BIODIVERSITAS
Volume 23, Number 8, August 2022
Pages: 4082-4089

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230828

Acclimation study of *Smilax nageliana* A.DC., a climber species endemic to East Java, Indonesia

SITI SOFIAH[1,3,♥], LUCHMAN HAKIM[2], SERAFINAH INDRIYANI[2], IYAN ROBIANSYAH[3]
[1]Graduate Program, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia. Tel./Fax. +62-341-551611, ♥email: siti.sofiah2291@gmail.com
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia
[3]Research Center for Plant Conservation, Botanic Garden and Forestry, National Agency For Research and Innovation. Jl. Raya Surabaya-Malang Km. 65, Pasuruan 67163, East Java, Indonesia

Manuscript received: 21 March 2022. Revision accepted: 25 July 2022.

Abstract. *Sofiah S, Hakim L, Indriyani S, Robiansyah I. 2022. Acclimation study of* Smilax nageliana *A.DC., a climber species endemic to East Java, Indonesia. Biodiversitas 23: 4082-4089.* *Smilax* L. species, popularly known in Indonesia as Canar, belong to the family Smilacaceae and have been used in folk medicine as a tonic against rheumatism and as anti-syphilitic. *Smilax nageliana* A.DC is an endemic plant to East Java, Indonesia which is only distributed in Ranu Darungan, Bromo Tengger Semeru National Park (BTSNP), and Mt. Kawi. In the effort for its conservation and sustainable management, information regarding the adaptation of *S. nageliana* to a new environment is necessary. The aim of this study was to investigate the acclimation of *S. nageliana* under three different treatments, namely (i) in hood application in a greenhouse; (ii) in its natural habitat under shade; (iii) in natural habitat in the open area. Each treatment had ten replications. Survival and growth performance were observed for six months in terms of survival rates, number of new shoots, shoot length, number of leaves and chlorophyll content. Environment factors were also measured including solar intensity, temperature, humidity and water content. Data were analyzed using a two-way Anova. The principal component analysis (PCA) was performed to determine the relationships between environmental components and *S. nageliana* acclimation performance. The result showed that *S. nageliana* had a one hundred percent survival rate in the hood application and natural habitat with shade. The best growth performances (i.e., shoot length, the number of leaves, shoots per root and chlorophyll content) were achieved under the hood application. The average temperature was quite influential on the first component (PC1), followed by solar intensity while water affected the second component (PC2). The findings of this study suggest that where sunlight/solar intensity and humidity were controlled, it gave the most optimal results for acclimation of *S. nageliana.*

Keywords: A climber, acclimation, East Java, endemic plant, *Smilax nageliana*

INTRODUCTION

Endemic plants are generally more vulnerable to extinction compared to common and widely distributed plant species. Işik (2011) and Nilawati et al. (2015) stated that there are several attributes that make endemic plants prone to extinction including environmental deterioration, only a few populations exist, small population size and low genetic variability, narrow distribution range, low reproductive potential, and the need for unique ecological niches as well as stable and nearly constant environmental conditions to grow. These factors make endemic plants more susceptible to both anthropogenic and natural threats, leading them to have a disproportionately high proportion of threatened species (Pitman and Jørgensen 2002). Therefore, endemic plants are top priorities in conservation and are often targeted in conservation efforts at various scales, ranging from single species to landscapes (McDonald et al. 2018; Vargas et al. 2020).

Smilax nageliana A.DC. is a climber species endemic to East Java, Indonesia. This species belongs to the family Smilacaceae and has a narrow geographical range that is known only from two locations: Ranu Darungan of Bromo Tengger Semeru National Park (BTSNP) and Mt. Kawi. *S. nageliana* habitat was found at an altitude of 670 m above sea level (masl) in Ranu Darungan, while in Mt. Kawi, the population occurred from 1100-1400 masl (Sofiah, in press). Locally, this plant is known as Grungungan or Riwono. Some people in Ranu Darungan know it as animal feed. *S. nageliana* might be considered as vulnerable to extinction due to its small population size, narrow distribution and needs for specific environmental conditions, yet there has been no assessment of the conservation status of *S. nageliana* on the IUCN Redlist.

Ex-situ conservation is now increasingly being recognized as an important strategy of conservation to complement *in-situ* approach. It contributes to plant conservation by collecting botanical specimens (e.g., living plants and seed banks) to support phenological observations, horticultural research, and the provision of materials for seed exchange and habitat restoration (Heywood 2017). A botanic garden is an ex-situ conservation institution that plays an important role in conserving various plant species to prevent them from extinction (Cannon and Kua 2017; Chen and Sun 2018; Mounce et al. 2017; O'Donnell and Sharrock 2017; Volis 2017). Globally, botanic gardens have at least 105,634 species of living collection, equating to 30% of all plant species on the Earth, and conserve over 41% of known threatened species (Mounce et al. 2017).

Acclimation is an important step in ex-situ conservation programs that allows plants to cope and adapt to new environmental conditions (Kleine et al. 2021). In the context of botanic garden, there are few studies on plants acclimation (e.g., Rascio et al. 2002; Sahai and Sinha 2020; Sun et al. 2022). Research conducted in botanic gardens has mainly focused on morphological characterization, phenological observation, reproduction technology and conservation assessment (Sun et al. 2022). Therefore, more research on plant acclimation at botanic gardens is required to provide a better understanding of the adaptation mechanisms of plants to the environment under the conditions of a botanical garden. Ideally, acclimation studies need to be carried out in both natural habitats and under controlled greenhouse conditions as a reference for its conservation, both in-situ and ex-situ.

Despite the endemic status with a narrow geographical distribution, *S. nageliana* has not been conserved ex-situ as a living collection in botanic gardens or arboretums (Sofiah 2021, unpublished data; BGCI 2022) nor in its natural habitat (*in-situ*). Research by Sulistyaningsih (2019) showed that the last collections of the species in herbarium were only known in 1942 and 1980, and there has been no recent evaluation. During our recent surveys, the climber was found in both known locations in East Java, and subsequently the living specimens were collected to be conserved in Purwodadi Botanic Gardens. This provides us opportunity to study the species since generally for the Smilax genus there is a lack of information about its germination and seedling growth due to its complex structure (Martins et al. 2012), possibly making them have a low ability to form shoots as well as branching below the soil surface. Thus, the present study aims to study the acclimation of *S. nageliana*. The results of our study will provide information regarding the method of collection, acclimation and cultivation of the climber to support the long-term survival and conservation of the species.

MATERIALS AND METHODS

Species studied

Smilax nageliana A.DC. is a woody climber with prickles on the stem (Figure 1.A). Branches are smooth or not scabrid, but often ornamented with sparse spines. The basal leaf is rounded to truncate with subacute to attenuate apex and 5–7 secondary nerves. The basal petiolar sheath is narrow. Inflorescence has peduncle < 3 cm. Flowers have free 6 stamens and tepals. The species is known only from Ranu Darungan of Bromo Tengger Semeru National Park (BTSNP) and Mt. Kawi in East Java (Figure 1.B) and was last collected in 1980.

Plant materials collection and acclimation

A total of 30 roots of *S. nageliana* were collected from Mt. Kawi in Malang Regency, East Java Province (Figure 1.B). The roots were about 20 cm long with at least two rhizome nodes (Figure 2.A). During the collection, the roots were wrapped with plastic bags containing topsoil and litter collected around the collected individuals. In the acclimation sites, the media of each sampled root was moistened using water and left overnight.

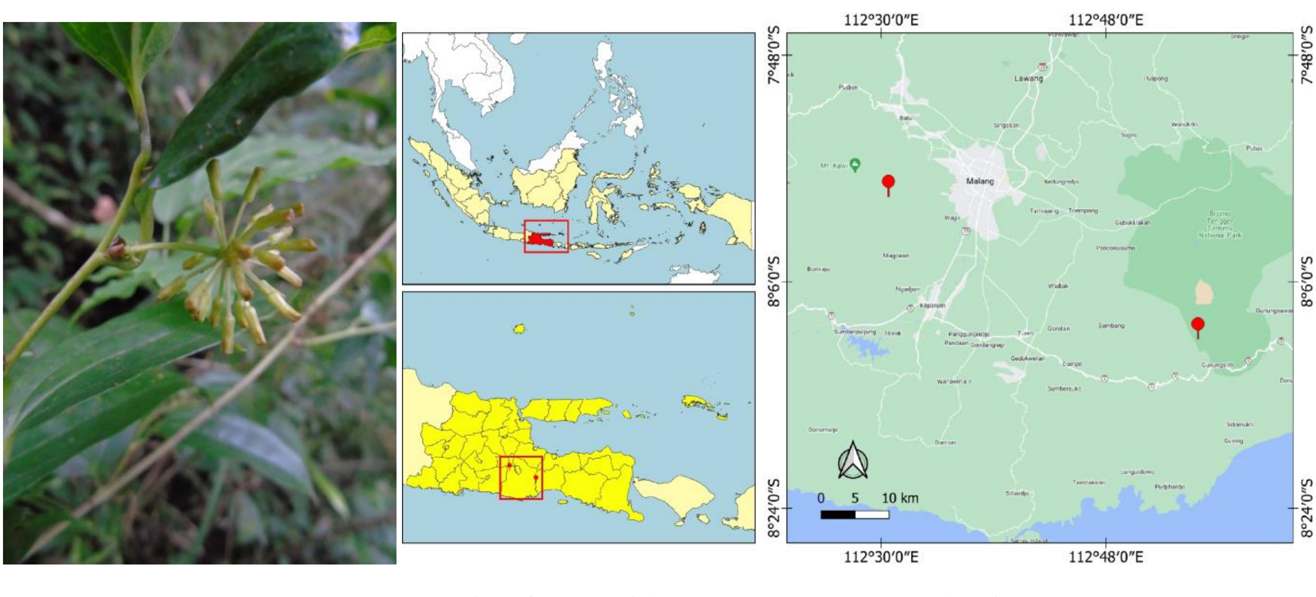

Figure 1. A. *Smilax nageliana* A.DC**.** B. Location of Mt. Kawi (S 07°59'06.9" E112°30'39.2") and Ranu Darungan, Mt Semeru (S 08°10'26.5" E 112°55'26.6"), East Java indicating the known populations of *S. nageliana*

Figure 2. A. Underground system of a single sample of *S. nageliana* plants in the field. Inside the circle, the hard tuberosity produces a root (R) and a rhizome (Rz). The apical bud of the rhizome produces a stem (Ap). B. The roots of *S. nageliana* in a hood application (Photo by Trimanto)

Prior to treatments, the roots of *S. nageliana* were transplanted into plastic bags containing sandy soil and organic fertilizer with a ratio of 1:1. There were three acclimation treatments namely: (i) planted in a hood application (Figure 2.B); (ii) planted in a natural habitat with shading; and (iii) planted in a natural habitat without shading. The first treatment was conducted in a greenhouse at the Purwodadi Botanic Gardens with controlled conditions of light, temperature and air humidity, and this is a standard procedure in the garden when preparing a plant specimen from the field for living collection in the garden. The last two treatments were conducted in Mt. Kawi near the collection site of the individuals. Each treatment has ten individuals. Six months after planting, the experiment was evaluated, and the following variables were measured: number and length of shoot, number of leaves, leaf chlorophyll content, and percentage of survived roots. In addition, the following environmental variables of each treatment were also recorded every two weeks: light intensity (lux), air humidity (%), temperature (°C) and water content.

Data analysis

To assess the effect of each treatment on root's development, data analysis was carried out using a two-way Anova. Significant difference test was carried out using Duncan's test. We also used the same analysis to assess the difference in environmental variables across acclimation sites. Principal Component Analysis (PCA) was used to analyze the relationship between *S. nageliana* acclimation performance and the environmental factors.

RESULTS AND DISCUSSION

Growth of *S. nageliana*

The results of this study indicated that the roots of *S. nageliana* could survive up to 100% when acclimated in shaded natural habitats and in hood application in greenhouses (Table 1). Based on Duncan's test, both treatments also gave the highest result for the number of shoots per root. Similarly, the hood application and shaded natural habitats gave significantly higher chlorophyll content compared to acclimation in the natural habitat without shade. The controlled light and air humidity in the hood application had a highly significant effect on shoot length and number of leaves, and gave the highest result for both parameters compared to the other two treatments.

We found that the growth of new shoots and leaves occurred at a different time in each treatment. The highest number of new shoots in the natural habitat without shade occurred in the fourth week, whereas the highest number of new leaves occurred in the twelfth week (Figure 3). In a natural habitat with shade, the number of new shoots increased in two, sixth, tenth, and twentieth weeks. Meanwhile, the highest number of new leaves occurred in the tenth week. In hood application, the growth of new shoots occurred almost every two weeks and peaked in the first two weeks, which also occurred with the number of new leaves.

Table 1. Survival and growth performance of *S. nageliana* under three acclimation treatments

Treatment	Survival rate	Number of new shoots	Shoot length (cm)	Number of leaves	Chlorophyll content (SPAD)
In natural habitat without shade	40%	0.2^{a}	0.8^{a}	3.46^{ab}	7.13^{a}
In natural habitat with shade	100%	1.7^{b}	3.2^{b}	2.80^{a}	13.92^{bc}
In hood application	100%	1.8^{bc}	6.8^{c}	7.30^{c}	13.25^{b}

Note: Means followed by different letters are significantly different at $p \leq 0.05$

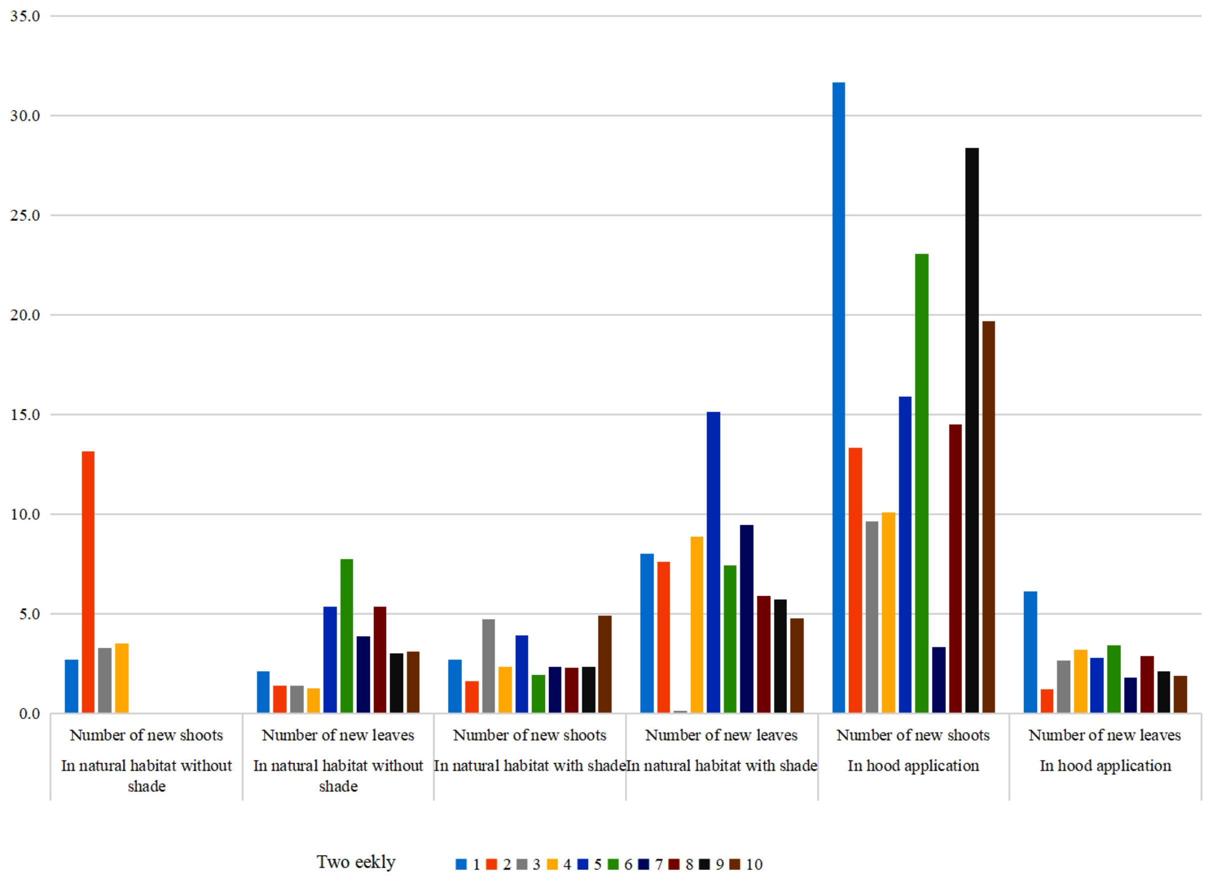

Figure 3. Number of new shoots and leaves in a two-week period across three acclimation treatments

Table 2. The results of the analysis of variance (ANOVE) on the effect of acclimation treatments on *S. nageliana* growth

ANOVA of *S. nageliana*	**Shoot length (cm)**			**Number of leaves**			**Chlorophyll content (SPAD)**		
Quartile Deviation	F calculate	F 0.05		F calculate	F 0.05		F calculate	F 0.05	
Replicate	0.16	2.46	NS	0.16	2.46	NS	0.38	2.46	NS
Treatment	10.06	3.55	HS	10.06	3.55	HS	4.27	3.55	S

Notes: NS = not significant; S = significant; HS = highly significant

Based on the analysis of variance, acclimation treatments had a highly significant effect on shoot height and number of leaves and a significant effect on chlorophyll content while replication had no significant effect on the three parameters (Table 2).

The measurement of environmental factors showed that all environmental factors had a significant effect on the acclimation of *S. nageliana* roots (Table 3). The solar intensity in habitats without shade certainly had the greatest value compared to that in natural habitats with shade or in the hood application. The average temperature also had a significant effect on the acclimation process of *S. nageliana* roots in which natural habitats without shade and hood application gave the most optimal results compared to habitats with shade. In acclimation of *S. nageliana* roots, the application of a plastic cap has given the effect of a warmer temperature compared to natural habitats with shade. Similarly, humidity also had a significant influence on the acclimation of *S. nageliana* roots in which hood application treatment gave the highest results for the effect of humidity on the acclimation of *S. nageliana*. Water content in open habitats had a significant effect, especially in areas of habitat without shade.

The PCA results of environmental factors on the acclimation of *S. nageliana* roots are shown in Figure 4. The PCA results shows that the environmental factor which had quite closely related to the acclimation of *S. nageliana* was water. This water factor had a close and positive relationship to the number of shoots and leaf chlorophyll content in their natural habitat, which was indicated by the acute angle between the water line and leaf chlorophyll content.

Table 3. Comparison of the mean value of environmental factors across three acclimation treatments of *S. nageliana*

Environmental Factors	Solar Intensity (Lux)			Temperature average (℃)			Humidity (%)			Water (mm)		
	In natural habitat without shade	In natural habitat with shade	In hood application	In natural habitat without shade	In natural habitat with shade	In hood application	In natural habitat without shade	In natural habitat with shade	In hood application	In natural habitat without shade	In natural habitat with shade	In hood application
Mean Square	24.063	4882	1897	82.38	26.48	82.22	9.8	69.3	504	3.362	53.5	4.011
Standard Deviation	0.553	9327	1581	3.93	1.996	6.7	16.25	6.68	2092	2.642	38.7	2.779
F-Value	13.41			1009.7			45.67			8.02		
P-Value	0			0			0			0.004		
	*			*			*			*		

Note *): The null hypothesis of the equal population mean is rejected when p-value ≤0.05

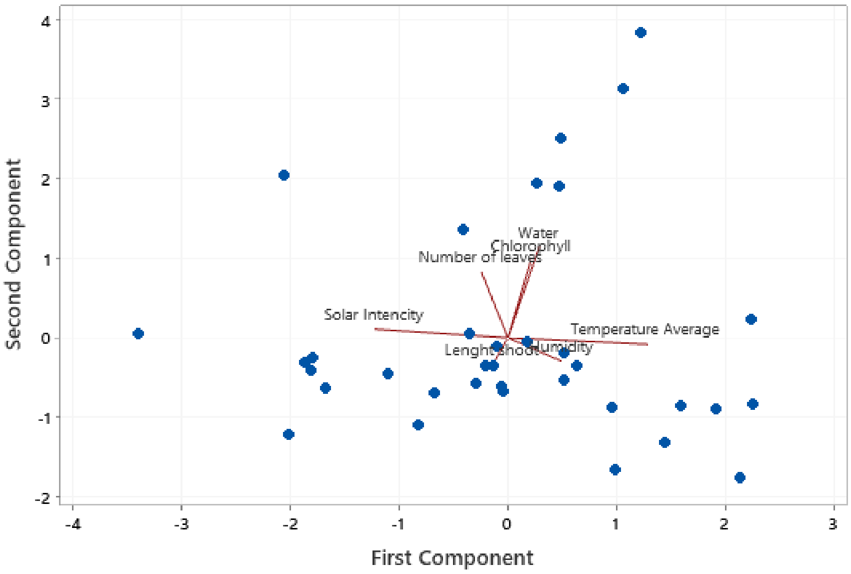

Figure 4. The results of Principal Component Analysis (PCA) of the relationship between *Smilax nageliana* acclimation performance and environmental factors

Table 4. Eigenvalues correlation matrix of environmental factors on the acclimation of *S. nageliana*

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	1.8945	1.7857	1.3041	0.931	0.7513	0.2025	0.1309
% Proportion	27.1	25.5	18.6	13.3	10.7	2.9	1.9
Light intensity	-0.647	0.06	0.099	0.271	-0.192	-0.677	-0.014
Temperature average	0.675	-0.045	0.082	-0.069	0.155	-0.706	-0.09
Humidity	0.261	-0.166	-0.046	0.93	-0.107	0.129	0.094
Water	0.153	0.641	0.162	0.077	-0.423	0.098	-0.587
Length Shoot	-0.073	-0.19	-0.824	0.017	0.076	-0.072	-0.519
Number of Leaves	-0.129	0.466	0.038	0.223	0.841	0.024	-0.086
Chlorophyll content	0.112	0.55	-0.525	-0.028	-0.192	-0.105	0.601

The Eigenvalues correlation matrix of environmental factors on the acclimation of *S. nageliana* is shown in Table 4. The first component explained 27.10% of all observed variables while the second component explained 25.50%. Table 4 shows that the proportional value of the first component is higher than that of the second component, meaning that the first component provides more information to describe *S. nageliana* acclimation performance than the second component. The average temperature was quite influential on the first component (PC1), followed by solar intensity while water affected the second component (PC2).

Based on Table 4, the model of the acclimation of *S. nageliana* can be formulated as PC1= -0.647 solar intensity + 0.675 temperature average + 0.261 humidity + 0.153 water - 0.073 shoot length - 0.129 number of leaves +

0.112 chlorophyll content. PC2= 0.060 solar intensity - 0.045 temperature average - 0.166 humidity + 0.641 water - 0.190 length shoot + 0.466 number of leaves + 0.550 chlorophyll content.

Discussion

Plant propagation can be done sexually or asexually. In the process that occurs in nature, most plants reproduce sexually (Roberto 2020). In plant propagation, acclimation is one of the most important stages, because it is the stage when plants must adapt to a different growing environment from the usual environment. In many plant species, the acclimation process is generally carried out in conditions of high air humidity with stable and constant temperature and intensity of sunlight. However, when the acclimation stage is completed, the plant will be in a new growing environment where the humidity, temperature and air temperature are no longer in a constant state (do Vale 2019). Acclimation of *S. nageliana* by the roots, which ensures retention of the good traits of the mother plant to the offspring (Tsaktsira 2021) and mitigating possible changes in the production of secondary metabolites, is one of the biggest challenges in the cultivation and management of this potentially medicinal *Smilax* species (Soares 2011).

Changes in plant morphology for management and conservation purposes, including changing plant stems, can be influenced by metabolic processes in plants and external factors such as temperature, humidity, stress, etc. (Sidhiq 2020; Hovenden 2012). In our acclimation trial, the result showed that the highest shoot length of *S. nageliana* was in hood application with an average 6.8 cm. In a dense canopy, the air humidity may be higher than in an open area. The relative humidity is very important for optimal plant growth since this is related to the rate of photosynthesis. In conditions of high relative humidity, it will reduce water pressure in the leaves, thereby increasing the stomatal conductance. The relative humidity is directly related to CO_2 acclimation through stomata response, which in turn is related to the process of nutrients produced so that it will affect plant growth. Ahmed et al. (2020) reviewed several studies and reported that a relative humidity of lower than 40% and higher than 85% causes malfunctioning of stomata, inhibiting plant growth rate and photosynthesis. In this study, the air humidity in the hood ranged from 64%-86% and this had a significant effect on the shoot length of *S. nageliana.*

It is well known that one of the most influencing environmental factors on shoot production success is the climate (light, temperature, moisture, etc.) (Garel 2014). Those factors caused changes in leaf structure, plant water status, and photosynthetic parameters (Table 3). The light was not a critical factor affecting the growth of seedlings of *S. nageliana* which usually occurs in shaded areas. *S. nageliana* likes places with high relative air humidity which ranges between 52% and 90%, with light shade (Sofiah, in press). Pogge & Bearce (1989) tested different light conditions for the germination of two *Smilax* species. They observed that species that occurred in open areas (*Smilax glauca*) needed more light to germinate than *Smilax rotundifolia*, which could be found in shaded areas. According to the authors, a light seemed to be a limiting factor for the germination of *Smilax glauca.*

The leaf is a starting point of net carbon-importing structure and remains so until photosynthetic activities are fully developed and the peak demand of photosynthates for the assembly of cells has subsided. Low light intensity decreases the chlorophyll content in plants (Park 2018), but not so with *S. nageliana*. With all the same age of shoots, chlorophyll content of *S. nageliana* roots gave the highest result in hood application and in natural habitat with light shaded, while in open natural areas was low. In mature and old leaves, chlorophyll content was significantly higher under low light than under optimal light (Sui 2012), which was mainly attributed to the slower unfolding of low-light-grown leaves. Many studies attributed the lower carbon assimilation capacity of young leaves to the absence of a fully functional photosynthetic apparatus, and low photosynthetic rates were associated with high rates of dark respiration indicating limited photosynthesis in the young leaves (Marino 2018). In this study, the gradual increase of photosynthesis (Photo System II/PS II) accompanied with an increase in chlorophyll content was clearly observed with leaf expanding under low light, suggesting the well development of photosynthetic apparatus, such as electron transport and carbon assimilation process. In this study, the existence of shady conditions with open air made a higher intake of carbon dioxide (CO_2), even though the sunlight is low, affecting the addition of ATP and NADPH in the photosynthesis process. The CO_2 concentration affects the rate of photosynthesis, metabolism, and physiological and chemical resistance of plants (Singh 2015), because CO_2 is one of the main components in the formation of plant nutritional energy. A lack of CO_2 not only results in low plant biomass, but reduces plant quality and resistance, because CO_2 is absorbed directly by plants through leaf stomata. The CO_2 also influences the transpiration process of plants. The treatment in hood application gave highly significant chlorophyll content of *S. nageliana*. The most direct impact of weak light is on changes in chlorophyll content that affect the rate of photosynthesis, because the light is a source of energy in the formation of plant nutrients (Sui 2012). Correspondingly, many reports, including this study, support the view that the decrease in PS II may represent a mechanism of down-regulated photosynthetic electron transport to match the decreased CO_2 assimilation capacity (Xiao-lei 2012).

Apparently, light is a limiting factor for the growth of *S. nageliana*. The highest chlorophyll content was found in the hood application and in a natural habitat with shade, indicating that light is a limiting factor in the growth of *S. nageliana*, but carbon dioxide (CO_2) and water (resulting from humidity) support the high leaf chlorophyll content, which is obtained through photosynthesis. So in its natural habitat in the forest (Mt. Kawi and Mt. Semeru), *S. nageliana* was mostly found in forests shaded by trees (Sofiah, in press). In the hood application in the greenhouse, the chlorophyll content was significantly different from the natural habitat (Table 1). Low light treatment leads to a decrease in the light compensation point and dark respiration rate, indicating that plants can adapt to a low light environment by improving efficiency in using low irradiance and reducing the consumption of photosynthates (Xiao-lei 2012). However, the effects of low light on photosynthetic gas exchange and photosynthetic efficiency, especially chlorophyll fluorescence related to photochemical efficiency during leaf development are unclear. The ability of *S. nageliana* to acclimate through the use of roots and shoots regeneration gave results 100% survival living in a natural habitat with light shade and in the hood application. This research shows that acclimation is a process of adjusting plant growth in leaves and shoots. Light conditions that can change over time will make plants have different responses to it (Liu 2016). The consequences for the process of photosynthesis, in particular, are sensitive to changes in light, and even other environmental factors such as humidity or air temperature, so the acclimation process will give a different response (Figure 3).

In this study, Principal Component Analysis (PCA) was used to investigate the influencing environmental factors, which consisted of four variables, namely solar intensity, temperature, humidity, and water. Two principal components can explain 52.60% of all environmental variables measured. Chlorophyll is the green coloring matter which enables photosynthesis to occur in the presence of light (Sarkar 2021). The leaf chlorophyll content is an important indicator of direct foliage damage and is strongly related to plant productivity (Du 2017). As a fundamental metabolism, leaf photosynthesis not only provides the necessary energy for plant survival and growth but also plays an important role in carbon fixation (Teng 2022). Certain species of *Smilax* are often found on the edge areas of forest openings (Sofiah 2019), for example *Smilax gigantea* Merr. The edge area is an intersection between two different types of environment or ecosystem which occurs between two different forms of habitat or landscape (Vinter 2013). *Smilax* species are also often found in areas with low light conditions and shaded trees (Havrilchak 2018), for instance, *Smilax rotundifolia* and *S. nageliana* (Sofiah, in press). These two species of *Smilax* are able to make optimal use of light sources even in low light conditions. In this study, it was seen that *S. nageliana* grew more optimally in shady areas.

This work has studied the acclimation of *S. nageliana* under three different types of treatment. The results showed that *S. nageliana* acclimation was most suitable in low-light areas. This finding suggests the possibility of developing the cultivation of *S. nageliana* as an endemic species, both in its natural habitat area and outside its habitat. In the treatment where light and humidity were controlled, it gave the most optimal results for the growth of *S. nageliana* roots.

ACKNOWLEDGEMENTS

The authors would like to acknowledge to National Agency For Research and Innovation for the funding support of the research through Saintek Scholarship.

REFERENCES

Ahmed HA, Yu-Xin T, Qi-Chang Y. 2020. Optimal control of environmental conditions affecting lettuce plant growth in a controlled environment with artifificial lighting: A review. South Afr J Bot 130: 75-89. DOI: 10.1016/j.sajb.2019.12.018.

BGCI. 2022. PlantSearch online database. Botanic Gardens Conservation International, Richmond, U.K. www.bgci.org/plant_search.php

Cannon CH, Kua CS. 2017. Botanic gardens should lead the way to create a “Garden Earth” in the Anthropocene. Plant Divers 39 (6): 331-7. DOI: 10.1016/j.pld.2017.11.003.

Chen G, Sun W. 2018. The role of botanical gardens in scientific research, conservation, and citizen science. Plant Divers 40 (4): 181-8. DOI: 10.1016/j.pld.2018.07.006.

Do Vale PAA, de Oliveira Júnior JB, da Silva Costa FH , Scherwinski-Pereira JE. 2019. Height and number of shoots on the survival and development of micropropagated bamboo plantlets during pre-acclimatization. Pesq Agropec Trop. Goiânia: 49. www.agro.ufg.br/pat. DOI: 10.1590/1983-40632019v4953751.

Du E, Dong D, Zeng X, Sun Z, Jiang X, de Vries W. 2017. Direct effect of acid rain on leaf chlorophyll content of terrestrial plants in China. Sci Tot Env 605-606. DOI: 10.1016/j.scitotenv.2017.06.044.

Garel M, Bouvet JM, Denis M, Saya A, Mankessi F, Vigneron P. 2014. Assessing the additive and dominance genetic effects of vegetative propagation ability in *Eucalyptus*—Influence of modeling on genetic gain. Tree Genet Gen. DOI: 10.1007/s11295-014-0757-6.

Havrilchak NA, Schedlbauer JL. 2018. Plant physiological changes along an encroachment gradient: an assessment of US Mid-Atlantic serpentine barrens. J Plant Ecol 11 (6): 853-865. DOI: 10.1093/jpe/rtx040.

Heywood VH. 2017. The future of plant conservation and the role of botanic gardens. Plant Divers 39 (6): 30. DOI: 10.1016/j.pld.2017.12.002.

Hovenden MJ, Vander JKV, Osanai Y. 2012. Relative humidity has dramatic impacts on leaf morphology but little effect on stomatal index or density in *Nothofagus cunninghamii* (Nothofagaceae). Austr J Bot 60 (8): 700-706. DOI: 10.1071/BT12110.

Işik K. 2011. Rare and endemic species: why are they prone to extinction?. Turkish J Bot 35 (4): 411-7. DOI: 10.3906/bot-1012-90.

Kleine T, Nägele T, Neuhaus HE, Schmitz-Linneweber C, Fernie AR, Geigenberger P, Grimm B, Kaufmann K, Klipp E, Meurer J, Möhlmann T. 2021. Acclimation in plants–the Green Hub consortium. Plant J 106 (1): 23-40. DOI: 10.1111/tpj.15144.

Liu W, Su J. 2016. Effects of light acclimation on shoot morphology, structure, and biomass allocation of two Taxus species in southwestern China. Sci Rep 6: 35384. https://rdcu.be/cOz8r. DOI: 10.1038/srep35384.

Marino G, Mantia ML, Caruso T, Marra FP. 2018. Seasonal dynamics of photosynthesis and total carbon gain in bearing and nonbearing pistachio (*Pistacia vera* L.) shoots. Photosynthetica 56 (3): 932-941. DOI: 10.1007/s11099-017-0752-x.

Martins AR, Soares AN, Bombo AB, Fidelis A, Novembre ADL, da Glória BA. Germination and seedling morphology of four South American *Smilax* (Smilacaceae). 2012. Intl J Trop Biol 60 (1): 495-504. DOI: 10.15517/rbt.v60i1.2784.

McDonald RI, Güneralp B, Huang CW, Seto KC, You M. 2018. Conservation priorities to protect vertebrate endemics from global urban expansion. Biol Conserv 224: 290-9. DOI: 10.1016/j.biocon.2018.06.010.

Mounce R, Smith P, Brockington S. 2017. Ex situ conservation of plant diversity in the world’s botanic gardens. Nat Plants 3 (10): 795-802. DOI: 10.1038/s41477-017-0019-3.

Nilawati TS, Surakusumah W, Annisya V. 2015. Land suitability for endemic plants in Kiara Payung Biodiversity Park, West Java. Nusantara Biosci 7 (2): 112-117. DOI: 10.13057/nusbiosci/n070209.

O'Donnell K, Sharrock S. 2017. The contribution of botanic gardens to ex situ conservation through seed banking. Plant Divers 39 (6): 373-8. DOI: 10.1016/j.pld.2017.11.005.

Park SG, Matsumoto MA. 2018. Study on the effects of light conditions on the longevity and characteristics of *Daphniphyllum macropodum* leaves. J Fac Agric 63: 15-19. DOI: 10.5109/1909897.

Pitman NCA, Jørgensen PM. 2002. Estimating the size of the world’s threatened flora. Science 298: 989. DOI: 10.1126/science.298.5595.989.

Pogge FL, Bearce BC. 1989. Germinating common and cat greenbrier. Tree Planters' Notes 40: 34-37.

Rascio N, Camani A, Sacchetti L, Moro I, Cassina G, Torres F, Cappelletti EM, Paoletti MG. 2002. Acclimatization trials of some *Solanum* species from Amazonas Venezuela at the botanical garden of Padova. Econ Bot 56 (4): 306-314. DOI: 10.1663/0013-0001(2002)056[0306:FAATOS]2.0.CO;2.

Roberto S, Colombo RC. 2020. Innovation in propagation of fruit, vegetable and ornamental plants. Horticulturae 6 (23): 1-8. DOI: 10.3390/horticulturae6020023.

Sahai PR, Sinha VB. 2020. Endangered *Taxus baccata* acclimitized in its non-native conditions pushing forward its ex situ conservation. Ann Agri Bio Res 25 (2): 194-7.

Sun Q, Lai L, Zhou J, Liu X, Zheng Y. 2022. Ecophysiological leaf traits of forty-seven woody species under long-term acclimation in a botanical garden. Plants 11 (6): 725. DOI: 10.3390/plants11060725.

Sarkar SD, Sarkar UK, Naskar M, Roy K, Bose AK, Nag SK, Karnatak G, Das BK. 2021. Effect of climato-environmental parameters on chlorophyll a concentration in the lower Ganga basin, India. Rev Biol Trop (Intl J Trop Biol) 69 (1): 60-76. DOI: 10.15517/rbt.v69i1.42731.

Sidhiq DF, Widiyastuti Y, Subositi D, Pujiasmanto B, Yunus A. 2020. Morphological diversity, total phenolic and flavonoid content of *Echinacea purpurea* cultivated in Karangpandan, Central Java, Indonesia. Biodiversitas 21 (3): 1265-1271. DOI: 10.13057/biodiv/d210355

Singh SK, Reddy VR, Sharma MP, Agnihotri R. 2015. Dynamics of plant nutrients, utilization and uptake, and soil microbial community in crops under ambient and elevated carbon dioxide. Nutrient Use Effificiency. Springer India, New Delhi, India. DOI: 10.1007/978-81-322-2169-2_24.

Soares AN, NovembreII ADLC, Martins AR, Piedade SMS, Appezzato-da-Gloria B. 2011. Propagation studies in *Smilax fluminensis* Steud. (Smilacaceae) Ciência Rural Santa Maria 41 (10): 1762-1768. DOI: 10.1590/S0103-84782011001000014.

Sofiah S, Sulistyaningsih LD. 2019. The diversity of *Smilax* (Smilacaceae) in Besiq-Bermai and Bontang Forests, East Kalimantan, Indonesia. Biodiversitas 20 (1): 379-387. DOI: 10.13057/biodiv/d200145.

Sulistyaningsih LD. 2019. The Systematic Study of Smilacaceae in Java Based on Morphology and Molecular Character. [Dissertation]. Universitas Indonesia, Depok. [Indonesian]

Sui XL, Mao SL, Wang LH, Zhang BX, Zhang ZX. 2012. Effect of low light on the characteristics of photosynthesis and chlorophyll a fluorescence during leaf development of sweet pepper. J Integr Agri 11: 1633-1643. DOI: 10.1016/S2095-3119(12)60166-X.

Tsaktsira M, Chavale E, Kostas S, Pipinis E, Tsoulpha P, Hatzilazarou S, Ziogou FT, Nianiou-Obeidat I, Iliev I, Economou A, Scaltsoyiannes A. 2021. Vegetative propagation and ISSR-based genetic identifification of genotypes of *Ilex aquifolium* 'Agrifoglio Commune'. Sustainability 13: 1-15. DOI: 10.3390/su131810345.

Teng L, Liul H, Chul X, Song S, Shi L. 2022. Effect of precipitation change on the photosynthetic performance of *Phragmites australis* under elevated temperature conditions. PeerJ 10: e13087 DOI: 10.7717/peerj.13087.

Vargas P, Jimenez-Mejias P, Fernandez-Mazuecos M. 2020. Endangered living fossils'(ELFs): Long-term survivors through periods of dramatic climate change. Environ Experimen Bot 170: 103892. DOI: 10.1016/j.envexpbot.2019.103892.

Vinter T. 2013. Edge effects on plant species diversity in forest landscapes. Plants and Ecology. The Department of Ecology, Environment and Plant Science, Stanford University, Stanford, CA.

Volis S. 2017. Conservation utility of botanic garden living collections: Setting a strategy and appropriate methodology. Plant Divers 39 (6): 365-72. DOI: 10.1016/j.pld.2017.11.006.

Xiao-lei S, Sheng-li M, Li-hao W, Bao-xi Z, Zhen-xian Z. 2012. Effect of low light on the characteristics of photosynthesis and chlorophyll a fluorescence during leaf development of sweet pepper. J Integr Agri 11 (10): 1633-1643 DOI: 10.1016/S2095-3119(12)60166-X.