MICROPROPAGATION OF *Andrographis paniculata*

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF BIOTECHNOLOGY (HONOURS)

FACULTY OF SCIENCE, TECHNOLOGY, ENGINEERING AND MATHEMATICS
INTI INTERNATIONAL UNIVERSITY
PUTRA NILAI, MALAYSIA

2015
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ACKNOWLEDGEMENT

I would like to express deepest appreciation to my supervisor, Dr. Thong Weng Hing. He always guided, motivated and helped me in this project. Thus, I was able to conduct this project successfully. Second, I would also like to thank my family members who always support me and thus I have motivation to finish my project. I would like to special thanks to Dr. Wong Ling Shing for borrowing me his camera so that I can take the photos of my cultures. Besides that, I would like to show appreciation to my friends who helped me in this project and they always supported me all the way. Lastly, I would like to thank all the lecturers and laboratory staff who assisted me with my project throughout this semester.
ABSTRACT

*Andrographis paniculata* belongs to Acanthaceae family. *A. paniculata* is a herbaceous plant that can be found in both tropical and subtropical countries. It is also a medicinal plant and possesses high medicinal values. Therefore, the objectives of this study were to determine the germination rate of the seed of *A. paniculata* and the effects of different concentrations of indole-3-butyric acid (IBA) and zeatin on the growth of *A. paniculata*. In this experiment, seeds of *A. paniculata* were grown in vitro on the Murashinge and skoog (MS) medium. After that, the cotyledonary leaves of this plant were subcultured on MS medium supplemented with 0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/L zeatin and IBA in single exposure or in combination and observed for multiple shoots and roots formation over a period of 8 weeks. Highest shoot formation and highest root formation was produced in MS medium supplemented with single exposure of 5.0 mg/L zeatin and 5.0 mg/L IBA respectively. Zeatin and IBA in combination was found to produce callus. Micropropagation could serve as an alternative reproduction system for *A. paniculata*. 

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<td>ANOVA</td>
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<td>BA</td>
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<td>BAP</td>
<td>6-benzylaminopurine</td>
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<td>HIV</td>
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<td>IAA</td>
<td>indole-3-acetic acid</td>
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<td>IBA</td>
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<td>mg/L</td>
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<td>mL</td>
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<td>MS</td>
<td>Murashinge and skoog</td>
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<td>NAA</td>
<td>1-naphthaleneacetic acid</td>
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<td>NaOCl</td>
<td>sodium hypochloride</td>
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<td>NaOH</td>
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<td>PGR</td>
<td>plant growth regulator</td>
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<td>TDZ</td>
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1.0 CHAPTER 1:

INTRODUCTION

Medicinal plants are important for human as food source and supplement to enhance their health condition. Medicinal plants are involved in herbalism and different parts of the plant can be used to treat many diseases (Hassan, 2012). The medicinal usages, ethnobotany, phytochemistry and pharmacology of medicinal plants are worth to be discovered and explored (Hossain, Urbi, Sule & Rahman, 2014). Nowadays, herbal-based medicine is gaining more attentions from people due to the realization of the adverse effects associated with modern medicine abuse. Hence, the number of herbal drug industries is increasing (Kataky & Handique, 2010; Verma & Singh, 2008). The demand for herbal medicine is increasing owing to their broad biological activities, low cost and safer than the synthetic drug (Kataky & Handique, 2010).

There are 28 species under genus *Andrographis*, but not all of them exhibiting medicinal properties. *Andrographis paniculata* is one of the species under genus *Andrographis* exhibiting medicinal properties (Kumar, Dora, Singh & Tripathi, 2012; Yadav & Singh, 2012). *A. paniculata* is a herbaceous plant with high medicinal values which is beneficial in many treatments and thus it is recommended for cultivation by National Medicinal Plant Board (Rawat & Vashistha, 2011). It is belonged to family of Acanthaceae and found in India, Pakistan, Sri Lanka and China (Kumar, Chakraborty & Kumar, 2010). This plant has been used for therapeutic purposes in Indian and Oriental medicine (Majee, Gupta, Mazumder & Chakraborty, 2011). There are a broad range of pharmacological effects of *A. paniculata*. Patients have to be administered with the proper proportion of the extracts from such medicinal plant (Tejavathi, Anitha, Murthy & Nijagunaiah, 2011). Any medicinal plant extract that is to be consumed by human must have proven safety and effectiveness in order to minimize or eliminate adverse effects. *A. paniculata* has been proven that it showed no toxicity when tested against animals (Niranjan, Tewari & Lehri, 2010; Subramanian, Asmawi & Sadikun, 2008).
The common name of *A. paniculata* is known as Kalmegh or King of bitters (Akhbar, 2011; Katakly & Handique, 2010). However, there are different specific names which are recognized in different countries or places. In northeastern India, the plant is known as Maha-tita. *A. paniculata* is also known as Bhui-neem. This plant is called as Hempedhu Bumi in Malaysia. This is because *A. paniculata* is one of the bitterest plants that have been used in traditional medicine (Kumar et al., 2012). The plant of *A. paniculata* generally have a dark green stem, leaves, small flower and yellow brown seeds located within the fruits (Yadav & Singh, 2012). Parts of the plants like roots and leaves have been used conventionally to treat different diseases (Jarukamjorn & Nemoto, 2008).

*A. paniculata* was originally propagated using seed but they might have different appearance in different locations and low germination rate (Rawat & Vashistha, 2011; Talei & Valdiani & Abdullah & Hassan, 2012; Tejavathi, et al., 2011). In order to ensure uniformity of the plants and enhance germination rate by using seed propagation, micropropagation is an alternative to cultivate this plant (Tejavathi et al., 2011). The demand for *A. paniculata* is increasing as it has high market potential (Katakly & Handique, 2010). Medicinal herb such as *Entada pursaetha* is one of the endangered species that facing extinction when overproduction of the plant parts for medicinal purpose (Vidya, Krishna, Manjunatha & Shankarmurthy, 2005). Hence, micropropagation is introduced to overcome the problem by rapidly producing the plants in higher yield, quality and uniformity (Debnath, Malik & Bisen, 2006). Hormonal treatment can increase seed germination rate as well (Karuppasamy & Kalimuthu, 2010; Kumar et al., 2010).

The aims of this experiment were:

1. To determine the germination rate of the seed of *A. paniculata*.
2. To determine the effects of different concentrations of indole-3-butyric acid (IBA) and zeatin on the growth of *A. paniculata*. 

2
2.0 CHAPTER 2:

LITERATURE REVIEW

2.1 Andrographis paniculata

A. paniculata belongs to Acanthaceae family and proved to have medicinal activities. A. paniculata plant has different names in different languages. In India, it is called as ‘Kalmegh’ whereas it is known as ‘Chuan-Xin-Lian’, Yijianxi or Lanhelian in China (Akhbar, 2011). In Malaysia, it is known as ‘hempedu bumi’. Besides that, it is also called as ‘Senshinren’ in Japan while in Scandinavian it is called as ‘green chireetta’ (Jayakumar, Hsieh, Lee & Sheu, 2013).

2.1.1 Physical characteristics of A. paniculata

A. paniculata is an erect annual herb which grow vertically to a height of 30 to 110cm (Jat, Singh, Choudhary & Maheshwari, 2014; Kumar et al., 2012). The stem of A. paniculata is dark green and 0.3 to 1.0 m in height with a diameter of 2 to 6 mm (Hannah, Olalekan & Bamidele, 2014). The stem is squared in cross section with longitudinal furrows and wings on the angles of the younger parts (Das & Srivastav, 2014; Yadav & Singh, 2012). Leaves are simple, lance shape, pinnate, 8 cm long and 2.5 cm wide (Kataky & Handique, 2010). The flowers are white (Deepak, Pawar & Shinde, 2014), small and spreading axillary and terminal racemes or panicles (Jarukamjorn & Nemoto, 2008). The fruits are capsule and it is erect, around 1 to 2 cm long and 2 to 5 mm wide (Kataky & Handique, 2010). The seeds of A. paniculata are very small, numerous and sub quadrature (Jarukamjorn & Nemoto, 2008; Kumar et al., 2012; Niranjan et al., 2010). The seeds are yellow brown in colour (Parashar, Upadhyay, Singh, Diwedi & Khan, 2011).

2.1.2 Distribution of A. paniculata

A. paniculata is found throughout tropical and subtropical Asia, Southeast Asia, and India (Jayakumar et al., 2013; Niranjan et al., 2010). It also grows abundantly in
China, Thailand, Cambodia, Caribbean islands, Laos, Hong Kong, Myanmar, and Sri Lanka. In addition, *A. paniculata* medicinal plant is also available in Malaysia, Pakistan, Indonesia, East and West Indies, Singapore, Americas and Vietnam (Hossain et al., 2014; Jarukamjorn & Nemoto, 2008; Kumar et al., 2012; Yadav & Singh, 2012).

It can be found in different areas, such as flat area, hill slopes, farms, moist or shady places and sea shore (Yadav & Singh, 2012). People can also grow *A. paniculata* in pine, forest areas, wastelands, roads and in villages. It can also be cultivated in gardens (Hossain et al., 2014). *A. paniculata* can also grow in variety of soil. The growth and yield of *A. paniculata* can be increased when it grow on the soil rich in organic matter (Rawat & Vashista, 2011).

### 2.1.3 Medical value of *A. paniculata*

The leaves and roots of *A. paniculata* were used for medicinal purposes. Researches have conducted varies studies on *A. paniculata* which proved that this plant have anti-diarrhoeal, antioxidant, anti-malarial, antiviral, anti-cancer, antibacterial, antifungal, and anti-inflammatory activity (Jayakumar et al., 2013; Niranjan et al., 2010). Leaves and roots used in general debility, dyspepsia, and dysentery (Akhbar, 2011; Kumar et al., 2012). This herb is used to remove heat because of its cooling property. The toxins can be removed from our body for the same reason (Hossain et al., 2014; Niranjan et al., 2010; Sudhakaran, 2012).

It is used to improve stomach function, bitter tonic, reduce muscle cramps and antiperistaltic (Anju, Jugnu, Kavita, Arun & Sandeep, 2012). It also exhibits carminative, pharyngolaryngitis, choleric, diuretic and adaptogenic. It has the effects of emollient, astringent, antipyretic and anthelmintic (Akhbar, 2011). The juice or chemical compounds from the fresh leaves of *A. paniculata* are extracted and used to release griping, irregular bowel movements and loss of appetite (Kumar et al., 2012). Furthermore, due to the ‘blood purifying’ activity of *A. paniculata*, it is recommended for leprosy, gonorrhea, scabies, boils and skin problems (Hossain et al., 2014). *A. paniculata* is also used to treat hypocholesterolemic, hypoglycemic, diarrhea and cough with thick sputum, carbuncle and sores (Kumar et al., 2012).
The modern uses of *A. paniculata* have been discovered for the prevention and treatment for common cold. It can reduce formation of blot and thus provide advantage in cardiovascular disease (Akhbar, 2011; Kumar et al., 2012). This plant also shows benefits in treatment of cancer and it exhibits a neutralizing activity against the human immunodeficiency virus (HIV) infection (Jarukamjorn & Nemoto, 2008). *A. paniculata* contains andrographolide and this compound was identified to have antiviral activity against HIV (Jayakumar et al., 2013). *A. paniculata* was reported to be effective in treatment of infectious disease and non-infectious diseases such as epidemic encephalitis B, mumps, neonatal subcutaneous annular ulcer, herpes zoster and chicken pox. It can also treat inflammatory disease acted on middle ear, neurodermatitis, and burns which mean the skin get heated. It can use in the treatment of female organ, vaginistis and cervical erosion (Akhbar, 2011). Other uses of *A. paniculata* included sinusitis, allergies, astringent, bacteria killing agent and painkiller (Hannah et al., 2014).

2.1.4 Chemical Compound of *A. paniculata*

The compounds that can be found in *A. paniculata*, including over 20 diterpenoids and over ten flavonoids, were identified and extracted using ethanol or methanol from plant parts like whole plant, leaf and stem (Chao & Lin, 2010; Chao & Lin, 2012). However, the important compound that was identified in *A. paniculata* was andrographolide (Dandin & Murthy, 2012). The leave of *A. paniculata* contain higher amount of andrographolide when compared to stem whereas seeds contain lowest amount of this compound (Alagesabooopathi, 2011; Yadav & Singh, 2012). It was also known as diterpene lactone, and it is colorless and crystalline (Niranjan et al., 2010). This compound gives *A. paniculata* its bitter taste (Chauhan, Tomar, Singh, Ali, Badoni, Debratati & Rana., 2009). Andrographolide becomes so important because it was identified to have multiple pharmacological properties (Chao & Lin, 2010), including anti-inflammatory, antiplate-aggregation and hepatoprotective (Jayakumar et al., 2013). Besides, the extract also exhibited activity of antiallergic, antivcancer and anti-human immunodeficiency virus (HIV) activities (Jha, Behar, Sharma, Mannade, Kumar & Sharma, 2012).
2.1.5 Toxicity of *A. paniculata*

*A. paniculata* is a safe and non-toxic plant and it has been used widely to treat many diseases (Jarukamjorn & Nemoto, 2008; Jayakumar et al., 2013). The toxicity study on *A. paniculata* was conducted in mice, rats, and rabbits. In one of the research, the rats were given high dosage of *A. paniculata* to determine the toxicity of plant. No toxicity was found out even in high dose of *A. paniculata* (Jayakumar et al., 2013). Another study was conducted on mice and rabbits which they were safe and alive (Niranjan et al., 2010). However, in one of the study, *A. paniculata* caused emesis due to its extreme bitterness (Hossain et al., 2014). When using over dosage of *A. paniculata* extract, it caused some of the adverse effect which included allergic reaction, fatigue, headache, instability of gastric and nausea (Hossain et al., 2014).

2.2 MICROPROPAGATION

Plants were conventionally propagated by using seed as seeds are the natural resources. High quality seeds were required to increase crop production (Chauhan et al., 2009). The problems of seed propagation included not enough crop production, low germination rates and low viability of seeds (Arunkumar & Jayaraj, 2011; Karuppusamy & Kalimuthu, 2010). *A. paniculata* was originally propagated using seed but it possessed problem of low germination rate and the seeds remain dormancy (Talei et al., 2012). Hence, micropropagation was introduced as it can save the endangered, rare and extinct plant species and increase production of crops. Micropropagation can also solve the problem which some of the plants may be difficult to be propagated through conventional method (Kapai, Kapoor & Rao, 2012; Mehrotra, Goel, Kukreja & Mishra, 2007).

Micropropagation was started with explants selections and then followed by aseptic culture techniques. Suitable method of multiplication was introduced to increase number of plantlets followed by rooting and acclimatization of the plantlets (Mihaljević et al., 2013). Micropropagation was carried out under controlled aseptic conditions and in a sterile area, for example laminar air flow cabinet (Ahsan, Amjad, Iqbal & Javed, 2013) to prevent contamination, to produce disease free plantlets and to conserve the germplasm (Anand, Nandagopalan & Doss, 2011; Ismail, Rani,
Batra, 2012; Kapai et al., 2010). Explant is an initial source of plant tissue culture. The leaves, shoot tips, roots and nodal segments of plants normally can be used as the sources of explant (Ahsan et al., 2013; Ismail et al., 2012). Micropropagation by using nodal segments as explants can produce multiple shoots, maintained genetic stability and genetic transformation of the plants (Jain & Bashir, 2010; Pattar & Jayaraj, 2012). Some studies suggested that nodal segment of explants produce higher shoot proliferation than leaf (Pattar & Jayaraj, 2012). Nodal explant was recommended to be used for some of the medicinal plants as it can induce multiple shoots (Ghanti, Kaviraj, Venugopal, Jabeen & Rao, 2004).

2.2.1 Sterilization of explants

Sterilization of explants is essential to eliminate any contamination on the plants during in vitro micropropagation. It is also the most important step in aseptic culture of explants. This is because microorganism can easily grow on the plant and therefore contaminated the plant. For example, viruses, bacteria, yeast and fungi can grow on plants (Mihaljević et al., 2013). Microbes can utilize the nutrients to grow and alter the environment of culture media such as pH, thus inhibiting the growing of explant (Odutayo, Amusa, Okutade, & Ogunsanwo, 2007).

Different sterilizing method was applied to different explants to achieve highest efficiency of sterilization. The type of sterilizing agent has to be chosen correctly in order to remain the biological activities of plants. Besides, duration of sterilization and concentration of sterilizing agent were crucial factors affecting the sterilization process (Odutayo et al., 2007; Tomaszewska & Figas, 2011). Hence, the contaminants can be eliminated entirely and sterile plant material would be obtained. Sodium hypochlorite, ethanol and mercuric chloride were suggested to be utilized to sterilize seeds of any plants (Talei, Saad, Yusop, Kadir & Valdiani, 2011). These sterilizing agents can eliminate different types of microbes from surface and objects (Mazzola, Jozala, Novaes, Moriel & Penna, 2009). Sodium hypochlorite was normally used when compared to mercuric chloride because mercuric chloride might be toxic to the plants and also users (Talei et al., 2011).