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PROPAGATION, GROWTH PERFORMANCE AND YIELD OF Piper betle L. AT DIFFERENT NITROGEN AND SHADE LEVELS

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By

QUSAY ABDULHAMZA MUTTALEB

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

February 2018

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DEDICATION

To my lovely country Iraq

To my parents, may God have mercy on them

To my wife, brothers, sisters and daughters whose encourage and gave me the power to achieve my goal

and

To my friends who boost me all those past years

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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February 2018

Chairman : Associate Professor Thohirah Lee Abdullah @ Lee Chin Chin, PhD
Faculty : Agriculture

Piper betle L., an evergreen perennial climber from the family *Piperaceae,* is an important medicinal plant in Malaysia. Despite its importance, knowledge on its propagation is still lacking. The present study was conducted to explore the feasibility of *in vitro* and *in vivo* systems of propagation. Sterilization of explants prior to *in vitro* procedures was carried out in 20% Clorox for 20 minutes. *In vitro* culture medium with 1.0 mg/L BAP was the best medium for shoot production. The study also examined *in vivo* rooting of cuttings and growth performance as affected by different indole 3 butyric acid (IBA) treatments and different nitrogen rates (0, 50 100, 150 kg/ha) and shade levels (0%, 30% and 50 %). Morphological parameters were recorded at 30, 60 and 90 days after planting. Semi-hardwood cuttings treated with 2000 mg/L IBA showed the best rooting performance (100%). Plants were significantly taller in treatment at 50 % shade and 100 kg N /ha. At first (H1), second (H2) and third (last) (H3) harvests, maximum height for each harvest was recorded at 70, 105.67 and 184.67 cm respectively. The number of branches was highest at 26.00 from H3, followed by H2 (10.33) and H1 (6.00). The number of leaves from H1, H2 and H3 harvests were 18.00, 46.33 and 171.00 respectively. The total leaf area recorded from H1, H2 and H3 were 653.17, 4108.28 and 10401.17 cm^2 respectively. The fresh weight of leaves were recorded at 18.12 g (H1), 91.07 g (H2) and 185.15 g (H3), while the dry weight of leaves were recorded at 21.55 g (H3) 10. 91 g (H2) and 2.40 g (H1), significantly higher at 30 % shade with 100 kg N/ha. With respect to physiological parameters, photosynthesis (PR) and transpiration (TR) rates and stomatal conductance (SC) increased with increasing light intensity with treatment at 30 % shade and 100 kg N/ha giving the highest PR of 10.13 µmol CO_2 / m^2 /s, SC 0.13 mol/m^2/s dan TR 1.60 µmol/m^2/s. Chlorophyll content (a and ab) did not show any significant difference in the 30% and 50 % shade levels (1.55 and 1.49 mg/cm^2) but was highest at 100 kg N/ha (1.76 mg/cm^2). Chlorophyll b was highest at 50% shade which suggests that chlorophyll-a biosynthesis in relation to chlorophyll-b was

significant in low light intensity. Total phenolic content (TPC) (highest at 1.19 mg (GAE)/g), total flavonoid content (TFC) (highest at 128.13 mg Quercetin/g) and antioxidant activities (DPPH) (highest at 85.15%) and FRAP 7.87 µmol Fe(ll)/g dry) were highly significant in the control treatment.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMBIAKAN, PRESTASI PERTUMBUHAN DAN HASIL *Piper betle* L. PADA PARAS NITROGEN DAN KETEDUHAN YANG BERBEZA

Oleh

QUSAY ABDULHAMZA MUTTALEB

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Piper betle L., satu spesies malar hijau pemanjat daripada keluarga Piperaceae, adalah satu spesies perubatan yang penting di Malaysia. Walaupon demikian, pengetahuan mengenai pembiakannya masih berkurangan. Kajian ini dijalankan untuk meneroka kebolehlaksanaan sistem pembiakan in vitro dan in vivo. Pengsterilan eksplan sebelum prosidur in vitro telah dilakukan dengan menggunakan 20% Clorox selama 20 minit. Medium kultur yang mengandungi 1.0 mg/L BAP didapati medium yang terbaik dari segi pengeluaran tunas. Kajian juga meneliti daya pengakaran keratan secara in vivo dan prestasi pertumbuhan ekoran rawatan dengan asid indol 3 butyric (IBA)serta kadar nitrogen (0, 50 100, 150 kg/ha) and paras keteduhan (0%, 30% and 50 %). Parameter morfologi telah dicatat pada 30, 60 and 90 hari selepas penanaman. Keratan separa-matang yang dirawat dengan 2000 mg/L IBA menunjukkan prestasi pengakaran yang terbaik (100%). Tanaman didapati lebih tinggi dengan ketara daripada rawatan 50% keteduhan dan 100 kg N/hektar. Pada tuaian pertama (H1), kedua (H2) dan ketiga (terakhir) (H3), ketinggian maksimum bagi setiap tuaian adalah masing-masing pada 70, 105.67 dan 184.67 cm. Bilangan dahan didapati tertinggi pada H3 (26.00) diikuti dengan H2 (10.33) dan H1 (6.00). Bilangan daun daripada H1, H2 dan H3 adalah masing-masing 18.00, 46.33 dan 171.00. Jumlah keluasan daun daripada H1, H2 and H3 adalah masing-masing 653.17, 4108.28 dan 10401.17 cm^2. Berat basah daun segar telah direkodkan pada 18.12 (H1), 91.07 (H2) dan 185.15 g (H3), sementara berat kering daun adalah pada 21.55 (H3), 10. 91 (H2) dan 2.40 g (H1), iaitu lebih tinggi dan ketara pada 30% keteduhan dengan 100 kg N/ha. Dalam kajian yang sama, keluasan daun spesifik (SLA) telah mencatatkan 272.44 (H1), 376.57 (H2) dan 483.82 cm^2/g. Kadar pertumbuhan tanaman (CGR) telah merekodkan 0.362 g daripada tuaian 30-60 hari diikuti oleh 1.794 daripada tuaian 60-90 hari dengan rawatan 100 N kg/hektar. Nisbah akar-pucuk (RSR) daripada H1 dan H2 adalah 0.79 g diikuti oleh H3 pada 0.43 g dengan ketinggian yang ketara pada keteduhan 30% dan 100 kg N/hektar. Bagi parameter fisiologi, kadar fotosintesis (PR)

dan transpirasi (TR), serta konduktans stomata (SC) meningkat dengan peningkatan keamatan cahaya dengan rawatan keteduhan 30% dan 100 kg N/hektar dengan mencatatkan nilai tertinggi PR pada 10.13 µmol CO_2 / m^2 /s, SC 0.13 mol/m^2/s dan TR 1.60 µmol/m^2/s. Kandungan klorofil (a dan b) tidak menunjukkan perbezaan yang ketara diparas keteduhan 30% dan 50% (1.55 dan 1.49 mg/cm^2), tetapi memuncak tinggi pada 100 kg N/hektar (1.76 mg/cm^2). Klorofil-b mecapai ketinggian maksimum pada keteduhan 50%, dan ini membawa erti bahawa biosintesis klorofil-a yang berkaitan dengan klorofil- b adalah rendah dengan ketara dalam keamatan cahaya yang rendah. Kandungan fenolik total (TPC) (tertinggi pada 1.19 mg(GAE)/g), kandungan flavonoid total (TFC) (tertinggi pada 128.13 mg Quercetin/g) dan aktiviti anti-oksidan (DPPH) (tertinggi pada 85.15%) dan FRAP pada 7.87 µmol Fe(ll)/g dry) adalah ketara pada rawatan kawalan.

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Qusay Abdulhamza Muttaleb, 2018

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LIST OF ABBREVIATIONS

DPPH	1,1-Diphenyl-2-picrylhydrazyl
TPTZ	2,4, 6-tri-(2-pyridyl)-striozine
AlCl3	Aluminum chloride
ANOVA	Analysis of Variance
BAP	benzylaminopurine
BDW	biomass dry weight
Ca	Calcium
CEC	Cation Exchange Capacity
cm mol/kg	centi mol per kilogram
cm	centimeter
cm^3	centimeter cubic
cm^2	centimeter square
cm^2/g	centimeter square per gram
Chl (a+b)	Chlorophyll (a + b)
Chl (a)	Chlorophyll (a)
Chl (b)	Chlorophyll (b)
pH	concentration of H+
CGR	Crop Growth Rate
DAT	Days After Transplanting
^oC	Degree centigrade
DLW	Dry Leaf Weight
F	Fertilization
FCR	Folin-Ciocalteau reagent
FAF	formalin: acitic acid

FRAP	Free Reducing Antioxidant Potential
FLW	Fresh Leaf Weght
GA	Gallic acid
g	gram
g/kg	gram per kilogram
g/L	gram per liter
H	Harvest
ha	hectare
h	hour
H_2O_2	Hydrogen peroxide
IBA	Indole-3-butyric acid
FeCl2	Iron (II) chloride
L	liter
Mg	Magnesium
µg	microgram
µg/L	microgram per liter
µml	micromillimeter
µmol/m^2/s	Micromole per meter square per second
µmol/mol	Micromole per mol
Mg	milligram
ml/L	milligram per liter
mm	millimeter
mm mole	millimole
mmolHCL	millimole Hydrochloric acid
mmol/m^2/s	Millimole per meter square per second
MS	Murashinge and Skoog

N	Nitrogen
NB	Number of Branches
NL	Number of Leaves
%shade	percentage of shade
P	Phesphorus
PR	Photosynthetic Rate
PH	Plant Hieght
K	Potassium
KCl	Potassium Chloride
RCBD	Rondomized Complete Block Design
RSR	Root-Shoot Ratio
NaOH	Sodium hydroxid
SLA	Specific Leaf Area
SD	Standard Deviation
SAS	Statistical Analysis Software
Gs	Stomata Conductance
TBA	tertiary butyl-alcohol
TFC	Total Flavanones Content
TLA	Total Leaf Area
TPC	Total Phenolics Content
E	Transpiration rate
V	volume

CHAPTER 1

INTRODUCTION

Human interest in herbal medicine is increasing day by day. Awareness regarding the potentiality of herbal medicine in curing certain physiological disorders in human has increase day by day after observing the side effect of synthetic drugs (Gopalakrishnan *et al.*, 2012). Herbal medicines are not new for health practices and it has been used since human civilization existed (Alsarhan *et al.*, 2014). It played an imperative role to treat the different types of human ailments (Sharma *et al.*, 2013). According to the report of World Health Organization (2003), 80% of the world population still relies on the traditional/ herbal medicine for their primary health care. These medicinal plants or phytomedicine refers to the use of any plant part such as seeds, leaves, bark or flowers for curing physiological disorders in human. The plants are found in different regions of the world, with a few of these have already been discovered and millions are still needed to be explored.

In Southern Asian region, Malaysia is a forest enrinch country possessing a variety of plants in its agroecosystem. These plants can be used as herbal medicine, however, few of these have already play a part in traditional Malaysian health care system due to their therapeutic efficacy (Alsarhan *et al.*, 2014). In this connection, *Piper betle* Linn from the Piperaceae family is one of the most precious medicinal herbs found in central and eastern Malaysia. It is commonly known as "Sirih" in the native language, and is second to tea and coffee based on daily consumption in Southern Asian region (Alsarhan *et al.*, 2014). Inspite of its medicinal properties, *P. betle* is commercially cultivated for its leaves. The leaves are used as masticator for chewing purpose in many parts of the world especially in the tropical and sub-tropical countries of the world (Pradhan *et al.*, 2014).

Piperaceae family is already very peculiar as it contains well-known plants such as black pepper and kava. There are about 100 varieties of *P. betle* found throughout the world and out of this 40 are found only in India and 30 in West Bengal (Guha, 1997; Samanta, 1994). However, the most probable place of origin of betel vine is Malaysia (Chattopadhyay and Maiti, 1967). This plant is perennial and dioecious in nature, with evergreen and shade loving creeping characteristics. Every part of the plant has high medicinal value and several attributes such as digestive, carminative, stimulant, antiseptic and antifungal activities have been described. A phenolic compound, hydroxyl-chavicol, with anti-carcinogenic property has also been identified in *P. betle* leaves (Verma *et al.*, 2004).

Stem cuttings either as semi hardwood or softwood cuttings are among the most probable techniques for vegetative propagation in most plants. (Tchoundjeu *et al.*, 2004; Zlesak, 2012). This technique can also play a significant role in the clonal propagation of *P. betle*. The triggerring of adventitious root initiation and development

in stem cuttings treated with auxins is well discussed in many plants (Erturk *et al*., 2010; Hartmann *et al.*, 2010: Bolat, 1995). Nevertheless, exogenous plant growth regulator such as auxin plays in important role in inducing rooting in stem cutting. Among the auxin that can induce rooting in stem cutting include indole 3 butyric acid (IBA) (Hartmann *et al.*, 2010; Yahya *et al.*, 2009). In *P. betle* no report has been made on the use of IBA in stimulating root formation from stem cutting.

On the other hand, in order to supply the active compounds for pharmaceutical and herbal industries, tissue culturing of medicinal plants is widely used. The culturing techniques now days has been often observed in conservation of genetic material of many vulnerable medicinal plants (Sidhu, 2011). In that case, micro-propagation is a good technique in order to increase the vegetative growth. Furthermore, this process insures a good regular supply of medicinal plants as multiplication from plants tissues or seeds occur with minimum space and time (Prakash and Van Staden, 2007). Furthermore, the less abundance of sunlight in Malaysia due to cloudy weather throughout the year can also reduce the phenol and flavonoid content of *P. betle* that do not only affect the yield but lower down the plant medicinal properties too. Due to this, efforts should be taken to increase the biomass by improving plant vegetative growth by application of high nutrient especially nitrogen to the soil at the vegetative growth phase of *P. betle*.

Currently, not much research has been carried out to solve the above problem. Therefore, this study was conducted with the objective:

i. To determine the effect of shade levels and nitrogen rates on the morphological traits, growth and yield of *P. betle.*
ii. To determine the role of shade and nitrogen on physiological traits and secondary metabolites of *Piper betle* L.
iii. To determine the types of cuttings and concentration of IBA in rooting of stem cuttings of *P. betle* and to develop micropropagation protocol for *P. betle*. using node explant.

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