represent released seeds and covered with leaf litter (10 mm of leaf litter). The third treatment buried the seed into the litter to a depth of 25 mm, to represent a historical seed release event. The leaf litter were used from *Eucalyptus cosmophylla* F.Muell. [Myrtaceae] (Cup Gum), with the leaves collected from nearby roadside reserves. These were then washed, dried, and exposed to UV light to reduce any possible contamination on the leaves. A fourth treatment sowed the seed on the surface of the growing media without any leaf litter. Each pot was given a number and then assigned to a random location within a four-block experimental design. Each treatment replication were carried out eight times, resulting in use of 24 square squats with six square squats per block. Response variables were the time of germination and survivorship over 100 days.

Growing media

Quarmby (2011) and Bickford et al. (2008) described the *A. robusta* populations' location as centred on glacial outwash. Bradford et al. (2008) described the Fleurieu Peninsula swamps originated from a perched water table. The experiment was conducted to determine which soil type would be conducive for *A. robusta* seed recruitment. The experiment used commercial growing media, which varied in the sand and organic matter ratio. Four treatments of varied soil media were used. These were propagation sand, orchid mix, all-purpose growing media and natural soil, which was determined using a mixture of the soils collected over three sites. Sites were Stipiturus Conservation Park (Site 7), Mt Billy Conservation Park (Site 5) and Hindmarsh Falls (A water reserve managed by the Yankalilla District Council, Site 10) (Figure 1). Sterilization treatment of natural soil were conducted to avoid any competition using solarisation and heating at 200^{o}C for 20 minutes. Twenty *A. robusta* seeds were sown into each punnet. The experiment was conducted in a single block design. Response variables were the time of germination and survivorship over 100 days.

Heat intensity

Fresh cones of *A. robusta* were collected, bagged and then exposed to 100^{o}C for five minutes to allow the fruits to release the seeds. When the seeds were released, they were batched into six groups then exposed to 100^{o}C for various durations.

Exposure length was at intervals of 0 minutes, 2 minutes, 4 minutes, 6 minutes, 8 minutes and 10 minutes. The seeds were sown in in sand-based growing media. The heat exposed groups of seed were replicated four times with thirty seeds sown in a 400 ml punnet. All six treatments were allocated a number and then randomly distributed into a growth box. Response variables included germination over the next 100 days and survivorship.

Heat shock/smoke

The experimental design simulates a fire's effects on the seed as described by Mackenzie et al. (2016). To simulate the impact of fire, seeds were sown in wet growing media where treatment was applied and then placed in a three-block design with each treatment replicated three times. Treatments of the seed *in situ* of the potting growing media included heat shock, smoke water, a combination of smoke water and heat shock and a control. The process of simulating heat shock to the seeds occurred by applying boiling water to the seed to act as a form of heat shock (Mackenzie et al. 2016). Heat shock does not necessarily mean exposure to fire in the form of flames but the heat generated by the fire (Mackenzie et al. 2016; Pounden et al. 2014). As *A. robusta* seed is protected in the fruit from any direct fire impacts (Clarke et al. 2010). The smoke water treatment comprised of a commercial product from Suregro and making up a solution of five litres at the concentration of 0.1 ml smoke water : 10 ml water and applied to punnets through overhead watering.

Seed depletion

Rates of seed loss from within the seed bank have traditionally been quantified by using mesh bags, enabling measurements of seed predation or unviable seeds (Van Mourik et al. 2005). Burying aggregate fruit

was considered inappropriate for *A. robusta*. Seed storage of *A. robusta* occurs in an above-ground seed bank.

To measure the seed bank depletion for the *A. robusta*, thirty cones were collected from the west to the east gradient at Hindmarsh Tiers (Figure 1). The cones were mixed to create a random source of seed. Grouping of fruits were made at site with six cones per paper bag, providing a comprehensive collection of five sites. Cones were dried and stored in temperatures ranging from 16.7ºC to 19.9ºC, with relative humidity ranging from 84% to 88%. The first bag of seed was sown two weeks after collection, with each subsequent bag sown every four weeks after the initial sowing. Sowing were carried out at a rate of 30 seeds per 400 ml plastic punnet with each treatment was replicated four times and then placed in one of four blocks in the growth boxes. The growing media used was a commercial sand-based growing media, including composted organic matter. These were monitored daily for germination and survival over 100 days.

Population viability

Seed collection was carried out at eleven of the twenty-four populations of *A. robusta*. Seeds were collected from individual *A. robusta* with 10% of a population sampled with ten cones selected from each individual. The cones were air dried without any environmental controls. Each bag was labelled with date and site information. The seeds were sown two weeks after collection. The seeds were sown in 200 ml punnets using sand-based growing media. Each punnet had thirty seeds sown, with each *A. robusta* collection site replicated four times. Watering of punnets were carried out before sowing and on completion of sowing of the blocks used in the growth boxes.

Growing condition of the seed treatments

Seeds were sown in 200 ml commercial nursery punnet using sand-based growing media unless specified elsewhere. The seed germination occurred in a growth box modelled on the progradation bed designed by Sage Horticulture using PSI lighting with purple globes. The growth boxes used were 149L clear plastic storage boxes with lighting fixed to the lid. Each box was filled to a depth of 20 mm of playground sand with a heating pad (Medium Seahawk Heat Pad) then covered with a further 20 mm of sand. Heat pads were applied whenever frost was forecasted. Daily data collection on sown seeds were recorded and they were watered every second day with approximately 150 ml of water applied via a mist system to each treatment. Other observational data included recording the seedling's survival viz. assigning a survival category, whether only cotyledons only were visible or central stem was visible with developed scale leaves. The seed collected from *A. robusta* along roadside corridors were carried out from random plants. The seed collection contained a limitation of no more than 10% of fruit collected from any individual and no more than 10% from a population. To meet the South Australian Scientific Permit (A26769-1) from the Department of Environment and Water requirements, watering used for the punnets consisted of using Adelaide mains water without any purification or treatments. No additional fertiliser or plant growth regulators were used to establish the *A. robusta* seeds unless it was part of the experimental design.

Statistical analysis

Descriptive analyses were carried out using XLSTAT (Mélard 2014). The descriptive statistics included looking at the data's central tendency (mean median, mode, standard deviation and variance). Each experiment was conducted with balance block design to enable ANOVA to identify statistical significance; after that further examination of the data was conducted using RStudio (R Development Core Team, 2010) for linear regression and frequency distribution plots. Single variables formed the basis of the investigation as each of the processes impacted germination due to being a pilot study.

The seed examination used a dissecting microscope at ×45 magnification. Data analysis from collected data was conducted in Delta Ver1.02 (Dallwitz 1993; Dallwitz et al. 2013) and PAUP Ver.4.0a (Swofford 2001). PAUP Ver.4.0a analysed data as an initial branch and bound tree based analysis on parsimony's informative characters. Topological constraints and trees that were unrooted were turned off.

RESULTS AND DISCUSSION

Seed examination

Examination of seed collected from *A. robusta* was conducted before use in the manipulative trials, from the criteria provenance and implications for environmental restoration. The character list design was used to identify any observable differences in morphology and establish a seed provenance for *A. robusta*. The parsimony data generated in PAUP Ver.4.0a showed insignificance for consistency index (0.5926) and homoplasy index (0.4783) towards morphology from the sampled populations.

Competition

A. robusta seed sown beneath the *D. glomerata* had only single germination, which is comparable to the *A. robusta* seed sown without competition which also had single germination. Whereas the *M. polymorpha*/*A. robusta* mixture had 12 germinated seeds of *A. robusta*. The number of seeds germinated in the growth box was not enough for statistical analysis, but they showed some interesting relationships with neighbouring vegetation. Not all seeds germinated simultaneously, with the first seed germinating on day 14 and the last seed germinating on day 55 (Table 1) in the *M. polymorpha*/*A. robusta* mixture. The results with *D. glomerata* had only single germination which occurred on day 23 and during the 100 days experimental period, that particular seedling died.

In Table 1, *A. robusta* competition with *M. polymorpha* only occurred in two of the replications, with the remainder having no *A. robusta* germination. Observation on these treatments showed that the punnets

with *M. polymorpha* appeared to be wetter compared to those with *D. glomerata*. Another observation between the two introduced species showed that *M. polymorpha* also had a less aggressive root system than *D. glomerata*. From Table 1, germination happened over 41 days and it decreased over time.

Surface litter

Eleven seeds germinated over 100 days. Germination began on day 36 and by day 88 it had concluded. Seeds buried to a depth of 25mm recorded no germination, yet the seed sown at the interface between the surface litter and the growing media began to germinate on day 65 and concluded by the 88th day. Seeds sown to simulate seed rain for germination began on day 36 and had concluded by day 85. Simulated seed rain without leaf litter had a more significant number of germinations than the seeds covered with leaf litter (F- value 2.631, DF 6, St. Dev. 19.292, P=0.061).

Growing media

A majority of the germination occurred in the propagation sand, with germination beginning on day 19. Longer germination times were taken on the general-purpose growing media, with limited germination occurring on the composted orchid growing media. In the natural soil mixture, only a single seedling appeared on day 42. The propagating sand had a significantly higher germination rate (F-value 4.494, DF 17, St. Dev. 5.916, P=0.009). The general-purpose growing media (10 germinations, F-value 4.494, DF 16, St. Dev. 1.182) and the composted orchid media (3 germinations, F-value 4.494, DF 18, St. Dev. 1.182) produced the same results with same statistical significance (P=0.01). Even though the composted orchid growing media did have statistically comparable results, the number of observed germinations were less.

Heat intensity

Exposure of the seed to 100^{o}C for 2 minutes intervals and finishing at 10 minutes resulted in 36 germinations. From the observation, 4-minute exposure time produced the highest number of germinations compared to other treatments. Germination began on day 25 and concluded by day 80. It was observed that the more significant exposure time to heat resulted in longer germination times. The control treatment began germinating after 19 days. The observational results indicate that 4 minutes exposure at 100^{o}C was optimum for maximising germination.

Heat shock / smoke

Only three of the punnets produced germinations over the 100 days. Seeds treated with smoke water began germinating 21 days after sowing. From the treatment, only 3/4 produced results, one being germination from the control treatment (Table 2).

From Table 2, the two-treatment exposed to smoke water had much higher germination compared to heat alone or no treatment.

Seed depletion

Sowing and storing seeds at an average ambient room temperature of 23 ^{o}C produced only one germination after 30 days. No other germinations occurred for the next 180 days. Seeds were sown every thirty days for up to 120 days, however, the observation continued upto 180th day.

Population viability

On examining eleven populations from west to east gradient of the *A. robusta* over 100-day period, only 31 germinations were found to occur. Population 2 produced 29.03% of germinants. Two other population produced seven germinants (22.58%), and other populations produced between 0-3 germinates. From a geographical interpretation of the data, all three sites with a high germination percentage occurred in the same valley system. Those sites which recorded no germination were in smaller and isolated valley systems.

Table 1. Germination of *Allocasuarina robusta* with *Medicago polymorpha*

Day	Germination	The germinated seedling withered at the cotyledon stage
14	4	0
14	3	0
27	0	1
27	2	0
35	2	0
55	1	0

Table 2. Treated *Allocasuarina robusta* seed germination responses

Treatment	Number of germinations
Smoke Water	3
Smoke Water and Heat Shock	3
Heat Shock	0
Control	1

Table 3. Table results from the population viability experiment

Site	No. of germinations
1	2
2	9
3	2
4	0
5	3
6	0
7	0
8	0
9	7
10	7
11	1

Discussion

From the present investigation, it could be said that the experimental design was flawed. However, the design flaws provide several vital points for directing future research and identifying the parameters requiring alteration (Chen 2010; Evans et al. 2020; Newman 2008). The notion of reporting results that are flawless or consistent with theory or expectation would weaken application to a meta-analysis (Palmer 2000). Chen (2010) discusses how scientific inquiry does not always occur in ideal circumstances but managed through appropriate variable controls. Reporting on which controls to manage or which variables should be analysed has, as Palmer (2000) indicated, resulted in selective reporting. When considered holistically, the scientific inquiry process and the reporting of either negative or positive results is necessary (Chen 2010; Palmer 2000). When a particular outcome does not occur in the investigative process, these areas allow learning to occur from reflecting on the experiment's hypothesis and design (Chen 2010; Evans et al. 2020; Symes et al. 2015).

The current experimental design contained contextual elements from Barritt and Facelli (2001), Abihudi et al. (2020) and Huang et al. (2021). The basis for scientific inquiry is the generation of new information and then explaining the results in the context of current theory (Chen 2010; Newman 2008). By piloting several methods can help identify and redefining the research question, which was the case for the current investigation. The aspect of piloting an experimental methodology and then reporting on the outcome is not new, as Evans et al. (2020) used to refine and identify research areas on seed recruitment. The results' perception indicates a general lack of significance from the current investigation, which supports the need to ensure pilot experiments or trials before larger experimentation is conducted.

From the simulated leaf litter effect on *A. robusta*, the results lacked significance (P=0.061). However, from a similar type of investigation performed by Barritt and Facelli (2001), the leaf litter would impact seed germination. Barritt and Facelli (2001) discussed how simulated, or natural forms would not hinder seedings' emergency. The *Casurarina* litter is like that of *A. robusta* which would be loose and provide no physical effect on seedling emergence (Barritt & Facelli 2001). In the litter experiment conducted for the *A. robusta* and in the investigation by Barritt and Facelli (2001), both experiments only measured a single factor of seedling emergence. Barritt and Facelli (2001) indicated that further investigation was required on the impact of the seedling emergence, including light availability, competition and soil community (e.g., fungi). From the experiment conducted, the p-value produced was not less than 0.05 and in the review of Newman (2008), showed that such result would require retesting or undertaking the experiment again. Nerveless in the context of Newman (2008), the results do not provide confidence towards the significance or the effect size. The lack of confidence from the results produced could, as Pennock (2004) discuss, arise from a lack of replication, but the results contain several similarities to the investigation of Barritt and Facelli (2001).

Replication can improve experimental precision, but replication does not always solve experimental design issues (Pennock 2004). The collection of samples to examine seed morphology of *A. robusta* occurred on an east to west transect through the population. Data collection on seed morphology may not be an accurate ecological indicator of the species' health. Additional data could be collected, including other morphological features, including phenological data related to the species. The addition of phenological data would explain how or when *A. robusta* seed development begins (McDonough MacKenzie et al. 2020). Phenological data would not resolve the sampling aspect, but McDonough MacKenzie et al. (2020) discussed how phenological data supports taxonomy and seed provenance questions. The caveat that needs to be applied is the number of sampling points, and the number of aggregate fruits selected which could allow pseudoreplication to occur (Pennock 2004). Phenological data is not solely focused on when a species flowers but can include when a species is actively growing. Phenological data can increase the taxonomic breath in an investigation by providing supportive information for taxonomy (McDonough MacKenzie et al. 2020). Analysing *A. robusta* seed morphology led to insignificance from the parsimony analysis. McDonough MacKenzie et al. (2020) indicated that phenological data can resolve the parsimony analysis's insignificance. The application of phenological data would increase the diversity in sample data. Applying phenological data would increase data diversity, but care needs to be applied equally to ensure a suitable and representative sample size.

Pennock (2004) indicates that when experimental design lacks sample diversity, it can give rise to pseudoreplication. The investigation focused only on one species (*A. robusta*), but the seed morphology experiment included comparing *A. robusta* relatives seed. Chen (2010) explains how this allows researchers to examine and develop an alternative research question. *A. robusta* surface litter results from current study were comparable to investigation of Barritt and Facelli (2001). Barritt and Facelli (2001) used a different species along with a different environmental habitat. However, future investigations should compare *A. robusta* to a common *Allocasuarina* species from the same environment. The similar type of concept was seen in the study of Abihudi et al. (2020). Identifying which common species to use could come from the investigation of Pearson (2020). The experimentation process tested heat shock, smoke and seed age which can provide vital information for managing a threatened or rare species. Considering study of Abihudi et al. (2020) and the data collected in the present investigation, could indicate a species' conservation trajectory in the Fleurieu Swamps. Germination of *A. robusta* was low in the current investigation. However, Dwyer (2017) conducted a study using species of *Acacia* and found survival of seedlings were low as 0.9% this could represent hundreds of

thousands of seeding facilitating the species survival on a per hectare basis. In this investigation a single germinant occurred, meaning that it would be difficult to come to the same conclusion as Dwyer (2017) on the number of seedlings required to produce a sustainable population.

The study of Abihudi et al. (2020) comparing a threatened species and a common species. Huang et al. (2021) extended the comparative concept to include an introduced species. The current study investigated the competition/nursery effect, which produced single germination with *M. polymorpha*. The assumption was that the competition for resources would only occur through the interaction between a native species and introduced species. Huang et al. (2021) indicated that competition could occur between two native species and the competition/nursery effects take place through the time taken for germination and the speed at which the species establishes. Observations from the current investigation can be related to concepts discussed in Catterall (2019), which highlights the role of nurse plants in restoration ecology and the current results will require further investigation to be more conclusive and definitive. Barritt and Facelli (2001) identified several related factors that impact germination (i.e. the interrelationships between a nurse plant and the species under study), all of which require further investigation. Understanding the role or relationship of a nurse plant with *A. robusta* will require further investigation. Lozano et al. (2020) explored the nurse plant relationship and role in the recruitment process. Lozano et al. (2020) identified that the main contributing factor for establishing a nurse plant relationship is soil, but in *A. robusta*, the evidence collected is inconclusive and requires further investigation. The current investigation had single germination, which poses several questions, did this occur by chance or was it through soil amelioration, as was the case in the study of Lozano et al. (2020). Alternatively, a single germinant's survival reflects long-term recruitment strategy of *A. robusta*, which also occurs in other species (Dwyer 2017; Navarro-Cano et al. 2019). Navarro-Cano et al. (2019) investigated the species' long term recruitment trajectory in a different genus.

The reporting of the investigation results and the comparison with other investigation (i.e. Abihudi et al. (2020) and Navarro-Cano et al. (2019) requires a caveat to avoid misinformation to give it a theoretical basis. Palmer (2000) termed investigation, which used different systems and species quasi replication. Identifying and comparing data against other species or different systems is often used, or comparative analysis from a data subset used as a means of justification (Palmer 2000). Palmer (2000) would not entirely dismiss the role that quasi replication can have in science. Chen (2010) identified that comparing but not analysing the investigation is not a true reflection of the hypothesis. For instance, the design of the heat intensity and heat shock/smoke experiments should demonstrate a significant germination event post fire for seed recruitment in *A. robusta*. The reported results were not conclusive to support or dismiss the hypothesis. A simulated fire used in the experimental design contained no comparative analysis with another species. Cury et al. (2020) compared species under the same condition which provided results with meaning and a theoretical context. In the current investigation, the absence of comparison between *A. robusta* and another species provides an opportunity for further investigation.

Replication needs to conducted under the same conditions, while comparison between common/ threatened or threatened/invasive can be made. The results from the current investigation examined the metapopulations of *A. robusta*. The results were not conclusive; the appearance of pseudoreplication could occur; as Pennock (2004) describes, the hypothesis was to determine the viability of metapopulations for natural regeneration. The method selected was like the method used by Costa e Silva et al. (2019) for examining the provenance of a Tasmanian *Eucalyptus* species. Costa e Silva et al. (2019) compared *Eucalyptus* provenances through replication within a controlled growing environment and a common garden experiment, while experiments on *A. robusta* was conducted only in a controlled growing environment. The current investigation requires a comparison to aid the recovery of *A. robusta* further.

From the data collected, several opportunities exist which can further aid the recovery of *A. robusta*. The community's role in collecting data or identifying new metapopulations of *A. robusta*, particularly on private land identified by Quarmby (2011). A strategy described by Breed et al. (2012) would provide the ideal means to achieve success through a tube stock program to improve seed recruitment practices. The caveat placed on the strategy needs to be evidence-based for the ecological community and each species used (Breed et al. 2012).

The relationship between community-driven action and science can support the recovery of *A. robusta*. From the investigation conducted, further investigation into seed recruitment of *A. robusta* is still required. Breed et al. (2012) discuss genetic diversity and variability as part of the seed collection strategy. Through population genetics, this would aid in understanding whether areas of seed provenance exist or not. The genetic data can aid and inform the community on how to progress the recovery efforts. Informing the professional's recovery efforts is not a one-way process, but a two-way process as the community can inform the professional to direct and support the research (Gollan et al. 2012). Gollan et al. (2012) identified that data collected from volunteers (community) is comparable to professionals' data. Using the findings from study of Gollan et al. (2012) and applying them to the *A. robusta* recovery project could mean that replication for common garden experiments could be made in various field locations where citizen science monitoring are taking place. For instance, the investigation involving a competition/nurse plant or leaf litter could be an ideal project that the community could undertake as a common garden experiment in collaboration with the professionals. The collaborative work undertaken as part of a citizen science project as part of the community engagement can produce valuable

data aiding the recovery efforts of a threatened species (Roger et al. 2020).

Defining the role of citizen science is essential for ensuring the success of the program. Community involvement in threatened species management would involve ensuring that some degree of quality control would be necessary over the data collection, which can be made through benchmarking activities for data collection (Gollan et al. 2012). Design consideration for increasing community involvement would, as Gollan et al. (2012) described, need to have simple and easy to use data collection tools. Gollan et al. (2012) described several case studies where presence/absence data collection tools or flipbooks were used to identify species present. Such ideas of Gollan et al. (2012) may be related to the *A. robusta* recovery project, particularly monitoring disturbance and natural regeneration. As can be seen, the experimental design problems allow space for rectification, which are a small investment that can guide the future direction of recovery efforts. Pilot studies can provide a means to refine or better identify the issues associated with species recovery. In the current investigation, the experimental design was refined from identifying problems at the pilot stage but more importantly, it led to the identification of new opportunities to aid *A. robusta* recovery.

In conclusion, interpreting the results provides some general inferences only but can provide an opportunity to reflect on the experimental design for *A. robusta* recruitment. Comparing *A. robusta* seed germination to a species of least concern would facilitate the species recovery process. The concept is no different from the investigation conducted by Abihudi et al. (2020). Abihudi et al. (2020) demonstrated the benefits of small discrete experimentation on a species could improve species management. *A. robusta* inhabits an environment considered to be prone to disturbance. The pilot investigation identified experimental design shortcomings, which translates to requiring further investigation but focussing on the essential environmental cues. For the recovery of *A. robusta* to be successful, community involvement is essential which can be applied from the lessons learned through experimentation and research at the pilot stage.

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ASIAN JOURNAL OF FORESTRY
Volume 5, Number 2, December 2021 E-ISSN: 2580-2844
Pages: 71-75 DOI: 10.13057/asianjfor/r050203

Estimating tree height and crown diameter of *Acacia auriculiformis* using diameter at breast height in Char Kukri-Mukri Island, Bangladesh

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Manuscript received: 13 April 2021. Revision accepted: 25 June 2021.

Abstract. *Dey T, Ahmed S, Islam MA. 2021. Estimating tree height and crown diameter of Acacia auriculiformis using diameter at breast height in Char Kukri-Mukri Island, Bangladesh. Asian J For 5: 71-75.* Measuring the height and crown diameter in the field is time-consuming and it needs more logistical efforts. Thus, in this study, we focused on evaluating the relationship between height and crown diameter with less effortlessly measured parameters such as diameter at breast height (DBH) to reduce the inventory costs and time. Different correlation-regression models for predicting tree height and crown diameter using DBH were developed for *Acacia auriculiformis* plantation in Char Kukri-Mukri Island, Bhola District, Bangladesh. Data of DBH, total height, and crown diameter of each tree were recorded. Coefficient of determination (R^2) and p-value was used for evaluating the models. The correlation coefficients between DBH and height and between DBH and crown diameter showed positive and significant relationships. The calculated p-value and R^2 value between DBH and height and between DBH and crown diameter in the correlation-regression analysis revealed that linear regression models were best fitted in both cases. The study concluded that the tree height could be estimated by the mean of DBH and vice versa, as well as crown diameter could be estimated by the mean of DBH and vice versa.

Keyword: *Acacia auriculiformis*, tree height, crown diameter, diameter at breast height, plantation

INTRODUCTION

Acacia auriculiformis A.Cunn. ex Benth (Akasmoni) is a fast-growing leguminous tree species and native to the savannas of Papua New Guinea, Northern Australia, and the Islands of the Torres Strait (Hawkins 1987). It has been introduced in several tropical countries such as Malaysia, India, Solomon Islands, Indonesia, the Philippines, Nigeria, Tanzania, and Bangladesh due to its highly adaptive capacity from rich to inferior sites (Kabir 2007). In Bangladesh, *A. auriculiformis* has been introduced in every agro-ecological zone of the country (Ghani 1990). The maximum height of this tree can reach 30 m with diameter of breast height (DBH) up to 60 cm under certain favorable conditions (Zabala 1990; Hawkins 1987). It prefers mean annual temperature from 26°C to 30°C, and mean annual rainfall ranges from 1500 mm to 2000 mm (Zabala 1990). It can grow in deep or shallow soils, eroding hillslopes, mining spoil, and highly acid to alkaline soils with pH ranges from 3.0 to 9.5 (Hawkins 1987) and altitudinal range from 0 up to 600 m above sea level (Zabala 1990).

One of the concerns and challenges of forest management is collecting accurate forest inventory information faster and more efficiently (Iizuka et al. 2017). This is because trees within a forest show considerable variation and flexibility regarding their height, crowns and diameter at breast height (Buba, 2013). Many forestry management activities involve information regarding diameter at breast height (DBH) and crown width. Therefore, any attempt to improve the accuracy of measuring, predicting, and analyzing these parameters should be considered (Ibrahim and Osman 2014). There are various technique, models, and other statistical tools that can quickly evaluate such parameters (Turan, 2009). The height-DBH relationship varies between tree species, even within the same forest stand (Mugasha et al. 2013) as well as within the same species with different tree sizes, stand densities, stand ages, compositions, species, and site conditions even over time (Poorter et al. 2006). In some studies, tree bole diameter is well correlated with tree crown diameter, where it can be used for determining the stock and stand density relationships (Ibrahim and Osman 2014). To characterize forest stands, the relationship between tree height and DBH is often used. However, in general, measuring height is more time-consuming than measuring DBH.

Thus, by developing predictive models of height from DBH, measurement costs can be reduced, keeping the accuracy of height at an acceptable level (Mugasha et al. 2013). By using the relationships between stem DBH and tree height, crown height, and crown length, it is possible to model growth and yield (Peper et al. 2001). As it is easy to measure DBH for the studies in ground-based forest inventory, total height, crown ratio, and crown length could be estimated through stem DBH (Turan 2009). The ability to predict crown diameter from DBH provides an efficient

method of obtaining an estimate of crown diameter (Gering and May 1995). Tree volume estimation, their development, and description of stands over time rely heavily on accurate height-diameter functions (Curtis 1967).

Estimating the current growing stock in large *A. auriculiformis* plantations through the traditional inventory system is both uneconomic and time-consuming. To reduce the inventory cost and time, modeling of DBH- height and DBH-crown diameter can be used as a proposed method to overcome higher inventory costs. This study aimed to evaluate the relationships between tree height and DBH as well as between crown width and DBH by developing different regression models, and to find out the best predicting models for *Acacia auriculiformis* tree plantation.

MATERIALS AND METHODS

Study area

The study was carried out in Char Kukri-Mukri Island, Charfashion Upazila, Bhola District, Bangladesh (Figure 1). Bangladesh Forest Department (BFD) made *A. auriculiformis* plantation under Char Kukri-Mukri Research Station of Bhola District in May 2011. The study area lies between 21°54 and 22°52 N and between 90°34 and 91°01 E. This area is part of the delta of the extended Himalayan watershed ecosystem, which forms the lowest landmass. Because of the presence of rivers of Meghna, Brahmaputra, and Ganges, the landscape has been developed into low-lying land, estuaries, and islands along the seacoast. Water salinity ranges from 3-27 ppt in monsoon, but it goes from 10-33 ppt in the dry season (Siddiqi and Khan 1990). The soil of the study area is silt-clay-loam, and soil salinity varies remarkably between the dry seasons and monsoon. Soil salinity ranges from 0.3-4.2 dS/m in December and reaches its peak is as high as 9 dS/m from April-May (Hasan 1987). Soil pH varies between 7.5-8.0 and is slightly alkaline (Siddiqi and Khan 2000). The the highest annual average temperature of the study area is 32.7°C, and lowest is 11.6°C, and yearly rainfall is 2360 mm (Bhola District statistics 2011).

Data collection

The suitable rotation age of *Acacia auriculiformis* in Bangladesh is 8 years and 13-15 years for fuelwood and timber, respectively (Islam et al. 2013). So, we selected 10 years old *Acacia auriculiformis* plantation for our study purpose. For data collection, the random sampling method was used where each tree has the possibility to be selected. After selecting trees randomly, DBH, crown diameter, and total tree height were measured. A total of 252 trees were measured randomly as the sample of the total population. The DBH, tree height, and crown width were taken as independent and dependent variables for data analysis.

Data were collected during March 2021 after ten years of planting the Akasmoni tree. All data needed for this study were collected by non-destructive measurements. Height was measured by Haga altimeter according to the formula for measuring tree height with Haga altimeter. Tree diameter at breast height (DBH) was measured by diameter tape (1.3 m from the ground level) with intensive care. The crown diameter was measured by taking the arithmetic average of the horizontal crown diameter on the north-south axis and on the east-west axis measured by measuring tape.

Data analysis

Data were compiled, and Pearson's correlations as well as regression models were employed using Microsoft Excel and SPSS of version 20. The complete dataset provided information for developing regression models between DBH and tree height as well as between DBH and crown diameter. Models were fitted using enter method in SPSS of version 20.

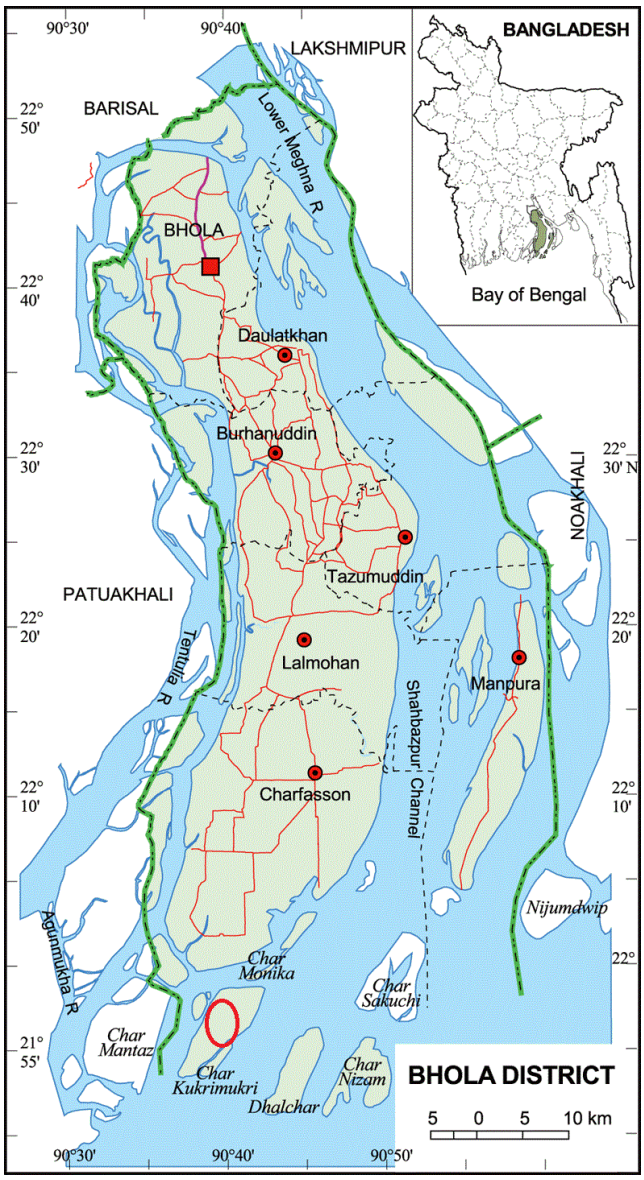

Figure 1. Map of the study area in Char Kukri-Mukri Island, Charfashion Upazila, Bhola District, Bangladesh (Source: Google image)

Table 1. Summary of the data of tree measurement of 10 years old *Acacia auriculiformis* plantation

Measure	Maximum	Minimum	Mean	Std. Deviation	CV value	N	Skewness	Kurtosis
DBH (cm)	22.345	8.294	15.363	3.425	22.293	252	0.221	-0.395
Height (m)	12.200	3.400	8.258	1.501	18.176	252	-0.173	0.290
Crown Diameter (m)	6.564	1.400	4.283	0.952	22.227	252	0.220	-0.249

RESULTS AND DISCUSSION

The summary of the raw data is presented in Table 1. The minimum, maximum, and mean DBH were 8.29 cm, 22.34 cm, and 15.36 cm, respectively. In contrast, the minimum, maximum, and mean tree heights recorded were 3.40 m, 12.20 m, and 8.26 m, respectively. The minimum, maximum, and mean crown diameters were 1.40 m, 6.56 m, and 4.28 m, respectively. The CV value of DBH, height, and crown diameter were 22.29, 18.18, and 22.23, respectively (Table 1). So, the growth rate of height in the *A. auriculiformis* tree is more homogeneous than the growth rate of crown diameter and growth rate of DBH, respectively (Table 1). Deb et al. (2012) found that the mean DBH and mean stem height of *A. auriculiformis* in the northeastern region of Bangladesh were 26.18 cm and 8.40 m, respectively. Rahman et al. (2018) found DBH, height and crown length of 10 years old *A. auriculiformis* strip plantations in Chattogram District were 19.2 cm, 14.86 m, and 5.8 m, respectively. Deb et al. (2012) also found that Skewness of DBH and stem height was 0.48 m and 0.30 m, respectively, and Kurtosis of DBH and stem height was -0.09 and -0.20, respectively for *A. auriculiformis* plantation in the northeastern region of Bangladesh.

The relationship between DBH and height (r = 0.789), the DBH and crown diameter (r = 0.74) showed a positive and strong correlation. Therefore, it can be concluded that there is a probability of increasing height and crown diameter with the increasing DBH and vice versa. In other words, trees with large DBH were taller and having more expansive canopies. Here, the highest R^2 value was found in linear equation (R^2=0.62) and the lowest R^2 found in exponential equation (R^2 =0.580) between Height and DBH as well as the highest R^2 value was found in linear equation (R^2=0.554) and the lowest R^2 value found in exponential equation (R^2 = 0.52) between crown diameter and DBH (Figs. 2 and 3).

Rahman et al. (2018) also found a positive and significant relationship between height and DBH for *A. auriculiformis* block, strip, and homestead plantation in the Chattogram district of Bangladesh. Arzai and Aliyu (2010) found a very strong linear relationship between DBH and height in *Parkia biglobosa*, *Khaya senegalensis*, and *Eucalyptus species*, as well as found a strong relationship between DBH and canopy width for *Parkia biglobosa*, *Khaya senegalensis*, *Eucalyptus species*, *Acacia digitate* and *Cassia siamea* species in the savanna zone of Nigeria. Kabir (2007) also found a positive and significant relationship between volume and DBH where height was constant for *A. auriculiformis* plantation in Dhaka Forest Division of Bangladesh.

The regression models are presented in Table 2. Here, the highest R^2 value found in linear regression analysis (R^2=0.62) with a p-value is .000*** between DBH and height is best fitted. On the other hand, the highest R^2 value found in linear regression analysis (R^2=0.55) with a p-value is .000*** between DBH and crown diameter is best fitted. Deb et al. (2012) found that all intercepts, regression coefficients, R^2 value, and p-value were significant in all models for estimating stem biomass of *A. auriculiformis* in the northeastern region of Bangladesh. In Chattogram District of Bangladesh, Rahman et al. (2018) found R^2 value (0.92), R^2 value (0.92), and R^2 value (0.93) in regression line for *A. auriculiformis* strip, block, and homestead plantation, respectively. Kabir (2007) found an R^2 value (0.95) in *A. auriculiformis* height vs. DBH linear regression model in Dhaka Forest Division of Bangladesh. Rahman et al. (2018) also found p-value (0.00225*), p-value (0.0024*), and p-value (0.0008*) in regression line for *A. auriculiformis* block, strip, and homestead plantation, respectively, in the Chattogram district of Bangladesh. Buba (2013) stated that one could use the prediction model to estimate the others by measuring either the DBH or the crown diameter. Acharya (2006) points out that the crown ratio can be calculated from stem DBH unless the crown ratio is defined differently.

In conclusion, a positive and moderate correlation was found between DBH and tree height as well as between DBH and crown diameter of *Acacia auriculiformis* trees in Char Kukri-Mukri Island, Bhola. The equations developed in this research will provide a method for predicting DBH or tree height and DBH or crown diameter depending on the data and the model used. This study concludes that it is easy to predict the tree height and canopy diameter from DBH as it is easy to measure for ground-based inventory and stand structure determination. The models developed by this study were based on data collected from Char Kukri-Mukri Island at Bhola district in Bangladesh. It should be used cautiously outside this area as plants show plasticity due to climatic and soil variability. With a greater variety of site and stand conditions, future research is needed in addition to a greater variety of tree sizes and ages.

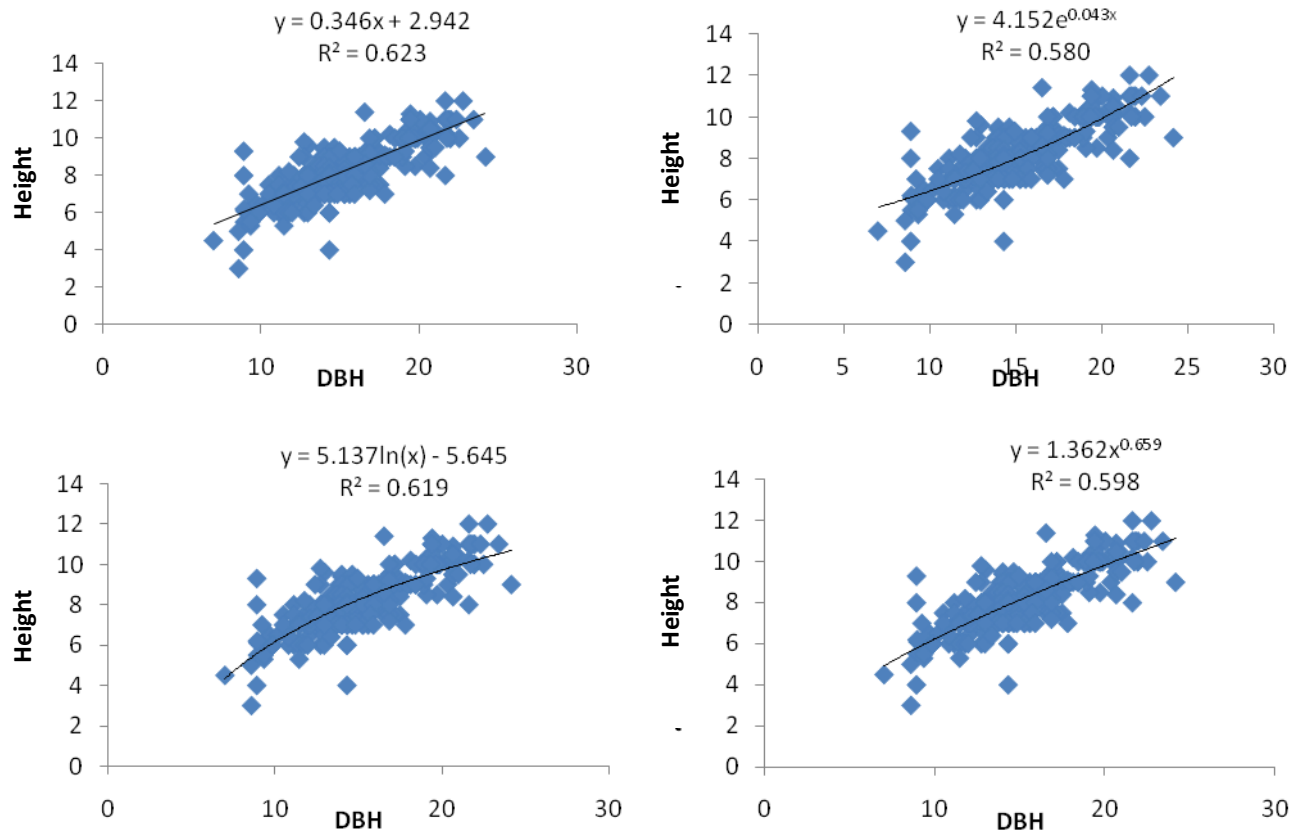

Figure 2. Relationship between Diameter at Breast Height (DBH) and Height

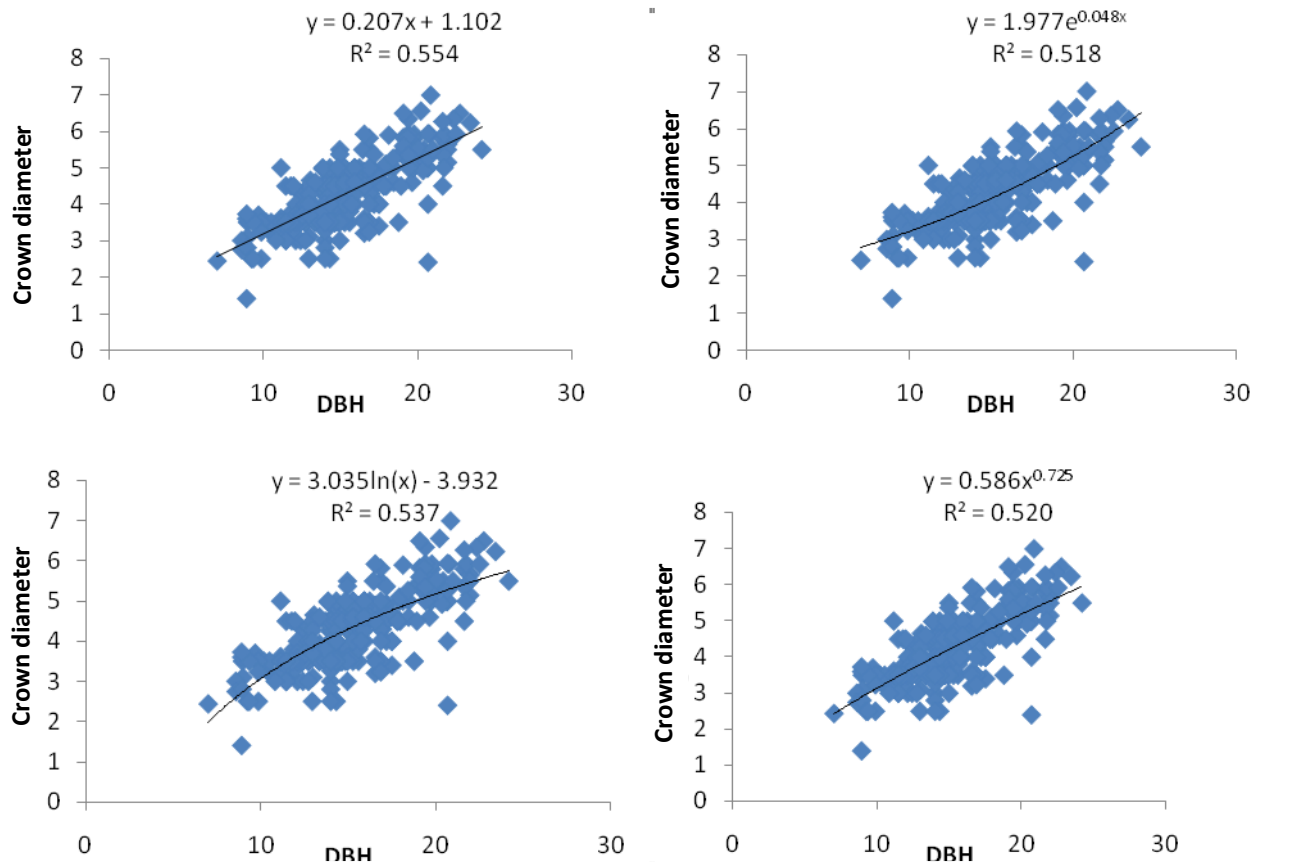

Figure 3. Relationship between Diameter at Breast Height (DBH) and Crown Diameter (CD)

Table 2. F-values, Regression prediction model, coefficient of determination (R^2), mean square error (MSE), standard error of coefficient (SEC), standard error of intercept (SEI), and p-value of the analysis of variance of the different tree variables

Model	Prediction model	R^2	MSE	SEC	SEI	F-value	P-value
Linear	DBH=0.486+1.801 (Height)	0.623	4.439	0.743	0.089	413.643	0.000***
	Height= 2.943+0.346 (DBH)	0.623	0.852	0.268	0.017	413.643	0 .000***
	DBH=3.897+2.677 (Crown Diameter)	0.554	5.253	0.666	0.152	310.767	0 .000***
	Crown Diameter=1.103+0.207 (DBH)	0.554	0.406	0.185	0.012	310.767	0.000***
Exponential	DBH = 5.530 e $^{0.121 \text{ (Height)}}$	0.620	0.020	0.277	0.006	407.329	0.000***
	Height = 4.153 e $^{0.044 \text{ (DBH)}}$	0.580	0.016	0.153	0.002	345.486	0.000***
	DBH = 7.016 e $^{0.177 \text{ (Crown Diameter)}}$	0.537	0.025	0.320	0.010	290.281	0.000***
	Crown Diameter = 1.978 e $^{0.049 \text{ (DBH)}}$	0.518	0.026	0.092	0.003	269.064	0.000***
Logarithmic	DBH= 13.313 ln (Height) – 12.508	0.580	4.947	1.506	0.716	345.486	0.000***
	Height = 5.137 ln (DBH) - 645	0.620	0.861	0.691	0.255	407.329	0.000***
	DBH= 10.660 ln(Crown Diameter) + 0.129	0.518	5.675	0.941	0.650	269.064	0.000***
	Crown Diameter= 3.036 ln(DBH) – 3.932	0.537	0.422	0.484	0.178	290.281	0.000***
Power	DBH = 2.237 (Height) $^{0.908}$	0.599	0.021	0.221	0.047	373.058	0.000***
	Height = 1.362 (DBH) $^{0.659}$	0.599	0.015	0.126	0.034	373.058	0.000***
	DBH = 5.374 (Crown Diameter) $^{0.717}$	0.520	0.026	0.339	0.044	270.926	0.000***
	Crown Diameter = 0.586 (DBH) $^{0.725}$	0.520	0.026	0.070	0.044	270.926	0.000***

ACKNOWLEDGEMENTS

We are grateful to the Suman Biswas, Scientific Officer, BARI, Gazipur, and field staff of Char Kukri-Mukri Forest Research Station of Plantation Trial Unit Division, Bangladesh Forest Research Institute, for sincere assistance in the execution of this experiment.

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ASIAN JOURNAL OF FORESTRY
Volume 5, Number 2, December 2021
Pages: 76-82

E-ISSN: 2580-2844
DOI: 10.13057/asianjfor/r050204

Allometric equation for aboveground biomass estimation of *Galiniera saxifraga* (Hochst.) Bridson in Gesha-Sayilem forest, southwestern Ethiopia

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Manuscript received: 10 June 2021. Revision accepted: 22 July 2021.

Abstract. *Bareke T, Addi A. 2021. Allometric equation for aboveground biomass estimation of* Galiniera saxifraga *(Hochst.) Bridson in Gesha-Sayilem forest, southwestern Ethiopia. Asian J For 5: 76-82.* There is limited information about the precise quantification of Aboveground Biomass (AGB) using allometric equations for shrubs and small trees. This study aimed to estimate aboveground biomass of *Galiniera saxifraga* to develop species-specific allometric equations for estimating biomass and carbon stock of shrub vegetation in Afromontane forest of southwest Ethiopia. Thirty *Galiniera saxifraga* plants were sampled to develop species-specific allometric biomass equations. Biometric parameters, including the diameter at the Breast Height (DBH), height and crown area, were predictive variables that were measured for each individual plant. AGB was measured through a destructive method. The AGB was correlated to biometric variables using regression analysis. The species-specific allometric models, with DBH and crown area as predictors (DBH-crown area models), accounted for 90% of the variation in the AGB of *G. saxifraga*. The DBH-crown area model was adequate for predicting the AGB for *G. saxifraga* with the adjusted R^2 value 0.9 and AIC values was 47.37. The specific allometric equation developed for the Gesha-Sayilem Afromontane forest can be used in similar moist forests in Ethiopia to estimate carbon stock.

Keywords: Allometric equation, aboveground biomass, biometric variables, Gesha and Sayilem

INTRODUCTION

Galiniera saxifraga (Hochst.) Bridson is a shrub up to 4-10 m high. It has branches that grow out in whorls from the trunk hanging down with regular rows of large opposite leaves. The leaves are shiny, ovate and the tip is clearly pointed with a hairy stalk. The flowers are small, white and fragrant like coffee flowers, and the fruit is a green berry, which is red when ripening. The species can be propagated from seeds and seedlings. It has a wide range of habitats, commonly growing in upland forest but sometimes also in secondary montane scrub, often near streams at altitudes between 1500 and 3000 m in most floristic regions of Ethiopia, and also in Eritrea and south to Zambia and Malawi (Addi et al. 2014).

The fragrance of the flowers attracts honeybees for nectar and pollen. It is one of the major honey source plants during September, October, and November in Gesha-Sayilem forest, southwest Ethiopia (Bareke and Addi 2020). It contributes to honey production along with other plants. The plant is also used for firewood, farm tools and the fruit is used as shot by children to make the sound of gun. *G. saxifraga* is one of the dominant shrub species in sub-canopy of Gesha-Sayilem forest. Gesha-Sayilem forest is designated as part of Bonga National Forest Priority Area and it is found under good conservation. All plant species which are found in this forest are under good conservation status including, *G. saxifraga*.

Forest ecosystem is a major component of the carbon reserves and it plays an important role in moderating global climate change through process of carbon sequestration (Addi et al. 2019; Tadesse et al. 2019). In forest ecosystems, the aboveground biomass (AGB) of shrubs and small trees comprises an essential component of total forest biomass. However, due to the lack of accurate quantification of aboveground biomass in shrubby vegetation, species-specific allometric equations for shrubs and small trees are relatively scarce (Cavanaugh et al. 2014; Ali et al. 2015).

The allometric equation estimates the whole or partial mass of a plant species from measurable tree dimensions, including trunk diameter, height, wood density, crown area, or their combination (Kuyah et al. 2012; Adrien et al. 2017; Tadese et al. 2019; Altanzagas et al. 2019). The most common allometric model used to predict biomass is the power function $Y = a \times Xb$, where Y is dry biomass weight, a is the integration factor, b is the scaling factor, and X is the diameter at breast height (Djomo et al. 2010). This function is considered the best applicable mathematical model for biomass studies because the growing plants maintain the different mass proportions between different parts. Allometric biomass equations have been developed for tree species in different ecological regions of the world, which are related to species-specific and strand-specific biomass models (Rebeiro et al. 2011).

The biomass models for moist Afromontane forest species of southwest Ethiopia are valuable tools for the estimation of carbon stocks in mitigation of climate change. Different authors have attempted to generate biomass equations for tropical forests for the estimation of

aboveground biomass (Henry et al. 2011; Chave et al. 2014; Edae and Soromessa 2019) and these equations may not accurately reveal the tree biomass in a specific region due to variability in wood density and the architecture of trees among and within species. The generic equation developed by Chave et al. (2005) may not adequately reveal the trees or shrubs biomass in a specific region in tropics including Ethiopia.

Therefore, species-specific equations are important to achieve higher levels of accuracy because trees of different species may differ greatly in tree architecture and wood density. No study was conducted to develop species-specific allometric equations to estimate the biomass for mitigating climate change effects, specifically developed for shrubs (Conti et al. 2013; Nogueira et al. 2018), let alone in tropical forest. Thus, the aim of this study was to estimate aboveground biomass of the *G. saxifraga* in order to develop species-specific allometric equations that could be used for biomass and carbon stock estimation in moist Afromontane forest of southwest Ethiopia, especially for shrub vegetation.

MATERIALS AND METHODS

Study area

The study area is located in the Southern Nations Nationalities Peoples Regional State (SNNPRS), Kaffa Zone in Gesha and Sayilem districts of Ethiopia. It is located between 6º24' to 7º70' N and 35º69' to 36º78' E (Figure 1). The topography of the landscape is undulating with valleys and rolling plateaus and some areas are flat in the plateaus. The altitude ranges from 1,600m to 3000m above sea level (Addi et al. 2020). The monthly mean maximum and minimum temperature for Gesha are 29.5ºC and 9.5ºC, respectively. On the other hand, the monthly maximum and minimum temperatures for Sayilem range 10ºC to 25ºC, and the annual rainfall for both districts ranges 1853-2004 mm.

Sampling design

A reconnaissance survey was carried out for the purpose of getting the overall impression of physiognomy of the forest, potential sampling sites, and accessibility. This helped to design the data collection methods prior to actual data collection. Because of the rugged and undulating terrain and its inaccessibility, collection of representative vegetation data using systematic sampling methods was not feasible, and therefore stratified random sampling methods were employed to collect vegetation data. For this purpose, altitudinal stratification was taken as criterion to divide the study area into different strata in order to get homogenous sampling units. Based on stratification principles, therefore the study area was divided into five elevational strata and the elevation distribution was extracted from the Digital elevation model (DEM) as indicated below starting from the lower to the highest altitude at intervals of 200m (Figure 2).

Species sampling

A direct destructive sampling method was applied for AGB measurements of individual trees. After measurements of shrub DBH and crown area, the plants were cut and the height of the felled plants was measured. The individual plants were partitioned into three components, namely, stem, branches, and leaves (including twigs with leaves having < 1 cm diameter). Six individual plants were randomly taken from each altitudinal strata to cover the widest possible range of plant sizes observed in the forest. A total of 30 individual plants were taken from the whole forest for AGB determination following methods developed by Maraseni et al. (2005) and Picard et al. (2012). Keeping climatic and soil conditions as constant as possible, the selected species were sampled across the study area.

Prior to destructive sampling, total height (H, meter), defined as the distance between the ground surface and the highest crown point; diameter at breast height (DBH, centimeter), maximum crown diameter (CD1, meter), and its perpendicular diameter (CD2, meter). Crown diameters were used to calculate crown area as follows:

$$CA = \pi \times (R_{1x} R_{2)}$$

Where,

CA: crown area (square centimeters)

R_1: Radius from the longest crown diameter (CD1) in centimeters

R_2: Radius from the crown diameter, perpendicular to CD1 (CD2) in centimeters (Conti et al. 2013).

The fresh weight of each stem, branch and leaves was measured on the site using a spring balance. To determine the dry matter content of the woods and leaves of all branches from each stem were taken from the thickest to the thinnest to make a composite sample and placed in sealed in plastic bags and transported to the laboratory. In the laboratory, fresh specimen samples were dried in the oven and weighted to estimate the water content. For stems and leaves dry biomass determination, the oven was set at temperature of 70ºC for 24 hours for leaves whereas for wood parts at 105ºC for 72 hours (Picard et al. 2012). AGB dry biomass per individual species was obtained by subtracting water content from individual fresh mass-weighted in the field. The carbon stock of a single shrub was obtained by multiplying the respective AGB by conversion factor or a default value of 0.5. This value is used when the following situation is happening. The wood density data for Ethiopian plant species is obtained from, the Ministry of the Environment and Climate Change. In cases where wood density of a species was not available, an average default value of 0.5 was used, as Chave et al. (2005) recommended for trees/shrubs from tropical forests.

Data analysis

R-software was used for data analysis. Data were analyzed using descriptive statistics, linear regression, and Pearson correlation analysis. All of the variables were log-transformed in order to apply linear models. Single and

multiple variable allometric equations were developed. Single variable refers to either diameter at the breast height (DBH), height (H), or crown area (CA), while multiple variables refer to the combination of two or three of these factors. Then, the selection of the best fit model was based on the goodness fit statistics (R^2) calculated for the species-specific equation such as adjusted coefficient of determination (R^2 adj), standard error of the mean *(*SE*)*, and Akaike Information Criterion (AIC).

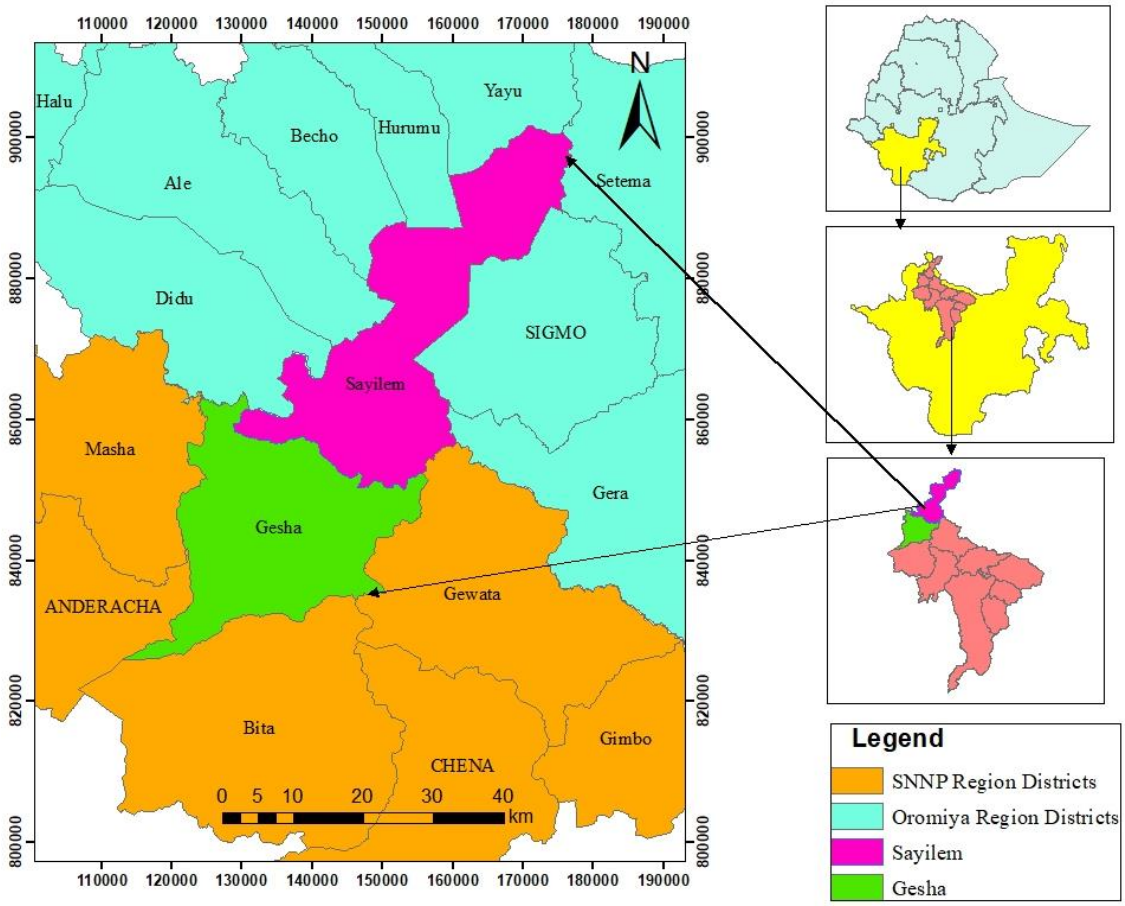

Figure 1. Map of Ethiopia, Oromia and SNNP Region, Kaffa zone, Gesha and Sayilem districts (Addi et al. 2020)

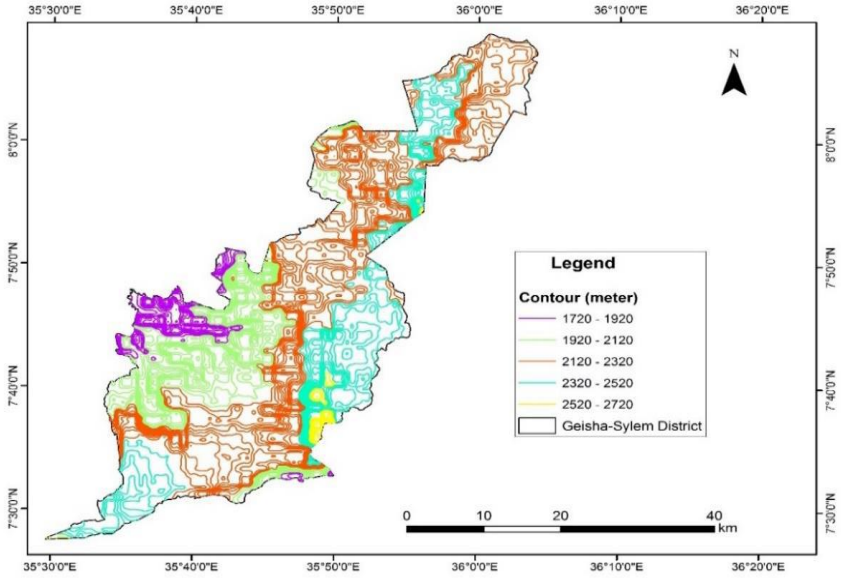

Figure 2. Gesha-Sayilem Digital Elevation model

Table 1. Models used to predict aboveground and components' biomass of *Galiniera saxifraga* in Gesha-Sayilem forest, Ethiopia

Model	Equation
M_1	$\log(AGB) = \beta_1 \log(DBH) + \varepsilon$
M_2	$\log(AGB) = \beta_1 \log(height) + \varepsilon$
M_3	$\log(AGB) = \beta_0 + \beta_1 \log(DBH) + \log(height) + \varepsilon$
M_4	$\mathrm{Log}(AGB) = \beta_1 log\,l(DBH) + \beta_2 \log(CA) + \beta_3 \log(DBH) : \log(CA) + \varepsilon$
M5	$\mathrm{Log(AGB)}=\beta_1 \log(\mathrm{DBH})+\beta_2 \log(\mathrm{Height})+\beta_3 log(CRA)$
M_6	$\mathrm{Log(AGB)} = \beta_1 \log(\mathrm{Height})+\beta_2(\mathrm{CRA})$

Note: AGB (aboveground biomass), $\beta_1\,(estimated\ parameters)$ BH (diameter at breast height), CRA (crown area), $\varepsilon\,(residual\ error)$

RESULTS AND DISCUSSION

Biomass measured variables

Allometric equations were developed by relating AGB against the predictive variables (DBH, height, and crown area) individually and in combination for *G. saxifraga* plant species. Data of the main variables were generated from direct field measurements. However, data for AGB was calculated from field and laboratory measurements. Descriptive summary for the main variables is presented in Table 2.

The aboveground biomass of *G. saxifarga* was positively correlated with the three variables (DBH, height, and crown area). The amount of aboveground biomass was highly affected by diameter at breast height (DBH) (R-squared 62.02%) followed by crown area (R-squared 48.45%) while less affected by the total height of the plant (Figure 3).

Pearson's correlation of biometric variables to biomass compartments

The Pearson's correlation analysis between aboveground biomass and biometric variables (DBH, height, and crown area) is shown in Table 3. The aboveground biomass was strongly correlated with DBH and it is the most influential factor affecting the biomass of the *G. saxifraga*. Crown area is the second important factor correlated strongly with biomass while height was poorly correlated with aboveground biomass. Furthermore, the analysis of sub-biomass (stem, big branch, small branches + leaves, and aboveground biomass) compartment of *G. saxifraga* showed that the biomass was strongly correlated with DBH in *G. saxifraga* but crown area was poorly correlated and no significant correlation was obtained with height.

The distribution of mean biomass fractions for the *G. saxifraga* showed that on average stem, branch and leaf biomass contributed to 6 and 13 kg of carbon/plant for foliage and wood respectively (Table 4). This indicates that the wood part of *G. saxifraga* stored more carbon than the foliage parts. The majority (68.42%) of the carbon of *G. saxifraga* was found in the stem and branch of the plant. The difference between branch and twig is that branch is a woody part of the tree or shrub arising from the trunk and usually dividing while twig is a small thin branch of a tree or shrubs.

Model selection and validation

This study explored several models with respect to the three primary biometric variables (DBH, H and CA) for estimating the AGB of *G. saxifraga* in Gesha-Sayilem forest. Selection of allometric equations was employed using statistical model performance. Equations with a higher coefficient of determination (adjusted R^2), lower residual standard error, and Akaike Information Criterion (AIC) values were found best-fitted. The DBH and crown area were found to be the best fit variables for *G. saxifraga* with the adjusted R^2 value 0.9 and AIC values was 47.37 for estimating the total AGB (Table 5). The coefficient of determination (R^2) explains the amount of percentage influence by independent variable on the dependent variables. In multiple regressions, adjusted R^2 considers the degrees of freedom which would be used instead of R^2 (Maraseni et al. 2005). Accordingly, model 4 was well performed in all parameter estimates and selected as the best to predict the aboveground biomass of *G. saxifraga* plant species. Allometric equations developed by Kuyah et al. (2012) based on crown area had a good fit with 85 % of the variation in aboveground biomass which was explained by crown area. Similarly, crown area explained a large fraction of the variability in each biomass component, with the greatest variability observed explained in branches.

Many authors have been explained that DBH is commonly used in allometric equations to estimate AGB. It can be used either alone or in combination with height, wood density or crown area depending on the nature of plant species (Ketterings et al. 2001; Chave et al. 2005; Kuyah et al. 2012). DBH can be measured easily with high accuracy and explains over 95% of the variability observed in the AGB (Kuyah et al. 2012). On the other hand, crown area could also be used as primary predictor variables, especially for highly branched crown plant species (Sah et al. 2004; Gibbs et al. 2007). The most important predictor of aboveground biomass is usually DBH (Nogueira et al. 2018). On the other hand, Conti et al. (2013) indicated that the crown area and crown shaped variables proved to be the variables with the best performance for both species-specific and multispecies shrubs models. A high proportion of biomass was accumulated in the stem and big branches of *G. saxifraga.* Similarly, Oliveira et al. (2011) study on coffee plants grown in agroforestry indicates that the woody component (stem + branch) accounted for 60-90% of aboveground biomass while the remainder being leaf biomass. A smaller biomass was accumulated in small branches and leaves.

Table 2. Summary of the measured variables and mean biomass of G*aliniera saxifraga* in Gesha and Sayilem forests

Parameter	Mean	Standard deviation	Minimum	Maximum
DBH (cm)	8.00	3.70	1.90	19.10
Height (m)	4.27	1.07	2.00	7.00
Crown area (m^2)	8.00	4.50	1.80	17.50
Aboveground biomass (kg/plant)	19.0	11.80	3.40	44.10

Table 3. Pearson's correlation coefficients between biomass compartments (stem, branches and above ground biomass) and dendrometric variables (diameter, height, and crown area) for *Galiniera saxifraga*

Biomass component	Dendrometric variables		
	DBH (cm)	Height (m)	CRA
Stem	0.69***	0.36ns	0.39ns
Big branch	0.54**	0.34ns	0.33ns
Small branches+ Leaves	0.58***	0.39ns	0.53**
Aboveground biomass	0.72***	0.62***	0.41*

Note: ns not significant, DBH diameter at breast height, CA Crown area. Significance level: * $p \leq 0.05$; ** $p \leq 0.001$;***$p \leq 0.001$

Table 4. Summary statistics of dry matter (kg/plant) of total aboveground biomass components and C contents of *Galiniera saxifraga* plant samples (n = 30)

Component	Dry matter (kg/plant)	Minimum	Maximum	Std. Deviation	Carbon (kg/plant)	% C
Foliage (leaf + twigs)	6	2.87	24.6	4.01	3	31.58
Wood (stem + branch)	13	7.27	23.44	3.87	6.5	68.42
Total aboveground biomass	19	10.14	48.04	7.88	9.5	100

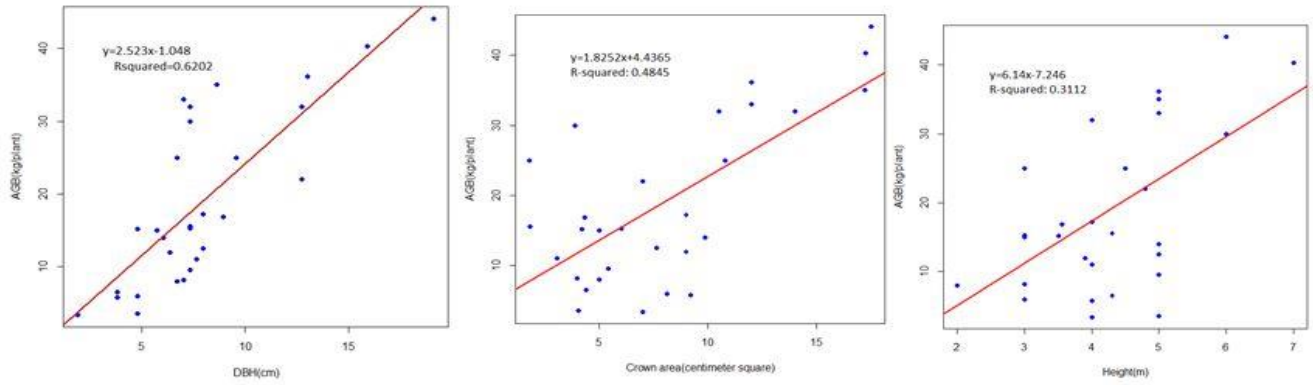

Figure 3. Correlation of DBH, crown area, and plant height on the aboveground biomass of *Galiniera saxifraga*

Table 5. Allometric equations and goodness of fit performance statistics for estimating aboveground biomass (kg dry matter/plant) of *Galiniera saxifraga* in Gesha-Sayilem forest (N=30).

Model	Model Equation	Parameter Estimates				Model Performance metrics	
		$\hat{\beta}_0$ (std.error)	$\hat{\beta}_1$ (std.error)	$\hat{\beta}_2$ (std.error)	$\hat{\beta}_3$ (std.error)	AIC	$Adj.R^2$
M_1	$\log(AGB) = \beta_1 \log(DBH) + \varepsilon$	-3.26(0.89)**	1.66 (0.269)	-	-	0.48	0.56
M_2	$\log(AGB) = \beta_1 \log(height) + \varepsilon$	-0.109(0.45)	1.67(0.33)***	-	-	52.6	0.49
M_3	$\log(AGB) = \beta_0 + \beta_1 \log(DBH) + \log(height) + \varepsilon$	-3.29(0.77)	1.21 (0.23)***	1.09(0.225)	-	34	0.49
M_4	$\mathrm{Log}(AGB) = \beta_1 log\,l(DBH) + \beta_2 \log(CA) + \beta_3 \log(DBH) : \log(CA) + \varepsilon$		2.56(0.076)***	2.15(0.682)**	-0.54(0.208)*	47.37	0.90
M_5	$\mathrm{Log(AGB)}=\beta_1 \log(\mathrm{DBH})+\beta_2 \log(\mathrm{Height})+\beta_3 log(CRA)$	-3.32(0.72)***	1.2125(0.241)***	1.0663(0.30)**	0.027 (0.13)	36.2	0.72
M_6	$\mathrm{Log(AGB)} = \beta_1 \log(\mathrm{Height})+\beta_2(\mathrm{CRA})$	-0.12(0.46)	1.75(0.38)**	-0.059(0.18)	-	54.6	0.47

Note: AGB (aboveground biomass), DBH (diameter at breast height), CRA (crown area), ε $(residual\ error)$ (Sign. code: * significant at 5%, ** significant at 1% and *** significant at 0.1%), AIC (Akaike Information Criterion), β0, β1, β2 and β3 are the coefficients

In conclusion, the total aboveground biomass of *G. saxifraga* plants occurred in Gesha and Sayilem forests had average of 19 kg of carbon per plant, with 68.42% was obtained from wood parts (stem + branches). This indicates that the wood part of *G. saxifraga* stored more carbon than the foliage parts. Each biomass component was found to be strongly correlated with DBH. Biometric variables DBH, and crown area model provided the best fit in *G. saxifraga*. The model developed in this study can be used to estimate forest carbon stocks, identify carbon sequestration capacity and establish carbon trade, and develop management value.

ACKNOWLEDGEMENTS

The authors are thankful to Oromia Agricultural Research Institute, Ethiopia for providing the required facilities and logistics. Our sincere thanks also to Holeta Agriculture Research Center, Ethiopia for helping us in the laboratory.

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ASIAN JOURNAL OF FORESTRY
Volume 5, Number 2, December 2021
Pages: 83-100

E-ISSN: 2580-2844
DOI: 10.13057/asianjfor/r050205

Quantitative evaluation of biological spectrum and phenological pattern of vegetation of a sacred grove of West Midnapore District, Eastern India

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Manuscript received: 26 April 2021. Revision accepted: 27 July 2021.

Abstract. *Sen UK, Bhakat RK. 2021. Quantitative evaluation of biological spectrum and phenological pattern of vegetation of a sacred grove of West Midnapore District, Eastern India. Asian J For 5: 83-100.* Sacred groves, small forests patches devoted to deities and ancestral spirits, are classic examples of community-based, culturally aware, natural resource management. They display rich biodiversity and provide ecological services to indigenous groups that care for them and have sustained the environment over the years, implying that sacred groves have cultural and spiritual significance. This study aimed to investigate the biological spectrum and phenological pattern of vegetation of sacred grove Kankabati Sitabala Than (KST) in West Midnapore District, India. The study revealed that the sacred forest hosts 312 plant species belonging to 257 genera under 78 families of 34 orders according to APG IV. Poales, 73, 23.40% and Poaceae, 48, 15.38% were the dominant order and family. Therophytes, cryptophytes and chamaephytes constitute a higher percentage 16.81%, 3.62% and 3.18% respectively than the normal spectrum exhibiting "thero-crypto-chamaephytic" phytoclimate. Leaf size spectra showed that the plant with leptophyll, 83, 26.60% and ovate, 59, 18.91% type's leaf lamina were dominant. The findings may have a heuristic value in developing future monitoring schemes and assessing the effects of environmental change in this varied but poorly studied area.

Keywords: Biodiversity, biological spectra, leaf size spectra, life form, sacred grove, West Midnapore

INTRODUCTION

Sacred groves have a wealth of history, traditions and ancient links between ecosystems and the local peoples (Anthwal et al. 2010). Across several countries of the world, sacred groves have been found to have a major role on biodiversity conservation and environmental protection because of the restrictions associated with them (Bhagwat et al. 2005). Traditional beliefs that limits the entry to sacred groves in otherwise deteriorated habitats have also contributed to well-preserved areas with high biodiversity (Tanyanyiwa and Chikwanha 2011; Rath and John 2018).

Sacred groves are remaining patches of virgin tropical forests, which are rarely destroyed by human activity but are conserved and protected by local people and serve as ecological and archaeological historical markers (Verschuuren et al. 2010). Many researchers have discussed their potential for conservation worldwide (Laird 2002). They are thought to be more effective than government-protected areas because they are community-managed and cover a wide variety of habitats. There was a general understanding among the ancients that the godly element is actively at work in places of natural sacred sites. These sites continue to exist today and play a significant role at various ecological levels (Wild et al. 2008).

The adaptation of a plant to certain ecological conditions determines a life form; hence, it is an important physiognomic feature that has been commonly used in the study of vegetation. This shows a certain area's macro and microclimate and human disturbance (Van der Maarel and Franklin 2012). The word "Biological Spectrum" was coined by Raunkiaer (1934) to describe the distribution of life-forms in flora as well as the phytoclimate in which the dominant life-forms evolved. Under this scheme, the plant species may be grouped into five main groups, i.e. phanerophytes, chamaephytes, hemicryptophytes, cryptophytes, and therophytes. The proportion of groups brought together in different life forms is called the biological continuum. Raunkiaer has developed a standard spectrum that can serve as a model against which spectra can be compared to different forms of life. Raunkiaer's standard range reveals a phanerophytes group, and the deviation (from that) defines the phytoclimate of an environment. Under a specific climate regime, climatic types can be characterized by the prevailing plant life forms in plant communities (da Costa et al. 2007).

There are many parts of India where indigenous communities live (Maffi and Woodley 2012). These diverse ethnic people has been documented as a unique example of traditional conservation practices including the co-existence of sacred groves within tribal communities. In these areas, the forest is considered sacred for the neighboring people.

The Indian region's biological spectrum is related to specific edaphic, altitudinal and climatic factors (Sen and Bhakat 2019; 2021). As a result, next to floristic composition, the analysis of life-form is a valuable method for describing vegetation. The biological continuum is also useful as an indicator of the state of health of the forest ecosystem (Ingegnoli 2015). Life type can also be graded

using the size of the leaf. It has some justification for using a leaf size to characterize different types of vegetation based on percentages of the different leaf sizes present (Dolph and Dilcher 1980). However, when performed at periodic intervals, the biological spectrum may set guidelines for the optimization and eco-restoration of a community. Life type can also be graded using the size of the leaf even within the same genotype (Alvarez-Clare et al. 2013).

Departing from the abovementioned rationale, the current study aimed to investigate the biological spectrum and phenological pattern of vegetation of sacred grove in India. The results of this study are expected to provide insights which may be used as a model for other sacred groves in general, and in particular for the study of phytoclimatics.

MATERIALS AND METHODS

Study site

This study was conducted in a sacred grove popularly known as "Kankabati Sitabala Than (KST)", located at latitude 22º25'15.12"- 22º25'15.55" N and longitude 87º15'11.90"- 87º15'12.16" E, at an altitude of 36.26 m asl (Figure 1). It is named after its presiding folk deity Sitabala or Sitala. It is situated 7 km from the West Midnapore district (India) headquarters town of Midnapore along the Midnapore-Jhargram road running east-west under the Midnapore Sadar block. The grove spreads over an area of 4 acres on public land at the common outskirts of the villages of Badhi, Kankabati and Lodhasai. This semi-evergreen, part-marshy, part-terrestrial of 800-year-old grove stands amid crop fields as an island of woodland. In addition to the regular worship given to the deity of the grove, local people, both tribal (Bhumij, Kora, Santal) and non-tribal from the surrounding villages visit the sacred forest en masse during the annual 'Makar Sankranti' (Mid-Month of January) during the two-day village fair. Strictly adhering to the taboos and ethics, people do not cut any grove plants or foul the area's serenity since the grove is the goddess's abode. Worshipping the goddess, according to folklore, grants immunity to pox, as well as village well-being and wealth to heralds.

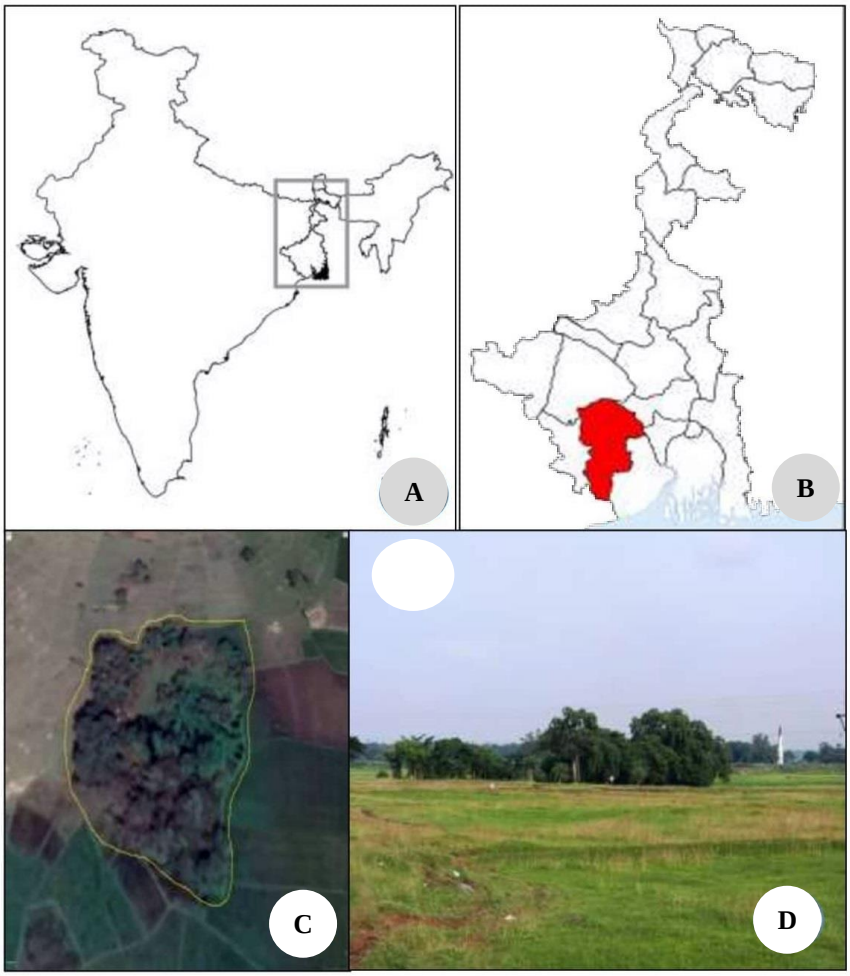

Figure 1. Location of the study area: (A) location of the state of West Bengal (boxed) within India; (B) location of West Midnapur district in West Bengal; (C) Google Earth image of Kankabati Sitabala Than (KST) and (D) Field picture of KST.

Field survey and data collection

The research area was extensively surveyed at different seasons during the period from September 2014 to October 2019 to examine botanical and social perspectives. Floristic surveys have been conducted based on "spot identification". For unknown plants, samples were collected of plants with flowers or fruits. After collection, the specimens were processed, stored and placed on herbarium sheets using traditional and modern herbarium techniques (Jain and Rao 1977). Photographs of some common, locally uncommon, endangered, and valuable plant species were taken at the sacred grove. Herbarium sheets were described by matching properly annotated materials available on the Herbarium at Vidyasagar University. For identification purposes, several related catalogs (Anderson 1862), regional floras (Hooker 1872-1897; Prain 1903; Haines 1921-1925; Bennet 1979; Sanyal 1994), monographs (Mitra 1958), revision works (Datta and Majumdar 1966) and other literature were consulted. The plant's scientific names were checked with the WCVP (World Checklist of Vascular Plant) (WCVP 2021) website and only accepted names were considered. The socio-cultural functions surrounding the grove were documented through information gathered from interviews and cross-interviews with devotees and local people during the Paus Sankranti festival.

Analysis of vegetation

In the systematic enumeration of taxa, the following terms were used: class, order, family, species along with voucher number, habit, life-span, nativity, flowering and fruiting time, life-form of Raunkiaer with subtype, leaf spectra, the shape of the lamina, IUCN Red List status (IUCN 2021) and plant growing seasons and then they were arranged according to the classification of Angiosperm Phylogeny Group IV (Chase et al. 2016) (Table 1). The total number of orders, families, genera and species in dicots and monocots were summarized (Table 2). All the species were categorized into different groups of Raunkiaer's life-form based on the location of regenerating parts or propagules in all the species collected and a biological spectrum was prepared for the grove, which was subsequently compared to the Raunkiaer's usual spectrum to determine the grove's phytoclimate (Raunkiaer 1934) (Table 1, 3). Knowledge of leaf size in understanding the physiological development of plants and plant communities was utilized to classify associations of plants. Diverse plant leaf sizes were arranged with their respective Raunkiaer life forms (Table 4). Plant's leaf sizes were divided into (i) leptophyll (< 25 mm^2), (ii) nanophyll (25-225 mm^2), (iii) microphyll (225-2025 mm^2), (d) notophyll (2025-4500 mm^2), (e) mesophyll (4500-18225 mm^2), (f) microphyll (18225-164025 mm^2) and (g) megaphyll (> 164025 mm^2) (Raunkiaer 1934).

RESULTS AND DISCUSSION

Different plant taxa

In this study, a total of 312 species belonging to 256 genera distributed among 78 families of 34 orders were reported from the sacred grove according to the APG IV (2016) classification. Rosids and Asterids were the top two clades. More than 80% of the flora was represented by orders from Eudicot and Core Eudicot, of which the major contributions (≥10 species) were from Poales, 73, (23.40%); Fabales, 39, (12.50%); Malpighiales, 20, (6.41%); Alismatales, 18, (5.77%); Lamiales, 16, (5.13%); Asterales, 14, (4.49%); Caryophyllales, 14, (4.49%); Malvales, 14, (4.49%) and Myrtales, 10, (3.21%) (Table 1; Figure 2).

Only sixteen out of the total families had ≥5 species, including Poaceae, 48 (15.38%); Fabaceae, 37 (11.86%); Cyperaceae, 23 (7.37%); Asteraceae, 14 (4.49%); Malvaceae, 14 (4.49%); Euphorbiaceae, 11 (3.53%); Araceae, 7 (2.24%); Cucurbitaceae, 7, (2.24%); Lamiaceae, 7, (2.24%); Commelinaceae, 6, (1.92%); Acanthaceae, 5, (1.60%); Dioscoreaceae, 5, (1.60%); Hydrocharitaceae, 5, (1.60%); Menispermaceae, 5, (1.60%); Rubiaceae, 5, (1.60%) and Vitaceae, 5, (1.60%) in descending array (Figure 3). Another four families had 4 species (1.28%); eight families had 3 species (0.96%) and eighteen families each had 2 species (0.64%) species, each, while thirty-two families were represented by just one species (Table 1).

The ten dominant plant families with ≥6 species comprised more than 51% genera, including Fabaceae, 14 (8.33%); Apocynaceae, 11 (6.55%); Asteraceae, 11 (6.55%); Lamiaceae, 9 (5.36%); Malvaceae, 9 (5.36%); Poaceae, 9 (5.36%); Acanthaceae, 6 (3.57%); Cyperaceae, 6 (3.57%); Euphorbiaceae, 6 (3.57%) and Rubiaceae, 6 (3.57%) (Table 1).

The eleven genera which were well represented are *Cyperus* (13 spp.), *Dioscorea* (4 spp.), *Fimbristylis* (4 spp.), *Setaria* (4 spp.), *Chrysopogon* (3 spp.), *Crotalaria* (3 spp.), *Euphorbia* (3 spp.), *Ficus* (3 spp.), *Panicum* (3 spp.), *Phyllanthus* (3 spp.) and *Sida* (3 spp.). The genera of *Agave*, *Annona*, *Cajanus*, *Chamaecrista*, *Commelina*, *Cyanotis*, *Eragrostis*, *Eriocaulon*, *Hygrophila*, *Jatropha*, *Murdannia*, *Potamogeton*, *Rhynchospora*, *Sacciolepis*, *Senna*, *Solanum*, *Tephrosia*, *Terminalia* and *Trichosanthes* were the nineteen well-represented genera with 2 species. There were only one species in another 224 genera (Table 1).

Species diversity in different growth form

The current sacred grove floristic study showed that it harbored a total of 312 plant species (dicots, 189, 60.58% and monocots, 123, 39.42%) of the genera 256 (dicots, 168, 65.63% and monocots, 88, 34.37%) of 78 families (dicots, 56, 71.80 % and monocots, 22, 28.20%) under 34 orders (dicots, 25, 73.53% and monocots 9, 26.47%). Of the reported species, 197, 63.14% were herbs. Other species reported were shrubs 38, 12.18%; trees 30, 9.62% and climbers 47, 15.06%. Herbs, shrubs, trees, and climbers made up 88, 35, 28, 38, and 109, 3, 2, 9 species respectively, accounting for 28.21%, 11.22%, 8.97%, 12.18%, and 34.94%, 0.96%, 0.64%, 2.88% of the total species (Table 2; Figure 4).

Table 1. Summary of the angiosperm taxa available in Kankabati Sitabala Than, eastern India

Clade	Order	Family	Habit			Raunkiaer's life-form						Leaf spectra						Total	
			H	S	T	C	Ph	Ch	He	Cr	Th	Le	Na	Mi	No	Me	Ma	Mg	Genus/ Genera	Species
	Nymphaeales	Nymphaeaceae	1							1								1	1	1
Mesangiosperms Magnoliids																				
	Piperales	Aristolochiaceae				1				1					1				1	1
	Magnoliales	Annonaceae			2		2									2			1	2
Independent Lineage Monocots																				
	Alismatales	Araceae	6			1				7		2	1	1			1	2	7	7
		Alismataceae	3						3					2			1		3	3
		Hydrocharitaceae	5							5		3	2						5	5
		Aponogetonaceae	1							1							1		1	1
		Potamogetonaceae	2						2							2			1	2
	Dioscoreales	Burmanniaceae	1								1	1							1	1
		Dioscoreaceae	1			4				5			1			4			2	5
	Pandanales	Pandanaceae		1			1											1	1	1
	Liliales	Colchicaceae				1	1									1			1	1
		Smilacaceae				1	1										1		1	1
	Asparagales	Orchidaceae	2				1			1				1	1				2	2
		Hypoxidaceae	1							1				1					1	1
		Xanthorrhoeaceae	2					1			1				2				2	2
		Amaryllidaceae	1							1								1	1	1
		Asparagaceae		2		1		2		1		1						2	2	3
	Arecales	Arecaceae			2	1	3							2				1	3	3
	Commelinales	Commelinaceae	6								6		4	2					3	6
	Zingiberales	Costaceae	1							1							1		1	1
		Zingiberaceae	4							4							3	1	4	4
	Poales	Eriocaulaceae	2								2	2							1	2
		Cyperaceae	23						23			19	4						7	23
		Poaceae	48						48			40	1		7				38	48
Eudicots	Ranunculales	Papaveraceae	2								2						2		2	2
		Menispermaceae				5	5									5			5	5
Rosids	Vitales	Vitaceae		1		4	4	1							3	2			5	5
	Fabales	Fabaceae	13	7	8	9	16	9			12		18	9	5	4	1		31	37
		Polygalaceae	2								2					2			2	2
	Rosales	Rhamnaceae				2	2								1	1			2	2
		Ulmaceae			1		1									1			1	1
		Moraceae			4		4							1		1	2		2	4
		Urticaceae	1								1	1							1	1
	Cucurbitales	Cucurbitaceae				7	7							1		6			6	7
	Celastrales	Celastraceae				1	1							1					1	1
	Oxalidales	Oxalidaceae	1								1		1						1	1
	Malpighiales	Hypericaceae	1								1	1							1	1
		Elatinaceae	1								1		1						1	1
		Violaceae	1								1		1						1	1
		Passifloraceae				1	1								1				1	1
		Salicaceae		1				1						1					1	1
		Euphorbiaceae	5	2	3	1	4	2			5	1	2	1	3	2	2		8	11
		Phyllanthaceae	3	1				1			3	1	2	1					2	4
	Myrtales	Combretaceae			2	1	3									1	2		2	3
		Lythraceae	4							1	3	3			1				3	4
		Onagraceae	1								1			1					1	1
		Myrtaceae			1		1									1			1	1
		Melastomataceae	1								1			1					1	1
	Sapindales	Sapindaceae		1		2	3								3				3	3
		Meliaceae			2		2								2				2	2
	Malvales	Malvaceae	9	5				4			10		1	7	4	2			12	14
	Brassicales	Capparaceae			1	1	2								1	1			2	2
		Cleomaceae	1								1			1					1	1

Superasterids	Santalales	Santalaceae		1						1	1							1	1
		Loranthaceae		2			2							2				2	2
	Caryophyllales	Polygonaceae	1			1	1			1		2						2	2
		Droseraceae	1							1	1							1	1
		Caryophyllaceae	3					2		1	2	1						3	3
		Amaranthaceae	3							3		1	2					3	3
		Aizoaceae	1							1			1					1	1
		Nyctaginaceae	1							1			1					1	1
		Portulacaceae	1							1			1					1	1
		Cactaceae		2				2			2							2	2
Asterids	Cornales	Cornaceae			1		1								1			1	1
	Ericales	Primulaceae	1							1			1					1	1
	Gentianales	Rubiaceae	2	2	1		2	1		2		2		2		1		5	5
		Loganiaceae	1		1		1			1		1			1			2	2
		Apocynaceae		1		2	2	1					1	1	1			3	3
	Boraginales	Boraginaceae	1							1				1				1	1
	Solanales	Convolvulaceae	2							2		1		1				2	2
		Solanaceae	1	1				1		1						2		1	2
	Lamiales	Plantaginaceae	2							2		2						2	2
		Acanthaceae	4	1				2		3			4	1				4	5
		Verbenaceae		2				2					1	1				2	2
		Lamiaceae	2	4	1		1	5		1		1	3		2	1		7	7
	Asterales	Asteraceae	13	1				1		13	2	2	6	3	1			14	14
	Apiales	Apiaceae	1							1				1				1	1
Total			197	38	30	47	75	38	76	30	93	83	52	55	48	44	21	9	256	312

Note: Habit: C: Climber, H: Herb, S: Shrub, T: Tree; Raunkiaer's Life: form and Sub: type: Ch: Chamaephytes, Cr: Cryptophytes, H: Hemicryptophytes, M: Mesophanerophyte, MM: Megaphanerophytes, N: Nanophanerophytes, Ph: Phanerophytes, T: Therophytes; Leaf spectra: Le: Leptophyll, Na: Nanophyll, Mi: Microphyll, No: Notophyll, Me: Mesophyll, Ma: Macrophyll, Mg: Megaphyll

Table 2. Taxonomic and habit distribution of angiosperm taxa in Kankabati Sitabala Than, eastern India

Group	Orders	Families	Genera	Species				
				Herbs	Shrubs	Trees	Climber	Total
Dicots	25	56	168	88	35	28	38	189
Monocots	9	22	88	109	3	2	9	123
Total	34	78	256	197	38	30	47	312

Table 3. Life-form analysis with different leaf sizes

Raunkiaer's life form	Leaf spectra							Total
	Le	Na	Mi	No	Me	Ma	Mg	
Ph	1	4	14	19	29	6	2	75
MM	0	0	2	0	5	4	1	12
M	0	0	1	6	3	1	0	11
N	1	4	11	13	21	1	1	52
Ch	3	7	9	8	4	5	2	38
He	59	5	2	7	2	1	0	76
Cr	6	4	3	2	4	6	5	30
Th	14	32	27	12	5	3	0	93
Total	82	52	55	48	44	21	9	312

Table 4. Biological spectrum (% of all life forms) of sacred grove and its comparison with Raunkiaer's normal spectrum

Life forms	Total no. of species	Biological spectrum (%) of the sacred grove	Raunkiaer's normal spectrum (%)	Deviation= (Raunkiaer's normal spectrum- Biological spectrum)
Phanerophytes (Ph)	75	24.04	46.00	-21.96
Megaphanerophytes (MM)	12	3.85	3.00	0.85
Mesophanerophyte (M)	11	3.53	28.00	-24.47
Nanophanerophytes (N)	52	16.66	15.00	1.67
Chamaephytes (Ch)	38	12.18	9.00	3.18
Hemicryptophytes (He)	76	24.36	26.00	-1.64
Cryptophytes (Cr)	30	9.62	6.00	3.62
Therophytes (Th)	93	29.81	13.00	16.81
Total	312	100	100	

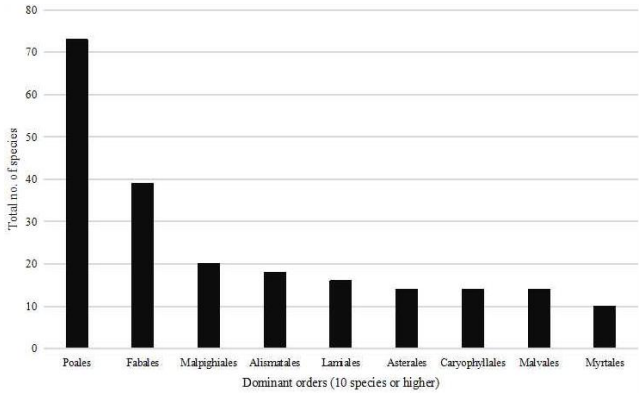

Figure 2. Dominant orders in the Kankabati Sitabala Than, eastern India

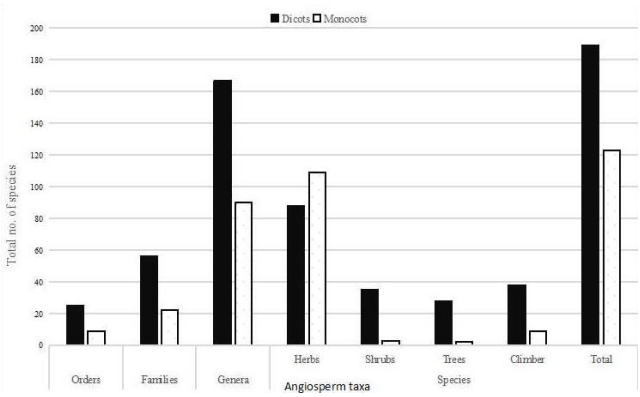

Figure 4. Diversity of different taxa in the Kankabati Sitabala Than, eastern India

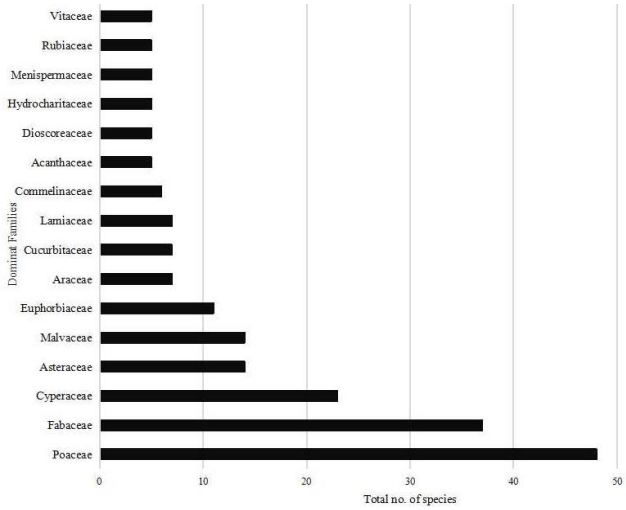

Figure 3. Dominant families in the Kankabati Sitabala Than, eastern India

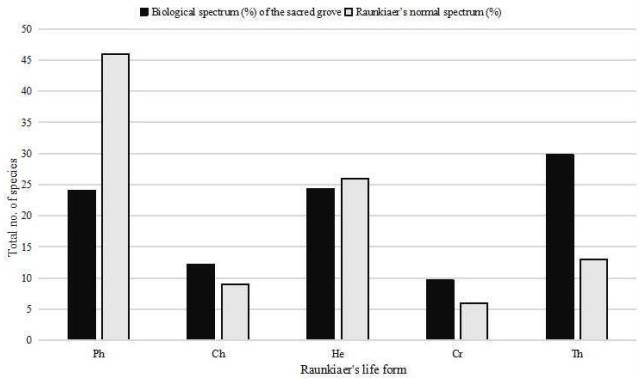

Figure 5. Comparison of biological spectrum of Kankabati Sitabala Than, eastern India with Raunkiaer's normal spectrum

Major seven herbaceous families (≥5 species) were Poaceae, 48 (24.37%); Cyperaceae, 23 (11.68%); Asteraceae, 13 (6.60%); Fabaceae, 13 (6.60%); Malvaceae, 9 (4.57%); Araceae, 6 (3.05%) and Commelinaceae, 6 (3.05%) held above 59% of the total herb population. The three major less-woody shrub families were Fabaceae, 7 (18.42%); Malvaceae, 5 (13.16%) and Lamiaceae, 4 (10.53%) held above 42% of the total shrubs population. Fabaceae, 8 (26.67%); Moraceae, 4 (13.34%) and Euphorbiaceae, 3 (10%) were three highly diversified families with over 50% of the total tree population. There were two trees in another four families, as well as seven families of single tree species. The five most speciose families in descending manner included Fabaceae, 9 (19.15%); Cucurbitaceae, 7 (14.89%); Menispermaceae, 5 (10.64%; Dioscoreaceae, 4 (8.51%) and Vitaceae, 4 (8.51%) clasp above 61% of the total liana population (Table 1).

Life span and nativity

In the sacred grove, in one growing season, 130 (41.67%) of annual plants would go through their life cycle. There were 1 (0.32%) biennial plants with a two-year life cycle and 181 (58.01%) perennial plants that could survive the most unfavorable conditions and stay alive for more than two years. In all, 225 (72.12%) species were native, while 87 (27.88%) species were exotic (Table 1).

Raunkiaer's life form and its distribution

One of Raunkiaer's life-form groups is phanerophyte, which is a plant whose perennial buds or shoot apices bore on aerial shoots, with the three most speciose families (≥5 species) mentioned in descending form including Fabaceae, 16 (21.34%); Cucurbitaceae, 7 (9.34%) and Menispermaceae, 5 (6.67%) containing more than 37% of the total phanerophytes. Three major descending chamaephyte families (≥4 species) were Fabaceae, 9 (23.68%); Lamiaceae, 5 (13.16%) and Malvaceae, 4 (10.53%); with a total population of (47.37%). Two leading hemicryptophytic families were Poaceae, 48 (63.16%) and Cyperaceae, 23 (30.26%); explicitly contained 93.42% of the total population. Araceae, 7 (23.34%); Dioscoreaceae, 5 (16.67%); Hydrocharitaceae, 5 (16.67%) and Zingiberaceae, 4 (13.34%) were four dominant descending

cryptophytes families total contained above 70% of the population. The five main therophyte families (≥5 species) were Asteraceae, 13 (13.98%); Fabaceae, 12 (12.90%); Malvaceae, 10 (10.75%); Commelinaceae, 6 (6.45%) and Euphorbiaceae, 5 (5.38%) of the total population of 49.46% (Table 1).

Life form and biological spectrum

The biological spectrum shows that therophytes, 93 (29.81%) were the dominant, followed by hemicryptophytes, 76 (24.36%); phanerophytes, 75 (24.04%); chamaephytes, 38 (12.18%) and cryptophytes, 30 (9.62%). Of the phanerophytes, nanophanerophytes, 52 (16.67%) was dominant than megaphanerophytes, 12 (3.85%) and mesophanerophytes, 11 (3.53%) (Table 4).

This study revealed that therophytes, cryptophytes and chamaephytes constitute the higher percentage 16.81%, 3.62% and 3.18% respectively than the normal spectrum exhibiting "thero-crypto-chamaephytic" phytoclimate. Further, the number of phanerophytes, 21.96% and hemicryptophytes, 1.64% is comparatively smaller in percentage than the Raunkiaer's normal spectrum. Out of the total phanerophytes, nanophanerophytes, 1.67% and megaphanerophytes, 0.85% was somewhat larger and mesophanerophyte, 24.47% was a comparatively smaller value than the Raunkiaer's normal spectrum (Table 4; Figure 5).

Leaf size spectra

The overall spectrum of leaf sizes showed that leptophyll, 83 (26.60%); nanophyll, 52 (16.67%); microphyll, 55 (17.63%); notophyll, 48 (15.38%); mesophyll, 44 (14.10%); macrophyll, 21 (6.73%) and megaphyll, 9 (2.88%) existed. As regards the spectrum of the leaf size, leptophyll was the highest followed by microphyll, nanophyll, notophyll, mesophyll, macrophyll and megaphyll. Poaceae, 40 (12.82%); Fabaceae, 18 (5.77%); Fabaceae, 9 (2.88%); Poaceae, 7 (2.24%); Cucurbitaceae, 6 (1.92%); Zingiberaceae, 3 (0.96%) and Araceae, 2 (0.64%) were dominant leptophyll, nanophyll, microphyll, notophyll, mesophyll, macrophyll and megaphyll families (Table 1, 3; Figure 6).

The shape of the leaf lamina and phenology

The leaf is generally a flat, green photosynthetic organ on the stem. As regards the shape of leaf lamina, ovate, 59 (18.91%) has been found to be the maximum followed by lanceolate, 46 (14.74%); cordate, 44 (14.10%); acicular, 39 (12.50%); linear, 41 (13.14%); sagitate, 16 (5.13%); obovate 14 (4.49%); subulate, 11 (3.53%); oblong, 9 (2.88%); hastate, 7 (2.24%); spathulate, 6 (1.92%); reniform, 5 (1.60%); orbicular, 4 (1.28%); cuneate, 3 (0.96%); palm like, 3 (0.96%); sabulate, 3 (0.96%); and lunate, 2 (0.64%) (Table 1).

The vegetation phenology observed during different seasons revealed that most of the species were dominant in rainy seasons, 311 (99.68%); followed by winter, 218 (69.87%) and summer, 93 (29.81%). Seasonally habit-wise species content varied; in the summer season, tree>climber>herb>shrub; rainy season, herb>climber> shrub>tree; winter season, herb>climber>shrub>tree, respectively (Table S1; Figure 7).

IUCN categories

230 plants have not yet been evaluated still now. There have been 80 species of Least Concerned (LC). *Cayratia pedata* was the vulnerable liana, whereas *Pterocarpus indicus* was the IUCN-species of a vulnerable tree (Table 1).

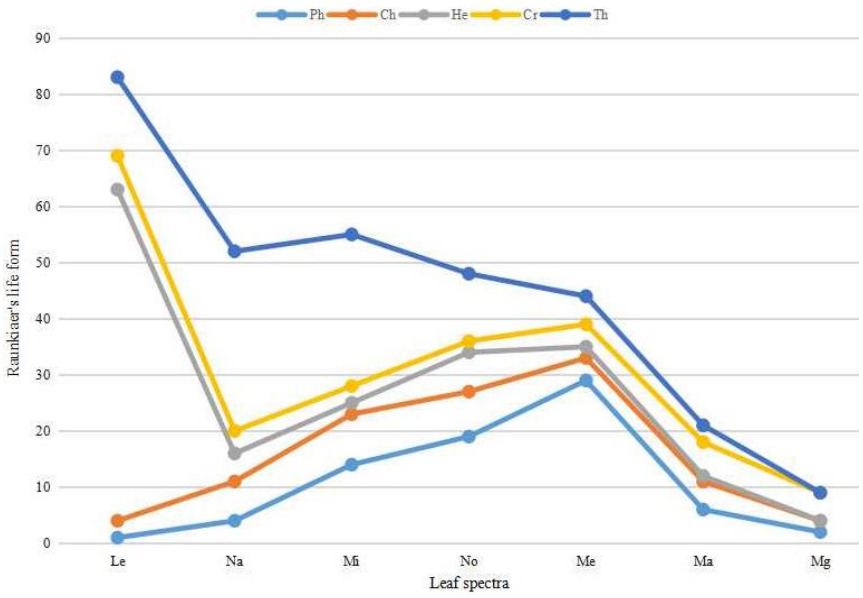

Figure 6. Leaf spectral variation in the Kankabati Sitabala Than, eastern India

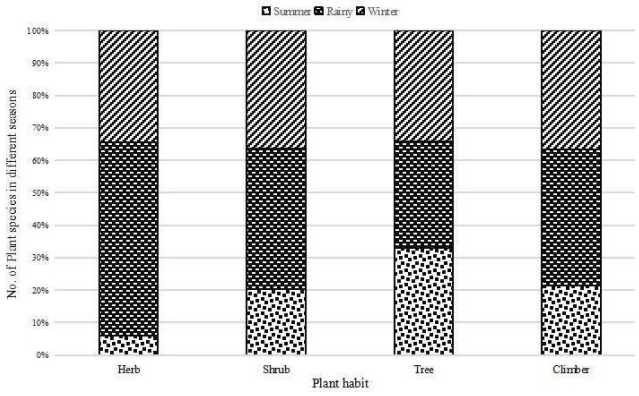

Figure 7. Vegetation phenology in the Kankabati Sitabala Than, eastern India

Discussion

Dominant taxa and climatic factors

The present study in the KST sacred grove which found 312 plant species belonging to 256 genera, 78 families and 34 orders indicates a considerable level of plant diversity. Such species have developed diverse societies adapted to their ecological needs and the management that human beings have implemented in recent years. High plant diversity in the area appeared to have been due to topographical, edaphic and physiographic conditions. Of course, the micro-climate factor was also effective in this respect, but variations in the area's climatic conditions were smaller than the other factors (Kargar-Chigani et al. 2017). From the analysis, it can be inferred that Rosids and Asterids were the dominant clades (Gastauer and Meira-Neto 2017). Poales, Fabales, Malpighiales, Alismatales, Lamiales, Asterales, Caryophyllales and Malvales were major contributing orders in terms of descending species number in the grove. The top ten families in descending form were Fabaceae, Apocynaceae, Asteraceae, Lamiaceae, Malvaceae, Poaceae, Acanthaceae, Cyperaceae, Euphorbiaceae and Rubiaceae (Sen and Bhakat 2018).

Biological spectrum

The phanerophytic life form had the third-highest percentage (24.04%), which was partially due to local security under some sacred grove taboos. The therophytes, cryptophytes and chamaephytes life forms had the highest 16.81%, 3.62% and 3.18% respectively, of the normal spectrum exhibiting "thero-crypto-chamaephytic" phytoclimate; phanerophytes (21.96%) were reasonably smaller in percentage than the normal spectrum probably partly due to the local threat. The dominant therophytes, cryptophytes and chamaephytes altogether constituted 51.61% of the life forms proportion. Therophytes showed the maximum divergence of the normal spectrum; a similar phytoclimatic association had also been reported by other workers for different tracks of vegetation (da Costa et al. 2007; De Mera and Vicente Orellana 2007; Sahu et al. 2012; Ceschin and Caneva 2013; Raju et al. 2014; Jakhar 2015; Yifru et al. 2015; Hamid and Raina 2019; Das et al. 2020; Zeb et al. 2020).

The highest percentage of therophytes taking place in the area was the trait of the subtropics and often related to soil and climatic conditions (Cornelissen et al. 2003). The prevalence of therophytes is accredited to diverse factors like widespread microclimate of the region united with anthropogenic activities like grazing, lopping, felling, deforestation, the introduction of annual weeds etc., was also reported by other workers (Khan et al. 2018; Sen and Bhakat 2018). In comparison to standard biological spectra, the present study shows that the vegetation was primarily sub-tropical in nature, with a higher percentage of therophytes and chamaephytes. Based on this study, the phytoclimate of the area, as per Raunkiaer's terminology, has been described as a "thero-crypto-chamaephytic" phytoclimate. This indicates the influence of anthropogenic activities in the study area which favors the chances of growth of short-lived annuals. It was also reported that therophytes stood next to phanerophytes. The prevalence of therophytes is also an indicator of biotic pressure (Halmy 2019). The growth of therophytes was much favored in disturbed areas (Lavorel et al. 1998). The bioclimate of the region, according to Meher-Homji (1964), reflected the life forms. Because of the favourable growing season, therophytes and nanophanerophytes are dominant throughout the year, particularly during the rainy season. During the start of the rainy season, there is always a flush of annual plants. The dominance of therophytes occurs due to unfavorable habitat conditions as suggested by others (Nazir and Malik 2006; Manhas et al. 2010; Sen and Bhakat 2020), and the findings agree with them.

Batalha and Martins (2004) and Ihsan et al. (2016) also considered therophytes, cryptophytes and chamaephytes as the major life forms in unfavorable conditions in the desert and open physiognomies. The hot, dry, and waterlogged conditions in the investigated region, combined with overgrazing, resulted in harsh conditions. The results also agree with those of Sher and Khan (2007), who also stated that therophytes and nanophanerophyes were characteristics of subtropical habitats. Sahu et al. (2012) discovered that therophytes and nanophanerophytes predominated in Odisha, India. Structurally and floristically the sub-tropical dry forests are less complex than wet forests, comprising about half or less of the tree species of the wet forest (Castro-Esau and Kalacska 2008). Cryptophytes are relatively fewer in number and are not a dominant life form of any particular climate (Box 2012). Cryptophytes, on the other hand, die back to underground storage organs in the Indian tropics to withstand unfavorable dry periods, fires, and other natural disasters. Cryptophytes are thought to be remnants of the paleoclimate that existed prior to the current extinction of the Indian subcontinent in the tropical ecosystem. According to Seward (2010), a fraction of the flora of a place may be in discordance with the present-day climate and could be the remnant of past climate. In this regard, the KST is floristically diverse and has the potential for future study. The dominance of therophytes, 93 species, 29.81% indicates that the investigated area was under moderate biotic pressure due to deforestation, overgrazing and agricultural land encroachment. Many plant species in the

region were on the decline. The local people will have a moral and ethical obligation to protect the plant resources. The majority of the medicinal plants were uprooted and grazed by livestock for burning purposes. Most of the fuelwood was extracted from the forests. The groves served as a haven for rare and precious animals and plants. More research is required to measure the data and propose conservation strategies for the sacred grove.

Patterns in leaf size spectra, leaf lamina and phenology

The present study recorded dominance of leptophyll during all seasons; microphyll and nanophyll were the next in order. Leaf spectra tell us about plant adaptation and association in a community. Small-sized leaves were present at the base while the large leaves were present at high altitudes as well as correlated with climatic warming and water availability in the soil (Tareen and Qadir 1993; Nicotra et al. 2011). The smaller type of leaf size indicates the climate was a sub-tropical type. Lepto and microphyllous elements were dominant in the sacred grove, which shows moisture and perennial water availability or wet condition. Seasonal changes are followed by changes in species diversity within the population. The vegetation phenology observed during different seasons revealed a substantial difference in vegetation among the seasons, owing to the study region's well-defined seasons. Most species dominated during the rainy season (99.68%), followed by winter (69.87%) and summer (29.81%). Expectedly, it may be attributed to the fact that a high proportion of therophytes and chamaephytes in the region appeared during the rainy and winter seasons.

In the present study, the proportion of different classes of leaf size was observed to change seasonally due to the presence of therophytes, cryptophytes, and chamaephytes. However, the nanophanerophytes and some chamaephytes (perennials and evergreens) almost retained the same status in all the seasons. Batalha and Martins (2004) also noted that the leaf size was related positively to drought and soil conditions. Badshah et al. (2010) identified the dominant leaf sizes of Kotli Azad Kashmir and Waziristan as being nanophyllous and microphyllous. This disparity was largely due to altitude variability in climate and ecosystem conditions. The size of the leaves alone could not be used to assess a specific leaf zone or climate. Other plant characteristics like habit and root system may also play a significant role. For the ecological study of a sacred grove in a region, the leaf spectra and biological spectrum alone are not ample, but quantitative studies such as vegetation structure and conservation are equally consequential.

IUCN categories

Given the above phytosociological analysis with ecological information about IUCN Red Listed plants reveals that the plants are still present and regenerate in the sacred groves but locally vanishing in nearby forests. Following the criteria devised by IUCN (2021), this report will highlight the status and distribution of the species in the study area, the ecological characteristics required for their survival, and the threats faced by some of the species designated. Various factors caused the increase in the numbers of vulnerable species in the area. Overgrazing was a major cause that led to the destruction of seedlings. In contrast, restricted population and low natural reproduction were determined to be the factors most effective on the vulnerability of *Cayratia pedata* and *Pterocarpus indicus*. Human behavior, such as overexploitation of the plant and land-use transition, was the most significant factor in the species' decline.

To conclude, sacred groves are the regenerated forest areas that surround places of worship. Sacred groves aid in the conservation of many rare and threatened species of plants and animals found in an area. Tribals in this area specifically forbid the practice of deforestation. Sacred groves are unquestionably hotspots of biological and socio-cultural diversity. It is also clear that many sacred groves are in jeopardy, whether they are managed by one or a few families or by entire communities. As sacred grove management is generally affected by a variety of social and economic factors, it is very difficult to pinpoint the specific strategies for their successful conservation. However, some common approaches to sacred grove management, that could be adopted with required changes to suit the needs of given sacred grove management, are as follows: self-imposition of a full ban on biomass removal for preserving ecosystem sustainability, creation of awareness among local people and stakeholder groups, identification of the type of contribution a stakeholder group can offer in management, and encouragement of all stakeholders to take part in sacred grove management, taking into account the wisdom and interests of the main stakeholder groups.

The present study denotes the possibility of using Raunkiaer's approach to ascertain the remarkable distinctions between the populations of angiosperm plants in a forested landscape or biome and their associations, the portion of species in the proportion of floristic life-forms that led by the current ecological parameters and environmental gradients. The biological spectrum of the sacred grove may be seen through the analysis of life forms. Dominant therophytes, cryptophytes and chamaephytes share, in the present study, the importance of depicting the phytoclimate "thero-crypto-chamaephytic." It may also be noted that in the future, the data obtained from this study will serve as a life-form database for change detection studies and bioclimatic or phytoclimate tenacity. It would also be helpful to compare and contrast the pattern of adjacent natural strands along the environmental gradients, revealing more than the mere forest covers in the ecosystem information, suggests that biotic factors play an important role in shaping a landscape's vegetation by directing successions. This indicates the presence in the sacred grove of anthropogenic disturbances promoting the development of more therophytes. Consequently, further disruption to the present sacred grove may encourage the potential changes to its present phytoclimate.

ACKNOWLEDGEMENTS

Special thanks to all informants who generously shared their knowledge of local plants and traditional medicinal

uses. We are grateful for the proper identification of plant species by Dr. G.G. Maity (Kalyani University, India). We thank the anonymous reviewers for their extremely useful comments.

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Table S1. List of angiosperm taxa of KST sacred grove

Name of the species	Voucher No.	Habit	Life-span	Nativity	Fl. & Fr. time	Raunkiaer's life-form	*Sub-type*	Leaf spectra	Shape of the lamina	IUCN Red List Status	Seasons		
											Summer	Rainy	Winter
Nymphaeales Salisb. ex Bercht. & J.Presl													
Nymphaeaceae Salisb.													
Nymphaea nouchali Burm. f.	USNY1	H	P	N	Aug.-Dec.	Cr		Mg	Or	LC	A	P	P
MESANGIOSPERMS													
MAGNOLIIDS													
Piperales Bercht. & J.Presl													
Aristolochiaceae Juss.													
Aristolochia indica L.	USAS1	C	A	N	Jul.-Jan.	Cr		No	La	NE	A	P	P
Magnoliales Juss. ex Bercht. & J.Presl													
Annonaceae Juss.													
Annona reticulata L.	USAN1	T	P	E	Jul.-Dec.	Ph	*N*	Me	La	Lc	P	P	P
Annona squamosa L.	USAN2	T	P	E	Mar.-Sep.	Ph	*N*	Me	La	Lc	P	P	P
INDEPENDENT LINEAGE: UNPLACED TO MORE INCLUSIVE CLADE													
MONOCOTS													
Alismatales R.Br. ex Bercht. & J.Presl													
Araceae Juss.													
Alocasia macrorrhizos (L.) G. Don	USAR1	H	P	E	Apr. -May	Cr		Mg	Sg	NE	P	P	P
Amorphophallus bulbifer (Roxb.) Blume	USAR2	H	P	N	May-Nov.	Cr		Mg	Ha	NE	A	P	P
Lemna perpusilla Torr.	USAR3	H	A	N	Jun.-Jan.	Cr		Le	Cu	LC	A	P	P
Pistia stratiotes L.	USAR4	H	A	E	Apr.-Oct.	Cr		Na	Cu	LC	A	P	P
Scindapsus officinalis (Roxb.) Schott	USAR5	C	P	N	-	Cr		Ma	Ov	NE	P	P	P
Typhonium trilobatum (L.) Schott	USAR6	H	A	N	Sep.-Oct.	Cr		Mi	Co	NE	P	P	P
Wolffia arrhiza (L.) Horkel ex Wimm.	USAR7	H	A	N	Jun.-Oct.	Cr		Le	Lu	LC	A	P	P
Alismataceae Vent.													
Butomopsis latifolia (D.Don) Kunth	USAL1	H	P	N	Sep.-Feb.	He		Mi	La	LC	A	P	P
Caldesia parnassifolia (Bassi) Parl.	USAL2	H	P	N	Apr.-Sep.	He		Mi	Re	LC	P	P	P
Sagittaria guayanensis Kunth	USAL3	H	P	N	Aug.-Nov.	He		Ma	Lu	LC	P	P	P
Hydrocharitaceae Juss.													
Blyxa octandra (Roxb.) Planch. ex Thwaites	USHD1	H	A	N	Nov.-Jan.	Cr		Le	La	LC	A	P	P
Hydrilla verticillata (L.f.) Royle	USHD2	H	A	N	Nov.-Mar.	Cr		Le	Li	LC	A	P	P
Najas graminea Delile	USHD3	H	A	N	Nov.-Jan.	Cr		Na	Ac	LC	A	P	P
Nechamandra alternifolia (Roxb. ex Wight) Thwaites	USHD4	H	A	N	Nov.-Feb.	Cr		Na	Li	LC	A	P	P
Ottelia alismoides (L.) Pers.	USHD5	H	A	N	All	Cr		Le	Sp	LC	A	P	P
Aponogetonaceae Planch.													
Aponogeton natans (L.) Engl. & K.Krause	USAN1	H	P	N	Aug.-Nov.	Cr		Ma	Li	LC	A	P	P
Potamogetonaceae Bercht. & J. Presl													
Potamogeton crispus L.	USPM1	H	A	N	Feb.-Apr.	He		Me	La	LC	A	P	P
Potamogeton nodosus Poir.	USPM2	H	A	E	Oct.-Dec.	He		Me	Oo	LC	A	P	P
Dioscoreales Mart.													
Burmanniaceae Blume													
Burmannia coelestis D.Don	USBU1	H	A	N	May.-Aug.	Th		Le	Li	LC	A	P	A
Dioscoreaceae R. Br.													
Dioscorea belophylla (Prain) Voigt ex Haines	USDI1	C	P	N	Sep.-Mar	Cr		Me	Re	NE	A	P	P
Dioscorea glabra Roxb.	USDI2	C	P	N	Sep.-Mar.	Cr		Me	Sg	NE	A	P	P
Dioscorea pentaphylla L.	USDI3	C	P	N	Sep.- Feb.	Cr		Me	Co	NE	A	P	P
Dioscorea pubera Blume	USDI4	C	P	N	Oct.-Jan.	Cr		Me	Co	NE	A	P	P
Tacca leontopetaloides (L.) Kuntze	USDI5	H	P	N	Aug.-Nov.	Cr		Na	Sp	LC	A	P	P
Pandanales R. Br. ex Bercht. & J. Presl													
Pandanaceae R. Br.													
Pandanus odorifer (Forssk.) Kuntze	USPN1	S	P	N	Jul.-May	Ph	*N*	Mg	Ob	LC	P	P	P
Liliales Perleb													
Colchicaceae DC.													
Gloriosa superba L.	USCO1	C	P	N	Jul.-Sep.	Ph	*N*	Me	Su	LC	A	P	A

Smilacaceae Vent.													
Smilax ovalifolia Roxb. ex D.Don	USSM1	C	P	N	Jun.-Dec.	Ph	*N*	Ma	Sg	NE	P	P	P
Asparagales Link													
Orchidaceae Juss.													
Geodorum recurvum (Roxb.) Alston	USOR1	H	P	N	Jul.-Aug.	Cr		Mi	La	LC	A	P	P
Vanda tessellata (Roxb.) Hook. ex G. Don	USOR2	H	P	N	Apr.-Jul.	Ph	*N*	No	Su	LC	P	P	P
Hypoxidaceae R. Br.													
Curculigo orchioides Gaertn.	USHP1	H	P	N	Aug.-Oct.	Cr		Mi	La	NE	A	P	A
Xanthorrhoeaceae Dumort.													
Aloe vera (L.) Burm.f.	USXA1	H	P	E	Dec.-Feb.	Ch		No	Su	NE	P	P	P
Asphodelus tenuifolius Cav.	USXA2	H	A	E	Jan.-Mar.	Th		No	La	NE	A	P	P
Amaryllidaceae J. St.-Hil.													
Crinum viviparum (Lam.) R.Ansari & V.J.Nair	USAY1	H	P	N	Aug.-Oct.	Cr		Mg	Li	LC	P	P	P
Asparagaceae Juss.													
Agave sisalana Perrine	USAP1	S	P	E	Mar.-Oct.	Ch		Mg	Su	NE	P	P	P
Agave vivipara L.	USAP2	S	P	E	Mar.-Oct.	Ch		Mg	Su	VU	P	P	P
Asparagus racemosus Willd.	USAP3	C	P	E	Aug.-Dec.	Cr		Le	Ac	NE	P	P	A
Arecales Bromhead													
Arecaceae Bercht. & J.Presl													
Borassus flabellifer L.	USAE1	T	P	E	Mar.-Oct.	Ph	*MM*	Mg	Pa	NE	P	P	P
Calamus viminalis Willd.	USAE2	C	P	N	Sep.-May.	Ph	*N*	Mi	Pa	NE	P	P	P
Phoenix sylvestris (L.) Roxb.	USAE3	T	P	N	Feb.-Jun.	Ph	*M*	Mi	Pa	NE	P	P	P
Commelinales Mirb. ex Bercht. & J.Presl													
Commelinaceae Mirb.													
Commelina benghalensis L.	USCM1	H	A	N	Aug.-Nov.	Th		Mi	Ov	LC	A	P	A
Commelina diffusa Burm.f.	USCM2	H	A	N	Aug.-Nov.	Th		Mi	Ov	LC	A	P	A
Cyanotis axillaris (L.) D.Don ex Sweet	USCM3	H	A	N	Sep.-Dec.	Th		Na	Su	LC	A	P	A
Cyanotis tuberosa (Roxb.) Schult. & Schult.f.	USCM4	H	A	N	Jul.-Sep.	Th		Na	Su	NE	A	P	A
Murdannia nudiflora (L.) Brenan	USCM5	H	A	N	Jul.-Nov.	Th		Na	Su	NE	A	P	A
Murdannia spirata (L.) G.Brückn.	USCM6	H	A	N	Sep.-Jan.	Th		Na	Su	LC	A	P	A
Zingiberales Grisebach													
Costaceae Nakai													
Hellenia speciosa (J.Koenig) S.R.Dutta	USCS1	H	P	E	Jul.-Sep.	Cr		Ma	Oo	LC	P	P	P
Zingiberaceae Martinov													
Alpinia calcarata (Andrews) Roscoe	USZI1	H	P	E	Apr.-Jun.	Cr		Ma	Li	NE	A	P	P
Curcuma aromatica Salisb.	USZI2	H	P	N	May-Jun.	Cr		Mg	Oo	NE	A	P	P
Globba marantina L.	USZI3	H	P	N	Aug.-Sep.	Cr		Ma	La	Lc	A	P	P
Zingiber capitatum Roxb.	USZI4	H	P	N	Jul.-Aug.	Cr		Ma	La	NE	A	P	P
Poales Small													
Eriocaulaceae Martinov													
Eriocaulon cinereum R.Br.	USER1	H	A	N	Oct.-Jan.	Th		Le	Ac	NE	A	P	P
Eriocaulon quinquangulare L.	USER2	H	A	N	Oct.-Feb.	Th		Le	Li	NE	A	P	P
Cyperaceae Juss.													
Bulbostylis barbata (Rottb.) C.B.Clarke	USCY1	H	P	E	Jul.-Oct.	He		Na	La	LC	A	P	A
Carex filicina Nees	USCY2	H	P	N	Sep.-Dec.	He		Na	Ac	LC	A	P	P
Cyperus brevifolius (Rottb.) Hassk.	USCY3	H	P	E	May-Oct.	He		Le	Ac	LC	A	P	A
Cyperus difformis L.	USCY4	H	P	E	Jul.-Nov.	He		Le	Li	LC	A	P	P
Cyperus rotundus L.	USCY5	H	P	E	Sep.-Dec.	He		Na	Ac	LC	A	P	P
Cyperus compactus Retz.	USCY6	H	P	N	Sep.-Nov.	He		Le	Ac	LC	A	P	P
Cyperus compressus L.	USCY7	H	P	N	Jul.-Nov.	He		Le	Ac	LC	A	P	P
Cyperus cyperoides (L.) Kuntze	USCY8	H	P	N	Aug.-Sep.	He		Le	Ac	LC	A	P	A
Cyperus distans L.f.	USCY9	H	P	E	Jul.-Sep.	He		Le	Ac	LC	A	P	A
Cyperus haspan L.	USCY 10	H	P	E	May-Jun.	He		Le	Ac	LC	A	P	P
Cyperus iria L.	USCY11	H	P	E	Aug.-Dec.	He		Le	Ac	LC	A	P	P
Cyperus laevigatus L.	USCY12	H	P	N	Aug.-Oct.	He		Le	Ac	LC	A	P	A
Cyperus pangorei Rottb.	USCY13	H	P	N	Oct.-Feb.	He		Le	Ac	LC	A	P	P
Cyperus paniceus (Rottb.) Boeckeler	USCY14	H	P	N	Jul.-Sep.	He		Na	Li	LC	A	P	A
Cyperus tenuispica Steud.	USCY15	H	P	E	May-Dec.	He		Le	Ac	LC	A	P	P
Fimbristylis aestivalis (Retz.) Vahl	USCY16	H	P	E	Feb.-May	He		Le	Ac	NE	A	P	P
Fimbristylis dichotoma (L.) Vahl	USCY17	H	P	E	Aug.-Oct.	He		Le	Ac	LC	A	P	A
Fimbristylis quinquangularis (Vahl) Kunth	USCY18	H	P	E	Aug.-Nov.	He		Le	Li	LC	A	P	P
Fimbristylis schoenoides (Retz.) Vahl	USCY19	H	P	N	Jul.-Oct.	He		Le	La	LC	A	P	A
Fuirena ciliaris (L.) Roxb.	USCY20	H	P	E	Sep.-Jan.	He		Le	Ac	LC	A	P	P
Rhynchospora colorata (L.) H.Pfeiff.	USCY21	H	P	E	May-Oct.	He		Le	Li	NE	A	P	A
Rhynchospora wightiana (Nees) Steud.	USCY22	H	P	N	Aug.-Oct.	He		Le	Li	NE	A	P	A
Schoenoplectiella articulata (L.) Lye	USCY23	H	P	N	Oct.-Feb.	He		Le	Li	LC	A	P	P

Poaceae Barnhart													
Alloteropsis cimicina (L.) Stapf	USPA1	H	A	E	Jul.-Oct.	He		Le	Co	NE	A	P	A
Apluda mutica L.	USPA2	H	P	N	Sep.-Nov.	He		Le	La	NE	A	P	P
Aristida setacea Retz.	USPA3	H	P	N	Aug.-Dec.	He		Le	Ac	NE	A	P	P
Arthraxon lancifolius (Trin.) Hochst.	USPA4	H	P	N	Sep.-Dec.	He		Le	Ac	NE	A	P	P
Cenchrus pedicellatus (Trin.) Morrone	USPA5	H	P	N	Oct.-Dec.	He		Le	Ac	LC	A	P	A
Chloris barbata Sw.	USPA6	H	P	E	Aug.-Nov.	He		Le	Li	NE	A	P	P
Chrysopogon aciculatus (Retz.) Trin.	USPA7	H	P	N	Sep.-Dec.	He		Le	Li	NE	A	P	P
Chrysopogon lancearius (Hook.f.) Haines	USPA8	H	P	N	Sep.-Dec.	He		Le	Ac	NE	A	P	P
Chrysopogon zizanioides (L.) Roberty	USPA9	H	P	N	Aug.-Dec.	He		Le	Li	NE	A	P	P
Coix lacryma-jobi L.	USPA10	H	A	N	Aug.-Jan.	He		No	Sg	NE	A	P	P
Cymbopogon martini (Roxb.) W.Watson	USPA11	H	A	N	Oct.-Dec.	He		No	Li	NE	A	P	P
Cynodon dactylon (L.)Pers.	USPA12	H	P	E	All	He		Le	Li	NE	P	P	P
Dactyloctenium aegyptium (L.) Willd.	USPA13	H	P	E	Jul.-Nov.	He		Le	La	NE	A	P	P
Desmostachya bipinnata (L.) Stapf	USPA14	H	P	E	Jun.-Oct.	He		Le	Ac	LC	A	P	A
Digitaria bicornis (Lam.) Roem. & Schult.	USPA15	H	P	N	Jul.-Oct.	He		Le	Ac	NE	A	P	A
Eleusine indica (L.) Gaertn.	USPA16	H	P	N	Aug.-Nov.	He		Le	Li	LC	A	P	P
Elytrophorus spicatus (Willd.) A.Camus	USPA17	H	P	N	Nov.-Jan	He		Le	Ac	LC	A	P	P
Eragrostiella brachyphylla (Stapf) Bor	USPA18	H	P	N	Aug.-Oct.	He		Le	Li	NE	A	P	A
Eragrostis viscosa (Retz.) Trin.	USPA19	H	P	E	Aug.-Feb.	He		Le	Li	NE	A	P	P
Eragrostis coarctata Stapf	USPA20	H	P	N	Aug.-Feb.	He		Le	Ac	LC	A	P	P
Hackelochloa granularis (L.) Kuntze	USPA21	H	P	N	Aug.-Nov.	He		No	Ac	NE	A	P	P
Hemarthria compressa (L.f.) R.Br.	USPA22	H	P	N	Jul.-Oct.	He		Le	Ac	LC	A	P	A
Heteropogon contortus (L.) P.Beauv. ex Roem. & Schult.	USPA23	H	P	N	Sep.-Jan.	He		Le	Ac	NE	A	P	P
Hygroryza aristata (Retz.) Nees ex Wight & Arn.	USPA24	H	A	N	Oct.-Mar.	He		No	La	NE	A	P	P
Hymenachne amplexicaulis (Rudge) Nees	USPA25	H	P	E	Oct.-Dec.	He		Le	Su	NE	A	P	P
Imperata cylindrica (L.) P.Beauv.	USPA26	H	P	E	Oct.-Dec.	He		Le	Li	LC	A	P	P
Isachne globosa (Thunb.) Kuntze	USPA27	H	P	N	Sep.-Feb.	He		Le	Li	LC	A	P	P
Leersia hexandra Sw.	USPA28	H	A	E	Sep.-Dec.	He		Le	Li	LC	A	P	P
Microchloa indica (L.f.) P.Beauv.	USPA29	H	P	N	Aug.-Oct.	He		Le	Li	NE	A	P	A
Miscanthus fuscus (Roxb.) Benth.	USPA30	H	P	N	Aug.-Oct.	He		Le	Ac	NE	A	P	A
Oplismenus burmanni (Retz.) P.Beauv.	USPA31	H	P	N	Sep.-Nov.	He		Le	Ov	NE	A	P	P
Oryza sativa L.	USPA32	H	P	N	Sep.-Dec.	He		No	Li	NE	P	P	P
Panicum curviflorum Hornem.	USPA33	H	P	N	Sep.-Dec.	He		Le	Ac	NE	A	P	P
Panicum notatum Retz.	USPA34	H	P	N	Sep.-Nov.	He		No	Li	NE	A	P	A
Panicum sumatrense Roth	USPA35	H	P	N	Aug.-Nov.	He		No	La	LC	A	P	A
Paspalum distichum L.	USPA36	H	P	N	Sep.-Nov.	He		Le	Li	LC	A	P	P
Perotis indica (L.) Kuntze	USPA37	H	P	N	Jul.-Nov.	He		Le	Ac	NE	A	P	A
Pogonatherum paniceum (Lam.) Hack.	USPA38	H	P	N	All	He		Le	La	LC	A	P	P
Sacciolepis myosuroides (R.Br.) Chase ex E.G.Camus & A.Camus	USPA39	H	P	N	Sep.-Dec.	He		Le	Ac	LC	A	P	P
Sacciolepis interrupta (Willd.) Stapf	USPA40	H	P	N	Sep.-Nov.	He		Le	Li	LC	A	P	P
Setaria flavida (Retz.) Veldkamp	USPA41	H	P	N	Aug.-Nov.	He		Le	Li	NE	A	P	A
Setaria parviflora (Poir.) Kerguélen	USPA42	H	P	N	Aug.-Nov.	He		Le	Li	LC	A	P	A
Setaria verticillata (L.) P.Beauv.	USPA43	H	P	N	Aug.-Nov.	He		Le	Li	NE	A	P	P
Setaria viridis (L.) P.Beauv.	USPA44	H	P	N	Jul.-Oct.	He		Le	Ob	NE	A	P	A
Sporobolus coromandelianus (Retz.) Kunth	USPA45	H	P	N	Aug.-Nov.	He		Na	Ac	NE	A	P	A
Tragus mongolorum Ohwi	USPA46	H	P	N	Aug.-Oct.	He		Le	Ac	NE	A	P	A
Urochloa ramosa (L.) T.Q.Nguyen	USPA47	H	P	N	Jul.-Nov.	He		Le	Co	LC	A	P	P
Urochloa reptans (L.) Stapf	USPA48	H	A	N	Aug.-Oct.	He		Le	La	LC	A	P	A
EUDICOTS													
Ranunculales Juss. ex Bercht. & J.Presl													
Papaveraceae Juss.													
Argemone mexicana L.	USPP1	H	A	E	Dec.-Apr.	Th		Ma	Sp	NE	P	A	P
Fumaria indica (Hausskn.) Pugsley	USPP2	H	A	E	Jan.-Mar.	Th		Ma	Sp	NE	A	P	P
Menispermaceae Juss.													
Cissampelos pareira L.	USMN1	C	P	N	Jul.-Jan.	Ph	*N*	Me	Co	NE	P	P	P
Cocculus hirsutus (L.) W.Theob.	USMN2	C	P	N	Aug.-Nov.	Ph	*N*	Me	Co	NE	P	P	P
Stephania japonica (Thunb.) Miers	USMN3	C	P	N	Jul.-Dec.	Ph	*N*	Me	Or	NE	P	P	P
Tiliacora acuminata (Lam.) Miers	USMN4	C	P	N	Nov.-May	Ph	*N*	Me	Ov	NE	P	P	P
Tinospora sinensis (Lour.) Merr.	USMN5	C	P	N	Feb.-Jun.	Ph	*N*	Me	Co	NE	P	P	P
ROSIDS													
Vitales Juss. Ex Berht. & Presl.													
Vitaeae Juss.													
Ampelocissus tomentosa (Roth) Planch.	USVT1	C	P	N	Aug.-Dec.	Ph	*N*	Me	Sg	NE	P	P	P

Causonis trifolia (L.) Mabb. & J.Wen	USVT2	C	P	N	Aug.-Dec.	Ph	*N*	No	Co	NE	A	P	P
Cayratia pedata (Lam.) Gagnep.	USVT3	C	P	N	Aug.-Feb.	Ph	*N*	No	Ov	VU	P	P	P
Cissus quinquangularis Chiov.	USVT4	C	P	N	Jul.-Jan.	Ph	*N*	No	Co	NE	P	P	P
Leea macrophylla Roxb. ex Hornem.	USVT5	S	P	N	Jul.-Sep.	Ch		Me	Sg	NE	P	P	P
Fabales Bromhead													
Fabaceae Lindl.													
Abrus precatorius L.	USFA1	C	P	N	Aug.-Mar.	Ph	*N*	Na	Ob	NE	A	P	P
Adenanthera pavonina L.	USFA2	T	P	N	Mar.-Jan.	Ph	*M*	No	Co	LC	P	P	P
Albizia lebbeck (L.) Benth.	USFA3	T	P	N	Mar.-Feb.	Ph	*MM*	Mi	Sb	NE	P	P	P
Alysicarpus monilifer (L.) DC.	USFA4	H	A	N	Aug.-Nov.	Th		Mi	Ob	NE	A	P	A
Brachypterum scandens (Roxb.) Miq.	USFA5	C	P	N	Jul.-Jan.	Ph	*N*	Na	Ob	NE	P	P	P
Cajanus cajan (L.) Huth	USFA6	S	P	E	Aug.-Feb.	Ch		Me	La	NE	A	P	P
Cajanus scarabaeoides (L.) Thouars	USFA7	C	A	N	Sep.-Feb.	Ph	*N*	Mi	Ov	NT	A	P	P
Cassia fistula L.	USFA8	T	P	N	Feb.-Dec.	Ph	*N*	No	Sb	NE	P	P	P
Chamaecrista absus (L.) H.S.Irwin & Barneby	USFA9	H	A	E	Aug.-Dec.	Th		Na	Ov	LC	A	P	A
Chamaecrista mimosoides (L.) Greene	USFA10	H	A	N	Mar. -Dec.	Th		Na	La	LC	A	P	A
Codariocalyx gyroides (Roxb. ex Link) Hassk.	USFA11	S	A	N	Aug.-Dec.	Ch		Na	La	NE	A	P	A
Crotalaria calycina Schrank	USFA12	S	A	N	Jul.-Nov.	Ch		Na	Li	NE	A	P	P
Crotalaria pallida Aiton	USFA13	S	A	E	Aug.-Jan.	Ch		Na	Ov	NE	A	P	P
Crotalaria retusa L.	USFA14	S	A	E	Jul.-Jan.	Ch		Mi	Ov	NE	P	P	P
Dalbergia sissoo Roxb. ex DC.	USFA15	T	P	E	Feb.-Aug.	Ph	*MM*	Mi	Ov	NE	P	P	P
Flemingia strobilifera (L.) W.T.Aiton	USFA16	H	A	N	Feb.-Sep.	Ch		Na	Ov	NE	A	P	P
Guilandina bonduc L.	USFA17	C	P	N	Aug.-Apr.	Ph	*N*	Me	Co	LC	P	P	P
Indigofera linifolia (L.f.) Retz.	USFA18	H	B	E	Aug.-Nov.	Th		Na	Oo	LC	P	P	P
Lablab purpureus (L.) Sweet	USFA19	C	A	E	Nov.-Mar.	Ph	*N*	Mi	Co	NE	A	P	P
Mimosa pudica L.	USFA20	H	P	E	Jul.-Nov.	Th		Na	La	LC	A	P	P
Mucuna pruriens (L.) DC.	USFA21	C	A	N	Sep.-May	Ph	*N*	No	Co	NE	A	P	P
Neptunia prostrata (Lam.) Baill.	USFA22	H	A	N	Sep.-Nov.	Th		Na	La	NE	A	P	P
Neustanthus phaseoloides (Roxb.) Benth.	USFA23	C	P	N	Aug.-Jan.	Ph	*N*	Mi	Co	NE	P	P	P
Pleurolobus gangeticus (L.) J.St.-Hil. ex H.Ohashi & K.Ohashi	USFA24	H	A	N	Oct.-Dec.	Th		Na	Ov	NE	A	P	A
Pongamia pinnata (L.) Pierre	USFA25	T	P	N	Apr.-Feb.	Ph	*M*	Me	Co	LC	P	P	P
Pseudarthria viscida (L.) Wight & Arn.	USFA26	H	P	N	Oct.- Jan.	Th		Mi	Ov	NE	A	P	A
Pterocarpus indicus Willd.	USFA27	T	P	N	Jul.-Dec.	Ph	*M*	No	Ov	EN	P	P	P
Samanea saman (Jacq.) Merr.	USFA28	T	P	E	Mar.-Feb.	Ph	*MM*	Me	Co	LC	P	P	P
Senegalia torta (Roxb.) Maslin, Seigler & Ebinger	USFA29	C	P	N	Feb.-Dec.	Ph	*N*	Na	Sb	NE	P	P	P
Senna alata (L.) Roxb.	USFA30	S	A	E	Aug.-Nov.	Ch		Ma	Ob	LC	A	P	P
Senna occidentalis (L.) Link	USFA31	S	P	E	Aug.-Dec.	Ch		No	Co	NE	A	P	P
Sesbania grandiflora (L.) Poir.	USFA32	T	P	N	Dec.-Mar.	Ch		Na	Ob	NE	P	P	P
Tephrosia candida DC.	USFA33	H	P	N	Sep.-Dec.	Th		Na	Ob	NE	A	P	P
Tephrosia pumila (Lam.) Pers.	USFA34	H	P	N	Jul.-Oct.	Th		Na	Oo	NE	A	P	P
Uraria rufescens (DC.) Schindl.	USFA35	H	P	N	Aug.-Dec.	Th		Na	Oo	NE	A	P	A
Vigna vexillata (L.) A.Rich.	USFA36	C	A	N	Jul.-Oct.	Ph	*N*	Mi	Co	NE	A	P	A
Zornia gibbosa Span.	USFA37	H	A	N	Aug.-Nov.	Th		Na	La	NE	A	P	A
Polygalaceae Hoffmanns. & Link													
Polygala crotalarioides Buch.-Ham. ex DC.	USPO1	H	A	N	Aug.-Nov.	Th		Me	Ov	NE	A	P	A
Salomonia ciliata (L.) DC.	USPO2	H	A	N	Aug.-Nov.	Th		Me	Li	NE	A	P	A
Rosales Bercht. & J.Presl													
Rhamnaceae Juss.													
Ventilago denticulata Willd.	USRH1	C	P	N	Nov.-Mar.	Ph	*N*	Me	La	NE	P	P	P
Ziziphus oenopolia (L.) Mill.	USRH2	C	P	N	Nov.-Mar.	Ph	*N*	No	Co	LC	P	P	P
Ulmaceae Mirb.													
Holoptelea integrifolia (Roxb.) Planch	USUL1	T	P	N	Jan.-Jun.	Ph	*MM*	Me	Ov	NE	P	P	P
Moraceae Gaudich.													
Ficus benghalensis L.	USMO1	T	P	N	Mar.-Sep.	Ph	*MM*	Ma	Co	NE	P	P	P
Ficus lacor Buch.-Ham.	USMO2	T	P	N	Mar.-Sep.	Ph	*MM*	Me	Co	NE	P	P	P
Ficus racemosa L.	USMO3	T	P	N	Mar.-Aug.	Ph	*M*	Ma	Co	Lc	P	P	P
Streblus asper Lour.	USMO4	T	P	N	Feb.-Jun.	Ph	*N*	Mi	Oo	Lc	P	P	P
Urticaceae Juss.													
Pouzolzia zeylanica (L.) Benn.	USUR1	H	A	N	Sep.-Jan.	Th		Le	Ov	NE	A	P	A
Cucurbitales Juss. ex Bercht. & J. Presl													
Cucurbitaceae Juss.													
Cayaponia laciniosa (L.) C.Jeffrey	USCU1	C	A	N	Jun.-Jan.	Ph	*N*	Mi	Sg	NE	A	P	A
Cucumis melo L.	USCU2	C	A	N	Jul.-Feb.	Ph	*N*	Me	Sg	NE	A	P	P
Diplocyclos palmatus (L.) C.Jeffrey	USCU3	C	P	N	Aug.-Oct.	Ph	*N*	Me	Sg	NE	A	P	P

Melothria trilobata Cogn.	USCU4	C	A	N	Jul.-Feb.	Ph	*N*	Me	Ov	NE	A	P	P
Solena amplexicaulis (Lam.) Gandhi	USCU5	C	A	N	Apr.-Dec.	Ph	*N*	Me	Sg	NE	A	P	P
Trichosanthes cucumerina L	USCU6	C	P	N	Aug.-Dec.	Ph	*N*	Me	Sg	NE	P	P	P
Trichosanthes tricuspidata Lour.	USCU7	C	A	N	Apr.-Sep.	Ph	*N*	Me	Ha	NE	P	P	P
Celastrales Link													
Celastraceae R.Br.													
Celastrus paniculatus Willd.	USCL1	C	P	N	Apr.- Dec.	Ph	*N*	Mi	Ov	NE	A	P	P
Oxalidales Bercht. & J. Presl													
Oxalidaceae R. Br.													
Oxalis corniculata L.	USOX1	H	A	E	All	Th		Na	Cu	NE	P	P	P
Malpighiales Juss. ex Bercht. & J.Presl													
Hypericaceae Juss.													
Hypericum japonicum Thunb.	USHY1	H	A	N	Feb.-Apr.	Th		Le	Co	NE	A	P	P
Elatinaceae Dumort.													
Bergia ammannioides Roxb.	USEL1	H	A	N	Nov.-Mar.	Th		Na	Ov	LC	A	P	P
Violaceae Batsch													
Afrohybanthus enneaspermus (L.) Flicker	USVI1	H	P	N	Jul.-Nov.	Th		Na	La	NE	A	P	A
Passifloraceae Juss. ex Roussel													
Passiflora foetida L.	USPS1	C	A	E	Aug.-Nov.	Ph	*N*	No	Sg	NE	P	P	P
Salicaceae Mirb.													
Flacourtia indica (Burm. f.) Merr.	USSA1	S	P	N	Sep.-May.	Ch		Mi	Ov	LC	P	P	P
Euphorbiaceae Juss.													
Acalypha lanceolata Willd.	USEU1	H	A	N	Aug.-Nov.	Th		No	Ov	NE	A	P	P
Chrozophora rottleri (Geiseler) Spreng.	USEU2	H	A	E	Jul.-Feb.	Th		Na	Co	NE	A	P	P
Croton bonplandianus Baill.	USEU3	H	P	E	All	Th		No	Co	NE	P	P	P
Euphorbia antiquorum L.	USEU4	T	P	N	Jan.-Apr.	Ph	*N*	Le	Oo	NE	P	P	P
Euphorbia hirta L.	USEU5	H	A	E	Feb.-Dec.	Th		Na	Co	NE	A	P	P
Euphorbia tithymaloides L.	USEU6	H	P	N	Mar.-Apr.	Th		No	Co	LC	P	P	P
Jatropha gossypiifolia L.	USEU8	S	P	E	Mar.-Aug.	Ch		Ma	Oo	LC	P	P	P
Jatropha curcas L.	USEU7	S	P	E	Mar.-Aug.	Ch		Ma	Sg	LC	P	P	P
Mallotus repandus (Willd.) Müll.Arg.	USEU9	T	P	N	Nov.-Apr.	Ph	*M*	Me	Co	NE	P	P	P
Suregada multiflora (A.Juss.) Baill.	USEU10	T	P	N	Mar.-Jul.	Ph	*N*	Mi	Ov	NE	P	P	P
Tragia involucrata L.	USEU11	C	P	N	Mar.-Jan.	Ph	*N*	Me	Ov	NE	A	P	P
Phyllanthaceae Martinov													
Breynia vitis-idaea (Burm.f.) C.E.C.Fisch.	USPY1	S	P	N	Apr.-Dec.	Ch		Mi	Ov	LC	P	P	P
Phyllanthus debilis J.G.Klein ex Willd.	USPY2	H	A	N	Apr.-Sep.	Th		Le	Ov	NE	A	P	P
Phyllanthus fraternus G.L.Webster	USPY3	H	A	N	Apr.-Sep.	Th		Na	Ov	NE	A	P	P
Phyllanthus virgatus G.Forst.	USPY4	H	A	N	Apr.-Sep.	Th		Na	La	NE	A	P	P
Myrtales Juss. ex Bercht. & J.Presl													
Combretaceae R.Br.													
Combretum roxburghii Spreng.	USCB1	C	P	N	Nov.-May	Ph	*N*	Me	Ov	NE	P	P	P
Terminalia arjuna (Roxb. ex DC.) Wight & Ar	USCB2	T	P	N	Apr.-Mar.	Ph	*MM*	Ma	Oo	NE	P	P	P
Terminalia catappa L.	USCB3	T	P	N	Apr.-Feb.	Ph	*MM*	Ma	Oo	LC	P	P	P
Lythraceae J. St.-Hil.													
Ammannia multiflora Roxb.	USLY1	H	A	N	Nov.-Mar.	Th		Le	Li	LC	A	P	A
Ammannia cordata Wight & Arn.	USLY2	H	A	N	Jun.-Feb.	Th		Le	Li	LC	A	P	A
Rotala rotundifolia (Buch.-Ham. ex Roxb.) Koehne	USLY3	H	A	N	Jan.-May	Th		Le	Li	LC	A	P	A
Trapa natans var. *bispinosa* (Roxb.) Makino	USLY4	H	A	N	Jun.-Nov.	Cr		No	Ha	NE	A	P	A
Onagraceae Juss.													
Ludwigia octovalvis (Jacq.) P.H.Raven	USON1	H	A	E	Sep.-Jan.	Th		Mi	Ov	LC	A	P	A
Myrtaceae Juss.													
Syzygium cumini (L.) Skeels	USMY1	T	P	N	Mar.-Jul.	Ph	*MM*	Me	La	LC	P	P	P
Melastomataceae Juss.													
Sonerila erecta Jack	USME1	H	A	N	Jun.-Dec.	Th		Mi	Ov	NE	A	P	A
Sapindales Juss. ex Bercht. & J.Presl													
Sapindaceae Juss.													
Allophylus cobbe (L.) Forsyth f.	USSP1	C	P	N	Jul.-Oct.	Ph	*M*	No	Ov	NE	A	P	A
Cardiospermum halicacabum L.	USSP2	C	A	N	Jul.-Dec.	Ph	*N*	No	Sp	LC	A	P	P
Dodonaea viscosa Jacq.	USSP3	S	P	N	Nov.-Apr.	Ph	*N*	No	Ob	LC	A	P	P
Meliaceae Juss.													
Azadirachta indica A. Juss.	USML1	T	P	N	Mar.-Jul.	Ph	*M*	No	La	LC	P	P	P
Melia azedarach L.	USML2	T	P	E	Feb.-Nov.	Ph	*M*	No	La	LC	P	P	P
Malvales Juss. ex Bercht. & J.Presl													
Malvaceae Juss.													
Abelmoschus crinitus Wall.	USMA1	S	A	N	Mar.-Sep.	Ch		No	Ov	LC	A	P	P

Azanza lampas (Cav.) Alef.	USMA2	S	A	N	Sep.-Dec.	Ch		No	Sg	NE	P	P	P
Byttneria herbacea Roxb.	USMA3	H	A	N	Sep.-Nov.	Th		No	Co	NE	A	P	P
Corchorus aestuans L.	USMA4	H	A	E	Jul.-Nov.	Th		Me	Ov	NE	A	P	A
Hibiscus mutabilis L.	USMA5	S	P	N	Aug.-Feb.	Ch		Me	Or	NE	P	P	P
Malachra capitata (L.) L.	USMA6	H	A	E	Sep.-Nov.	Th		Mi	Ha	NE	A	P	A
Malvastrum coromandelianum (L.) Garcke	USMA7	H	A	E	Jul.-Nov.	Th		Mi	Co	NE	A	P	A
Melochia corchorifolia L.	USMA8	H	A	E	May.-Jun.	Th		Na	Co	LC	A	P	A
Sida cordata (Burm.f.) Borss.Waalk.	USMA9	H	A	N	Aug.-Feb.	Th		Mi	Co	NE	A	P	A
Sida cordifolia L.	USMA10	S	A	N	Aug.-Dec.	Th		Mi	Co	NE	A	P	A
Sida mysorensis Wight & Arn.	USMA11	H	A	N	Sep.-Dec.	Th		Mi	Co	NE	A	P	A
Triumfetta rhomboidea Jacq.	USMA12	H	A	E	Sep.-Jan.	Th		Mi	Ha	NE	A	P	A
Urena lobata L.	USMA13	S	A	E	Sep.-Dec.	Ch		No	Ha	LC	A	P	P
Waltheria indica L.	USMA14	H	P	E	Aug.-Nov.	Th		Mi	Co	NE	A	P	A
Brassicales Bromhead													
Capparaceae Juss.													
Capparis zeylanica L.	USCP1	C	P	N	Mar.-Oct.	Ph	*M*	No	La	NE	P	P	P
Crateva magna (Lour.) DC.	USCP2	T	P	N	Mar.-Jul.	Ph	*M*	Me	Ov	NE	P	P	P
Cleomaceae Bercht. & J.Presl													
Cleome monophylla L.	USCE1	H	A	E	Aug.-Oct.	Th		Mi	Co	NE	A	P	P
SUPERASTERIDS													
Santalales R.Br. ex Bercht. & J.Presl													
Santalaceae R. Br.													
Viscum multinerve (Hayata) Hayata	USSN1	S	P	N	Mar.-Jul.	Th		Le	La	NE	P	P	P
Loranthaceae Juss.													
Dendrophthoe falcata (L.f.) Ettingsh.	USLO1	S	A	N	Nov.-Mar.	Ph	*N*	No	Ov	NE	A	P	P
Macrosolen capitellatus (Wight & Arn.) Danser	USLO2	S	A	N	Mar.-Sep.	Ph	*N*	No	Li	NE	A	P	P
Caryophyllales Juss. ex Bercht. & J.Presl													
Polygonaceae Juss.													
Antigonon leptopus Hook. & Arn.	USPL1	C	A	E	Aug.-Jan.	Ph	*N*	Na	Co	NE	P	P	P
Persicaria hydropiper (L.) Delarbre	USPL2	H	A	N	May-Jan.	Th		Na	La	LC	A	P	P
Droseraceae Salisb.													
Drosera burmanni Vahl	USDR1	H	A	N	Nov.-Apr.	Th		Le	Or	LC	A	P	A
Caryophyllaceae Juss.													
Polycarpon prostratum (Forssk.) Asch. & Schweinf.	USCR1	H	A	N	Dec.-Apr.	Ch		Na	Co	LC	A	P	A
Spergula arvensis L.	USCR2	H	A	N	Jan.-Mar.	Ch		Le	Ac	NE	A	P	A
Vaccaria hispanica (Mill.) Rauschert	USCR3	H	A	N	Jan.-Mar.	Th		Le	Su	NE	A	P	A
Amaranthaceae Juss													
Achyranthes aspera L.	USAM1	H	A	N	Sep.-Feb.	Th		Mi	Ov	NE	A	P	A
Alternanthera sessilis (L.) R. Br. ex DC.	USAM2	H	A	E	Jul.-Feb.	Th		Mi	Ov	LC	P	P	P
Amaranthus spinosus L.	USAM3	H	A	E	All	Th		Na	Ov	NE	P	P	P
Aizoaceae Martinov													
Trianthema portulacastrum L.	USAI1	H	A	E	Apr.-Oct.	Th		Mi	Oo	NE	P	P	P
Nyctaginaceae Juss.													
Boerhavia diffusa L.	USNC1	H	A	N	Jun.-Dec.	Th		Mi	Re	NE	A	P	A
Portulacaceae Juss.													
Portulaca oleracea L.	USPR1	H	A	E	Jun.-Dec.	Th		Mi	Oo	NE	P	P	P
Cactaceae Juss.													
Cereus pterogonus Lem.	USCC1	S	P	N	Jun.-Jul.	Ch		Le	Ac	LC	P	P	P
Opuntia stricta (Haw.) Haw.	USCC2	S	P	E	Apr.-Aug.	Ch		Le	Ac	LC	P	P	P
ASTERIDS													
Cornales Link													
Cornaceae Bercht. & J.Presl													
Alangium salviifolium (L.f.) Wangerin	USCN1	T	P	N	Mar.-Jul.	Ph	*N*	Me	Ov	LC	P	P	P
Ericales Dumortier													
Primulaceae Batsch ex Borkh.													
Lysimachia arvensis (L.) U.Manns & Anderb.	USPI1	H	A	E	Jan.-Mar.	Th		Mi	Ov	NE	A	P	P
Gentianales Juss. ex Bercht. & J.Presl													
Rubiaceae Juss.													
Benkara malabarica (Lam.) Tirveng.	USRU1	S	P	N	Apr.-Nov.	Ch		No	La	NE	P	P	P
Gardenia resinifera Roth	USRU2	S	P	N	Feb.-Jun.	Ph	*N*	No	Ov	NE	P	P	P
Neolamarckia cadamba (Roxb.) Bosser	USRU3	T	P	N	Jul.-Nov.	Ph	*MM*	Ma	Ov	NE	P	P	P
Scleromitrion pinifolium (Wall. ex G.Don) R.J.Wang	USRU4	H	A	N	Sep.-Nov.	Th		Na	Li	NE	A	P	A
Spermacoce brachystema R.Br. ex Benth.	USRU5	H	A	E	Jul.-Dec.	Th		Na	Ov	NE	A	P	A

Loganiaceae R. Br. ex Mart.													
Mitrasacme prolifera R.Br.	USLO1	H	A	N	Aug.-Dec.	Th		Na	Li	NE	A	P	A
Strychnos nux-vomica L.	USLO2	T	P	N	Mar.-Jan.	Ph	*MM*	Me	Ov	NE	P	P	P
Apocynaceae Juss.													
Gymnema sylvestre (Retz.) R.Br. ex Sm.	USAO1	C	P	N	Apr.-Mar.	Ph	*N*	Mi	La	NE	A	P	P
Pergularia daemia (Forssk.) Chiov.	USAO2	C	P	N	Sep.-Jan.	Ph	*N*	Me	Co	NE	A	P	P
Rauvolfia tetraphylla L.	USAO3	S	P	N	Feb.-Dec.	Ch		No	La	NE	P	P	P
Boraginales Juss. ex Bercht. & J.Presl													
Boraginaceae Juss.													
Heliotropium indicum L.	USBO1	H	A	N	Oct.-Jan.	Th		No	Co	NE	A	P	P
Solanales Juss. ex Bercht. & J.Presl													
Convolvulaceae Juss.													
Evolvulus alsinoides (L.) L.	USCV1	H	A	N	Jul.-Feb.	Th		Na	La	NE	A	P	P
Ipomoea aquatica Forssk.	USCV2	H	P	N	All	Th		No	Co	LC	P	P	P
Solanaceae Juss.													
Solanum americanum Mill.	USSO1	H	A	E	Dec.-Jun.	Th		Ma	Ov	NE	A	P	A
Solanum sisymbriifolium Lam.	USSO2	S	A	E	Jul.-Mar.	Ch		Ma	Ov	NE	A	P	P
Lamiales Bromhead													
Plantaginaceae Juss.													
Bacopa monnieri (L.) Wettst.	USPT1	H	A	N	Sep.-Jan.	Th		Na	Re	LC	A	P	A
Limnophila indica (L.) Druce	USPT2	H	A	N	Sep.-Jan.	Th		Na	Ac	LC	A	P	A
Acanthaceae Juss.													
Andrographis paniculata (Burm.f.) Nees	USAC1	H	A	N	Sep.-Apr.	Th		No	Ov	NE	A	P	A
Barleria prionitis L.	USAC2	S	P	N	Dec.-Apr.	Ch		Mi	La	NE	A	P	A
Ecbolium viride (Forsk.) Alston	USAC3	H	P	N	Dec.-Apr.	Ch		Mi	Ov	NE	A	P	A
Hygrophila auriculata (Schumach.) Heine	USAC4	H	A	N	Sep.-Jan.	Th		Mi	La	LC	A	P	A
Hygrophila polysperma (Roxb.) T.Anderson	USAC5	H	A	N	Sep.-Jan.	Th		Mi	La	NE	A	P	A
Verbenaceae J.St.Hil.													
Lantana camara L.	USVE1	S	P	E	Nov.-Feb.	Ch		No	Ov	NE	P	P	P
Lippia javanica (Burm.f.) Spreng.	USVE2	S	P	N	Sep.-Apr.	Ch		Mi	Ov	NE	P	P	P
Lamiaceae Martinov													
Anisomeles indica (L.) Kuntze	USLA1	H	A	N	Sep.-Jan.	Ch		Mi	Ov	NE	A	P	A
Clerodendrum infortunatum L.	USLA2	S	P	N	Feb.-Jul.	Ch		Ma	Co	NE	A	P	P
Leonotis nepetifolia (L.) R.Br.	USLA3	S	A	E	Apr.-Jul.	Th		Me	Co	NE	A	P	A
Leonurus sibiricus L.	USLA4	S	A	N	Sep.-Feb.	Ch		Mi	La	NE	A	P	A
Mesosphaerum suaveolens (L.) Kuntze	USLA5	S	A	E	Sep.-Jan.	Ch		Me	Ov	NE	A	P	A
Ocimum basilicum L.	USLA6	H	P	N	May-Jul.	Ch		Na	Ov	NE	A	P	A
Vitex negundo L.	USLA7	T	P	N	Mar.-Jun.	Ph	*N*	Mi	Ov	NE	P	P	P
Asterales Link													
Asteraceae Bercht. & J.Presl													
Ageratum conyzoides (L.) L.	USAT1	H	A	E	Nov.-Mar.	Th		Mi	Ov	LC	A	P	P
Ayapana triplinervis (Vahl) R.M.King & H.Rob.	USAT2	H	A	N	Sep.-Feb.	Th		No	La	NE	A	P	A
Blumea lacera (Burm.f.) DC.	USAT3	H	A	E	Aug.-Feb.	Th		Mi	La	NE	A	P	P
Chromolaena odorata (L.) R.M.King & H.Rob.	USAT4	S	A	E	Mar.-Sep.	Ch		Mi	Ov	NE	A	P	P
Cyanthillium albicans (DC.) H.Rob.	USAT5	H	A	N	Aug.-Mar.	Th		Mi	Li	NE	A	P	P
Eclipta prostrata (L.) L.	USAT6	H	A	E	All	Th		Mi	La	LC	A	P	P
Elephantopus scaber L.	USAT7	H	A	N	Sep.-Jan.	Th		No	Oo	NE	A	P	A
Enydra fluctuans Lour.	USAT8	H	A	N	Dec.-Mar.	Th		Mi	La	LC	A	P	P
Grangea maderaspatana (L.) Poir.	USAT9	H	A	E	Dec.-May	Th		Le	Sp	LC	A	P	P
Sonchus oleraceus L.	USAT10	H	A	N	Sep.-Jan.	Th		Na	Ha	NE	A	P	P
Sphaeranthus senegalensis DC.	USAT11	H	A	E	Nov.-Apr.	Th		Le	Ov	LC	A	P	P
Synedrella nodiflora (L.) Gaertn.	USAT12	H	A	E	Sep.-Jan.	Th		No	Ov	NE	A	P	P
Tridax procumbens L.	USAT13	H	A	E	All	Th		Na	Sg	NE	A	P	A
Xanthium strumarium L.	USAT14	H	A	E	Sep.-Apr.	Th		Me	Sg	NE	A	P	A
Apiales Nakai													
Apiaceae Lindl.													
Centella asiatica (L.) Urb.	USAP1	H	A	N	Jul.-Jan.	Th		No	Re	LC	A	P	A

Note**:** *Habit*: C: Climber, H: Herb, S: Shrub, T: Tree. *Life: Span*: A: Annual, B: Biennial, P: Perennial. *Nativity*: E: Exotic, N: Native. *Flowering and Fruiting time*: Jan.: January, Feb.: February, Mar.: March, Apr.: April, Jun.: June, Jul.: July, Aug.: August, Sep.: September, Oct.: October, Nov.: November, Dec.: December, All: All season. *Raunkiaer's Life: form and Sub: type*: Ch: Chamaephytes, Cr: Cryptophytes, H: Hemicryptophytes, M: Mesophanerophyte, MM: Megaphanerophytes, N: Nanophanerophytes, Ph: Phanerophytes, T: Therophytes. *Leaf spectra*: Le: Leptophyll, Na: Nanophyll, Mi: Microphyll, No: notophyll, Me: Mesophyll, Ma: Macrophyll, Mg: Megaphyll. *IUCN Status*: EN: Endangered, LC: Least Concern, NE : Not Evaluated, NT: Nearly Threatened, VU: Vulnerable. *Leaf Lamina*: Ac: Acicular, Co: Cordate, Cu: Cuneate, Ha: Hastate, La: Lanceolate, Li: Linear, Lu: Lunate, Ob: Oblong, Oo: Obovate, Or: Orbicular, Ov: Ovate, Pa: Palm like, Re: Reniform, Sb: Sabulate, Sg: Sagitate, Sp: Spathulate, Su: Subulate. *Seasons*: A: Absent, P: Present

ASIAN JOURNAL OF FORESTRY
Volume 5, Number 2, December 2021 E-ISSN: 2580-2844
Pages: 101-110 DOI: 10.13057/asianjfor/r050206

Orchids diversity on six ecosystem types in Wasur National Park, Merauke, Papua, Indonesia

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Manuscript received: 24 June 2021. Revision accepted: 19 August 2021.

Abstract. *Kusumastuti NK, Suratman, Pitoyo A. 2021. Orchids diversity on six forest types in Wasur National Park, Merauke, Papua, Indonesia. Asian J For 5: 101-110.* Orchidaceae is one of the two largest families of flowering plants. Wasur National Park, Papua, Indonesia is an important habitat of many orchid species. This research aimed to explore the diversity of orchid species in *Seksi Pengelolaan Taman Nasional Wilayah* III (SPTN III) Wasur, Wasur National Park, Merauke District, Papua Province, Indonesia and to investigate the distribution of orchid species in six different types of ecosystem. This research was conducted using exploratory method. A total of 25 orchid species belonging to 11 genera were found in the studied area. Monsoon forest was the ecosystem type with the highest number of orchid species with 15 orchid species, followed by savanna with ten species, *Melaleuca* forest with ten species, woodland forest with nine species, riparian forest with six species, and *Melaleuca-Eucalyptus* forest with 5 species. *Dendrobium smillieae* and *Dendrobium rigidum* were the most widely distributed epiphytic orchid species, and occurred in almost all ecosystem types. Two terrestrial orchids, *Geodorum densiflorum* and *Apostasia wallichii*, were only found in the monsoon forest.

Keywords: exploratory method, orchids, SPTN III Wasur, Wasur National Park

INTRODUCTION

Indonesia is one of the three mega-biodiversity countries and has the largest area of tropical forest after Brazil and Congo (Turubanova et al. 2018). Indonesia is estimated to have 20,000 species of flowering plants (Kusmana and Hikmat 2015). The family Orchidaceae is one of the two largest families of flowering plants in number of species, and includes 736 genera in five subfamilies (Chase 2015). One of the islands in Indonesia that has a high diversity of orchids is New Guinea. Most of the orchids in this area are wild orchid species with some of them are endemic to New Guinea. According to Cámara-Leret et al. (2020), New Guinea has 2,856 species of orchids.

Merauke District is one of the districts in Papua (Indonesian New Guinea) with a large potential for orchid diversity. Pammai et al. (2014) who surveyed 11 sub-districts in Merauke District (Merauke, Naunkenjerai, Sota, Elikobel, Jagebob, Okaba, Muting, Animha, Kimaam, Ilwayab, and Kaptel) found a total of 41 species of orchids that belong to 8 genera. Another study by Arobaya et al. (2020) in Wasur National Park found a total of 11 species recorded from *Dendrobium* section *Spathulata* alone. Wasur National Park is an important orchid habitats in Merauke District since the environment of the national park is still preserved. The diversity of vegetation in Wasur National Park is spread over several ecosystems, including *Melaleuca* forest, co-dominant *Melaleuca-Eucalyptus* forest, woodland forest, coastal forest, monsoon forest, riparian forest, mangrove forest, savanna, grassland, swampy grassland, *Eucalyptus* forest, lowland forest, and *Exoecaria* forest (Wasur National Park 2014). Wasur National Park, especially the *Seksi Pengelolaan Taman Nasional Wilayah* III (SPTN III), is one of the areas that is less explored, particularly regarding the diversity of orchid species. This research aimed to investigate orchid species diversity in SPTN III Wasur, Wasur National Park, Merauke District, Papua Province, Indonesia and to study the distribution of orchid species in six different ecosystem types.

MATERIALS AND METHODS

Study period and area

This research was conducted during July-August 2019 in the SPTN III Wasur, Wasur National Park, Merauke District, Papua Province, Indonesia (Figure 1). Six ecosystem types were observed, namely (i) *Melaleuca-Eucalyptus* forest, (ii) *Melaleuca* forest, (iii) woodland forest, (iv) monsoon forest, (v) riparian forest and (vi) savanna (Figure 1).

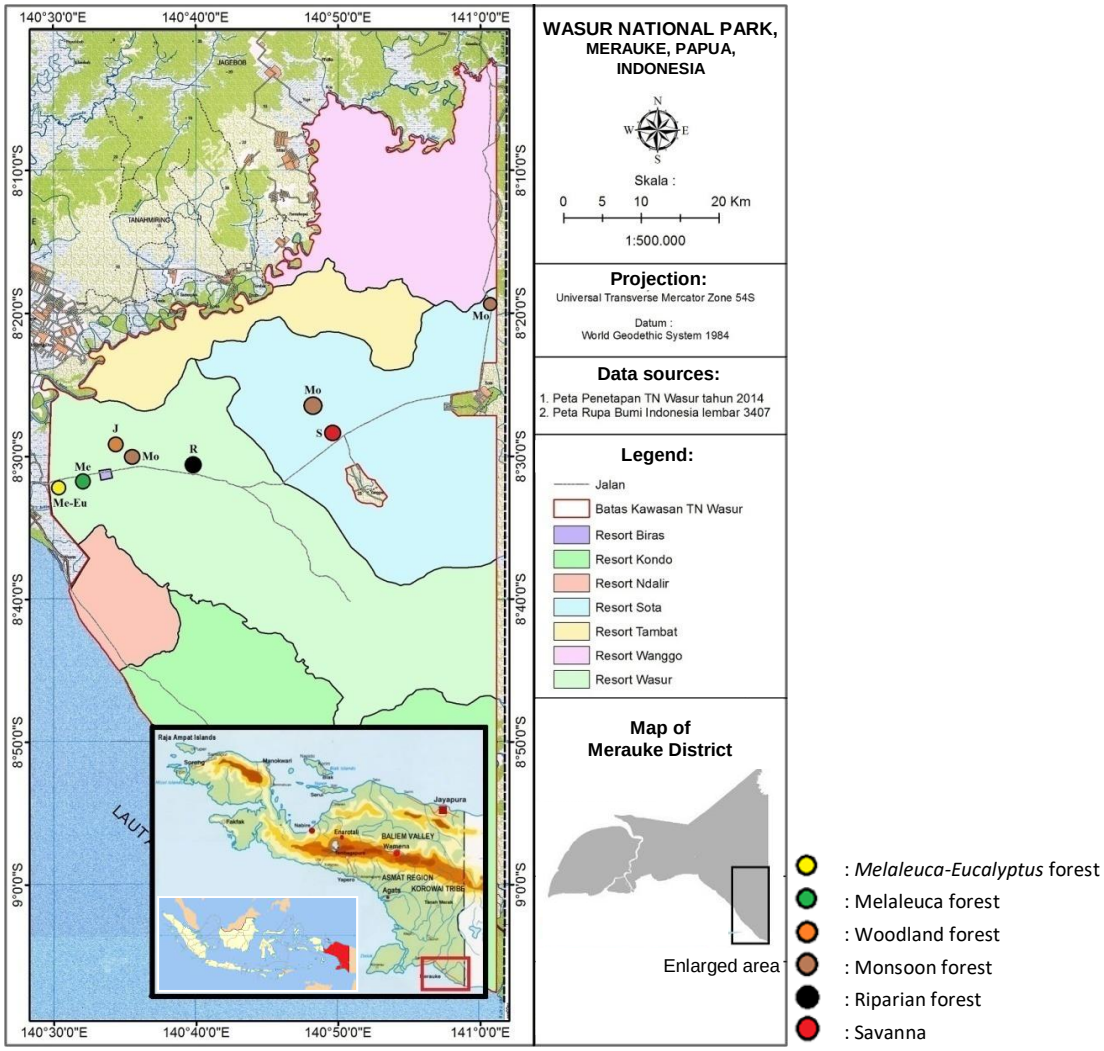

Figure 1. Map of research locations in the SPTN III Wasur, Wasur National Park, Merauke District, Papua Province, Indonesia (Wasur National Park 2014)

Procedures

The research was carried out by exploratory methods by exploring accessible forest areas in the six ecosystem types. The orchid inventory and the making of herbarium specimens were carried out by following the Balgooy method (1987). Orchid collections were preserved in Copenhagen mix (ethanol 70% and 5% glycerol). Data recorded include collection number, date, name(s) of collector(s), location (latitude and longitude), habitat and ecology information, morphological features that will be lost when the specimen is preserved (the colour of the orchid stems, leaves and flowers), local names and the uses (Balgooy, 1987).

Epiphytic orchids were observed based on the distribution of orchids in host tree zoning following the Johansson method (1974) which divides the host tree into five zones. Identification of orchids followed determination keys and some specific scientific papers (Minderhpud and de Vogel, 1986; Watthana 2007; Chowlu and Rao 2015; de Vogel, 1988), using the characters described in Millar (1990) and Schuiteman (2013), and asking Wasur National Park staff and local communities.

At each location, abiotic factors were measured following the Guinness and Walpole (2012) method, including elevation (m asl) and geographical location, light intensity (lux), air temperature (◦C), soil temperature (◦C), humidity (%), soil humidity (%) and soil pH. These abiotic factors were measured three times and then averaged.

Data analysis

The orchid species found and environmental data (biotic and abiotic factors) were analyzed descriptively.

RESULTS AND DISCUSSION

Based on the results of our field exploration, orchids (family Orchidaceae) in *SPTN III* Wasur, Wasur NP, comprised 25 species belonging to 11 genera (Table 1).

Orchids distribution across ecosystem types

Based on the results of field exploration, the diversity of orchid species varied in each forest type. Monsoon forest was the type of forest with the highest number of orchid species with 15 species, followed by savanna with 10 species, *Melaleuca* forest with 10 species, woodland forest with 9 species, riparian forest with 6 species, and *Melaleuca-Eucalyptus* forest with 5 species (Table 1). *Dendrobium smillieae* and *Dendrobium rigidum* were the most widely distributed epiphytic orchid species, found in both open and shady habitats where light intensity can vary from low light intensity to full sunlight.

The differences in orchid species found in each ecosystem type are influenced by several factors including forest structure, tree species composition, and micro-climate such as light intensity, temperature, humidity, and proximity to water sources. Tree species composition affects the epiphytic vegetation through substrate characteristics provided by each tree species, giving rise to epiphytic host specificity (Frei, 1973; Johansson, 1974; Dressler, 1981). Other substrate factors include texture (roughness) and bark porosity (water retention).

Table 1. Orchids distribution and host tree in each forest type in the SPTN III Wasur, Wasur National Park, Merauke District, Papua Province, Indonesia

No.	Species	Host Tree	Mo	S	Me	W	R	Me-Eu
1	*Acriopsis liliifolia* (J.Koenig) Ormerod	*Melaleuca* sp.	+	+	+		+	
2	*Apostasia wallichii* R.Br.	-	+					
3	*Bulbophyllum baileyi* F.Muell.	*Syzygium* sp.	+					
4	*Dendrobium antennatum* Lindl.	*Melaleuca* sp.			+	+		+
5	*Dendrobium canaliculatum* R.Br.	*Melaleuca* sp.		+	+			
6	*Dendrobium carronii* Lavarack & P.J.Cribb	*Melaleuca* sp. *Xanthostemon crenulatus* C.T. White		+	+	+		
7	*Dendrobium discolor* Lindl.	*Alstonia scholaris* (L.) R.Br. *Bombax ceiba* L *Melaleuca* sp.	+		+			+
8	*Dendrobium glabrum* J.J.Sm.	*Dillenia alata* (D.C) Martelli *Eucalyptus pellita* F. Muell.		+		+		
9	*Dendrobium goldfinchii* F.Muell.	*Alstonia scholaris* (L.) R.Br. *Barringtonia acutangula* (L.) Gaertn.	+			+	+	
10	*Dendrobium insigne* (Blume) Rchb.f. ex Miq.	*Barringtonia acutangula* (L.) Gaertn.					+	
11	*Dendrobium johannis* Rchb.f.	*Dillenia alata* (D.C) Martelli *Eucalyptus pellita* F. Muell. *Melaleuca* sp.		+	+	+		+
12	*Dendrobium lacteum* Kraenzl.	*Buchannia* sp.	+					
13	*Dendrobium pruinosum* Teijsm. & Binn.	*Barringtonia acutangula* (L.) Gaertn.	+					
14	*Dendrobium rigidum* R.Br.	*Barringtonia acutangula* (L.) Gaertn. *Melaleuca* sp. *Planchonia* sp. *Xanthostemon paradoxus* F. Muell.		+	+	+	+	+
15	*Dendrobium smillieae* F.Muell.	*Barringtonia acutangula* (L.) Gaertn. *Eucalyptus pellita* F. Muell. *Maranthes corymbosa* Bl. *Melaleuca* sp. *Xanthostemon paradoxus* F. Muell.	+	+	+	+	+	
16	*Dendrobium trilamellatum* J.J.Sm.	*Eucalyptus pellita* F. Muell. *Melaleuca* sp.		+				+
17	*Dendrobium* sp.	*Maranthes corymbosa* Bl.	+					
18	*Geodorum densiflorum* (Lam.) Schltr.	-	+					
19	*Luisia tristis* (G.Forst.) Hook.f.	*Melaleuca* sp.		+	+	+		
20	*Oberonia* sp.	*Decaspermum* sp.	+					
21	*Pholidota imbricata* Hook.	*Barringtonia acutangula* (L.) Gaertn.	+				+	
22	*Pinalia fitzalanii* (F.Muell.) Kuntze	*Melaleuca* sp. *Xanthostemon paradoxus* F. Muell.		+	+	+		
23	*Pomatocalpa macphersonii* (F.Muell.) T.E.Hunt	*Endiandra* sp.	+					
24	*Pomatocalpa marsupiale* (Kraenzl.) J.J.Sm.	*Syzygium* sp.	+					
25	*Thrixspermum platystachys* (F.M.Bailey) Schltr.	*Decaspermum* sp.	+					

Note: Mo: Monsoon forest, S: Savanna, Me: Melaleuca forest, W: Woodland forest, R: Riparian forest, Me-Eu: *Melaleuca-Eucalyptus* forest

One important factor that also affects the distribution and abundance of terrestrial orchids is soil pH. In this study, terrestrial orchids were found in soil pH 6-6.5. The pH gradient can be considered a resource gradient since soil reactions control mineral uptake either directly or through mycorrhizal associations. However, in highly

alkaline soils as well as in highly acidic soils, mycorrhizae cannot survive, which can lead to a reduction in the number of orchids (Dijk et al. 1997). Most orchids can adapt to a broad range of light environments in different habitats (Zhang et al. 2018). In this study, several orchids, such as *Dendrobium discolor*, *D. carronii*, *D. canaliculatum*, and *D. glabrum*, were found growing in the tree canopy as an epiphyte tolerant of high light intensity than terrestrial orchids i.e. *Apostasia wallichii* and *Geodorum densiflorum* found on shady forest floors.

Habitat and ecology of orchids in SPTN III Wasur, Wasur NP

Orchids grew at an elevation of 7.8-26.7 m above sea level in SPTN III Wasur, with a varied range of habitats and vegetation. In our field exploration, orchids were collected from 6 different ecosystems types in SPTN III Wasur, namely monsoon forest, savanna, *Melaleuca* forest, woodland forest, riparian forest, and *Melaleuca-Eucalyptus* forest (Figure 2.A-F)..

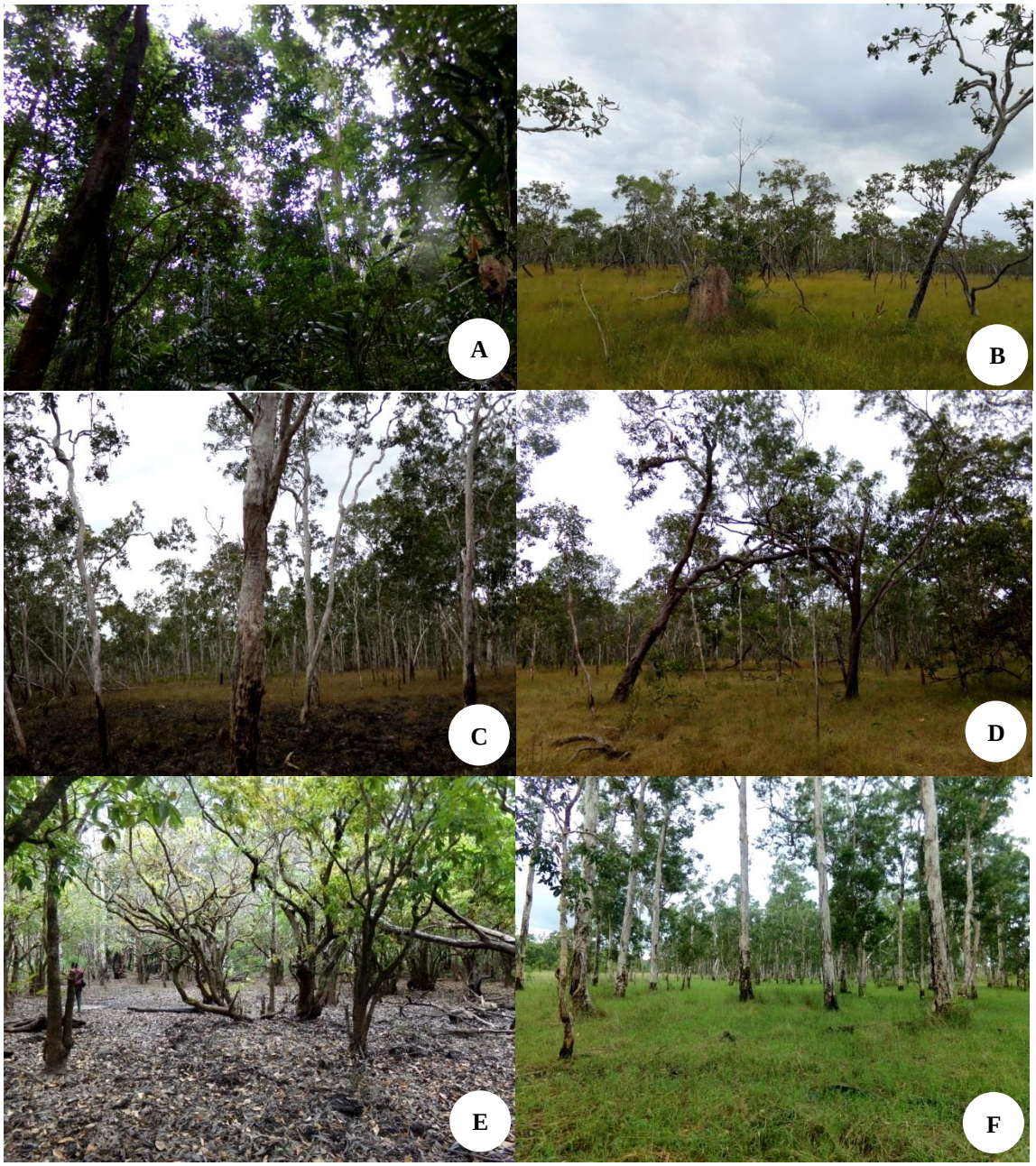

Figure 2. Six ecosystem types in SPTN III Wasur, Wasur NP, Merauke, Indonesia. A. Monsoon forest, B. Savanna, C. *Melaleuca* forest, D. Woodland forest, E. Riparian forest, F. *Melaleuca-Eucalyptus* forest

Monsoon forest

Monsoon forest is a forest type in tropical and subtropical regions with warm climate throughout the year. However, it has a longer dry season for several months than the rainy season. The trees in the monsoon forest usually shed their leaves during the dry season. Lianas (woody vines) and herbaceous epiphytes (such as orchids) are found in this forest. Monsoon forest in this study, which

is located in Yanggandur and Sota at an elevation between 12-27.2 m asl with an average air humidity of 82.6% and an average temperature of 27ºC, was dominated by species like *Eucalyptus* sp., *Acacia auriculiformis, Acacia mangium, Banksia dentata, Rhodomyrtus* sp. and others (Wasur National Park 2014). Trees such as *Syzygium* sp., *Buchanania* sp., *Maranthes corymbosa, Alstonia scholaris, Bombax ceiba, Barringtonia acutangula, Terminalia* sp., *Endiandra* sp. and *Decaspermum* sp. are common hosts of epiphytic orchids in this type of forest.

Monsoon forest was the ecosystem type with the highest number of orchid species with 15. Both epiphytic and terrestrial orchids were found in this habitat. This forest had a good environment for orchid survival because its habitat conditions (moisture and nutrient availability) were suitable for the growth of orchids. The dense forest conditions with high canopy cover mean that there was not too much sunlight in this habitat, therefore many orchid species preferred shade or not to be exposed to direct sunlight. In addition, the presence of diverse and healthy host trees provided good support for epiphytic orchids. Forest floors that have much lower light reception conditions, higher air humidity and soil moisture, and lower wind influence than canopy areas can support the development of terrestrial orchids that are compatible with these conditions (Kromer and Gradstein 2007).

Savanna

Savannas are grasslands interspersed with several types of trees that grow sparsely. The savanna was located in Yanggandur at an elevation of ± 14.5 m above sea level with an average air humidity of 55.7% and an average temperature of 31.7ºC. It was dominated by species like *Melaleuca cajuputi, Banksia dentata, Asteromyrtus symphyocarpa, Eucalyptus* sp. and *Melaleuca* sp. (Wasur National Park 2014). In addition, trees such as *Melaleuca* sp., *Planchonia* sp., *Timonius timon, Eucalyptus pellita* and *Dillenia alata* were common hosts of epiphytic orchids in this habitat.

Savannas and *Melaleuca* forests had the second highest number of species after monsoon forests, i.e. ten species. Dry savanna conditions with open canopies mean that only a few species of orchids can grow in these environments with high sunlight intensity. In this habitat, many epiphytic orchids have effective adaptations to drought and high levels of sunlight. One of the special adaptations of epiphytic orchids to drought conditions is to have pseudobulbs, making them more tolerant of the microclimate such as drought (Werner and Gradstein 2009). In this study, terrestrial orchids were not found in savanna, possibly because terrestrial orchids are prevented from growing into the savanna by fire. According to Eden (1974), in parts of southern Papua there is clear evidence of anthropogenic disturbance of the vegetation, principally due to shifting cultivation and burning. Burning occurs regularly in the savanna and grassland during the dry season. However, it cannot be assumed that the savanna and grassland are the result of human activity.

Melaleuca forest

Melaleuca forest was located at an elevation of ± 16 m above sea level with an average air humidity of 50% and an average temperature of 32.6ºC. Species like *Melaleuca* sp dominate it., *Lophostemon lactifluus, Xanthostemon* sp., *Acacia leptocarpa, Asteromyrtus symphyocarpa, Eucalyptus* sp. and others (Wasur National Park 2014). *Melaleuca* sp. were the common hosts of epiphytic orchids in this habitat.

The ecology of the *Melaleuca* forest was similar to the ecology of the savanna, but the canopy cover was slightly more closed than in the savanna. In addition, what distinguishes these two habitats was that the host trees in the savanna were more diverse than the host trees in the *Melaleuca* forest, which were all *Melaleuca* sp. As many as 8 out of 10 species of orchids found in these two habitats shared the same species. The high number of epiphytic orchid species was not only due to the microclimate that supports it, but also because of the host tree factor. Most of the host trees in both habitats were trees with broken or cracked bark. In the research of Hernández-Pérez et al. (2018), the highest epiphyte richness was also recorded in trees with cracked or broken bark. Other studies have also shown that trees with broken bark can increase water absorption and increase the rate of epiphyte formation, because these characteristics prevent seeds of epiphytes from easily separating from the host (Tupac-Otero et al. 2007; Cascante-Marín et al. 2009; Wagner et al. 2015). In contrast, hosts with smooth and peeled bark have low levels of epiphyte richness, which may be related to increased seed mortality and limited formation of epiphytic plant groups due to unstable substrates (Zimmerman and Olmsted, 1992; López-Villalobos et al. 2008; Woods et al. 2015).

Woodland forest

Woodland forest is a low-density forest that forms open habitats with lots of sun and limited shade. It has sparse tree cover (10-30%) and has undergrowth and herbaceous vegetation including grasses. The forest was located at an elevation of ± 12 m above sea level with an average air humidity of 75.5% and an average temperature of 28.6ºC. It was dominated by species like *Vitex pinnata, Melaleuca* sp., *Xanthostemon* sp., *Trichospermum* sp., *Dillenia alata, Eucalyptus* sp., *Asteromyrtus symphyocarpa* and at the bottom grow various shrubs (Wasur National Park 2014). Trees such as *Melaleuca* sp., *Xanthostemon paradoxus*, and *Xanthostemon crenulatus* were common hosts of epiphytic orchids in woodland forests. Woodland forest conditions were similar to savanna, but the canopy cover was more closed and had more diverse host trees compared to savanna and *Melaleuca* forest.

Woodland forest was the ecosystem type with the third-highest number of species after savanna and *Melaleuca* forest. *Dendrobium smillieae* was found more frequently in this forest than in other forest types. Besides being found as an epiphyte, *D. smillieae* was sometimes also found as a lithophyte in mossy or littered rocks. The high number of *D. smillieae* in the woodland forest was likely because the orchid had more diverse host trees than in the savanna and

Melaleuca forests. The host of *D. smillieae* in savanna was recorded only as *Eucalyptus pellita*. The host in *Melaleuca* forest was only *Melaleuca* sp. The host of this orchid in the woodland forest was *Melaleuca* sp. and *Xanthostemon paradoxus. X. paradoxus* is a tree commonly found in woodland forests, therefore the orchids that are suitable on this host will grow well and also more abundant, such as *D. smillieae*.

Riparian forest

Riparian forests located on the edge of swamp waters, lakes, water sources, or rivers. This type of forest has a unique character, due to the combination of the aquatic and terrestrial environment i.e. the presence of plants that can adapt to the waters. The forest was located at an elevation of ±19 m above sea level with an average air humidity of 70% and an average temperature of 30ºC. It was dominated by species like *Barringtonia acutangula, Trichospermum* sp., *Bamboo* sp., *Nypa fruticans* and *Graminae* spp. (Wasur National Park 2014). *B. acutangula* is a common host tree for epiphytic orchids in this habitat.

Riparian forests were the habitat with the second lowest number of species. In this type of habitat, only six species of orchids can be found. The condition of riparian forest with high canopy cover and high humidity causes many shade-loving species to grow well in this habitat. Although the environmental conditions of the riparian forest are favorable for the growth of shade-loving orchid species, only the host tree *Barringtonia acutangula* was found at the study site. The slight variation in host trees at the study site may have resulted in the low number of orchid species found.

Melaleuca-Eucalyptus forest

Melaleuca-Eucalyptus forest is a forest type dominated by *Melaleuca* and *Eucalyptus* species. The forest was located at an elevation of ± 10 m asl with an average humidity of 70% and an average temperature of 30.6ºC. It was dominated by *Melaleuca cajuputi, Eucalyptus alba, Eucalyptus pellita, Eucalyptus* sp., *Asteromyrtus symphiocarpa, Rhodomyrtus* sp. and others (Wasur National Park 2014). *Melaleuca* sp. was the common host tree for orchids in the *Melaleuca-Eucalyptus* forest.

Melaleuca-Eucalyptus forest was the habitat with the lowest number of orchid species among six ecosystem types, i.e. only 5 species. The condition of this forest was similar to the conditions in the *Melaleuca* forest. The host trees found were also the same as those recorded in the *Melaleuca* forest, specifically *Melaleuca* trees. However, humidity in the *Melaleuca-Eucalyptus* forest was higher than in the *Melaleuca* forest. The species of orchid commonly found in *Melaleuca-Eucalyptus* forests was *Dendrobium discolor*. The habitat conditions in this type of forest were almost identical to those in the *Melaleuca* forest. Nevertheless, fewer orchids were found. This is probably because the higher humidity in this habitat only allowed a few orchids with high light requirements and relatively high humidity requirements to thrive. It may also be caused by scattering the seeds of some orchid species that do not reach this habitat. In orchid reproduction, orchid seeds are blown by the wind. The seeds then attach to the trunk of a suitable host tree. Orchid seeds will germinate, grow, develop and regenerate continuously if the host tree is suitable, supported by temperature, humidity, and light intensity (Sadili 2013).

Host trees and vertical distribution of epiphytic orchids

This study found 16 host tree species of 23 epiphytic orchid species in all six different ecosystem types (Table 1). *Dendrobium smillieae* had the most diverse host range, as it was found growing on five host tree species, followed by *Dendrobium rigidum* which was found growing on four host tree species. It was possibly because these species can adjust, via morphological and physiological changes, to a wide range of environments. Meanwhile, 74% of the total recorded epiphytic orchids were found on less than 2 host tree species.

Of the 16 host tree species, *Melaleuca* sp. is the most common host of orchids, supporting up to 11 species of epiphytic orchids. Other important host tree species are *Xanthostemon paradoxus, Eucalyptus pellita, Barringtonia acutangula, Syzygium* sp. and *Maranthes corymbosa*, each supporting 2 to 7 species of epiphytic orchids. Although, in all six ecosystem types, the lowest number of epiphytic orchid species was found at the base of the host tree (Zone 1/Z1) and the outer part of the branching of the host tree (Zone 5/Z5), only one species of orchid was found growing in each of these two zones. Meanwhile, the highest number of orchid species was found at Z2, Z3 and Z4 (Figure 3).

The large number of orchid species recorded in the middle part of the tree (Z2, Z3 and Z4) can be caused by the fact that the area is characterized by relatively stable environmental conditions (humidity, light intensity and temperature). In contrast, the small number of orchid species in Z1 and Z5 was caused by the zone being the most shaded (Z1) and most open (Z5) part of the tree. This can be related to the intensity of sunlight, temperature and humidity. The lower part of the tree will be more humid than the outermost part, while the outer and most exposed parts of the tree are usually the driest. Light intensity and temperature will change at different tree heights (Hernández-Pérez et al. 2018). Some species of orchids can only tolerate specific ranges of light intensity, temperature and humidity.

Zone 1 is the part that receives less sunlight because it is shaded by the canopy of the host tree and other surrounding vegetation. The only orchid found growing in this zone is *Pomatocalpa marsupiale*. Whereas zone 5 is the part that receives more direct sunlight, causing the evapotranspiration rate to be very high (Marsusi et al. 2001), the only orchid that was found to be able to adapt to this zone is *Dendrobium johannis*.

The study indicates that 91.3% of epiphytic orchid species grew in Zone 3 of the host tree. These results are similar to those obtained by other researchers in that the number of epiphytic orchid species is primarily found in Zone 3 (Marsusi et al. 2001; Managanta and Pangli 2014; Nurfadilah 2015). This is because Zone 3 has the largest branch size and the smallest degree of inclination compared to Zones 1 and 2, which are tilted 90º or perpendicular

(Marsusi et al. 2001). Whereas in Zone 5 the air humidity is lower coupled with high temperatures and strong winds, so that epiphytes are rarely found in this zone (Kromer and Gradstein 2007).

Dendrobium is a genus that was common in SPTN III Wasur, Wasur NP. This is because *Dendrobium* can grow in various types of environments such as high and mountainous areas, at moderate temperatures, in humid and foggy environments, in lowlands and in environments with high temperatures. *Dendrobium* is widespread in Asia, Australia and Europe, India, Sri Lanka, China, Japan, Korea, New Guinea, the Pacific Islands and New Caledonia. *Dendrobium* is the second largest genus of orchids in the world with about 1500 species. About 614 species from this genus have been recorded in New Guinea, making *Dendrobium* also the second largest orchid genus in New Guinea after *Bulbophyllum* (Cámara-Leret et al. 2020). *Bulbophyllum* is the largest orchid genus in New Guinea with 658 species (Cámara-Leret et al. 2020). However, only one species of *Bulbophyllum* was found in this study, possibly because of using a purposive sampling method by exploring accessible forest areas. More *Bulbophyllum* species may be found in unreached areas and in other ecosystem types that have not been studied. Some species of orchids of *SPTN III* Wasur are presented in Figure 4.

Taxonomy treatment

Acriopsis liliifolia (J. Koenig) Ormerod., Opera Bot. 124 (1995) 58

Epiphytic sympodial. Pseudobulbs ovate, 2.2-5 × 0.5-2.2 cm, glossy apple-green, 2-4 leaved. Leaves narrowly oblong, 10-18 × 1-1.5 cm, apex acute, green. Flowers 0.8-1.4 cm long, greenish-white to yellowish-white, with purple spots at the tips of sepals and petals. Lip 3-lobed.

Apostasia wallichii R.Br. in Wall., Pl. Asiat. Rar. 1 (1830) 75, pl. 84

Terrestrial. Leaves linear-lanceolate, 4-14 × 0.5-0.7 cm, apex acute. Inflorescences pendant, sometimes erect with 1-6 branches, 2-5 flowers on each branch, appearing at the ends of the stems. Flowers 1-2 × 2 cm long, pale yellow. Sepals oblong-lanceolate, 4-6.5 × 6-1.7 mm. Petals and lip similar to each other, 3.7-6 × 0.5-1.5 mm, the lip sometimes broader and more convex. Two fertile anthers with an unequally bilobed tip.

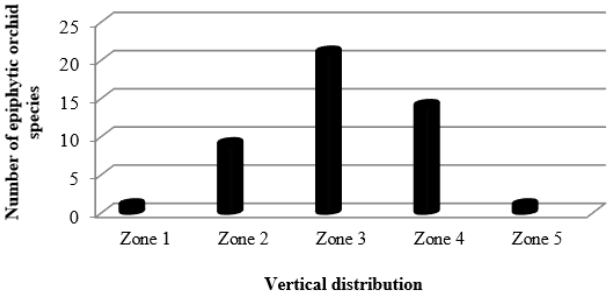

Figure 3. Vertical distribution and number of epiphytic orchid species on host trees

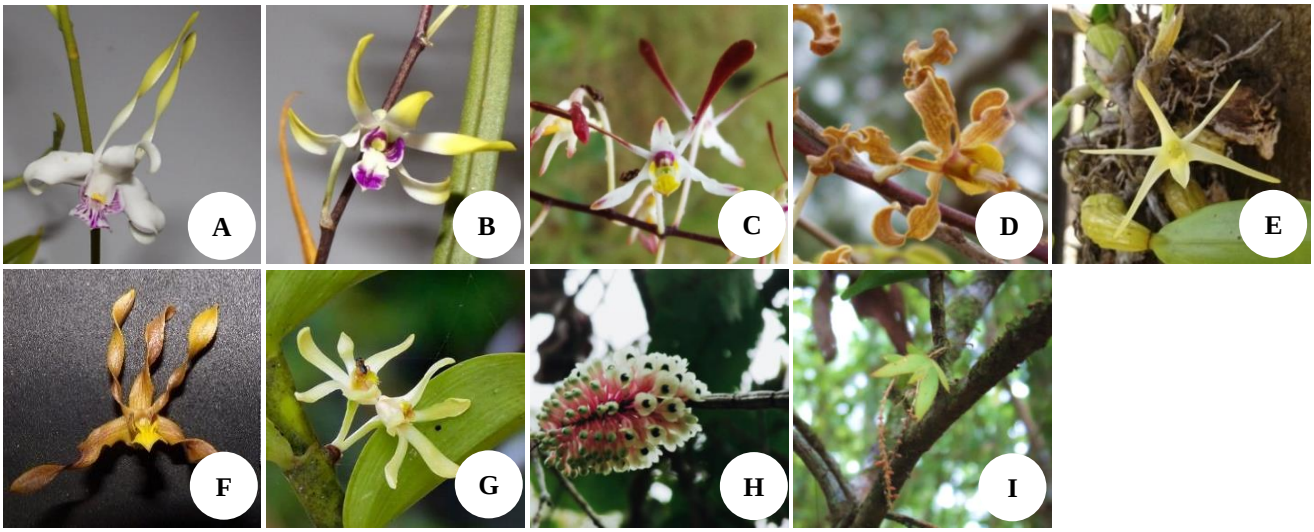

Figure 4. Several species of orchids found in SPTN III Wasur, Merauke, Indonesia. A. *Dendrobium antennatum* Lindl., B. *Dendrobium canaliculatum* R.Br., C. *Dendrobium carronii* Lavarack & P.J.Cribb., D. *Dendrobium discolor* Lindl., E. *Dendrobium glabrum* J.J.Sm., F. *Dendrobium johannis* Rchb.f., G. *Dendrobium pruinosum* Teijsm. & Binn., H. *Dendrobium smillieae* F.Muell., I. *Oberonia* sp.

Bulbophyllum baileyi F. Muell., Fragm. 9 (1875) 5

Epiphytic sympodial. Pseudobulbs ovate, 1.5-3 × 0.3-1.5 cm. Leaves obovate, 5.5-24 × 1.6-5 cm, apex rounded. Flowers mostly moderately opening. Dorsal sepal elliptic to ovate-triangular, 15-24 × 4-7.5 mm, apex acute, curved downwards at the top. Lateral sepals ovate triangular, 13-24 × 6-8.5 mm, apex acute. Petals elliptic, 13-20 × 3-4.5

mm, shortly acuminate, curved at the top. Flowers cream to yellowish-green with red spots.

Dendrobium antennatum Lindl., London J. Bot. 2 (1843) 236

Epiphytic sympodial. Stem fleshy, erect, 15-75 × 1-1.5 cm. Leaves distichous, thick-fleshy, oblong-lanceolate to ovate-elliptic, 4-7 × 0.5-3 cm, apex acuminate. Flowers fragrant, ±6 cm diameter, white with green or greenish-yellow petal tip. Dorsal sepal oblong-lanceolate, 1.6-2.3 × 0.6-0.7 cm, apex acute, twisted. Lateral sepal, oblong-lanceolate, 1.6-2.5 × 0.7 cm, apex acute, twisted. Petal linear, 2.5-5 × 0.2-0.3 cm, acute, spirally twisted. Lip 3-lobed, purple or violet.

Dendrobium canaliculatum R.Br., Prodr. (1810) 333.

Epiphytic sympodial. Pseudobulbs fusiform to ovoid, 3-12 × 1.5-3 cm, 2-6 leaved. Leaves flattened cylindrical, 10-17 × 0.5-1 cm, apex acute. Flowers small, ±3 cm diameter. Sepals and petals white with yellow tips. Lip three-lobed, 1.5 × 0.9 cm, white. Midlobe ovate, callus of 3 ridges, apex acute with purple spot, recurved. Side-lobes obovate, apex rounded with purple spot.

Dendrobium carronii Lavarack & P.J. Cribb., Austrobaileya 1 (1982 publ. 1983) 497.

Epiphytic orchid. Pseudobulbs fusiform to rounded, 1.5-4 × 1-2 cm, 2-4 leaved. Leaves terete, 7-11 × 0.5-1 cm, apex acute. Inflorescences ± 10 cm long, rachis purple-brown, producing ± 8 flowers. Sepals triangular, 0.5-1 × 0.3-0.5 cm, apex acute, white with purple stripes. Petals oblanceolate, 1.8-2 × 0.2-0.3 cm, apex acute, dark brown, maroon to dark purple with white underside. Lip 3-lobed, 1-1.4 × 0.8 cm, callus of 3 ridges, bright yellow color.

Dendrobium discolor Lindl., Edwards's Bot. Reg. (1841) t. 52 Misc. 21.

Epiphytic sympodial. Pseudobulb up to 2.5 m long, with cane-like stems. Leaves distichous, elliptic or ovate-elliptic, fleshy, 5-10 × 2.5-5 cm, apex obtuse-bilobed. Flowers with convoluted and crisped segments, brownish-yellow. Dorsal sepal oblong, 1.5-3 × 0.3-0.5 cm, apex obtuse, spirally twisted. Lateral sepals linear, 2.5-4 × 0.8-1.2 cm, apex acute, spirally twisted. Petals oblanceolate, margin undulate, 2-5 × 0.4-0.8 cm, apex obtuse. Lips 3-lobed, 1-2 cm × 0.3-1.5 cm, with whitish callus.

Dendrobium glabrum J.J.Sm., Bull. Dép. Agric. Indes Néerl. 5 (1907) 4; Nova Guinea 8, 1 (1909) 56, t. 20, fig. 64.

Epiphytic sympodial, creeping. Pseudobulbs ellipsoid, 3 × 1 cm, 1-leaved, yellowish-green. Leaves erect, lanceolate, 5.5 × 1.5 cm, apex acute, stiff. Inflorescence 1-flowered, emerging from a pseudobulb. Flowers ± 3.5 cm diameter, yellowish-white to pale yellow. Dorsal sepal lanceolate-linear, 1 × 0.1-0.15 cm, apex acute. Lateral sepals lanceolate- linear, 1.6 × 0.1-0.15 cm, apex acute. Petals lanceolate-linear, 1.6 × 0.1 cm, apex acute to acuminate. Lips 3- lobed, ± 8 mm.

Dendrobium goldfinchii F. Muell., S. Sci. Rec. 3 (1883) 4.

Epiphytic sympodial. Stems upwards almost flat. Leaves alternate, distichous, lanceolate-linear, bilaterally flattened, fleshy, 3-7.5 × 0.3 cm, apex acute. Flowers ± 0.9 cm in diameter, inflorescence terminal, glabrous, white or pale green with a faint yellow tinge and a pinkish-purple stripe. Stalklets at the base are closely surrounded by bracts, passing gradually into the narrow and short ovarigerous calyx-tube. Upper calyx-lobe ovate-lanceolate, inner lobes nearly as long as the other lanceolate. Lip rounded, yellowish-white.

Dendrobium insigne (Blume) Rchb.f. ex Miq., Fl. Ned. Indië 3 (1859) 640.

Epiphytic orchids. Stems slender, erect, ±45 cm long. Leaves alternate, ovate-oblong, 5-6.5 × 2-2.5 cm, fleshy, somewhat stiff, apex unequally bidentate. Inflorescences lateral, 2-flowered pair. Flowers ±3 cm diameter, pale yellow to cream-yellow with a light brown pattern. Dorsal sepal lanceolate, 2 × 0.53 cm, apex acute. Lateral sepals oblong-triangular, falcate, 1.3-1.8 × 0.95 cm, apex obtuse. Petals lanceolate, 1.65 × 0.4 cm, apex acute. Lip 3-lobed, 1.6 × 0.6 cm, apex acute, whitish-yellow with pale brown to red spots.

Dendrobium johannis Rchb.f., Gard. Chron. (1865) 890.

Epiphytic sympodial. Stems fusiform, 8-35 × 0.7-1.2 cm, 7 leaved. Leaves lanceolate, 6-11 × 1 cm, thick, fleshy, apex acute. Flowers ±3.5 cm diameter, brownish-yellow. Dorsal sepal oblong-lanceolate, 2.5 × 0.5 cm, apex acute, twisted. Lateral sepals oblong-lanceolate, 2.5 × 0.5 cm, apex acute, twisted. Petals oblong-lanceolate, 2.5 × 0.5 cm, apex acute, twisted. Lip 3-lobed, 1.5 × 0.5 cm, callus of 3 ridges, bright yellow.

Dendrobium lacteum Kraenzl., Österr. Bot. Zeitschr. 44 (1894) 334.

Epiphytic sympodial. Stems slender, clavate, ± 17.5 cm tall, 4-angled, 2-leaved. Leaves lanceolate-ovate, 11-13 × 2-2.5 cm, thin, stiff, acute to acuminate. Inflorescences ephemeral, 2-3 flowered on each stem, appearing from the swollen upper internodes. Flowers ±5 cm across, white. Dorsal sepal ovate-lanceolate, 2.5-3.2 cm long, apex acuminate. Lateral sepal ovate-triangular, falcate, 2.5-3 cm long, apex acuminate. Petal lanceolate, 2.5-3 cm long, apex acuminate. Lips clasping the column, 1.2-1.5 × 1.9-2.2 cm, white inside with orange-red-brown or purple-brown stripes, callus fleshy, golden yellow with brown spots.

Dendrobium pruinosum Teijsm. & Binn., Natuurk. Tijdschr. Ned.-Indië 24 (1862) 314.

Epiphytic sympodial. Stems ±100 cm long. Leaves elliptic, apex unequally bilobulate. Inflorescences lateral, flowers born in pairs, opposite each other. Flowers ±5 cm across, cream colors. Dorsal sepal ligulate, 2.6 cm long, glabrous, apex apiculate. Lateral sepal ligulate, 2.6 cm long, apex apiculate. Petal ligulate, a little shorter than the sepals, apex apiculate. Lip 3-lobed, callus yellowish creamy.

Dendrobium rigidum R.Br., Prodr. (1810) 333.

Epiphytic sympodial, creeping. Stems ±1.5 cm long, 1-leaved. Leaves lanceolate, 1.3-4.5 × 0.7-1.1 cm, fleshy, stiff, apex acute. Inflorescences arising from the apex of the stems behind the leaves. Flowers ±1.5 cm across, yellowish cream with purplish spots outside. Dorsal sepal lanceolate-ovate, apex obtuse. Lateral sepals triangular, apex obtuse. Petals lanceolate, apex obtuse. Lip 3-lobed, apex acute, yellow with purplish spots.

Dendrobium smillieae F. Muell., Fragm. 6 (1867) 94.

Epiphytic sympodial, sometimes lithophytic. Stems fusiform, 19-35 × 1.5 cm long. Leaves lanceolate, 2-9 × 1-2.5 cm, thin, shiny, green, dark purple when young, apex acute. Inflorescences arising from leafless stems, pedicel and mentum pink to pale purple. Flowers ± 1.5-2.5 cm across, fleshy, sepals and petals greenish-white, apex green. Lip narrow, 1.5-2 cm, concave, fleshy, shiny dark green.

Dendrobium trilamellatum J.J.Sm., Bull. Dép. Agric. Indes Néerl. 19 (1908) 21; Nova Guinea 8, 1 (1909) 69, t. 24, fig. 76

Epiphytic sympodial. Stems fusiform, 35-54 × 1.5 cm. Leaves lanceolate, 2-17 × 0.8-4 cm, fleshy, apex acute. Flowers yellow to yellow-brown. Dorsal sepal lanceolate, 2-4 × 0.5 cm, apex acute, twisted. Lateral sepals lanceolate, 2-4 × 0.5 cm, apex acute, twisted. Petals lanceolate, 2-4 × 0.5 cm, apex acute, twisted. Lip 3-lobed, 2.3 × 1.5 cm, callus of 3 ridges, yellow with reddish-brown stripes.

Geodorum densiflorum (Lam.) Schltr., Repert. Spec. Nov. Regni Veg. Beih. 4 (1919) 259.

Terrestrial. Pseudobulbs rounded, ±3 cm across. Leaves lanceolate, 20-24 × 6-7 cm, plicate, thin, glabrous. Inflorescences arising from the pseudobulbs. Flowers pink with red or purple lines. Dorsal sepal ovate, 1.4 × 0.3-0.4 cm. Lateral sepal ovate, 1.4 × 0.3-0.4 cm. Petals ovate, 1.4 × 0.3 cm. Lip oblong, 3-lobed.

Luisia tristis (G. Forst.) Hook.f., Fl. Brit. Ind. 6 (1890) 25

Epiphytic. Stems erect or ascending, ± 20 × 0.5 cm, terete, stiff, fleshy. Leaves terete, 4-14 × 0.5 cm, apex acute. Inflorescences arising lateral, 0.8 × 1 cm. Flowers 1-1.2 cm across, green to greenish-yellow. Dorsal sepal elliptic, cucullate, apex obtuse. Lateral sepal ovate, concave, apex apiculate. Petals oblong, subspathulate, apex apiculate. Lip 3-lobed, fleshy, maroon, covered with minute brown hairs inside.

Pholidota imbricata Hook., Exot. Fl. 2 (1825) t. 138

Epiphytic. Pseudobulbs elliptic to ovate-elongate, 3-4.5 × 1-6 cm, glabrous, sheathing at the base, 1-leaved. Leaf obovate-oblong to lanceolate, ± 30 × 3.5-6 cm, thin, apex acute. Inflorescences pendent, densely many-flowered. Flowers 0.4-0.5 cm across, creamy white with dark yellow-orange lips. Dorsal sepal ovate, 5-6 × 3-4 mm, top obtuse to acute, tip acuminate. Lateral sepals ovate to ovate-oblong, 5-7 × 2.5-3.5 mm, top acute. Petal falcate, 4.5-6 × 1.3-2.2 mm, top acute, tip rounded. Lip broadly inserted.

Pinalia fitzalanii (F. Muell) Kuntze., Rev. Gen. Pl. (1891) 679.

Epiphytic. Pseudobulb ovate to elliptic, 4-10 × 2-3.5 cm, covered with scales, 2-4 leaved. Leaves elliptic-ligulate, 10.5-20 × 2-2.5 cm, apex acute. Inflorescence arising near the apices of the pseudobulbs, densely many-flowered. Flowers ± 6 mm across, erect-patent, white. Dorsal sepal oblong, apiculate, 0.6 cm long. Lateral sepal oblique, 0.25 cm long. Petal obliquely oblong, broadly obtuse, glabrous, somewhat shorter than the sepals. Lip obovate-cuneate, glabrous, 0.65 cm long.

Pomatocalpa macphersonii (F. Muell.) T.E. Hunt., Queensland Naturalist 16 (1958) 27.

Epiphytic monopodial. Stem short. Leaves subfalcate-oblique, ligulate, base half twisted, 15-29 × 3-4 cm, fleshy, shiny, apex unequally-bilobed. Inflorescences pointing downwards. Flowers small, ±0.8 cm across, non-resupinate. Dorsal sepal, lateral sepal and petals obovate, fleshy, apex rounded to obtuse, yellow with reddish spots. Lip 3-lobed, thickened at the base, white to pale yellow. Spurs bucket-shaped, rounded to obtuse, pale yellow with brown spots.

Pomatocalpa marsupiale (Kraenzl.) J.J.Sm., Natuurk. Tijdschr. Ned.-Indië 72 (1912) 105.

Epiphytic monopodial. Stem ±25-100 cm long. Leaves scattered along the stem, strap-shaped, 13-22 × 2.5 cm, falcate, apex obtuse, unequally-bilobed. Flowers non-resupinate, ±0.4 cm across, yellow to yellowish-green. Dorsal sepal, lateral sepal and petals obovate-oblong, slightly falcate, apex obtuse to rounded. Lip 3-lobed. Spur bucket-shaped.

Thrixspermum platystachys (F.M.Bailey) Schltr., Orchis 5 (1911) 55.

Epiphytic. Leaves lanceolate, 14 × 2.5 cm, ligulate, glabrous, apex unequally-bilobed. Inflorescences with long, flat and elongated peduncle.

Dendrobium sp. (Section Diplocaulobium)

Epiphytic. Stems slender, 1-2.5 × 0.15 cm, 1-leaved. Leaves erect, ligulate, 2-4.5 × 0.3-0.5 cm, fleshy, slightly stiff, apex bilobed.

Oberonia sp.

Epiphytic, very small. Stems very short, covered with 3-5 leaved. Leaves lanceolate-elongate, 0.5-2 × 0.1-0.4 cm, arranged like a fan, laterally flattened, thin, apex acute, pale green. Flower buds very small, 1.5-4 cm across, arranged in whorls, reddish-brown. Flowers not seen.

In conclusion, a total of 25 orchid species belonging to 11 genera were found in SPTN III Wasur, Wasur National Park. Monsoon forest was the ecosystem type with the highest number of orchid species with 15 orchid species, followed by savanna with 10 species, *Melaleuca* forest with 10 species, woodland forest with 9 species, riparian forest with 6 species, and *Melaleuca-Eucalyptus* forest with 5 species. *Dendrobium smillieae* and *Dendrobium rigidum*

were the most widely distributed epiphytic orchid species, and their distribution was almost in all ecosystem types. Whereas terrestrial orchids, *Geodorum densiflorum* and *Apostasia wallichii*, were only found in the monsoon forest.

ACKNOWLEDGEMENTS

The authors would like to thank Wasur National Park for issuing a research permit in SPTN III Wasur, Wasur National Park, Merauke, Papua. In addition, the first author is thankful to La Hisa for helping identify plant specimens and guidance during the research.

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ASIAN JOURNAL OF FORESTRY
Volume 5, Number 2, December 2021 E-ISSN: 2580-2844
Pages: 111-122 DOI: 10.13057/asianjfor/r050207

Filtering multi-collinear predictor variables from multi-resolution rasters of WorldClim 2.1 for Ecological Niche Modeling in Indonesian context

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Manuscript received: 8 August 2021. Revision accepted: 16 September 2021.

Abstract. *Pradhan P, Setyawan AD. 2021. Filtering multi-collinear predictor variables from multi-resolution rasters of WorldClim 2.1 for Ecological Niche Modeling in Indonesian context. Asian J For 5: 111-122.* WorldClim is one of the popular environmental datasets which hosts multi-resolution interpolated gridded climate raster surfaces and derived bioclimatic variables for both the immediate past, present and future scenarios. Bioclimatic variables along with other environmental factors like solar radiation, wind speed, water vapour pressure etc. have been used as primary set of explanatory variables for mapping and spatial modeling of many biological processes, including defining environmental niche of a species and identifying potential areas for its distribution through machine learning methods like Ecological Niche Modeling or Species Distribution Modeling or Habitat Suitability Modeling. However, the interpolated explanatory datasets are known to cause over-fitting of the models mainly due to multi-collinearity or redundancy within the variables. In the present study, 58 bioclimatic and environmental variables of Indonesian extent extracted from WorldClim 2.1 are screened to investigate the presence of multi-collinearity or redundancy. From the total 3364 variable pairs per raster resolution, 174 variable pairs were known to be affected by multicollinearity, from which temperature related bioclimatic variables, water vapour pressure and elevation associated variables were highly notable. For all the raster resolutions, bioclimatic variable 2, 3, 4, 15, 18 and 19, as well as slope, aspect, solar radiation for January, April, May, September, wind speed for August and November were found to be non-collinear. While, solar radiation for March and July were found to be non-collinear for 30s, 2.5m and 5m raster resolutions; Wind speed of July was non-collinear for 30s and 2.5m; Solar radiation for February and June were non-collinear for 10m; water vapour pressure for August for 2.5m and wind speed for January was non-collinear for 30s raster resolutions. The results of this study might serve as a convenient reference for investigators of the region for selection of bioclimatic and other environmental variables for conducting ecological niche modeling studies.

Keywords: Bioclimatic variables, elevation, habitat suitability modeling, MaxEnt, R, raster resolution, solar radiation, species distribution modeling, variance inflation factor, water vapour pressure, wind speed

INTRODUCTION

Bioclimatic variables along with other environmental variables represent important explanatory/predictor variables to understand species distribution (Busby 1986; Nix 1986). Bioclimatic variables express spatial variation in annual means, seasonality and extreme or limiting climatic factors, and represent biologically meaningful parameters for characterizing species distributions (Saatchi et al. 2008; O'Donnell and Ignizio 2012; Pradhan 2015). The advent of machine learning based ecological niche modeling (ENM)/species distribution modeling (SDM)/habitat suitability modeling (HSM) – from now on in this paper it is called ecological niche modeling – has opened an array of utility of bioclimatic variables and other climatic surfaces.

Among several climate and environmental databases, WorldClim is one of the popular environmental datasets used for mapping and spatial modeling of many biological processes due to availability of multi-resolution interpolated gridded climate raster surfaces and derived bioclimatic variables for both the immediate past, present and future scenarios. WorldClim version 1.4 was developed by Hijmans et al. (2005) with the present 'year 1960-1990' and future climate surfaces based on Coupled Model Intercomparison Project Phase 5 (CMIP5). The updated and expanded WorldClim version 2 was released during 2017 by Fick and Hijmans (2017) and it was further upgraded to version 2.1 (released on January 2020) with the present 'year 1970-2000' and future climate surfaces based on CMIP6, as well as monthly environmental variables such as solar radiation, wind speed and water vapour pressure. However, one of the major drawback linked to such interpolation derived environmental datasets is reported to be redundancy or multicollinearity and overfitting of resultant models (Pradhan 2016).

In machine learning process, optimal training by minimal set of explanatory variables (low training error) is very important for building optimal model, which could perform well against testing variables (low testing error).

However, training based on redundant, multicollinear, more than necessary and less relevant inputs may lead to learning of 'noise' of training data to the resultant model making it fit close to the training data (overfitted/low training error) and make it more complex and less sensitive to testing data (high testing error) (Anderson and Gonzales 2011; van Gils et al. 2014).

However, there are methods to counter such anomaly by selecting few non-collinear explanatory/predictor variables, making the resultant models less-overfitted and are simpler and parsimonius/based on minimally selected predictor variables. Variance Inflation Factor (VIF) analysis is a widely used method to identify problematic collinearity/redundancy among the variables. VIF is an indicator of the degree to which the standard errors are inflated due to the levels of multicollinearity (Montgomery and Peck 1992). In R, VIF can be calculated in packages like *car* (Fox and Weisberg 2019), *faraway* (Faraway 2016), *usdm* (Naimi et al. 2014), *vegan* (Oksanen et al. 2016), etc. However, these packages provide only individual VIFs per variable when the VIF itself being a derivative of correlation, pair-wise calculation of VIF would have provided more insight into which two variables are collinear and at which level of VIF.

Ecological niche modeling has been increasingly employed in Indonesia for modeling present habitat suitability and in some cases future potential distribution. Some notable studies from the region employing ENM techniques using WorldClim data are: Proboscis Monkey (*Nasalis larvatus*) (Suwarto et al. 2016), Javan hawk-eagle *Nisaetus bartelsi* (Nursamsi et al. 2018), Zebra Wood (*Guettarda speciosa*) (Yudaputra et al. 2019), *Baccaurea macrocarpa* trees (Gunawan et al. 2021), *Selaginella* spp. (Setyawan et al. 2017, 2020a, 2020b, 2021) and so on. While such studies provide strong basis for the development of ecological niche modeling application in Indonesia, methodological improvements to minimize the presence of multicollinearity might be useful for future application of ENM.

The current analysis aimed to investigate the presence of multicollinearity among 19 bioclimatic variables and monthly environmental variables of solar radiation, wind speed and water vapour pressure available in WorldClim version 2.1 in Indonesian context. In doing so, we undertake from the scratch approach in R for studying pair-wise multicollinearity in terms of Variance Inflation Factor amongst such variables. This analysis suggests variable pairs which are not to be used in combination together and also to identify the purely non-collinear variables which may be used for easy reference for future studies applying ecological niche modeling in the region.

MATERIALS AND METHODS

Data requirements

WorldClim version 2.1 website hosts multi-resolution continuous raster surfaces of monthly climate data of minimum, mean, and maximum temperature (°C), precipitation (mm), solar radiation (kJ m^{-2} day^{-1}), wind speed (m s^{-1}), water vapour pressure (kPa) for the 'current/present' period of 1970-2000 in *.tiff (Geotiff) format, besides hosting rasters of 19 bioclimatic variables for the above-mentioned period. For the current study, rasters of bioclimatic variables, solar radiation, wind speed, water vapour pressure were downloaded from WorldClim version 2.1 website (Fick and Hijmans 2017) in four raster resolutions viz. 30 seconds, 2.5 minutes, 5 minutes and 10 minutes. Raster resolution here is referred to the cell size/ spatial resolution which is the dimension of the area covered on the map and represented by a single pixel.

Raster handling workflow in R

By default, getData function of *raster* package still downloads obsolete WorldClim version 1.4. Hence, the updated 2.1 version raster files were downloaded, sorted resolution-wise, loaded in R, then resolution-wise raster *stack* was prepared with *raster* (Hijmans 2020) and *rgdal* (Bivand et al. 2020) packages, and projected to WGS84 coordinate reference system. Rasters of slope and aspect were created from Digital Elevation Model (DEM) with *terrain* function of *raster* package. Shape file of the world in 1:1 million scale was downloaded from EUROSTAT (2020) and the rasters were cropped and masked to the extent of Indonesia. Rasters were renamed as Bio1-19 for bioclimatic variables, Elev for DEM raster, Srad1-12 respectively for twelve monthly solar radiation rasters, Vapr1-12 respectively for twelve monthly water vapour pressure rasters, Wind1-12 respectively for monthly wind speed rasters, while names of slope and aspect rasters were kept unchanged.

Statistical analysis

The cropped rasters were converted to matrix through *as.matrix* function, and to obtain Pearson r value, pair-wise correlation of matrices (N) were conducted through *cor* function. After getting r value of the matrix, r^2 was obtained by multiplying N with N. Pair-wise Variance Inflation Factor (VIF) was calculated through the formula $VIF<-(1/(1-r^2))$. After VIF calculation, all cells with *Inf* values were replaced with 1 and the table values were formatted to one digit after zero through *round* function. Further, the VIF matrix was converted to dataframe, eliminating diagonal and duplicates to get pair-wise VIF. Shapiro test for assessing normality of data, Kruskal-Wallis and Friedman rank sum test, as well as post hoc Pair-wise Wilcox test were carried out in R with *shapiro.test()*, *kruskal.test()*, *friedman.test()* and *pairwise.wilcox.test()* functions respectively.

Data visualization

Density plot and Boxplot were prepared through *ggplot2* library. Heatmap of the pair-wise VIF matrix (used only for comparison of result of *usdm* package) was created with *heatmaply* package (Galili 2017), the resultant plot was saved as webpage through *htmlwidgets* package (Vaidyanathan 2019), and the webpage was converted to jpeg through *webshot2* package (Chang 2020). For visual comparison of four raster cell sizes with reference to zoomed inset of Gunung Leuser National Park region,

northern Sumatra, cropped and masked rasters of four resolutions of mean temperature of warmest quarter (Bio10) were visualized in R with plot function and exported as *.pdf. Four individual maps were compiled in Adobe Photoshop.

RESULTS AND DISCUSSION

In this study, gridded rasters of 58 explanatory variables available at WorldClim 2.1 were analyzed for presence of multicollinearity among them at the masked extent of Indonesia. The studied variables include 19 bioclimatic variables, elevation and its derivatives of slope and aspect, monthly variables of solar radiation (Srad), wind speed and water vapour pressure (Vapr). It was found that 174 pairs of above environmental variables had multicollinear relationship in one raster resolution or the other. The highest VIF for all raster resolutions belonged to the variable pair Bio1-Bio10 263±51.2

The environmental variables which showed multicollinearity were Bio1 (annual mean temperature), Bio5 (max temperature of warmest month), Bio6 (min temperature of coldest month), Bio8 (mean temperature of wettest quarter), Bio9 (mean temperature of driest quarter), Bio10 (mean temperature of warmest quarter), Bio11 (mean temperature of coldest quarter), Bio13 (precipitation of wettest month), Bio14 (precipitation of driest month), Bio16 (precipitation of wettest quarter), Bio17 (precipitation of driest quarter), elev (elevation/altitude), solar radiation for October (Srad10) and November (Srad11), water vapour pressure for January-December (Vapr1-12), wind for January-July (Wind1-Wind7), wind for September, October and December (Wind9, Wind10 and Wind 12). The detailed pair-wise comparison of multicollinear environmental variables are discussed below.

VIF pairs corresponding to Bio 1 (Annual Mean Temperature)

Bio 1 or annual mean temperature approximates the total energy inputs for an ecosystem. Results suggest that for four raster resolutions (30s, 2.5m, 5m and 10m), Bio1 is not to be used along with Bio10, Bio11, Bio5, Bio6, Bio8, Bio9, Elev, Vapr1, Vapr2, Vapr3, Vapr4, Vapr5, Vapr11, Vapr12. In addition, for 2.5m, 5m and 10m resolution rasters, Bio1 is not to be used along with Vapr10, and for 10m resolution raster, Bio1 is to be avoided to be used together with Vapr6.

VIF pairs corresponding to Bio 5 (Maximum Temperature of the Warmest Month)

Bio5 or maximum temperature of warmest month indicates the maximum monthly temperature occurrence over a given year (time-series) or averaged span of years (normal). This information is useful when examining whether species distributions are affected by warm temperature anomalies throughout the year. Results suggest that for four raster resolutions (30s, 2.5m, 5m and 10m) rasters, Bio5 is not to be used along with Bio1 (Table 1), Bio10, Bio11, Bio6, Bio8, Bio9, Elev, Vapr1, Vapr2, Vapr3, Vapr12 (Table 2). For 5m and 10m resolution rasters, Bio5 is not to be used along with Vapr11, while for 10m resolution raster, Bio5 is not to be used along with Vapr5.

VIF pairs corresponding to Bio 6 (Minimum Temperature of the Coldest Month)

Bio 6 or minimum temperature of coldest month takes account of the minimum temperature value across all months within a given year. This index is useful when examining whether species distributions are affected by cold temperature anomalies throughout the year. Results suggest that for four raster resolutions (30s, 2.5m, 5m and 10m), Bio6 is not to be used along with Bio10, Bio11, Bio8, Bio9, Elev, Vapr1, Vapr2, Vapr3, Vapr4, Vapr5, Vapr6, Vapr10, Vapr11, Vapr12 (Table 3), Bio1 (Table 1), Bio5 (Table 2). For 10m resolution raster, Bio6 is not to be used along with Vapr7, Vapr8, Vapr9.

Table 1. Distribution of VIF in variable pair of Bio1 (annual mean temperature) with other variables corresponding to 30 arc second, 2.5 minutes, 5 minutes and 10 minutes raster resolution. VIF values with (*) indicate values <10

VarPair	30s	2.5m	5m	10m
Bio1-Bio10	212.9	237.3	271.6	330.9
Bio1-Bio11	94.5	102.6	112.6	130.2
Bio1-Bio5	40.8	45.5	52.2	64
Bio1-Bio6	41.8	46.5	51.3	58.3
Bio1-Bio8	77.4	83.1	88.6	97.9
Bio1-Bio9	55.3	60.3	65.8	75
Bio1-Elev	28.9	30.5	31.4	32.2
Bio1-Vapr1	17.2	19.5	21.8	26.4
Bio1-Vapr10	9.5*	10.3	11.1	12.7
Bio1-Vapr11	13.3	14.3	15.3	17.2
Bio1-Vapr12	16.2	18.1	20.1	23.8
Bio1- Vapr2	17.7	20.1	22.7	27.2
Bio1-Vapr3	13.6	15	16.5	19.1
Bio1-Vapr4	12.6	13.9	15.3	17.9
Bio1-Vapr5	11.1	12.2	13.4	15.8
Bio1-Vapr6	8*	8.7*	9.6*	11.3

Table 2. Distribution of VIF in variable pair of Bio5 (maximum temperature of warmest month) with other variables corresponding to 30 arc second, 2.5 minutes, 5 minutes and 10 minutes raster resolution. VIF values with (*) indicate values <10. Reverse variable pairs of Bio5 available in other tables are not mentioned here.

VarPair	30s	2.5m	5m	10m
Bio5-Bio10	57.3	64	71.3	84.2
Bio5-Bio11	22.2	24.4	27.8	33.6
Bio5-Bio6	11.8	13.1	14.8	17.4
Bio5-Bio8	24.7	26.7	29.4	34.2
Bio5-Bio9	22.1	24.8	28.5	34.6
Bio5-Elev	14.9	16.2	17.4	19.1
Bio5- Vapr1	12.1	13.9	15.8	19.6
Bio5-Vapr12	10.7	12.1	13.6	16.6
Bio5-Vapr2	12.5	14.5	16.6	20.5
Bio5-Vapr3	10.3	11.6	13.1	15.9
Bio5-Vapr11	8.5*	9.4*	10.3	12.1
Bio5-Vapr4	8.6*	9.8*	11	13.4
Bio5-Vapr5	7*	7.8*	8.9*	10.9

VIF pairs corresponding to Bio 8 (Mean Temperature of the Wettest Quarter)

The quarterly index of Bio 8 or mean temperature of wettest quarter approximates average temperature for the three months with the highest cumulative precipitation. This index provides mean temperatures during the wettest three months of the year, which can be useful for examining how such environmental factors may affect species seasonal distributions.

Results suggest that for four raster resolutions (30s, 2.5m, 5m and 10m), Bio8 is not to be used along with Bio10, Bio11, Bio9, Elev, Vapr1, Vapr2, Vapr3, Vapr11, Vapr12 (Table 4), Bio1 (Table 1), Bio5 (Table 2), Bio6 (Table 3). For 5m and 10m resolution rasters, Bio8 is not to be used along with Vapr4, while for 10m resolution raster, Bio8 is not to be used along with Vapr5.

VIF pairs corresponding to Bio 9 (Mean Temperature of the Driest Quarter)

This quarterly index of Bio 9 or mean temperature of driest quarter approximates mean temperature for three months of the year with the lowest cumulative precipitation, which can be useful for examining how such environmental factors may affect species seasonal distributions. Results suggest that for four raster resolutions (30s, 2.5m, 5m and 10m), Bio9 is not to be used along with Bio10, Bio11, Elev, Vapr1, Vapr2, Vapr3, Vapr4, Vapr5, Vapr10, Vapr11, Vapr12 (Table 5), Bio1 (Table 1), Bio5 (Table 2), Bio6 (Table 3), Bio8 (Table 4). In addition, for 10m resolution raster, Bio9 is not to be used along with Vapr6.

VIF pairs corresponding to Bio 10 (Mean Temperature of the Warmest Quarter)

The quarterly index of Bio10 or mean temperature of warmest quarter approximates mean temperatures that prevail during the warmest three months of the year, which can be useful for examining how such environmental factors may affect species seasonal distributions. Results suggest that for four raster resolutions (30s, 2.5m, 5m and 10m), Bio 10 or mean temperature of warmest quarter is not to be used along with Bio11, Elev, Vapr1, Vapr2, Vapr3, Vapr4, Vapr11, Vapr12, Bio1 (Table 1), Bio5 (Table 2), Bio6 (Table 3), Bio8 (Table 4), Bio9 (Table 5). In addition, for 2.5m, 5m and 10m resolution rasters, Bio10 is not to be used along with Vapr5. Further, for 10m resolution raster, Bio10 is to be avoided to be used together with Vapr6 and Vapr10.

VIF pairs corresponding to Bio 11 (Mean Temperature of the Coldest Quarter)

The index of Bio 11—mean temperature of coldest quarter provides mean temperatures during the coldest three months of the year, which can be useful for examining how such environmental factors may affect species seasonal distributions. Results suggest that for four raster resolutions (30s, 2.5m, 5m and 10m), Bio11 is not to be used along with Elev, Vapr1, Vapr10, Vapr11, Vapr12, Vapr2, Vapr3, Vapr4, Vapr5, Bio1 (Table 1), Bio10 (Table 6), Bio5 (Table 2), Bio6 (Table 3), Bio8 (Table 4), Bio9 (Table 5). In addition, for 5m and 10m resolution rasters, Bio10 is not to be used along with Vapr6.

Table 3. Distribution of VIF in variable pair of Bio6 (min temperature of coldest month) with other variables corresponding to 30 arc second, 2.5 minutes, 5 minutes and 10 minutes raster resolution. VIF values with (*) indicate values <10. Reverse variable pairs of Bio6 available in other tables are not mentioned here

VarPair	30s	2.5m	5m	10m
Bio6-Bio10	26.1	29.3	33.2	39.5
Bio6-Bio11	44	48.8	52.3	58.4
Bio6-Bio8	24.7	27.4	30.2	33.9
Bio6-Bio9	31.7	33.7	35.4	38.5
Bio6-Elev	20	21.3	22.6	24.7
Bio6-Vapr1	13.3	14.9	16.6	19.3
Bio6-Vapr10	12.5	13.1	14	15.6
Bio6-Vapr11	14.7	15.7	16.8	18.7
Bio6-Vapr12	14.4	16.1	17.9	20.8
Bio6-Vapr2	13.3	14.7	16.5	18.9
Bio6-Vapr3	11.3	12.2	13.4	15
Bio6-Vapr4	13.2	14.2	15.4	17.3
Bio6-Vapr5	15.3	16.3	17.3	19.4
Bio6-Vapr6	10.3	10.9	11.6	12.8
Bio6-Vapr7	8.1*	8.6*	9.3*	10.9
Bio6-Vapr8	7.6*	8.2*	8.9*	10.4
Bio6-Vapr9	8.4*	8.8*	9.4*	10.6

Table 4. Distribution of VIF in variable pair of Bio8 (mean temperature of wettest quarter) with other variables corresponding to 30 arc second, 2.5 minutes, 5 minutes and 10 minutes raster resolution. VIF values with (*) indicate values <10. Reverse variable pairs of Bio8 available in other tables are not mentioned here

VarPair	30s	2.5m	5m	10m
Bio8-Bio10	63.8	67.8	72	80.2
Bio8-Bio11	33.6	36.5	39.7	44.4
Bio8-Bio9	20.4	22.3	24.4	28.1
Bio8-Elev	17.8	18.8	19.3	20
Bio8-Vapr1	13.5	14.9	16.3	18.8
Bio8-Vapr11	10	10.7	11.3	12.4
Bio8-Vapr12	12.1	13.2	14.3	16.3
Bio8-Vapr2	13.6	15.1	16.6	19
Bio8-Vapr3	10.4	11.3	12.3	13.8
Bio8-Vapr4	9.1*	9.9*	10.7	12.2
Bio8-Vapr5	8*	8.7*	9.5*	11

Table 5. Distribution of VIF of variable pair of Bio9 (mean temperature of driest quarter) with other variables corresponding to 30 arc second, 2.5 minutes, 5 minutes and 10 minutes raster resolution. VIF values with (*) indicate values <10. Reverse variable pairs of Bio9 available in other tables are not mentioned here.

VarPair	30s	2.5m	5m	10m
Bio9-Bio10	34.5	38.5	43.7	52.4
Bio9-Bio11	56.5	59.1	61.1	65
Bio9-Elev	19.8	20.8	21.8	23.3
Bio9-Vapr1	12.7	14.2	15.9	19.3
Bio9-Vapr10	10.4	11.1	12	13.7
Bio9-Vapr11	12	13	14	15.9
Bio9-Vapr12	13.1	14.6	16.2	19.4
Bio9-Vapr2	12.6	14.2	15.9	19.1
Bio9-Vapr3	11	12.1	13.3	15.5
Bio9-Vapr4	12.1	13.2	14.3	16.5
Bio9-Vapr5	13	13.9	14.9	17
Bio9-Vapr6	9.5*	10.2	11	12.6

VIF pairing between Bio13-Bio16 and Bio14-Bio17

Bio 13 or precipitation of wettest month identifies the total precipitation that prevails during the wettest month, which may be useful if extreme precipitation conditions during the month is known to influence potential range of a species. Bio 16 or precipitation of wettest quarter is quarterly index approximates total precipitation that prevails during the wettest three months of the year, which can be useful for examining how such environmental factors may affect species seasonal distributions.

Bio 14 or precipitation of driest month identifies the total precipitation that prevails during the driest month, which may be useful if extreme precipitation conditions during the month is known to influence potential range of a species. The quarterly index of Bio 17 or precipitation of driest quarter approximates total precipitation that prevails during the driest three months of the year, which can be useful for examining how such environmental factors may affect species seasonal distributions.

Results suggest that for four resolution (30s, 2.5m, 5m and 10m) rasters, Bio13 is not to be used along with Bio16, while Bio14 is not to be used along with Bio17 (Table 8).

VIF pairs corresponding to Elevation (Elev)

Digital Elevation Model is important raster variable used to make bioclimatic variables (Fick and Hijmans 2017). Results suggest that for four raster resolutions (30s, 2.5m, 5m and 10m), Elev is not to be used along with Vapr1, Vapr2, Vapr3, Vapr4, Vapr11, Vapr12 (Table 9), Bio1 (Table 1), Bio10 (Table 6), Bio11 (Table 3), Bio5 (Table 2), Bio6 (Table 3), Bio8 (Table 4), Bio9 (Table 5). In addition, for 2.5m, 5m, 10m resolution rasters, Elev is not to be used along with Vapr5. Further, for 10m resolution raster, Elev is not to be used along with Vapr10.

VIF pairs corresponding to Solar Radiation (Srad)

Results suggest that for four raster resolutions (30s, 2.5m, 5m and 10m), Srad10 is not to be used along with Srad11 and vice versa (Table 10).

VIF pairs corresponding to Water Vapour Pressure (Vapr)

Vapr1

For four raster resolutions (30s, 2.5m, 5m and 10m), Vapr1 or water vapour pressure for the month of January has VIF values of >10 with Vapr2, Vapr3, Vapr4, Vapr5, Vapr6, Vapr10, Vapr11, Vapr12, Bio1 (Table 1), Bio10 (Table 6), Bio11 (Table 7), Bio5 (Table 2), Bio6 (Table 3), Bio8 (Table 4), Bio9 (Table 5), and Elev (Table 9), hence Vapr1 is not to be used together with these variables. For 10m raster resolution, Vapr1 is not to be used along with Vapr7 and Vapr9.

Vapr2

For four raster resolutions (30s, 2.5m, 5m and 10m), Vapr2 or water vapour pressure for the month of February has VIF values of >10 with Vapr1, Vapr3, Vapr4, Vapr5, Vapr6, Vapr10, Vapr11, Vapr12, Bio1 (Table 1), Bio5 (Table 2), Bio6 (Table 3), Bio8 (Table 4), Bio9 (Table 5), Bio10 (Table 6), Bio11 (Table 7) and Elev (Table 9), hence Vapr2 is not to be used together with these variables. For 10m raster resolution, Vapr2 is not to be used along with Vapr7 and Vapr9.

Vapr3

For four raster resolutions (30s, 2.5m, 5m and 10m), Vapr3 or water vapour pressure for the month of March has VIF values of >10 with Vapr1, Vapr2, Vapr4, Vapr5, Vapr6, Vapr10, Vapr11, Vapr12, Bio1 (Table 1), Bio5 (Table 1), Bio6 (Table 3), Bio8 (Table 4), Bio9 (Table 5), Bio10 (Table 6), Bio11 (Table 7) and Elev (Table 9), hence Vapr3 is not to be used together with these variables. For 10m raster resolution, Vapr3 is not to be used along with Vapr7 and Vapr9.

Table 6. Distribution of VIF in variable pair of Bio10 (mean temperature of warmest quarter) with other variables corresponding to 30 arc second, 2.5 minutes, 5 minutes and 10 minutes raster resolution. VIF values with (*) indicate values <10. Reverse variable pairs of Bio10 available in other tables are not mentioned here

VarPair	30s	2.5m	5m	10m
Bio10-Bio11	37.1	40.7	45.5	53.9
Bio10-Elev	29.9	32.7	34.9	37.2
Bio10-Vapr1	17.1	19	21.1	24.9
Bio10-Vapr2	18.2	20.4	22.7	26.8
Bio10-Vapr3	13.7	15	16.4	18.9
Bio10-Vapr4	11.6	12.7	13.9	16.3
Bio10-Vapr11	12.1	12.8	13.7	15.4
Bio10-Vapr12	15.5	17	18.7	21.8
Bio10-Vapr5	9.2*	10.1	11.1	13.2
Bio10-Vapr6	6.9*	7.6*	8.4*	10.1
Bio10-Vapr10	8.3*	8.9*	9.8*	11.3

Table 7. Distribution of VIF in variable pair of Bio11 (mean temperature of coldest quarter) with other variables corresponding to 30 arc second, 2.5 minutes, 5 minutes and 10 minutes raster resolution. VIF values with (*) indicate values <10. Reverse variable pairs of Bio11 available in other tables are not mentioned here

VarPair	30s	2.5m	5m	10m
Bio11-Elev	20.2	21.1	21.7	22.6
Bio11-Vapr1	13.4	15.4	17.5	21.9
Bio11-Vapr10	10.2	11.1	12.1	14.2
Bio11-Vapr11	12.7	14	15.5	18.5
Bio11-Vapr12	13.6	15.6	17.7	22
Bio11-Vapr2	13.3	15.2	17.3	21.4
Bio11-Vapr3	11.1	12.4	13.8	16.4
Bio11-Vapr4	12.3	13.8	15.5	18.7
Bio11-Vapr5	13	14.5	16.1	19.5
Bio11-Vapr6	8.7*	9.5*	10.3	11.9

Table 8. Distribution of VIF in variable pair of Bio13-Bio16 and Bio14-Bio17 corresponding to 30 arc second, 2.5 minutes, 5 minutes and 10 minutes raster resolution

VarPair	30s	2.5m	5m	10m
Bio13-Bio16	21	21.3	21.6	22
Bio14-Bio17	78.7	77.9	77.4	78.5

Vapr4

For four raster resolutions (30s, 2.5m, 5m and 10m), Vapr4 or water vapour pressure for the month of April has VIF values of >10 with Vapr1, Vapr2, Vapr3, Vapr5, Vapr6, Vapr7, Vapr9, Vapr10, Vapr11, Vapr12, Bio1 (Table 1), Bio6 (Table 3), Bio9 (Table 5), Bio10 (Table 6), Bio11 (Table 7) and Elev (Table 9), hence Vapr4 is not to be used together with these variables. For 5m and 10m raster resolutions, Vapr4 is not to be used along with Vapr8, Bio5 (Table 2) and Bio8 (Table 4).

Vapr5

For four raster resolutions (30s, 2.5m, 5m and 10m), Vapr5 or water vapour pressure for the month of May has VIF values of >10 with Vapr1, Vapr2, Vapr3, Vapr4, Vapr6, Vapr7, Vapr8, Vapr9, Vapr10, Vapr11, Vapr12, Bio1 (Table 1), Bio11 (Table 7), Bio6 (Table 3) and Bio9 (Table 5), hence Vapr5 is not to be used together with these variables. For 2.5m, 5m and 10m raster resolutions, Vapr5 is not to be used along with Bio10 (Table 6) and Elev (Table 9). For 10m raster resolutions, Vapr5 is not to be used along with Bio5 (Table 2) and Bio8 (Table 4).

Vapr6

For four raster resolutions (30s, 2.5m, 5m and 10m), Vapr6 or water vapour pressure for the month of June has VIF values of >10 with Vapr1, Vapr2, Vapr3, Vapr4, Vapr5, Vapr7, Vapr8, Vapr9, Vapr10, Vapr11, Vapr12 and Bio6 (Table 3), hence Vapr6 is not to be used together with these variables. For 2.5m, 5m and 10m raster resolutions, Vapr6 is not to be used along with Bio9. For 5m and 10m raster resolutions, Vapr6 is not to be used along with Bio11. For 10m raster resolution, Vapr6 is not to be used along with Bio1 and Bio10.

Vapr7

For four raster resolutions (30s, 2.5m, 5m and 10m), Vapr7 or water vapour pressure for the month of July has VIF values of >10 with Vapr4, Vapr5, Vapr6, Vapr8, Vapr9, Vapr10 and Vapr11, hence Vapr7 is not to be used together with these variables. For 5m and 10m raster resolutions, Vapr7 is not to be used along with Vapr12. For 10m raster resolution, Vapr7 is not to be used along with Bio6, Vapr1, Vapr2, Vapr3.

Vapr8

For four raster resolutions (30s, 2.5m, 5m and 10m), Vapr8 or water vapour pressure for the month of August has VIF values of >10 with Vapr5, Vapr6, Vapr7, Vapr9, Vapr10 and Vapr11, hence Vapr8 is not to be used together with these variables. For 5m and 10m raster resolutions, Vapr8 is not to be used along with Vapr4. For 10m raster resolution, Vapr8 is not to be used along with Vapr12 and Bio6 (Table 3).

Vapr9

For four raster resolutions (30s, 2.5m, 5m and 10m), Vapr9 or water vapour pressure for the month of September has VIF values of >=10 with Vapr4, Vapr5, Vapr6, Vapr7, Vapr8, Vapr10 and Vapr11, hence Vapr9 is not to be used together with these variables. For 2.5m, 5m and 10m raster resolutions, Vapr9 is not to be used along with Vapr12. For 10m raster resolution, Vapr9 is not to be used along with Bio6 (Table 3), Vapr1, Vapr2 and Vapr3.

Vapr10

For four raster resolutions (30s, 2.5m, 5m and 10m), Vapr10 or water vapour pressure for the month of October has VIF values of >=10 with Vapr1, Vapr2, Vapr3, Vapr4, Vapr5, Vapr6, Vapr7, Vapr8, Vapr9, Vapr11, Vapr12, Bio11 (Table 7), Bio6 (Table 3) and Bio9 (Table 5), hence Vapr10 is not to be used together with these variables. For 2.5m, 5m and 10m raster resolutions, Vapr10 is not to be used along with Bio1 (Table 1). For 10m raster resolution, Vapr10 is not to be used along with Elev (Table 9) and Bio10 (Table 6).

Vapr11

For four raster resolutions (30s, 2.5m, 5m and 10m), Vapr11 or water vapour pressure for the month of November has VIF values of >=10 with Vapr12, Bio1 (Table 1), Bio10 (Table 6), Bio11 (Table 7), Bio6 (Table 3), Bio8 (Table 4), Bio9 (Table 5), Elev (Table 9), Vapr1, Vapr10, Vapr2, Vapr3, Vapr4, Vapr5, Vapr6, Vapr7, Vapr8 and Vapr9, hence Vapr11 is not to be used together with these variables. For 5m and 10m raster resolutions, Vapr11 is not to be used along with Bio5 (Table 2).

Vapr12

For four raster resolutions (30s, 2.5m, 5m and 10m), Vapr12 or water vapour pressure for the month of December has VIF values of >=10 with Bio1 (Table 1), Bio10 (Table 6), Bio11 (Table 7), Bio5 (Table 2), Bio6 (Table 3), Bio8 (Table 4), Bio9 (Table 5), Elev (Table 9), Vapr1, Vapr10, Vapr11, Vapr2, Vapr3, Vapr4, Vapr5 and Vapr6, hence Vapr12 is not to be used together with these variables. For 2.5m, 5m and 10m raster resolutions, Vapr12 is not to be used along with Vapr9. For 5m and 10m raster resolutions, Vapr12 is not to be used along with Vapr7. For 10m raster resolutions, Vapr12 is not to be used along with Vapr8.

Table 9. Distribution of VIF in variable pair of Elevation with other variables corresponding to 30 arc second, 2.5 minutes, 5 minutes and 10 minutes raster resolution. VIF values with (*) indicate values <10. Reverse variable pairs of Elev available in other tables are not mentioned here

VarPair	30s	2.5m	5m	10m
Elev-Vapr1	14.6	15.6	16.5	18
Elev-Vapr11	11.4	11.8	12.3	13.1
Elev-Vapr12	14.2	15.2	16.1	17.7
Elev-Vapr2	16.6	17.8	18.8	20.3
Elev-Vapr3	13.7	14.5	15.4	16.7
Elev-Vapr4	12.5	13.1	13.9	15.2
Elev-Vapr5	9.5*	10	10.5	11.7
Elev-Vapr10	8.4*	8.7*	9.1*	10

Table 10. Distribution of VIF in variable pair of Srad10 and Srad11 corresponding to 30 arc second, 2.5 minutes, 5 minutes and 10 minutes raster resolution

VarPair	30s	2.5m	5m	10m
Srad10-Srad11	13.5	13	12.7	12.7

Table 11. Distribution of VIF among variable pair of water vapour pressure variables corresponding to 30 arc second, 2.5 minutes, 5 minutes and 10 minutes raster resolution. VIF values with (*) indicate values <10. Reverse variable pairs of water vapour pressure available in other tables are not mentioned here.

VarPair	30s	2.5m	5m	10m
Vapr1-Vapr10	16.9	18.7	20.8	24.2
Vapr1-Vapr11	38.6	40.5	42.6	45.7
Vapr1-Vapr12	195.4	216.5	233.6	264.7
Vapr1-Vapr2	186.2	201.5	221.8	250
Vapr1-Vapr3	102	111.1	121.5	138.5
Vapr1-Vapr4	40.8	45.7	50.8	61.5
Vapr1-Vapr5	16.5	18.8	21.4	26.9
Vapr1-Vapr6	11.4	13.4	15.6	20.3
Vapr1-Vapr7	7*	8*	9.1*	11.6
Vapr1-Vapr9	7.8*	8.6*	9.6*	11.3
Vapr10-Vapr11	55.9	63.2	73	91.6
Vapr10-Vapr12	25.7	28.8	32.5	38.7
Vapr11-Vapr12	80.7	84.4	88.1	92.6
Vapr2-Vapr10	14.4	15.6	17.1	19.4
Vapr2-Vapr11	30.6	31.6	32.8	34.4
Vapr2-Vapr12	93	101	111.4	126.2
Vapr2-Vapr3	226.5	246.7	261.2	292.6
Vapr2-Vapr4	48.5	53.2	57.8	66.8
Vapr2-Vapr5	15.7	17.7	19.9	24.4
Vapr2-Vapr6	11.2	13	15.2	19.7
Vapr2-Vapr7	6.7*	7.5*	8.5*	10.6
Vapr2-Vapr9	7.3*	8*	8.7*	10
Vapr3-Vapr10	16.4	17.5	19	21.1
Vapr3-Vapr11	33.3	33.8	34.6	35.1
Vapr3-Vapr12	83.7	89.7	97.2	107
Vapr3-Vapr4	78.1	83.6	88.3	95.4
Vapr3-Vapr5	18.4	20.4	22.7	26.8
Vapr3-Vapr6	13.9	16.3	19.3	25.3
Vapr3-Vapr7	7.6*	8.5*	9.7*	11.9
Vapr3-Vapr9	8*	8.7*	9.4*	10.6
Vapr4-Vapr10	30.5	32.9	36.3	40.8
Vapr4-Vapr11	65.9	71.8	79	91.2
Vapr4-Vapr12	63.2	73	84.4	108.7
Vapr4-Vapr5	47.3	53.9	61.9	78
Vapr4-Vapr6	21.2	24.1	27.3	32.6
Vapr4-Vapr7	11.4	12.8	14.4	17.7
Vapr4-Vapr9	11.9	12.8	13.9	15.5
Vapr4-Vapr8	8.7*	9.6*	10.7	12.5
Vapr5-Vapr10	51.9	56	60.7	69.7
Vapr5-Vapr11	42.1	50.5	60.4	83.8
Vapr5-Vapr12	22.6	26.4	30.5	39.6
Vapr5-Vapr6	43.9	44.5	44.3	42.9
Vapr5-Vapr7	27.9	30.2	32.8	37.6
Vapr5-Vapr8	18.3	20	21.8	24.9
Vapr5-Vapr9	20.8	21.9	23.1	25.1
Vapr6-Vapr10	28.4	30.9	33	36.2
Vapr6-Vapr11	16.7	18.9	21	24.3
Vapr6-Vapr12	13.5	15.9	18.6	24
Vapr6-Vapr7	48.5	50.3	52.3	56
Vapr6-Vapr8	16.5	16.9	17.2	17.4
Vapr6-Vapr9	21.4	22.1	22.8	23.6
Vapr7-Vapr10	23.4	26.5	30.1	38.1
Vapr7-Vapr11	12	13.8	15.8	20.1
Vapr7-Vapr8	49.9	51.1	52.2	53.7
Vapr7-Vapr9	37	39.2	41.6	47
Vapr7-Vapr12	8.3*	9.6*	11	14.1
Vapr8-Vapr10	20.6	23.7	27	33.7
Vapr8-Vapr11	10.1	11.7	13.5	17.4
Vapr8-Vapr9	60	65.7	70.4	79.9
Vapr8-Vapr12	6.8*	7.7*	8.7*	10.7
Vapr9-Vapr10	45.3	50.8	57	67.3
Vapr9-Vapr11	15.4	17.3	19.5	23.5
Vapr9-Vapr12	9.7*	10.9	12.2	14.6

VIF pairs corresponding to Wind Speed (Wind)

For four raster resolutions (30s, 2.5m, 5m and 10m), Wind1 or wind speed for the month of January has VIF values of >=10 with Wind2, and conversely Wind2 (wind speed for the month of February) has VIF values of >=10 with Wind1. Similarly, for all of these data resolutions, i) Wind2 has VIF values of >=10 with Wind3 (wind speed for the month of March) and vice versa, ii) Wind3 has VIF values of >=10 with Wind4 (wind speed for the month of April) and vice versa, iii) Wind4 has VIF values of >=10 with Wind5 (wind speed for the month of May) and vice versa, iv) Wind5 has VIF values of >=10 with Wind6 (wind speed for the month of June) and vice versa, hence these variable pair combinations should be avoided (Table 11).

For the raster resolutions (5m and 10m), i) Wind2 has VIF values of >=10 with Wind12 (wind speed of December) and vice versa, and ii) Wind4 has VIF values of >=10 with Wind10 (wind speed of October) and vice versa, hence these variable pair combinations should be avoided.

For the 10m raster resolution, i) Wind3 has VIF values of >=10 with Wind5 and vice versa, and ii) Wind6 has VIF values of >=10 with Wind7 (wind speed of July) and vice versa, iii) Wind9 (wind speed of September) has VIF values of >=10 with Wind10 (wind speed of October) and vice versa, hence these variable pair combinations should be avoided.

Comparison with VIF output by *usdm* package

The VIF pairs identified for studied explanatory variables in Indonesian extent from present study was compared with the VIF of the said explanatory variables for the same extent derived from *vifstep* function of *usdm* package. The output of *vifstep* function provides total number along with names of variables from the input variables that have collinearity problem, but it doesn't provide the variable pairs having the collinearity problem. After excluding the collinear variables, the function provides value for variable pair with minimum and maximum values of linear correlation coefficients. Next, it provides VIFs of the remained variables, but here VIF is associated with a particular variable, and not the variable pair (Table 12).

The *usdm* package output enlisted 38 variables for 30s and 2.5m resolution and 39 variables for 5m and 10m resolution to have problematic collinearity (VIF>10) (Table 13). The output also mentioned i) Bio7, Bio8, Srad8 and Srad 12 for all resolutions, ii) Srad2 and Srad6 for 30s, 2.5m and 5m resolutions, and iii) Srad3 for 10m resolution to have VIF>10, but from the present study, said variables were found to have VIF<10 (Tables 13,14).

Interestingly, there were disparity between the VIF status of some 'non-collinear' variables resulting from *usdm* and their collinearity indicated from present study. Viz. for 30s resolution rasters, *usdm* ascribed VIF of 3.46 to Bio13 and VIF of 4.28 to Vapr8, indicating their non-collinearity, but for the said resolution, present study found that Bio13 is linked to Bio16 with VIF 21, and Vapr8 is linked to Vapr9 (VIF 60), Vapr10 (VIF 20.6), Vapr11 (VIF 10.1). Similarly, for 2.5m resolution rasters, *usdm* ascribed

VIF of 3.47 to Bio13 and VIF of 7.14 to Wind1, but for the said resolution, present study found that Bio13 is linked to Bio16 with VIF 21.3, and Wind1 is linked to Wind2 (VIF 10.6). For 5m resolution rasters, *usdm* ascribed VIF of 3.53 to Bio13, 4.83 to Vapr8 and 7.95 to Wind1, but the present study revealed for the said resolution, Bio13 to be linked to Bio16 with VIF 21.6, Vapr8 to be linked to Vapr9 (VIF 70.4), Vapr10 (VIF 27), Vapr11 (VIF 13.5) and Wind1 to be linked to Wind2 with VIF 11.9. In case of 10m resolution rasters, *usdm* attributed VIF of 3.47 to Bio13, 5.05 to Vapr8 and 9.96 to Wind1, but the present study revealed for the said resolution, Bio13 to be linked to Bio16 with VIF 22, Vapr8 to be linked to Vapr9 (VIF 79.9), Vapr10 (VIF 33.7), Vapr11 (VIF 17.4) and Vapr12 (VIF 10.7), while Wind1 was found to be associated with Wind2 with VIF 13.8.

Effect of data (raster) resolution

At equator (0° latitude), 30 arc-seconds (0.5-minute) spatial resolution corresponds to about 0.86 km^2 cell size (commonly referred to as '1-km' spatial resolution), 2.5 minutes raster resolution is equivalent to around 21.44 km^2 cell size, 5 minutes raster resolution is equivalent to around 85.75 km^2 cell size and 10 minutes raster resolution is equivalent to around 342.99 km^2 cell size. Species distribution are scale dependent hence it is pertinent to understand the changes in VIF values corresponding to data (raster) resolution.

Table 12. Distribution of VIF among variable pair of wind variables corresponding to 30 arc second, 2.5 minutes, 5 minutes and 10 minutes raster resolution. VIF values with (*) indicate values <10

VarPair	30s	2.5m	5m	10m
Wind1-Wind2	8.6*	10.6	11.9	13.8
Wind2-Wind3	8.9*	10.9	12.4	13.7
Wind3-Wind4	8.8*	10.8	12.3	14.5
Wind4-Wind5	8.4*	10.4	12.3	16.4
Wind5-Wind6	8.3*	10.1	11.5	13.8
Wind2-Wind12	7.7*	9.3*	10.3	11.9
Wind4-Wind10	7.3*	9.2*	10.7	12.5
Wind3-Wind5	5.9*	7.1*	8.4*	11.2
Wind6-Wind7	7.7*	8.9*	9.6*	10.7
Wind9-Wind10	6.9*	8.6*	9.6*	10.9

Table 13. Variables with collinearity problem for Indonesian extent derived from *vifstep* function of *usdm* package. Variables which were found to have VIF>10 in usdm output but having VIF<10 in the present study are bold italicized and (*) marked

30s	2.5m	5m	10m
Bio1,	Bio1,	Bio1,	Bio1,
Bio10,	Bio10,	Bio10,	Bio10,
Bio11,	Bio11,	Bio11,	Bio11,
Bio12*,	***Bio12****,	***Bio12****,	***Bio12****,
Bio14,	Bio14,	Bio14,	Bio14,
Bio16,	Bio16,	Bio16,	Bio16,
Bio17,	Bio17,	Bio17,	Bio17,
Bio5,	Bio5,	Bio5,	Bio5,
Bio6,	Bio6,	Bio6,	Bio6,
Bio7*,	***Bio7****,	***Bio7****,	***Bio7****,
Bio8,	Bio8,	Bio8,	Bio8,
Bio9,	Bio9,	Bio9,	Bio9,
Elev,	Elev,	Elev,	Elev,
Srad10,	Srad10,	Srad10,	Srad10,
Srad11,	Srad11,	Srad11,	Srad11,
Srad12*,	***Srad12****,	***Srad12****,	***Srad12****,
Srad2*,	***Srad2****,	***Srad2****,	***Srad3****,
Srad6*,	***Srad6****,	***Srad6****,	Srad7,
Srad8*,	***Srad8****,	***Srad8****,	***Srad8****,
Vapr1,	Vapr1,	Vapr1,	Vapr1,
Vapr10,	Vapr10,	Vapr10,	Vapr10,
Vapr11,	Vapr11,	Vapr11,	Vapr11,
Vapr12,	Vapr12,	Vapr12,	Vapr12,
Vapr2,	Vapr2,	Vapr2,	Vapr2,
Vapr3,	Vapr3,	Vapr3,	Vapr3,
Vapr4,	Vapr4,	Vapr4,	Vapr4,
Vapr5,	Vapr5,	Vapr5,	Vapr5,
Vapr6,	Vapr6,	Vapr6,	Vapr6,
Vapr7,	Vapr7,	Vapr7,	Vapr7,
Vapr9,	Vapr9,	Vapr9,	Vapr9,
Wind10,	Wind10,	Wind10,	Wind10,
Wind12,	Wind12,	Wind12,	Wind12,
Wind2,	Wind2,	Wind2,	Wind2,
Wind3,	Wind3,	Wind3,	Wind3,
Wind4,	Wind4,	Wind4,	Wind4,
Wind5,	Wind5,	Wind5,	Wind5,
Wind6,	Wind6,	Wind6,	Wind6,
Wind9	Wind9	Wind7,	Wind7,
		Wind9	Wind9

Table 14. The highest VIF value of the variables for Indonesian extent taken as collinear by *usdm* package but found to be non-collinear in present study

30s		2.5m		5m		10m	
VarPair	**VIF**	**VarPair**	**VIF**	**VarPair**	**VIF**	**VarPair**	**VIF**
Bio7-Bio2	1.8	**Bio7**-Bio2	1.8	**Bio7**-Bio2	1.9	**Bio7** – Vapr8	1.3
Bio12-Bio17	4.9	**Bio12**-Bio17	4.7	**Bio12**-Bio17	4.6	**Bio12**-Bio17	4.6
Srad8-Srad7	3.3	**Srad8**-Srad7	3.3	**Srad8**-Srad7	3.4	**Srad8**-Srad7	3.6
Srad6-Srad7	3.1	**Srad6**-Srad7	2.9	**Srad6**-Srad7	2.8		
Srad2-Srad3	7.7	**Srad2**-Srad3	8.1	**Srad2**-Srad3	8.6	**Srad3**-Srad2	9
Srad12-Srad11	8.5	**Srad12**-Srad11	7.9	**Srad12**-Srad11	7.5	**Srad12**-Srad11	6.9

It was observed from the summary statistics (Table 15) that going from the gradient of fine resolution (30s) towards coarser data resolution (10 m), there was increase in variance, increase in large value VIFs (outliers), increase in median, mean and corresponding standard deviation of mean. Figure 1 depicts visual comparison of four raster cell sizes with example of mean temperature of warmest quarter (Bio10) map in Indonesia, with zoomed inset of Gunung Leuser National Park region, Northern Sumatra, which will be helpful to understand the grain size/ raster resolution at the scale of Indonesia and in preliminarily selection of preferred raster resolution as per study objective.

To study the significance of pair-wise raster differences, firstly, data distribution (VIF) was assessed with Shapiro-Wilk normality test which resulted in W = 0.54311, p-value < 2.2e-16, meaning that p-value <0.05 showed strong evidence of the data being non-normal. It was further corroborated with the density distribution plot of raster resolution-wise VIF, which showed strong skewed values towards left side (Figure 2), with the presence of large value VIFs as outliers (Figure 3).

As the distribution was found to be non-normal, Kruskal-Wallis rank sum test (non-parametric version of One-way ANOVA) and Friedman rank sum test (non-parametric version of one-way repeated measures ANOVA) were carried out.

Kruskal-Wallis rank sum test showed chi-squared = 29.203, df = 3, p-value = 2.03e-06 and Friedman rank sum test showed chi-squared = 494.47, df = 3, p-value < 2.2e-16. As both the results showed p-value <0.05, the null hypothesis was rejected and significant difference between VIF values of raster resolutions were considered. To understand particularly the pairs of different individual raster resolutions, post hoc Wilcoxon rank sum test with pair-wise comparisons with bonferroni continuity correction was conducted. It was observed that 30s resolution raster was significantly different (p-value <0.05) than 5m and 10m, while 2.5m resolution raster was significantly different (p-value <0.05) than 10m resolution raster (Table 16).

Table 15. Summary statistics of resolution-wise variance inflation factor values

Res	Min.	Max.	Mean	SD	Median	Variance
30s	5.90	226.50	26.68	33.81	13.80	1142.95
2.5m	7.10	246.70	29.16	36.90	15.60	1361.27
5m	8.40	271.60	31.87	40.29	17.15	1623.43
10m	10.00	330.90	36.68	46.36	19.85	2149.37

Table 16. Post hoc Wilcoxon rank sum test with pair-wise comparisons of rasters with *bonferroni* continuity correction

Resolution	30s	2.5m	5m
2.5m	0.6588	-	-
5m	0.0155	0.7003	-
10m	4.50E-06	0.0011	0.0917

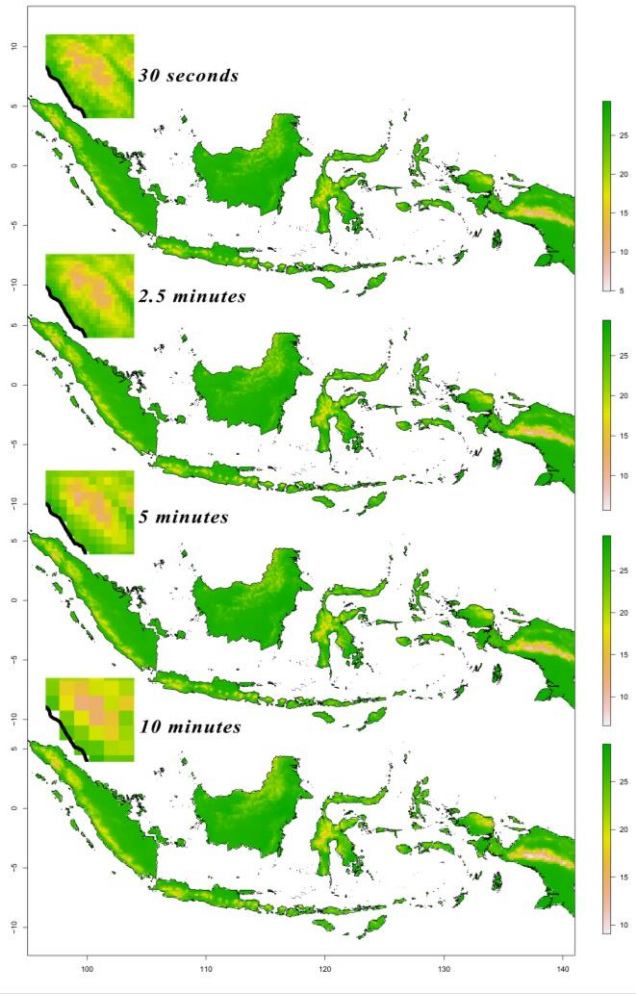

Figure 1. Visual comparison of four raster cell sizes with example of mean temperature of warmest quarter (Bio10) map in Indonesia, with zoomed inset of Gunung Leuser National Park region, northern Sumatra.

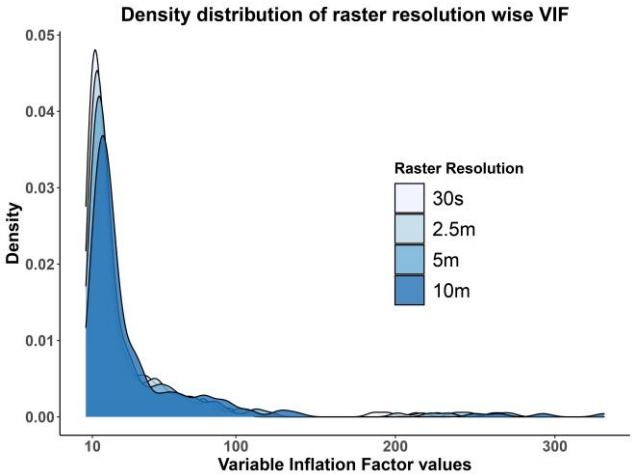

Figure 2. Density distribution of VIF with respect to various raster resolution

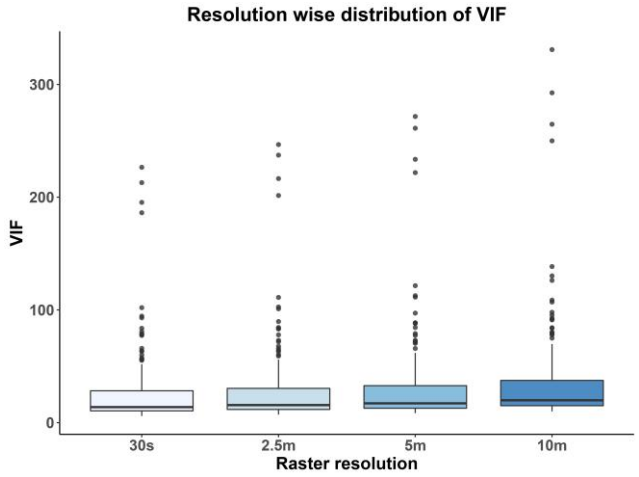

Figure 3. Distribution of VIF with respect to various raster resolution

Tentative list of non-collinear variables

For all the raster resolutions, Bio2, Bio3, Bio4, Bio15, Bio18, Bio19, slope, aspect, Srad1, Srad4, Srad5, Srad9, Wind8, Wind11 were found to be non-collinear. While, Srad3 and Srad 7 were found to be non-collinear for 30s, 2.5m and 5m raster resolutions; Wind 7 was non-collinear for 30s and 2.5m; Srad2 and Srad6 were non-collinear for 10m; Vapr8 for 2.5m and Wind1 was non-collinear for 30s raster resolutions.

Discussion

From the analysis of 58 explanatory/predictor variables and their corresponding 3364 variable pairs, 174 resolution wise variable pairs were known to be affected by multicollinearity, out of which temperature related bioclimatic variables, water vapour pressure and elevation associated variables were highly notable. Resolution-wise, 10m rasters had the highest variance, indicating more data noise and presence of greater number of collinear variables, while 30s rasters had the lowest variance, meaning these datasets deviate less significantly than mean, and have a smaller number of collinear variables.

Regarding bioclimatic variables, temperature variables mainly affected by multicollinearity include mean temperatures of warmest (Bio10), wettest (Bio8), coldest (Bio11) and driest quarter (Bio9), annual mean temperature (Bio1), as well as limiting factors like maximum temperature of warmest month (Bio5) and minimum temperature of coldest month (Bio6). With respect to the precipitation related factors, precipitation of wettest month (Bio13) was found to be collinear with precipitation of wettest quarter (Bio16). Further, precipitation of driest month (Bio14) was found to be collinear with precipitation of driest quarter (Bio17). Water vapour pressure also had high multicollinearity among themselves, with elevation and temperature related bioclimatic variables as water vapour pressure itself is calculated from dew-point temperature/ mean relative humidity and mean temperature (Fick and Hijmans 2017). Higher multicollinearity of elevation with temperature related variables may be due to dependence of temperature with gradients of latitude and elevation, and less multicollinearity among precipitation related variables may be explained by the fact that precipitation can be highly variable in time and space and some regions have abrupt changes (Fick and Hijmans 2017). Wind speed was found to be interesting in a sense that none of the 30 second rasters were multicollinear (VIF values <10), while gradually VIF values of concerned variable pairs increased with the increase in resolution.

While selecting explanatory variables for ecological niche modeling, composite variables based on the precipitation of the coldest or warmest period or temperature of the driest or wettest period could be avoided as these datasets are hinted to be internally flawed. Therefore, limiting factors like the maximum temperature of the warmest month, minimum temperature of the coldest period, temperature variability, precipitation variability, precipitation of the wettest and driest periods and so on may be used in combination unless otherwise VIF values restrict them (Pradhan 2016). However, working only with non-redundant ones may not always yield good results as some of the redundant variables may act as good ecological descriptors, which could be used in ENM process without its corresponding variable pair (VIF>10). It should also be noted that sometimes overfitting may be the result of selecting too much aggregate sampling sites (sample/observation bias) which may be corrected (Pradhan 2016).

After VIF screening, the variables may be preliminarily run in MaxEnt (minimum of triplicate runs), which may be helpful to identify the least significant variables based on jackknife test for evaluating relative importance of variables, % contribution to the model as well as individual response to the variable (Jueterbock et al. 2016; Gunawan et al. 2021). In case, multiple models are built for the same species utilizing multiple sets of non-collinear variables, the final model is suggested to selected based upon lowest AICc score, highest AUC value and incorporating lesser number of correlated variables (VIF <10) (Warren et al. 2010). *ENMeval* (Muscarella et al. 2014; Kass et al. 2021) and *Maxentvariableselection* packages in R offer various evaluation metrics for selecting explanatory variables which may be consulted prior to model building (Jueterbock et al. 2016) and the overall workflow of ecological niche modeling may be conducted following Overview, Data, Model, Assessment and Prediction (ODMAP) protocol outlined by Zurell et al. (2020).

Further, under current climatic conditions, performance and prediction likelihood of models based on CHELSA climatic database are reported to outperform than that of WorldClim climatic database, especially for high mountain regions (Bobrowski 2021). Besides, it may also be kept in mind that all temperature variables of WorldClim 2.1 are based upon the higher global correlation coefficient (ρ) between estimated and observed values of 0.99, and similarly solar radiation and water vapour pressure both have correlation coefficients higher than 0.95; however, accuracy was lowest for wind speed (ρ=0.76) and precipitation (ρ=0.86) (Fick and Hijmans 2017), which is

important in understanding how realistic explanatory variables are we using for ENM studies as regional/ local scale (Pradhan 2019).

In conclusion the study presented a primer for selection of various explanatory/predictor variables based upon WorldClim 2.1 datasets available in four raster resolutions. At the extent of Indonesia, out of 58 explanatory variables and their corresponding 3364 variable pairs, 174 variable pairs were known to be affected by multicollinearity, from which temperature related bioclimatic variables, water vapour pressure and elevation associated variables were highly notable. For all the raster resolutions, Bio2, Bio3, Bio4, Bio15, Bio18, Bio19, slope, aspect, Srad1, Srad4, Srad5, Srad9, Wind8, Wind11 were found to be non-collinear. While, Srad3 and Srad 7 were found to be non-collinear for 30s, 2.5m and 5m raster resolutions; Wind 7 was non-collinear for 30s and 2.5m; Srad2 and Srad6 were non-collinear for 10m; Vapr8 for 2.5m and Wind1 was non-collinear for 30s raster resolutions. In a gradient smaller, resolution raster had smaller data variance e.g. 30s than the larger resolution raster e.g. 10m. VIF output of *usdm* package of R was compared with present study, and some disparities were noted, necessitating validation of VIF of screened variables who rely solely on such packages. Besides WorldClim, other climatic databases such as CHELSA is to be compared and explored for regional ENM studies.

ACKNOWLEDGEMENTS

Authors acknowledge the precise perusal of the manuscript and thoughtful comments by the anonymous reviewers which were very helpful in improvement of the manuscript. First author is thankful to the Indian Statistical Institute, Kolkata for their 2018 workshop on economic and ecological impacts of Invasive Alien Species, during the course of which possibilities of R program was introduced; further, the first author is indebted to miscellaneous insights in R and R codes available at Stackoverflow threads (https://stackoverflow.com/), GIS Stackexchange threads (https://gis.stackexchange.com/), Statistical tools for high-throughput data analysis (http://www.sthda.com/english/) and Facebook group *Ecology in R*.

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