RECENT TECHNOLOGY IN CULTIVATING PIPER BETLE VAR NIGRA. A COMPREHENSIVE REVIEW
/
TEKNOLOGI PEMBIAKAN TERKINI PIPER BETLE VAR NIGRA. SEBUAH REVIEW LENGKAP

Dewanto HARJUNOWIBOWO*[1)], Anif JAMALUDDIN[1)], Yudi RINANTO[2)], Fajar PRASETYA[3)] [1]

[1)]Physics Education, Universitas Sebelas Maret/ Indonesia;
[2)]Biology Education, Universitas Sebelas Maret/ Indonesia
[3)]Faculty of Pharmacy, Universitas Mulawarman/ Indonesia
Tel: (62) 82258919195; E-mail: dewanto_h@staff.uns.ac.id
Corresponding author: Dewanto Harjunowibowo
DOI: https://doi.org/10.35633/inmateh-69-25

Keywords: *Betel vine, Piper betle var. Nigra, cultivation, human health, herbs*

ABSTRACT
The betel vine, a species of Piper, has organic impacts on human health through its secondary metabolites. Historically, it has been used to treat various illnesses including liver and stomach problems, inflammation, and as an antioxidant, antipyretic, and antimicrobial. Despite its medicinal benefits, the black betel vine (Piper betle var. Nigra) is not widely cultivated. This review summarises traditional and advanced methods of betel cultivation, including the habitats and diseases affecting Piper Betle L. There are two cultivation systems: closed-type and open-type, each with its own advantages and disadvantages. The modern method of propagation through callus induction is gaining popularity, but requires specialised knowledge and is more costly. Traditional cultivation is less complicated, but exposes young plants to disease. Implementing smart farming practices, maintaining optimal growth conditions, and utilizing a screen house with artificial shading and lighting may lead to higher-quality crops with reduced losses. Before planting, the cuttings should be soaked in a fungicide mixture and grown as individual plants to avoid bacterial leaf blight from occurring.

ABSTRAK
Tanaman betel, spesies dari Piper, memiliki dampak alami pada kesehatan manusia melalui metabolit sekundernya. Sejarahnya, tanaman ini digunakan untuk mengatasi berbagai penyakit termasuk masalah hati dan lambung, inflamasi, dan sebagai antioksidan, antipiretik, dan antimikroba. Meskipun memiliki manfaat obat, tanaman sirih hitam (Piper betle var. Nigra) tidak banyak dibudidayakan. Tinjauan ini menguraikan metode budidaya betel tradisional dan modern, termasuk habitat dan penyakit yang mempengaruhi Piper Betle L. Ada dua sistem budidaya: tipe tertutup dan tipe terbuka, masing-masing memiliki kelebihan dan kekurangan. Metode modern reproduksi melalui induksi callus sedang populer, tetapi membutuhkan pengetahuan khusus dan lebih mahal. Budidaya tradisional lebih sederhana, tetapi membuat tanaman muda rentan terhadap risiko penyakit. Penerapan praktik pertanian cerdas, menjaga kondisi pertumbuhan yang optimal, dan memanfaatkan screen membran dengan pencahayaan buatan dapat menghasilkan tanaman berkualitas tinggi dengan kerugian yang lebih sedikit. Sebelum ditanam, potongan tanaman harus direndam dalam campuran fungisida dan harus ditanam sebagai satu tanaman untuk menghindari terjadinya penyakit bakteri layu.

INTRODUCTION
Recently, the concept of utilising natural medication (*Kano et al., 2017)* and preserving food has gained increased popularity (*Salehi et al. 2019; Sahlan, Mandala et al., 2020*), particularly in the era of the COVID-19 pandemic. Science has developed new methods for evaluating herbs utilised in different cultures *(Salehi et al., 2018; Madhumita, Guha and Nag 2020*). The pharmacological properties of plant life, such as food, medicine *(Daud et al., 2020)*, energy *(Ayadi et al., 2020; Sahlan, Muryanto et al. 2020; Kuncoro and Purwanto, 2020*), industrial *(Izzah et al., 2020*), and religious functions, have been demonstrated through improved analysis methods over the centuries (*Dwivedi and Tripathi, 2014; Shukla et al., 2015*).

[1] *Dewanto Harjunowibowo, Assistant Professor; Anif Jamaluddin, Assistant Professor; Yudi Rinanto, Associate Professor; Fajar Prasetyo, Associate Professor.*

The secondary metabolites of Piper betle L. plants *(Prasetya et al., 2022)* such as flavonoids, polyphenols, tannins, saponins, alkaloids, and steroids have significant impacts on human health, exhibiting antimicrobial (*Prasetya et al., 2021*), anti-rheumatic *(Murugesan et al., 2020)*, anti-diabetic, anti-thrombin and Cytotoxic bioactive *(Yogeswari et al., 2020*), anti-cancer (*Wang et al., 2014*), anti-tumour *(Kangralkar and Kulkarni, 2013*), anti-asthma *(Hajare et al., 2011)*, anti-oxidant and anti-fungal *(Li Y. et al. 2020)*, gastroprotective (*Arawwawala, Arambewela, and Ratnasooriya, 2014*), and anti-bacterial *Bintang et al.* (2014);*Taukoorah et al.* (2016) (in mouth) properties (*Teanpaisan et al, 2016)*.

The genus Piper is an abundant group of plants found in pantropical regions such as India (*Sengupta, 2019*), Indonesia (*Patra et al., 2016*), and Sri Lanka (*Arambewela et al., 2011*). These species are classified as aromatic plants due to the presence of essential oil-producing cells (*Dyer and Palmer, 2004*). Chemical analysis of a fresh leaf revealed that it consists of 85.4% moisture, 6.1% carbohydrates, 3.1% protein, 2.3% minerals, 2.3% fibre, and 0.8% fat. Additionally, the leaf contains various minerals and vitamins, including magnesium, iron, carotene, calcium, niacin, riboflavin, vitamin C, and thiamine *(Shah et al., 2016*). Betel leaves are consumed by 2 billion people in India annually and have a significant economic impact, contributing to the national income to the tune of Rs 7000 million annually (*Das et al., 2016*).

In fact, the information about the various biological activities of piper plants comes from in vitro *(Muttaleb et al., 2018; Ghazali et al., 2016; Prasetya et al., 2021; Murugesan et al., 2020; Kangralkar and Kulkarni, 2013*), while cultivation studies are still limited. However, these plants have potential for multiple applications, and the mechanisms of their fundamental cultivation are yet to be investigated. Among the species, cultivation of Piper betle is the most challenging (*Raja et al., 2018*). In particular, the cultivation of betel vine, especially black betel vine, is not yet done effectively and efficiently. Therefore, this current work reviews available literature on traditional and advanced methods of cultivation, specifically for *Piper betle var Nigra*.

MATERIALS AND METHODS

The manuscript has been compiled through a comprehensive literature review. To determine the current understanding of the potency and cultivation of black betel vines, over a hundred articles from various sources around the world were analysed. The literature review commenced with an examination of the potential and utilisation of black betel vines, followed by a general overview of the factors affecting plantation growth. This was followed by a discussion of traditional and contemporary techniques for farming Piper betle. For the reader's convenience, brief findings were provided in each sub-discussion, leading to a comprehensive conclusion based on the results obtained.

RESULTS AND DISCUSSION

1. General factors influencing the plant's growth

The quality of medicinal plants is verified by their prevalent hereditary properties and also large biomass, with reliable and high content of secondary metabolites (*Mosaleeyanon et al., 2005b*). The concentration and chemical profile of secondary metabolites in plants grown outdoors are affected by environmental conditions such as temperature, light quality and intensity *(Mosaleeyanon et al., 2005b; Kozai, Afreen, and Zobayed, 2005*).

The characteristics of in vitro plants in terms of physiology and anatomy are related and primarily governed by the microclimate of the culture container, which includes factors such as relative humidity, light, air movement within the container, carbon dioxide, and the nutrient composition of the medium. All of these factors interact and influence the growth and output of seedlings *(Salehi et al., 2019*).

1.1. Light

All organisms that possess chlorophyll require sunlight to synthesise organic compounds such as carbohydrates. These organic compounds serve as an energy source for cellular mechanisms in plants and for other forms of life. Photosynthesis, the process by which plants synthesise organic compounds from light, occurs in the visible light spectrum (*Singh et al., 2015*) between 400 nm-700 nm (*Bhatia, 2014*). Hence, light is a crucial factor in the production of fruits and vegetables in greenhouse farming (*Messinger and Lauerer, 2015; Choi, Moon, and Kang, 2015; Cossu et al., 2014; Mosaleeyanon et al., 2005a; Rahman, Prihantini, and Nasruddin, 2020)*. In greenhouse cultivation, the choice of cladding material for the facade is a critical factor in providing natural light. Some authors have recommended the use of glass, polyethylene, acrylic, and polycarbonate cladding materials, which have transmission percentages of Photosynthetically Active Radiation (PAR) of 90%, 88%, 86%, and 75%, respectively *(Both, 2013)*.

Under controlled environmental conditions, an increase in photosynthetic photon flux (PPF) and CO_2 concentration leads to an increase in the leaf net photosynthetic rate (*Mosaleeyanon et al., 2005b*). The addition of artificial light in controlled environments enables the maximisation of plant output while minimising the release of resources and pollutants into the environment, thus reducing environmental degradation (*Hoque, 2022*).

Additionally, it facilitates the planning of plant growth, scheduling, and production, and reduces the risk of contamination from diseases, metals, insects, and other harmful substances *(Toyoki Kozai, 2020*).

Studies have shown that environmental factors such as CO_2 concentration and light intensity can have a significant impact on metabolite concentrations. For example, elevated light intensity of 400 mmol $m^{-2}s^{-1}$ leads to a significant increase in hypericin concentration in St. John's *wort* plants *(Briskin and Gawienowski, 2001*). Kurata et al. found that high light irradiation enhances the production of purine alkaloids in *Coffea arabica* cell suspension cultures (*Kurata, Matsumura, and Furusaki, 1997*), and the production of anthocyanin in *Perilla frutescens* cell culture (*Zhong et al., 1991*).

LED lighting has been shown to enhance lettuce growth when using a Red-Blue-White (RBW) spectrum (*Lin et al., 2013*). The highest yield of volatile terpenoids in vegetable plants (*Gynura bicolour*) was obtained using RB20 LED lighting under ambient CO_2 (*Ren, Guo, Xin et al., 2014*). The quality of broccoli seedlings was found to be improved more by red LED light compared to white or blue light *(Wilson, Iwabuchi, & Rajapakse, 1998; Wilson, Iwabuchi, Rajapakse, et al., 1998)*. Despite this, LED lighting is regarded as the most suitable light source for crop cultivation *(Bula et al., 1991; Singh et al., 2015)*.

Ouzounis et al. conducted a study to examine the impact of light spectrum on plant growth and secondary metabolites in *Chrysanthemum morifolium*, *Campanula portenschlagiana*, and *Rosa* hybrida, with a focus on photosynthesis. The findings showed that the levels of flavonoids and phenolic acids increased when the plants were exposed to higher ratios of blue light from LED sources *(Ouzounis et al. 2015; Matysiak, 2021*). It is well established that these compounds play a crucial role in plant stress defence and pathogen resistance, as well as contributing to pigmentation (*Jensen et al., 2011*). Furthermore, the study also revealed that a 40% blue LED light ratio was less supportive of terpene accumulation in the roots of *Gynura bicolour* compared to a 20% ratio *(Ren, Guo, Cheng, et al., 2014)*.

1.2. Brief findings on light effect against plants

Blue light helps plants better handle stress due to the function of secondary metabolites. Additionally, blue LED lighting is more practical for low-temperature storage due to its smaller light fixture size and lower reduction in irradiance compared to fluorescent lamps. If cost-effective, using blue LED lights under dim conditions in low-temperature environments would be ideal for various agricultural operations, including tissue culture production.

1.3. Carbon dioxide (CO_2) concentration

Seedling growth in vitro is reliant on both the CO_2 levels in the growing microclimate (endogenous source) and the sugars in the growth medium (exogenous source), known as *photomyxotrophic* growth. Photoautotrophic growth occurs when CO_2 serves as the sole carbon source through photosynthesis in an air microclimate culture *(Afreen, Zobayed, and Kozai, 2001*). As plants grow, their CO_2 demand increases, leading to enhanced net photosynthetic rate, growth, rooting, and development through CO_2 enrichment. Stomata density also increases significantly in photoautotrophic conditions with CO_2 enrichment *(Kirdmanee, Kitaya, and Kozai, 1995*).

A recent study showed that growing St. John's *wort* in 1500 mmol/mol CO_2-enriched conditions boosted secondary metabolite production and net photosynthetic rate (*Zobayed and Saxena, 2004*). Kim et al. similarly increased the production of secondary metabolites (berberine) in Thalictrum rugosum non-photosynthetic cell culture grown in a CO_2-enriched air transport bioreactor (*Kim, Pedersen, and Chin, 1991*). Plant secondary metabolites are valuable natural compounds and play a critical role in stress defence and pathogen resistance (*Yang et al., 2018*).

The development and survival of in-vitro generated plants require a substantial root system. CO_2 increase (450 to 1200) enhances phenol production including anthocyanin, flavonoids, and phenolics. However, further increase in CO_2 (1200 to 2000) seems to negate this promotion *(Ren, Guo, Xin, et al., 2014*). Modifying storage atmosphere (low O_2 and CO_2) can effectively preserve vegetable soybeans' green colour and mass (*Makino et al., 2020*). The CO_2 storage fertiliser (CO_2SMs), derived from captured CO_2, applied as an energy source has been shown to significantly increase stem diameter, height, and root growth of vegetable crops (*Zhao et al., 2018*).

Increased CO_2 (827 mmol mol^{-1}) can result in 34% higher vegetable yields and reduce drought stress (*Dong et al., 2020*). However, elevated CO_2 enrichment has no effect on the constant ratio of night respiration to day photosynthesis (*Nomura et al., 2021*). Moreover, increased CO_2 can increase DDT absorption in the shoots of B. Lee and B. Bailey, posing a risk to human health upon consumption (*Wu et al., 2019*).

1.4. Brief findings on CO_2 effect against plants

Elevated CO_2 levels improve photosynthetic rate, resulting in increased biomass and nutrient content and improved tolerance to drought stress in plants. However, prolonged exposure to high levels of CO_2 may negatively impact human health and reduce plant secondary metabolite production. Therefore, using the climate change effect (*Lestari, 2019*) could be a good step for cleaner vegetable production.

1.5. Relative humidity (RH)

The relative humidity (RH) during the light period has been found to have a greater effect on the growth of rice seedlings than during the dark period (*Hirai et al., 2000*). When high RH is combined with an increase in CO_2, it can also promote photosynthesis and growth of tomato plants. However, it is important to exercise caution in controlling air moisture levels (*Sultan et al., 2014*) and monitoring transpiration rates, as crops grown in high relative humidity can be prone to instability (*Suzuki et al., 2015*). For tropical crops, such as watermelon, which are subject to cold stresses of 10°C (during the day) and 5°C (at night), increasing RH may be beneficial in reducing cold tolerance (*Lu et al., 2021*). The increase in RH reduces the potential for water loss in leaves, reduces electrolyte leakage, increases photosynthetic stability, and reduces chilling damage.

A study by *Mortensen (1986)* found that the dry weight of several flower plants including *Saintpaulia ionantha* (17-36 percent), *Lycopersicon esculentum* (20 percent), *Euphorbia pulcherrima* (31 percent), *Chrysanthemum* (31 percent), *Begonia* (47 percent), and *Nephrolepis exaltata* (17-36 percent) showed a significant increase in dry weight with increasing RH (from the lowest level to the highest level, by 68%). However, RH had no effect on the dry weight of *Rosa*, *Campanula isophylla*, *Cucumis sativa*, or *Lactuca sativa*, and negatively affected *Soleirolia soleirolii*.

In conventional micropropagation systems, high relative humidity levels, resulting from sealing culture vessels, can pose challenges for plant growth. When the relative humidity is too high or air circulation is inadequate, plants are unable to extract nutrients or allow water to evaporate from the soil. To mitigate this, lowering relative humidity through forced ventilation can significantly aid in the normal functioning of plant stomata (*Kozai, Afreen, and Zobayed, 2005*).

1.6. Brief findings on relative humidity effect against plants

The relative humidity within a plantation is crucial for maintaining optimal plant growth. It is imperative to note that both excessively low and excessively high levels of relative humidity can prove detrimental to the health of plants, regardless of the growing method being utilised, be it traditional or modern. It is therefore imperative to exercise caution when adjusting the relative humidity within a plantation, and to ensure that any changes made are based on the specific needs of the plants in question.

1.7. Temperature

Numerous studies have explored the impact of temperature on plant growth within a plantation setting *(Ito, 2014)*. The utilization of forced ventilation has been shown to aid in maintaining a suitable culture environment by reducing relative humidity. As temperatures rise, plants undergo morphological, anatomical, physiological, and biochemical changes that ultimately influence growth and agricultural yields *(Wahid et al., 2007)*. The role of temperature in root plasticity against pathogen and biocontrol agent inoculation has also been explored, with Seflo et al. finding that temperature plays a critical role in this process *(Sefloo et al., 2021)*. Results from the study by Seflo et al. indicated that at 24°C, there was no significant change in the morphological properties of *Fusarium oxysporum f.sp. lycopersici* roots, but at 28°C, inoculation with *Fusarium oxysporum f.sp. lycopersici* reduced root volume, surface, and length.

MacAlister et al., (2020), investigated the effects of temperature on the growth and stress tolerance properties of A. linearis, and found that, with the exception of phenolics content, increasing temperature and altering soil nutrients had a negative impact on plant biomass. Additionally*, Li et al., (2020),* examined the impact of temperature fluctuations, including day/night cycles and heat waves, on *Gossypium hirsutum* L., and found that higher daytime temperatures reduced leaf carbon production and yield, while nighttime warming did not compensate for these negative effects.

Temperature has a direct impact on photosynthesis, a highly temperature-dependent biochemical process regulated by enzymes *(Afreen, 2005, p. 65)*. As depicted in Fig. 1, temperatures that are too low or too high can inhibit photosynthesis by affecting enzyme activity. Furthermore, decreases in temperature can indirectly reduce the net photosynthetic rate by reducing the rate of CO_2 diffusion. Plant species that are more resistant to heat stress tend to have a higher content of secondary metabolites *(Wahid et al., 2007)*, increased carbohydrate availability (*Liu and Huang, 2000*), higher photosynthesis rates *(Scafaro, Haynes, and Atwell, 2010; Bita and Gerats, 2013*), and lower chlorophyll concentrations (*MacAlister et al., 2020*).

1.8. Brief findings on temperature effect against plants

In order to maximize the effect of temperature on plantation growth, it is crucial to maintain temperatures within the range of heat stress tolerance. This can be challenging in traditional growing methods, particularly in regions with extreme climates. In these instances, the utilization of a glasshouse equipped with a temperature control system can greatly aid the growth process.

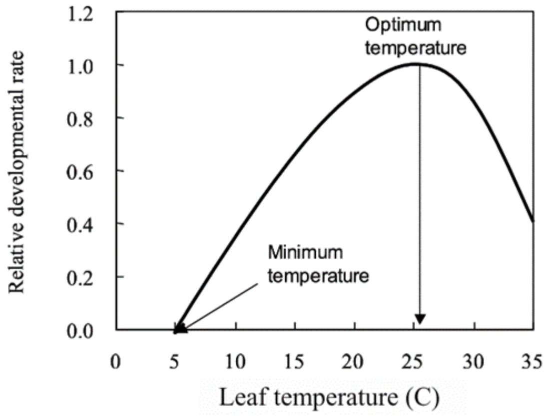

Fig. 1 – General plant developmental rate response to temperature (*Afreen, 2005*)

Fig. 2 – Piper betle var Nigra (*Harjunowibowo, 2021*)

1.9. Substrate or supporting medium

Water and fertiliser are essential factors that influence agricultural production (*Du et al., 2015*). Under photoautotrophic conditions, using fibrous planting media materials with high air porosity, such as florialite *(Nguyen & Kozai, 2001)*, vermiculite *(Feng & Zhang, 2021)*, humic acid *(Feng & Zhang, 2021),* and rockwool *(Acuña et al., 2013)*, promotes root development and enhances plant growth. The use of hydrogels as a planting medium can also improve soil biological properties and enhance the growth of water-stressed tomato plants *(Nassaj-Bokharaei et al., 2021)*. In a study by *Sefloo, et al. (2021)*, root growth was found to be high in perlite soil, with an increase of only 2% in root growth observed in the sucrose treatment.

Soilless culture involves growing seedlings without soil as the rooting medium. This technique is divided into substrate culture and water culture, with floating hydroponics being a widely used aquaculture technique for the cultivation of fresh vegetables and aromatic plants *(Tüzel et al., 2019)*. In soilless agriculture, all essential plant nutrients are supplied through a nutrient solution, with the exception of carbon which is taken up from the air as CO_2. The soil in greenhouses must be periodically changed to address soil structure, fertility, and pest issues. However, soilless agriculture is not yet a widely accepted alternative in organic farming. The recycling and biological treatment of municipal waste to produce fertilizer is preferable for supporting plant growth and reducing greenhouse gas emissions *(Berisha & Osmanaj, 2021)*.

1.10. Brief findings on Substrate or supporting medium effect against plants

The reduction of water consumption and the optimization of nutrient utilization are becoming increasingly recognised as critical components of sustainable agriculture globally. The implementation of these strategies in growing media can lead to improved soil moisture management, increased plant growth, and enhanced soil microbial activity and nutrient availability.

2. Ecology of *Piper Betle L.*

Betel (Piper betle) is a vine species belonging to the family Piperaceae that thrives in warm and humid environments with diffused sunlight *(Sengupta, 2019)*. It is often cultivated under an artificial structure known as Barouj, which provides high humidity and moisture levels *(Dasgupta and , 2017)*. Due to its widespread use, betel has earned the nickname "green gold" in Southeast Asia and India *(Vikas Bajpai, 2020)*.

For optimal cultivation, betel requires porous and fertile soil in upland sites, as it is sensitive to waterlogging. In India, there are three distinct cultivation systems, ranging from natural to fully controlled, with varying degrees of anthropogenic interventions aimed at regulating light, temperature, and humidity. To ensure proper growth, saplings should be planted in parallel rows with a two-foot spacing, with reeds providing support as they mature and twined around upright sticks of split bamboo to avoid reciprocal shading *(Jane, Deshmukh and S., 2014)*.

The cultivation of Piper betle L. (betel), a vine of the Piperaceae family, is influenced by various factors, including temperature and light. Cold temperatures, particularly low frost, can result in crop damage, particularly in subtropical regions. The sensitivity to cold varies among landraces, with some, such as *Madras Paan Kapoori* and *Kapoori Vellaikodi*, being more sensitive than others, such as *Deshi Bangla*, *Kaker*, and *Bangla Mahoba (Tripathi, Khare, and Kumar, 2000; Gupta et al., 2012)*. This sensitivity is more pronounced in male landraces than in female landraces, as demonstrated by molecular studies using DNA markers *(Ranade et al., 2002; Samantaray and Phurailatpam, 2012)*.

The growth of Piper betle L is influenced by the presence of shade and the application of nitrogen. Phytochemical production in the plant is positively impacted by these factors *(Muttaleb et al., 2018)*. Betel is a commercially valuable plant that is propagated asexually and exhibits a range of cultivars *(Patra, Mukherjee and Acharya, 2011).* Cultivation of betel nut in partial shade, with a 30 percent to 50 percent canopy and 100 kg/ha nitrogen application, has been shown to result in increased chlorophyll content. Conversely, full sun exposure with 0 kg/ha nitrogen application is unfavourable for the growth of betel plants, resulting in leaf scorch, stunted growth, and the accumulation of secondary metabolites and antioxidants.

Secondary metabolites produced by the betel plant serve important ecological functions, such as providing resistance against diseases and herbivores. The biosynthesis of these metabolites is influenced by environmental factors, including temperature, light, soil fertility, groundwater, and soil salinity *(Yang et al., 2018).* These environmental factors can impact the concentration of specific compounds required for plant survival. However, changes in phytoconstituents cannot be distinguished through morphological features or traditional methods, highlighting the need for chemical-based discrimination methods for quality control purposes.

The Black betel (*Piper betle L. var. nigra*), an endemic species of Indonesia, has potential as a medicinal plant *(Junairiah et al., 2021)*. Unlike other closely related species, Black betel thrives in shady environments, climbing and clinging to nearby plants, and is able to grow on most trees. The plant is characterized by its dark green, stiff, and smooth heart-shaped leaves with a distinctive aroma, black stems, and a growing habit at altitudes of 500-700 m above sea level *(Suriani et al., 2020)*, as shown in Fig. 2. Black betel is a rapidly growing perennial herb that can reach heights of 5 to 15 meters, leaving a ring-shaped mark on the stem when its fallen leaves decompose.

The plant species, Black betel, is a source of secondary metabolites, including flavonoids, alkaloids, steroids, tannins, terpenoids, and saponins *(Junairiah, Ni'matuzahroh et al., 2019; Junairiah et al., 2021).* It has been investigated for its antimicrobial activity, with studies demonstrating its efficacy against tooth decay and gum inflammation *(Prasetya and Cipta Narsa, 2013).* Additionally, two amide derivatives, piperenamide A-B, have been shown to act against oral pathogens *(Prasetya, Salam, Rahmadani, Haikal, et al., 2021)*. Fatty acid derivatives, *2-octenoic acid* and *2-hexenoic acid*, have also been identified in this species and have potential health benefits *(Prasetya, Salam, Rahmadani, Kuncoro et al., 2021)*. The health-related benefits of betel leaves are largely attributed to its bioactive phenolic compounds, the content and composition of which vary according to genotype, agronomic practices, and climatic conditions *(Gagnon and Berrouard, 1994)*. Extracts from this perishable product have potential applications in the food, beverage, organic synthesis, and medicinal industries to address environmental problems *(Madhumita, Guha, and Nag, 2020).* Despite its potential, research on the cultivation of black betel vine is limited, and further studies are required to fully understand the method of cultivation for this species.

2.1. Traditional Cultivation

In a closed-type cultivation system, the common betel vine is grown in square or rectangular man-made structures constructed from locally available and economical materials such as bamboo, bamboo sticks, and jute sticks. These structures are designed to provide ideal growing conditions for the betel vine in terms of light, humidity, temperature, and soil moisture.

The betel vine is well-suited for cultivation in well-drained fertile soil in both wet and dry climates. A well-drained sandy loam soil or loam soil with a pH of 6.8-7.5, as well as a porous substrate, is ideal for betel culture *(Sengupta, 2019)*. The soil must be properly prepared and arranged in furrows 75 cm wide, 10-15 m long, and 75 cm deep. Soil that has been tilled with solarisation and dry soils are most suitable for planting.

However, freshly tilled or recently collected soil should not be used directly, and instead, should be opened and stacked for at least 6-10 months to sterilise the soil and eliminate soil-borne pathogens. Single node vine cuttings are planted with proper dressing between February and June, depending on the location and prior to the onset of the monsoon season and mild winter *(Das et al., 2016)*.

The cultivation of betel vine is negatively impacted by alkaline soil and waterlogging, thus saline or alkaline soil is unsuitable for its growth. Chemical fertilisers, particularly those containing nitrogen, have been shown to exacerbate leaf disease and reduce the shelf life and durability of leaves. Leptolyngbya HS-16 demonstrated better growth in NPK medium at a concentration of 80 ppm *(Prihantini et al., 2020)*. The combination of *Synechococcus sp*. HS-9 and NPK media with a tubular photobioreactor (tPBr) system has the potential to produce biomass feedstock for biofuels *(Ardiansyah et al., 2019)*, highlighting the cost-effectiveness of using NPK media as a growth medium. However, some studies have indicated that while nitrogen from organic sources may reduce disease incidence, it does not improve storage quality *(Sengupta, 2019).*

The combination of organic fertilisers derived from algae *(Kaur et al., 2013)* and nitrogenous fertilisers has been found to be effective in increasing betel leaf yields. Vermicompost and cow dung have also been shown to be suitable for betel cultivation. Scientific studies have indicated that phosphorous (100 kg P_2O_5/ha) and potassium (100-125 kg K_2O/ha) have a positive effect on crop yields, maintenance quality, and the spread of disease outbreaks. Additionally, the administration of zinc (0.25-0.5%), manganese (0.5%), and molybdenum (0.1%) has been shown to increase leaf yields through studies on micronutrients.

Betel is best cultivated under the ecologically shady conditions found in tropical forests, with a relative humidity ranging from 40% to 80%, rainfall between 2250 and 4750 millimetres, and a mild temperature range between 15°C and 40°C *(Vikas Bajpai, 2020)*. Irrigation throughout the year is necessary for the optimal growth of the plant *(Sengupta, 2019)*. The cultivation of betel vine in India demonstrates the plant's ability to thrive in both mild and highly variable climatic conditions. However, the quality of betel leaves is generally better in wet and medium environments compared to dry areas. Extremely hot and dry winds can have a negative impact on the growth of the vines, as hot air causes wilting and burning of the soft leaves, while cold waves lead to yellowing.

Black betel is propagated through cuttings. The cuttings should be taken from healthy, high-yielding mother vines, with large, dark green leaves. The cuttings should be 15-50 cm in length, with 2-8 internodes, and taken from the top of the betel tree *(Sengupta, 2019)*. They can be planted directly in the field or as rooted cuttings in a polybag mixture of mother soil, cow dung, coconut dust, and sand (Fig. 3).

Fig. 3 – Cultivating from stem cutting

Fig. 4– Betel vine planting field

Betel is typically planted in indented beds (Fig. 4). The soil must be level and free from any contamination by bacterial leaf blight for at least two years prior to planting. Beds measuring 1.2 meters by 7.5 meters are created to facilitate maintenance. Spacing between beds should be cleared to prevent the spread of illnesses and facilitate management. The beds should be sterilised by burning straw. A seepage canal, 30 cm in width and 60 cm in depth, should be constructed around the cluster of beds. A stake should be provided for the betel vine to climb, with two cuttings planted close to each stake at a 45 cm^2 spacing.

Before planting, the cuttings should be soaked in a fungicide mixture for two minutes. They are then planted in 30 cm^2 holes in the beds. The beds should be covered with shading material for 4-6 weeks to support the growth of seedlings from the cuttings, which take 20-45 days to establish. Intercropping is not recommended, as it may serve as a host plant for diseases *(Sengupta, 2019)*. Hence, betel should be grown as a single plant, in addition to the indented beds.

After planting the cuttings, the initial watering schedule should be twice daily for the first 3-4 days, once daily for the next 3 days, and once every 2 days for the following 6 days. Subsequently, irrigation water should be applied judiciously and slowly, for which the drip irrigation method is recommended as the ideal option *(Sengupta, 2019)* (Fig. 5). The flood method of irrigation should be avoided to prevent the spread of disease pathogens.

Fig. 5 – Drip Irrigation

Fig. 6 – Lowering the vines *(Dr Ysrhu Hortiportal, 2021)*

When the betel vines reach a height of approximately 2 meters, they come close to touching the roof of the barouj. The lowering of the vines should be performed regularly, approximately 5-6 times per year, with due care taken during the monsoon months as most pathogens are soil-borne. Once lowered, the vine can be covered with soil or kept coiled just above the ground (Fig. 6). When lowering the vine, it is necessary to spray a copper fungicide solution on the bottom and roll up parts of the vine, then tie it to the support sticks *(Sengupta, 2019).*

2.2. Finding for traditional cultivation on black betel

There are two methods of cultivating betel vine: the closed-type system and the open-type system. Each method has its own advantages and disadvantages. While the open-type system may be cost-effective, it is susceptible to disease and lacks the ability to make necessary adjustments. The closed-type system requires a higher initial investment in facilities and control systems, but helps to avoid harmful diseases and offers the potential to produce high quality and quantity of leaves at low operating costs.

3. Recent Cultivation Technology

Closed greenhouses provide an environment that facilitates the cultivation of insect and pesticide-free transplant seedlings, with a 40% reduction in production time as compared to conventional greenhouses that employ optimal environmental controls, including CO_2 enrichment *(Kozai, 2020).*

3.1. Callus induction

The *Piper betle L. var Nigra* plant is a valuable source of secondary metabolites, which can be obtained through the extraction of various plant parts. However, this approach requires a large supply of fresh herbs and incurs significant costs associated with isolation, purification, and extraction. As an alternative, callus culture technology has been proposed as a means of maintaining the availability of secondary metabolites while also increasing production in a more time-efficient manner.

Junairiah et al. has investigated the combination of growth regulators on callus induction of black betel leaf explants *(Junairiah et al., 2017)*. Based on their research, the concentration of *Indole-3-Acetic Acid* (IAA) 1.0 mg/L and 1.5 mg/L of kinetin is the best recipe for callus induction from black betel leaf. The resulting fresh weight was 0.2972 grams with a dry weight of 0.1660 grams. Furthermore, the growth regulator 2.4-D given at kinetin (2.5 mg/L) was able to induce callus with an average induction time of 14 days *(Junairiah, Mahmuda et al., 2019)*. Meanwhile, a combination for callus induction of P. betle L. var. Nigra leaf explants in the form of addition of 2.0 mg/l BAP and 0.5 mg/L 2.4 D combined with coconut water (5%) resulted in callus induction time of 13 days *(Junairiah et al., 2021)*. Furthermore, the growth regulators IBA (2 mg/L) and BAP (2 mg/L) is combined and resulted in a callus induction time of nine days *(Junairiah, Rahmawati et al., 2020).* While callus of black betel had a compact texture in all the treatments (brownish yellow colour), in the eighth week the callus mostly turned black (

Fig. 7).

In a study by *Junairiah et al. (2018),* the objective was to identify the bioactive compounds present in black betel fruit through the use of ethyl acetate, n-hexane, and methanol extracts. The n-hexane extract was found to contain three main components: *3-butenoic acid*, *1-pentene*, and *furan*.

Further research conducted by *Junairiah et al. (2020)* explored the impact of varying concentrations of cobalt (II) chloride ($CoCl_2$) on black betel leaf callus biomass and terpenoid profiles. It was found that the addition of 1.0 mg/L $CoCl_2$ to the callus resulted in a 5.95% production of terpenoids, which was higher compared to the control. Additionally, the fresh and dry weights of the callus increased over three weeks of growth. However, it should be noted that this technique requires specialised skills and must be conducted under strict sterile conditions. It also takes up to 8 weeks for induction, and even longer to cultivate plantlets.

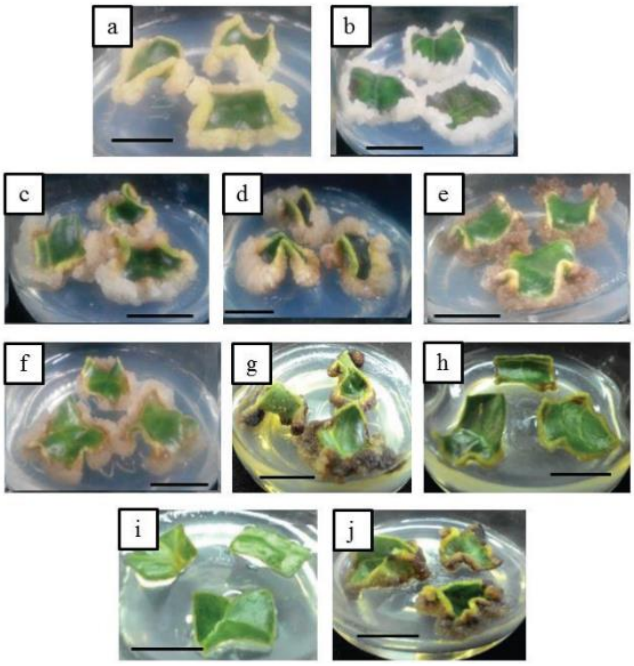

Fig. 7 – Callus morphology of Piper betle L. Var Nigra in some treatments *(Junairiah et al., 2017)*

3.2. Finding for callus method of black betel

The cultivation of betel vine and black betel vine shares similar physical requirements for optimal growth, including adequate light, temperature, relative humidity, irrigation, and substrate nutrition. Both traditional and modern cultivation methods can be utilised, although the traditional method of soil-based cultivation may require a longer period of time to produce high-quality crops in significant quantities. Callus induction technology may provide a solution to these challenges.

However, it should be noted that callus induction technology is delicate and sensitive to changes in growth parameters, and requires careful attention to ensure successful outcomes. Nevertheless, it is well suited for large-scale production or for cultivating species that are limited in availability, such as black betel vine. Typically, callus induction takes between 8 to 14 weeks before the plantlets are ready for transplantation into soil.

CONCLUSIONS

Betel vine holds significant potential both for its health-promoting secondary metabolites and as a source of income, making the cultivation of high-quality crops in economic quantities a crucial goal. The recent discovery of a new genus of *Piper betle L*., known as "*Piper betle var Nigra*", adds further interest to this species. However, cultivating betel vine presents several challenges.

Traditional cultivation techniques, including open- and closed-type systems, have been used, while the modern method of callus induction is becoming increasingly popular, particularly for mass production. Each of these technologies has its own advantages and limitations, with the modern technique requiring specialized skills, being more time-consuming, and more expensive, while traditional techniques are simpler but face the risk of disease.

By utilising a closed system, such as a greenhouse, it may be possible to produce higher-quality crops than with open systems. Through the implementation of smart farming practices and maintaining optimal conditions for betel vine growth, any losses can be reduced, particularly through the use of appropriate lighting. As betel vine requires low levels of sunlight, tropical temperatures, and protection from insects, a screen house with artificial shading and lighting may be an appropriate choice for subtropical regions.

ACKNOWLEDGEMENTS

The authors thank Ministry of Research and Technology/National Agency for the funding support through Research and Innovation (RISTEK-BRIN) Grant no 11/E1/KP.PTNBH/2021, Productive Innovative Research (RISPRO) PRJ-52/ LPDP/2019 and ESMART for the facilities given.

REFERENCES

[1] Acuña, R. A.Bonachela, S.Magán, J. J.Marfà, O.Hernández, J. H.& Cáceres, R. (2013). Reuse of rockwool slabs and perlite grow-bags in a low-cost greenhouse: Substrates' physical properties and crop production. *Scientia Horticulturae*, *160*, 139–147. https://doi.org/https://doi.org/10.1016/j.scienta.2013.05.031

[2] Afreen, F. (2005). Physiological and anatomical characteristics of in vitro photoautotrophic plants. In *Photoautotrophic (sugar-free medium) micropropagation as a new propagation and transplant production system* (pp. 61–90). Springer Netherlands.

[3] Afreen, F.Zobayed, S. M. A.& Kozai, T. (2001). Mass-Propagation of Coffee from Photoautotrophic Somatic Embryos. In N. Morohoshi & A. Komamine (Eds.), *Molecular Breeding of Woody Plants* (1st ed., Vol. 18, pp. 355–364). Elsevier Masson SAS. https://doi.org/10.1016/S0921-0423(01)80092-7

[4] Arambewela, L. S. R.Arawwawala, L. D. A. M.Kumaratunga, K. G.Dissanayake, D. S.Ratnasooriya, W. D.& Kumarasingha, S. P. (2011). Investigations on Piper betle grown in Sri Lanka. *Pharmacognosy Reviews*, *5*(10), 159–163. https://doi.org/10.4103/0973-7847.91111

[5] Arawwawala, L. D. A. M.Arambewela, L. S. R.& Ratnasooriya, W. D. (2014). Gastroprotective effect of Piper betle Linn . leaves grown in Sri Lanka. *Journal of Ayurveda & Integrative Medicine*, *5*(1), 38–42. https://doi.org/10.4103/0975-9476.128855

[6] Ardiansyah, S. R.Orlando, A. M.Rahman, A.Prihantini, N. B.& Nasruddin. (2019). Tubular photobioreactor: A preliminary experiment using synechococcus sp. (cyanobacteria) cultivated in NPK media for biomass production as biofuel feedstock. *Evergreen*, *6*(2), 157–161. https://doi.org/10.5109/2321011

[7] Ayadi, M.Ahou, S.Awad, S.Abderrabba, M.& Andres, Y. (2020). Production of biogas from olive pomace. *Evergreen*, *7*(2), 228–233. https://doi.org/10.5109/4055224

[8] Berisha, A.& Osmanaj, L. (2021). Kosovo scenario for mitigation of greenhouse gas emissions from municipal waste management. *Evergreen*, *8*(3), 509–516. https://doi.org/10.5109/4491636

[9] Bhatia, S. C. (2014). *Advanced renewable energy systems* (1st ed.). Woodhead Publishing India Pvt. Ltd.

[10] Bintang, M.Safira, U. M.Kusumawati, D. E.Pasaribu, F. H.Template, A. D. N. A.& Isolate, B. S. (2014). *Analysis of 16S rRNA Sequence of Endophytic Bacteria Isolate BS1 from Piper betle [L.] Stem*. 69–70.

[11] Bita, C. E.& Gerats, T. (2013). Plant tolerance to high temperature in a changing environment: Scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science*, *4*(JUL), 1–18. https://doi.org/10.3389/fpls.2013.00273

[12] Both, A. J. (2013). *Measuring LED Lighting Systems and Developing l Guidelines d l for f Evaluation , Comparison and Use*. 1–8.

[13] Briskin, D. P.& Gawienowski, M. C. (2001). Differential effects of light and nitrogen on production of hypericins and leaf glands in Hypericum perforatum. *Plant Physiology and Biochemistry*, *39*(12), 1075–1081.

[14] Bula, R. J.Morrow, R. C.Tibbitts, T. W.Barta, D. J.Ignatius, R. W.& Martin, T. S. (1991). Light-emitting Diodes as a Radiation Source for Plants. *HortScience*, *26*(2), 203–205. https://journals.ashs.org/hortsci/downloadpdf/journals/hortsci/26/2/article-p203.pdf

[15] Choi, H. G.Moon, B. Y.& Kang, N. J. (2015). Effects of LED light on the production of strawberry during cultivation in a plastic greenhouse and in a growth chamber. *Scientia Horticulturae*, *189*, 22–31. https://doi.org/10.1016/j.scienta.2015.03.022

[16] Cossu, M.Murgia, L.Ledda, L.Deligios, P. a.Sirigu, A.Chessa, F.& Pazzona, A. (2014). Solar radiation distribution inside a greenhouse with south-oriented photovoltaic roofs and effects on crop productivity.

Applied Energy, *133*, 89–100. https://doi.org/10.1016/j.apenergy.2014.07.070
[17] Das, S.Parida, R.Sriram Sandeep, I.Nayak, S.& Mohanty, S. (2016). Biotechnological intervention in betelvine (Piper betle L.): A review on recent advances and future prospects. *Asian Pacific Journal of Tropical Medicine*, *9*(10), 938–946. https://doi.org/10.1016/j.apjtm.2016.07.029
[18] Dasgupta, B.& Sarkar, S. (2017). Changes in crop canopy architecture on the incidence of major foliar diseases of betelvine (Piper betle L.). *Journal of Applied Horticulture*, *19*(2), 135–138. https://doi.org/10.37855/jah.2017.v19i02.24
[19] Daud, D.Yang, S. C.Balaja, C. C.Ja'afar, F.Yasin, H. M.Kusrini, E.Prihandini, W. W.& Usman, A. (2020). Radical scavenging activity assay and red fluorescence microscopy studies: Antioxidant properties of selected young and mature leaves for application in pharmaceutical industry. *Evergreen*, *7*(2), 216–220. https://doi.org/10.5109/4055222
[20] Dong, J.Gruda, N.Li, X.Tang, Y.Zhang, P.& Duan, Z. (2020). Sustainable vegetable production under changing climate: The impact of elevated CO2 on yield of vegetables and the interactions with environments-A review. *Journal of Cleaner Production*, *253*, 119920. https://doi.org/10.1016/j.jclepro.2019.119920
[21] Dr YSRHU Hortiportal. (2021). *betelvine.pdf*. Dr YSRHU Hortiportal. www.drysrhu.edu.in/crops/betelvine.html
[22] Du, T.Kang, S.Zhang, J.& Davies, W. J. (2015). Deficit irrigation and sustainable water-resource strategies in agriculture for China's food security. *Journal of Experimental Botany*, *66*(8), 2253–2269. https://doi.org/10.1093/jxb/erv034
[23] Dwivedi, V.& Tripathi, S. (2014). Review study on potential activity of Piper betle. *Journal of Pharmacognosy and Phytochemistry JPP*, *93*(34), 9398.
[24] Dyer, L. A.& Palmer, A. D. N. (2004). *Piper : A Model Genus for Studies of Phytochemistry , Ecology , and Evolution Piper* (Kluwer Academic (ed.)). Plenum Publishers. https://doi.org/10.1007/978-0-387-30599-8
[25] Feng, X.& Zhang, L. (2021). Vermiculite and humic acid improve the quality of green waste compost as a growth medium for Centaurea cyanus L. *Environmental Technology & Innovation*, *24*, 101945. https://doi.org/https://doi.org/10.1016/j.eti.2021.101945
[26] Gagnon, B.& Berrouard, S. (1994). Effects of several organic fertilizers on growth of greenhouse tomato transPlants. *Canadian Journal of Plant Science*, *74*(1), 167–168. https://doi.org/10.4141/cjps94-035
[27] Ghazali, N. A.Elmy, A.Yuen, L. C.Sani, N. Z.Das, S.Suhaimi, F.Yusof, R.Yusoff, N. H.& Thent, Z. C. (2016). Piper betel leaves induces wound healing activity via proliferation of fibroblasts and reducing 11β hydroxysteriod dehydrogenase-1 expression in diabetic rat. *Journal of Ayurveda and Integrative Medicine*, *7*(4), 198–208. https://doi.org/10.1016/j.jaim.2016.08.008
[28] Gupta, S.Gupta, S. M.Sane, A. P.& Kumar, N. (2012). Chlorophyllase in Piper betle L . has a role in chlorophyll homeostasis and senescence dependent chlorophyll breakdown. *Molecular Biology Reports*, *39*(6), 7133–7142. https://doi.org/10.1007/s11033-012-1545-8
[29] Hajare, R.Darvhekar, V. M.Shewale, A.& Patil, V. (2011). *Evaluation of antihistaminic activity of Piper betel leaf in guinea pig*. *5*(February), 113–117. https://doi.org/10.5897/AJPP09.376
[30] Harjunowibowo, D. (2021). *Pembuatan Protected Crop untuk Pemeliharaan Sirih Hitam*. Kompasiana. https://www.kompasiana.com/dewantoharjunowibowo9651/611e06ea06310e2d85167502/pembuatan-protected-crop-untuk-pemeliharaan-sirih-hitam?page=all#section1
[31] Hirai, G. I.Okmura, T.Takeuchi, S.Tanaka, O.& Chujo, H. (2000). Studies on the effect of the relative humidity of the atmosphere on the growth and physiology of rice plants: Effects of relative humidity during the light and dark periods on the growth. *Plant Production Science*, *3*(2), 129–133. https://doi.org/10.1626/pps.3.129
[32] Hoque, M. J. (2022). Causes, Mechanisms and Outcomes of Environmental Degradation in Bangladesh: a Study in Sylhet. *Evergreen*, *9*(2), 310–325. https://doi.org/10.5109/4793670
[33] Ito, K. (2014). Micro- and macro-scale measurement of fungal growth under various temperature and humidity conditions. *Evergreen*, *1*(1), 32–39. https://doi.org/10.5109/1440974
[34] Izzah, N.Zamri, I.Fields, M.Izzah, N.Zamri, I.Lailatul, S.Zulmajdi, N.& Kusrini, E. (2020). *Rhodamine B Photocatalytic Degradation using CuO Particles under UV Light Irradiation for Applications in Industrial and Medical Fields Rhodamine B Photocatalytic Degradation using CuO Particles under UV Light Irradiation for Applications in Industrial an*. *7*(2), 280–284.
[35] Jane, N. S.Deshmukh, A. P.& S, J. M. (2014). Review Of Study Of Different Diseases On Betelvine Plant and Control Measure. *International Journal of Application or Innovation in Engineering &*

Management (IJAIEM), *3*(3), 560–563. https://www.ijaiem.org/volume3issue3/IJAIEM-2014-03-31-157.pdf
[36] Jensen, B.Shetty, N. P.Jensen, J. D.Shetty, R.Frette, X.Jørgen, H.Jørgensen, L.Newman, M.& Christensen, L. P. (2011). Silicon-Induced Changes in Antifungal Phenolic Acids , Flavonoids , and Key Phenylpropanoid Pathway Genes during the Interaction between Miniature Roses and the Biotrophic Pathogen Podosphaera pannosa 1 [W]. *Plant Physiology*, *157*(4), 2194–2205. https://doi.org/10.1104/pp.111.185215
[37] JunairiahEly, T. W.Manuhara, Y. S. W.Ni'Matuzahroh& Sulistyorini, L. (2021). Optimation of callus induction from piper betle l . Var . nigra explants with various concentrations of coconut water and addition of 2 , 4-d and bap. *Annals of Biology*, *36*(2), 324–328. http://agribiop.com/optimation-of-callus-induction-from-piper-betle-l-var-nigra-explants-with-various-concentrations-of-coconut-water-and-addition-of-24-d-and-bap/
[38] Junairiah, J.Mahmuda, A.Manuhara, Y. S. W.Ni'Matuzahroh& Sulistyorini, L. (2019). Callus Induction and Bioactive Compounds from Piper betle L. var nigra. *IOP Conference Series: Earth and Environmental Science*, *217*(012026), 1–8. https://doi.org/10.1088/1755-1315/217/1/012026
[39] JunairiahNi'matuzahroh& Sulistyorini, L. (2018). GCMS analysis of bioactive compounds in n-hexane, ethyl acetate, and methanol extract of P iper betle L.var. nigra. *Bioscience Research*, *15*(3), 472–1479. https://www.isisn.org/BR15(3)2018/1472-1479-15(3)2018BR18-204.pdf
[40] JunairiahNi'matuzahrohZuraidassanaaz, N. I.& Sulistyorini, L. (2019). Isolation and identification of secondary metabolites of black betel (Piper betle L. var Nigra). *Jurnal Kimia Riset*, *3*(2), 131. https://doi.org/10.20473/jkr.v3i2.12064
[41] JunairiahRahmawati, R. K.Manuhara, Y. S. W.Ni'matuzahrohPramudya, M.& Sulistyorini, L. (2020). Induction and identification of bioactive compounds from callus extract of Piper betle L . var . Nigra. *Malaysian Journal of Analytical Sciences*, *24*(6), 1024–1034.
[42] JunairiahZuraidassanaaz, N. I.Izdihar, F. N.& Manuhara, Y. S. W. (2017). Callus induction of leaf explant Piper betle L . Var Nigra with combination of plant growth regulators indole-3-acetic acid (IAA), benzyl amino purin (BAP) and kinetin Callus Induction of Leaf Explant Piper betle L . Var Nigra with Combination of Plan. In H. Y. Abdullah (Ed.), *6th International Conference And Workshops On Basic And Applied Sciences* (Vol. 1888, pp. 020028.1-11). AIP Publishing. https://doi.org/10.1063/1.5004305
[43] JunairiahZuraidassanaaza, N. I.Manuhara, Y. S. W.Ni'matuzahroha& Sulistyorini, L. (2020). Biomass and terpenoids profile of callus extract of Piper betle L . var . Nigra with abiotic elicitor cobalt (II) chloride. *Moroccan Journal of Chemistry*, *1*(1), 88–98. https://revues.imist.ma/index.php/morjchem/article/download/19129/10475
[44] Kangralkar, V. A.& Kulkarni, A. R. (2013). In vitro antitumor activity of alcoholic extract of piper betel leaf. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *4*(4), 1558–1561.
[45] Kano, A.Kamita, M. K.Iwasaki, T.& Shindo, M. (2017). Bongkrekic acid induces selective cytotoxicity in tumor cells, revealed by CCK-8. *Evergreen*, *4*(2–3), 23–27. https://doi.org/10.5109/1929661
[46] Kaur, G.Singh, S.Kuppan, P.Goto, M.Sugiura, N.Johari, M.Mohd, M.& Ujang, Z. (2013). Physical Water Quality and Algal Density for Remediation of\nAlgal Blooms in Tropical Shallow Eutrophic Reservoir. *Journal of Novel Carbon Resource Science*, *7*(feb 2013), 33–41. http://ncrs.cm.kyushu-u.ac.jp/assets/files/JNCRS/JNCRS_Vol.7_33-41.pdf
[47] Kim, D.-I.Pedersen, H.& Chin, C.-K. (1991). Cell Suspension in an Improved Airlift Bioreactor: Stimulatory Effect of Carbon Dioxide and Ethylene on Alkaloid Production. *Biotechnology and Bioengineering*, *38*(4), 331–339. https://doi.org/10.1002/bit.260380403
[48] Kirdmanee, C.Kitaya, Y.& Kozai, T. (1995). Effects of CO2 Enrichment and Supporting Material In vitro on Photoautotrophic Growth of Eucalyptus Plantlets In vitro and Ex vitro. *In Vitro Cellular & Developmental Biology. Plant*, *31*(3), 144–149. http://www.jstor.org/stable/4293078
[49] Kozai, T. (2020). Transplant production in closed systems. In M. T. Toyoki Kozai, Genhua Niu (Ed.), *An Indoor Vertical Farming System for Efficient Quality Food Production* (2nd ed., pp. 299–332). Academic Press. https://doi.org/10.1016/B978-0-12-816691-8.00022-4
[50] Kozai, T.Afreen, F.& Zobayed, S. M. A. (2005). *Photoautotrophic (sugar-free medium) Micropropagation as a New Micropropagation and Transplant Production System* (T. KOZAI, F. AFREEN, & S. M. A. ZOBAYED (eds.)). Springer Netherlands. https://link.springer.com/content/pdf/10.1007%2F1-4020-3126-2.pdf
[51] Kuncoro, A.& Purwanto, W. W. (2020). Analysis of energy-water nexus palm oil biodiesel production in riau using life cycle assessment and water footprint methods. *Evergreen*, *7*(1), 104–110.

https://doi.org/10.5109/2740965

[52] Kurata, H.Matsumura, S.& Furusaki, S. (1997). Light irradiation causes physiological and metabolic changes for purine alkaloid production by a Coffea arabica cell suspension culture. *Plant Science*, *123*(1), 197–203. https://doi.org/https://doi.org/10.1016/S0168-9452(96)04588-8

[53] Lestari, N. A. (2019). Reduction of CO2 emission by integrated biomass Gasific ation-solid oxide fuel cell combined with heat recovery and in-situ CO2 utilization. *Evergreen*, *6*(3), 254–261. https://doi.org/10.5109/2349302

[54] Li, X.Shi, W.Broughton, K.Smith, R.Sharwood, R.Payton, P.Bange, M.& Tissue, D. T. (2020). Impacts of growth temperature, water deficit and heatwaves on carbon assimilation and growth of cotton plants (Gossypium hirsutum L.). *Environmental and Experimental Botany*, *179*(June), 104204. https://doi.org/10.1016/j.envexpbot.2020.104204

[55] Li, Y.Zhang, C.Pan, S.Chen, L.Liu, M.Yang, K.Zeng, X.& Tian, J. (2020). LWT - Food Science and Technology Analysis of chemical components and biological activities of essential oils from black and white pepper (Piper nigrum L .) in five provinces of southern China. *LWT - Food Science and Technology*, *117*(September 2019), 108644. https://doi.org/10.1016/j.lwt.2019.108644

[56] Lin, K. H.Huang, M. Y.Huang, W. D.Hsu, M. H.Yang, Z. W.& Yang, C. M. (2013). The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (Lactuca sativa L. var. capitata). *Scientia Horticulturae*, *150*, 86–91. https://doi.org/10.1016/j.scienta.2012.10.002

[57] Liu, X.& Huang, B. (2000). Carbohydrate accumulation in relation to heat stress tolerance in two creeping bentgrass cultivars. *Journal of the American Society for Horticultural Science*, *125*(4), 442–447. https://doi.org/10.21273/jashs.125.4.442

[58] Lu, J.Shireen, F.Cheng, F.& Bie, Z. (2021). High relative humidity improve chilling tolerance by maintaining leaf water potential in watermelon seedlings. *Plant Physiology and Biochemistry*, *166*(May), 818–826. https://doi.org/10.1016/j.plaphy.2021.06.037

[59] MacAlister, D.Muasya, A. M.Crespo, O.Ogola, J. B. O.Maseko, S. T.Valentine, A. J.Ottosen, C. O.Rosenqvist, E.& Chimphango, S. B. M. (2020). Effect of temperature on plant growth and stress tolerant traits in rooibos in the Western Cape, South Africa. *Scientia Horticulturae*, *263*(December 2019), 109137. https://doi.org/10.1016/j.scienta.2019.109137

[60] Madhumita, M.Guha, P.& Nag, A. (2020). Bio-actives of betel leaf (Piper betle L.): A comprehensive review on extraction, isolation, characterization, and biological activity. *Phytotherapy Research*, *34*(10), 2609–2627. https://doi.org/10.1002/ptr.6715

[61] Makino, Y.Nishizaka, A.Yoshimura, M.Sotome, I.Kawai, K.& Akihiro, T. (2020). Influence of low O2 and high CO2 environment on changes in metabolite concentrations in harvested vegetable soybeans. *Food Chemistry*, *317*(June 2019), 126380. https://doi.org/10.1016/j.foodchem.2020.126380

[62] Matysiak, B. (2021). The effect of supplementary led lighting on the morphological and physiological traits of miniature rosa × hybrida 'aga' and the development of powdery mildew (Podosphaera pannosa) under greenhouse conditions. *Plants*, *10*(2), 1–14. https://doi.org/10.3390/plants10020417

[63] Messinger, J.& Lauerer, M. (2015). Solanum quitoense, a new greenhouse crop for Central Europe: Flowering and fruiting respond to photoperiod. *Scientia Horticulturae*, *183*, 23–30. https://doi.org/10.1016/j.scienta.2014.11.015

[64] Mortensen, L. M. (1986). Effect of relative humidity on growth and flowering of some greenhouse plants. *Scientia Horticulturae*, *29*(4), 301–307. https://doi.org/10.1016/0304-4238(86)90013-0

[65] Mosaleeyanon, K.Zobayed, S. M. A.Afreen, F.& Kozai, T. (2005a). Relationships between net photosynthetic rate and secondary metabolite contents in St . John ' s wort. *Plant Science*, *169*, 523–531. https://doi.org/10.1016/j.plantsci.2005.05.002

[66] Mosaleeyanon, K.Zobayed, S. M. A.Afreen, F.& Kozai, T. (2005b). Relationships between net photosynthetic rate and secondary metabolite contents in St. John's wort. *Plant Science*, *169*(3), 523–531. https://doi.org/10.1016/J.PLANTSCI.2005.05.002

[67] Murugesan, S.Ravichandran, D.Lakshmanan, D. K.Ravichandran, G.Arumugam, V.Raju, K.Geetha, K.& Thilagar, S. (2020). Evaluation of anti rheumatic activity of Piper betle L. (Betelvine) extract using in silico, in vitro and in vivo approaches. *Bioorganic Chemistry*, *103*(May), 104227. https://doi.org/10.1016/j.bioorg.2020.104227

[68] Muttaleb, Q. A.TL, A.SA, H.AA, R.S, T.OA, A.& DA, A. (2018). The Role of Shade and Nitrogen on Physiological Traits and Secondary Metabolites of Piper betle L. *Journal of Horticulture*, *05*(02), 1–8.

https://doi.org/10.4172/2376-0354.1000230
[69] Nassaj-Bokharaei, S.Motesharezedeh, B.Etesami, H.& Motamedi, E. (2021). Effect of hydrogel composite reinforced with natural char nanoparticles on improvement of soil biological properties and the growth of water deficit-stressed tomato plant. *Ecotoxicology and Environmental Safety*, *223*, 112576. https://doi.org/10.1016/J.ECOENV.2021.112576
[70] Nguyen, Q. T.& Kozai, T. (2001). Photoautotrophic Micropropagation of Tropical and Subtropical Woody Plants. In N. Morohoshi & A. B. T.-P. in B. Komamine (Eds.), *Molecular Breeding of Woody Plants* (Vol. 18, pp. 335–344). Elsevier. https://doi.org/https://doi.org/10.1016/S0921-0423(01)80090-3
[71] Nomura, K.Yasutake, D.Kaneko, T.Takada, A.Okayasu, T.Ozaki, Y.Mori, M.& Kitano, M. (2021). Long-term compound interest effect of CO2 enrichment on the carbon balance and growth of a leafy vegetable canopy. *Scientia Horticulturae*, *283*, 110060. https://doi.org/10.1016/j.scienta.2021.110060
[72] Ouzounis, T.Fretté, X.Rosenqvist, E.& Ottosen, C. O. (2015). Corrigendum to 'Spectral effects of supplementary lighting on the secondary metabolites in roses, chrysanthemums, and campanulas' [J. Plant Physiol. 171 (16) (2014) 1491-1499] 10.1016/j.jplph.2014.12.006. *Journal of Plant Physiology*, *175*, 192. https://doi.org/10.1016/j.jplph.2014.12.006
[73] Patra, A. P.Mukherjee, A. K.& Acharya, L. (2011). Comparative Study of RAPD and ISSR Markers to Assess the Genetic Diversity.pdf. *American Journal of Biochemistry and Molecular Biology*, *1*(2), 200–211. https://doi.org/10.3923/ajbmb.2011.200.211
[74] Patra, B.Das, M. T.& Dey, S. K. (2016). A review on Piper betle L. *Journal of Medicinal Plants Studies*, *4*(6), 185–192.
[75] Prasetya, F.& Cipta Narsa, A. (2013). Aktivitas gel mulut berbahan aktif ekstrak daun sirih hitam kalimantan sebagai antimikroba penyebab radang gusi (Gingivitis) dan gigi berlubang (Caries). *Journal Of Tropical Pharmacy And Chemistry*, *2*(3), 146–151. https://doi.org/10.25026/jtpc.v2i3.60
[76] Prasetya, F.Salam, S.Rahmadani, A.Haikal, K.Febrina, L.Anshory, H.Arifuddin, M.Siregar, V. O.Narsa, A. C.Herman, H.Ahmad, I.Indriyanti, N.Ibrahim, A.Rusli, R.Rijai, L.& Kuncoro, H. (2021). Novel Amides Derivative with Antimicrobial Activity of Piper betle var . nigra Leaves from Indonesia. *Molecules*, *26*(335), 1–8. https://doi.org/10.3390/molecules26020335
[77] Prasetya, F.Salam, S.Rahmadani, A.Kuncoro, H.Rusli, R.Ruchaemi, A.& Sukartiningsih. (2021). Chemical constituents from Piper betle L. Var Nigra (Piperaceae). *Journal of Tropical Pharmacy and Chemistry*, *5*(3), 229–232. https://doi.org/10.25026/jtpc.v5i3.322
[78] Prasetya, F.Salam, S.Rijai, H. R.Kuncoro, H.& Rusli, R. (2022). Pharmacognostic Profile of Simplicia and Ethanolic Leaves Extract from Indonesian Piper betle var . nigra. *Pharmacogn J.*, *14*(5), 610–618. https://doi.org/10.5530/pj.2022.14.143
[79] Prihantini, N. B.Rakhmayanti, N.Handayani, S.Sjamsuridzal, W.Wardhana, W.& Nasruddin. (2020). Biomass production of indonesian indigenous leptolyngbya strain on npk fertilizer medium and its potential as a source of biofuel. *Evergreen*, *7*(4), 593–601. https://doi.org/10.5109/4150512
[80] Rahman, A.Prihantini, N. B.& Nasruddin. (2020). Biomass production and synthesis of biodiesel from microalgae synechococcus hs-9 (Cyanobacteria) cultivated using bubble column photobioreactors. *Evergreen*, *7*(4), 564–570. https://doi.org/10.5109/4150507
[81] Raja, M. B.Parthiban, S.Anandhan, M.Venkadeswaran, E.Pandi, K.Suganthi, S.& Prakash, M. S. (2018). Rooting and sprouting performance of nodal cutting of cultivated and wild inter specific Piper rootstocks. *International Journal of Chemical Studies*, *6*(2), 20–24.
[82] Ranade, S.Verma, A.Gupta, M.& Kumar, N. (2002). RAPD profile analysis of betel vine cultivars. *Biologia Plantarum*, *45*(4), 523–527. https://bp.ueb.cas.cz/pdfs/bpl/2002/04/09.pdf
[83] Ren, J.Guo, S.-S.Xin, X.-L.& Chen, L. (2014). Changes in volatile constituents and phenols from Gynura bicolor DC grown in elevated CO2 and LED lighting. *Scientia Horticulturae*, *175*, 243–250. https://doi.org/10.1016/j.scienta.2014.06.023
[84] Ren, J.Guo, S.Cheng, Q.Shen, Y.& Feng, H. (2014). Synergistic Effects of CO2 and LED Lighting on Accumulation of Terpenes in Roots of Gynura bicolor. *Chinese Herbal Medicines*, *6*(3), 228–236. https://doi.org/10.1016/S1674-6384(14)60033-2
[85] Sahlan, M.Mandala, D. K.Pratami, D. K.Adawiyah, R.Wijarnako, A.Lischer, K.& Fauzi, A. (2020). Exploration of the antifungal potential of indonesian propolis from Tetragonula biroi bee on Candida sp. and Cryptococcus neoformans. *Evergreen*, *7*(1), 118–125. https://doi.org/10.5109/2740968
[86] Sahlan, M.MuryantoHermansyah, H.Wijarnako, A.Gozan, M.Lischer, K.Ahmudi, A.& Pujianto, P. (2020). Ethanol production by encapsulated rhizopus oryzae from oil palm empty fruit bunch. *Evergreen*, *7*(1),

92–96. https://doi.org/10.5109/2740963
[87] Salehi, B.Javier Hernández-Álvarez, A.Del Mar Contreras, M.Martorell, M.Ramírez-Alarcón, K.Melgar-Lalanne, G.Matthews, K. R.Sharifi-Rad, M.Setzer, W. N.Nadeem, M.Yousaf, Z.& Sharifi-Rad, J. (2018). Potential Phytopharmacy and Food Applications of Capsicum spp.: A Comprehensive Review. *Natural Product Communications*, *13*(11), 1543–1556. https://ndb.nal.usda.gov/ndb/;
[88] Salehi, B.Zakaria, Z. A.Gyawali, R.Ibrahim, S. A.Rajkovic, J.Shinwari, Z. K.Khan, T.Sharifi-Rad, J.Ozleyen, A.Turkdonmez, E.Valussi, M.Tumer, T. B.Fidalgo, L. M.Martorel, M.& Setzer, W. N. (2019). Piper Species : A Comprehensive Review on Their and Applications. *Molecules*, *24*(1364), 1–118. https://doi.org/10.3390/molecules24071364
[89] Samantaray, S.& Phurailatpam, A. (2012). *Identification of sex-specific DNA markers in betel vine (Piper betle L .)*. 645–653. https://doi.org/10.1007/s10722-011-9707-4
[90] Scafaro, A. P.Haynes, P. A.& Atwell, B. J. (2010). Physiological and molecular changes in Oryza meridionalis Ng., a heat-tolerant species of wild rice. *Journal of Experimental Botany*, *61*(1), 191–202. https://doi.org/10.1093/jxb/erp294
[91] Sefloo, N. G.Steinkellner, S.& Hage-Ahmed, K. (2021). The bioprotective effect of root endophytic Serendipita herbamans against Fusarium wilt in tomato and its impact on root traits are determined by temperature. *Rhizosphere*, 100453. https://doi.org/10.1016/J.RHISPH.2021.100453
[92] Sengupta, K. (2019). Agronomy of Betelvine Crop. In M. Hasanuzzaman (Ed.), *Agronomic Crops: Volume 1: Production Technologies* (1st ed., Vol. 1, pp. 533–543). Springer Singapore. https://doi.org/10.1007/978-981-32-9151-5_2
[93] Shah, S. K.Garg, G.Jhade, D.Patel, N.& Sciences, M. (2016). *Review Article Piper Betle: Phytochemical, Pharmacological and Nutritional Value in Health Management*. *38*(34), 181–189.
[94] Shukla, R.Sachan, S.Mishra, A.& Kumar, S. (2015). A scientific review on common chewing plant of Asians : Piper Betle Linn. *Journal Of Harmonized Research in Pharmacy*, *4*(1), 01–10.
[95] Singh, D.Basu, C.Meinhardt-Wollweber, M.& Roth, B. (2015). LEDs for energy efficient greenhouse lighting. *Renewable and Sustainable Energy Reviews*, *49*, 139–147. https://doi.org/10.1016/j.rser.2015.04.117
[96] Sultan, M.El-Sharkawl, I. I.Miyazaki, T.Saha, B. B.& Koyama, S. (2014). Experimental study on carbon based adsorbents for greenhouse dehumidification. *Evergreen*, *1*(2), 5–11. https://doi.org/10.5109/1495157
[97] Suriani, N. L.Suprapta, D. N.Nazir, N.Made, N.& Parwanayoni, S. (2020). Potential of Bioactive Substances of Piper caninum and Piper betle Var . Nigra Leaves as Biopesticide in Organic Bali Rice. *Preprints*, *August*, 1–19.
[98] Suzuki, M.Umeda, H.Matsuo, S.Kawasaki, Y.Ahn, D.Hamamoto, H.& Iwasaki, Y. (2015). Effects of relative humidity and nutrient supply on growth and nutrient uptake in greenhouse tomato production. *Scientia Horticulturae*, *187*, 44–49. https://doi.org/10.1016/j.scienta.2015.02.035
[99] Taukoorah, U.Lall, N.& Mahomoodally, F. (2016). South African Journal of Botany Piper betle L . (betel quid) shows bacteriostatic , additive , and synergistic antimicrobial action when combined with conventional antibiotics. *South African Journal of Botany*, *105*, 133–140. https://doi.org/10.1016/j.sajb.2016.01.006
[100] Teanpaisan, R.Kawsud, P.& Pahumunto, N. (2016). Screening for antibacterial and antibio film activity in Thai medicinal plant extracts against oral microorganisms. *Journal of Traditional Chinese Medical Sciences*, 6–11. https://doi.org/10.1016/j.jtcme.2016.06.007
[101] Tripathi, R. Khare, G.& Kumar, N. (2000). Effect of low temperature stress on betel vine (Piper betle L.) types , Bangia and Desavari. *Journal of Spices and Aromatic Crops*, *9*(2), 141–143. https://updatepublishing.com/journal/index.php/josac/article/download/4564/4064
[102] Tüzel, Y.Gül, A.Tüzel, I. H.& Öztekin, G. B. (2019). Different Soilless Culture Systems and Their Management. *Journal of Agricultural, Food and Environmental Sciences*, *73*(3), 7–12.
[103] Vikas Bajpai, N. K. and B. K. (2020). *Phytochemistry of Piper betle Landraces* (B. Kumar (ed.); 1st ed). Taylor & Francis Group, LLC.
[104] Wahid, A.Gelani, S.Ashraf, M.& Foolad, M. R. (2007). Heat tolerance in plants: An overview. *Environmental and Experimental Botany*, *61*(3), 199–223. https://doi.org/10.1016/j.envexpbot.2007.05.011
[105] Wang, Y. H.Natschke, S. L. M.Yang, J.Niu, H. M.Long, C. L.& Lee, K. H. (2014). Anticancer Principles from Medicinal Piper (胡椒 Hú Jiāo) Plants. *Journal of Traditional and Complementary Medicine*, *4*(1),

8–16. https://doi.org/10.4103/2225-4110.124811
[106] Wilson, S. B.Iwabuchi, K.& Rajapakse, N. C. (1998). Responses of broccoli seedlings to light quality during low-temperature storage in vitro.pdf. *HortScience*, *33*(7), 1253–1257. https://irrecenvhort.ifas.ufl.edu/microprop/HortScience 1998-microprop-I.pdf
[107] Wilson, S. B.Iwabuchi, K.Rajapakse, N. C.& Young, R. E. (1998). Responses of Broccoli Seedlings to Light Quality during Low-temperature Storage in Vitro_ II. *HortScience*, *33*(7), 1258–1261. https://journals.ashs.org/hortsci/downloadpdf/journals/hortsci/33/7/article-p1258.xml
[108] Wu, X. R.Li, Y. P.Tu, S. X.Ding, Y. Z.Wang, R. G.Rensing, C.& Feng, R. W. (2019). Elevated atmospheric CO2 might increase the health risk of long-term ingestion of leafy vegetables cultivated in residual DDT polluted soil. *Chemosphere*, *227*, 289–298. https://doi.org/10.1016/j.chemosphere.2019.04.024
[109] Yang, L.Wen, K.-S.Ruan, X.Zhao, Y.-X.Wei, F.& Wang, Q. (2018). Response of Plant Secondary Metabolites to Environmental Factors. *Molecules*, *23*(762), 1–26. https://doi.org/10.3390/molecules23040762
[110] Yogeswari, S.Bindu, K. H.Kamalraj, S.Ashokkumar, V.& Jayabaskaran, C. (2020). Antidiabetic, Antithrombin and Cytotoxic bioactive compounds in five cultivars of Piper betle L. *Environmental Technology and Innovation*, *20*, 101140. https://doi.org/10.1016/j.eti.2020.101140
[111] Zhao, L.Liu, C.Yue, X.Ma, L.Wu, Y.Yang, T.& Zhang, J. (2018). Application of CO2-storage materials as a novel plant growth regulator to promote the growth of four vegetables. *Journal of CO2 Utilization*, *26*(January), 537–543. https://doi.org/10.1016/j.jcou.2018.06.011
[112] Zhong, J.Seki, T.Kinoshita, S.& Yoshida, T. (1991). *Effect of Light Irradiation on Anthocyanin Production by Suspended Culture of Perilla frutescens*. 0–5.
[113] Zobayed, S.& Saxena, P. K. (2004). Production of St . John ' s wort plants under controlled environment for maximizing biomass and secondary metabolites. *In Vitro Cell. Dev. Biol.*, *40*(February), 108–114. https://doi.org/10.1079/IVP2003498