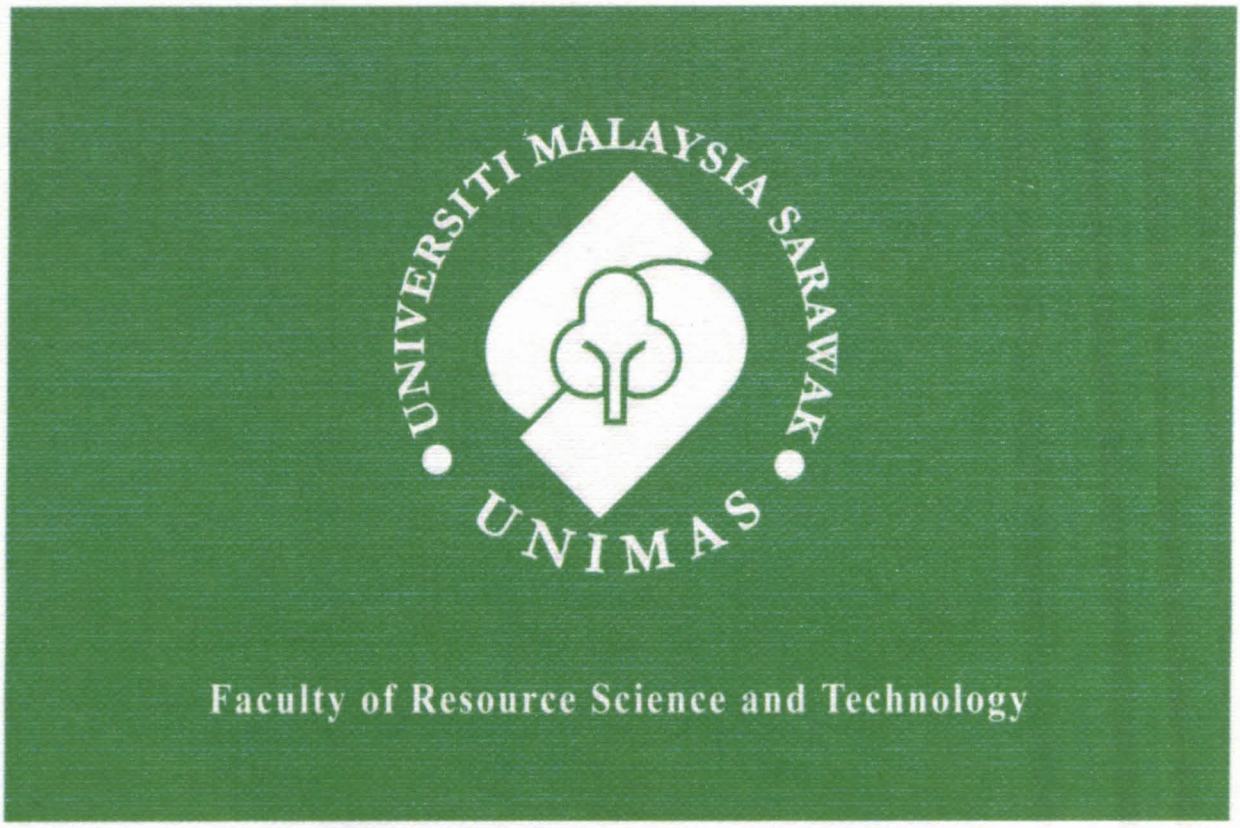

EFFECT OF BAP ON *IN VITRO* SHOOT INDUCTION OF *NEOLAMARCKIA MACROPHYLLA* (RED KELAMPAYAN)

CHIA CHUN MIN
(51452)

Bachelor of Science with Honours
(Resource Biotechnology)
2018

EFFECT OF BAP ON *IN VITRO* SHOOT INDUCTION OF *NEOLAMARCKIA MACROPHYLLA* (RED KELAMPAYAN)

CHIA CHUN MIN
(51452)

A thesis submitted in fulfillment of the requirement for the degree of
Bachelor Science with Honours

Supervisor: AP Dr Ho Wei Seng

Resource Biotechnology

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2018

UNIVERSITI MALAYSIA SARAWAK

Grade: _____

Please tick (√)

Final Year Project Report	√
Masters	
PhD	

DECLARATION OF ORIGINAL WORK

This declaration is made on the ....04..... day of ....06.....2018.

Student's Declaration:

I, Chia Chun Min, 51452, Faculty of Resource Science and Technology hereby declare that the work entitled **'Effect of BAP on *In Vitro* Shoot Induction of *Neolamarckia macrophylla* (Red Kelampayan)'** is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

04.06.2018
Date submitted

Chia Chun Min (51452)

Supervisor's Declaration:

I, Assoc. Prof. Dr. Ho Wei Seng hereby certifies that the work entitled **'Effect of BAP on *In Vitro* Shoot Induction of *Neolamarckia macrophylla* (Red Kelampayan)'** was prepared by the above named student, and was submitted to the "FACULTY" as a * partial/full fulfillment for the conferment of Bachelor Of Science With Honours In Resource Biotechnology, and the aforementioned work, to the best of my knowledge, is the said student's work.

Received for examination by: _____ Date: 04.06.2018
(Assoc. Prof. ~~Dr. Ho Wei Seng~~)

I declare that Project/Thesis is classified as (Please tick (√)):

☐ **CONFIDENTIAL** (Contains confidential information under the Official Secret Act 1972)*
☐ **RESTRICTED** (Contains restricted information as specified by the organization where research was done)*
☑ **OPEN ACCESS**

I declare this Project/Thesis is to be submitted to the Centre for Academic Information Services and uploaded into UNIMAS Institutional Repository (UNIMAS IR) (Please tick (√)):
☐ **YES**
☐ **NO**

Validation of Project/Thesis

I therefore duly affirm with free consent and willingly declare that this said Project/Thesis shall be placed officially in the Centre for Academic Information Services with the abiding interest and rights as follows:

- This Project/Thesis is the sole legal property of University Malaysia Sarawak (UNIMAS).
- The Centre for Academic Information Services has the lawful right to make copies for the purpose of academic and research only and not for other purpose.
- The Centre for Academic Information Services has the lawful right to digitalize the content for the Local Content Database.
- The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis for academic exchange between Higher Learning Institute.
- No dispute or any claim shall arise from the student itself neither third party on this Project/Thesis once it becomes the sole property of UNIMAS.
- This Project/Thesis or any material, data and information related to it shall not be distributed, published or disclosed to any party by the student except with UNIMAS permission.

Student signature _____ Supervisor signature: _____

Current Address:
Fakulti Sains dan Teknologi Sumber,
Universiti Malaysia Sarawak,
94300 Kota Samarahan,
Sarawak.

Notes: * If the Project/Thesis is **CONFIDENTIAL** or **RESTRICTED**, please attach together as annexure a letter from the organization with the period and reasons of confidentiality and restriction.

[The instrument was prepared by The Centre for Academic Information Services]

Acknowledgements

I have gained wonderful experiences and knowledge in completing this research. First of all, I would like to express my deepest gratitude to my supervisor, Associate Professor Dr Ho Wei Seng for giving me advises and sharing his expert tissue culture knowledge. Moreover, I would like to thank to my post-graduate students, Sew Jun Wu, Mok Pei Kieng, Eng Wee Hiang and Delvino Disonneo Anak Dalin for their kind and guidance throughout this study. I also wish to thank lab assistant, Ms. Kamalia which provided adequate materials and tools throughout the study. I would also like to express my appreciation to my parents and siblings for moral support. Finally, I wish to thank my friends, Low Yong Chun and Ooi Ka Beng and my lab-mates, Lee Tian Wee and Ong Li Ying for their helps and opinions.

Table of contents

List of Abbreviations

ANOVA	Analysis of Variance
B5	Gamborg B5 media (1976)
BAP	6-Benzylaminopurine
DMRT	Duncan's Multiple Range Test
PGR	Plant growth regulators
SPSS	Statistical Package for the Social Sciences

List of Figures

List of Tables

Effect of BAP on *In Vitro* Shoot Induction of *Neolamarckia macrophylla* (Red Kelampayan)

Chia Chun Min

Resource Biotechnology
Faculty of Resource Science and Technology
University Malaysia Sarawak

ABSTRACT

Overpopulation and increase in demand of timer have caused the depletion of timber resources. Therefore, *Neolamarckia macrophylla* (red kelampayan) belongs to family Rubiaceae is selected as one of the species for forest plantation and reforestation programmes in Sarawak due to its fast growing characteristic. However, conventional propagation of *N. macrophylla* is time consuming and possessed several problems mentioned. Therefore, *in vitro* shoot induction of *N. macrophylla* should be explored for mass propagation purposes in order to meet the global demands for timber resources. Effect of BAP on *in vitro* shoot induction of *N. macrophylla* was investigated. The optimum concentration of BAP on *in vitro* shoot induction of *N. macrophylla* was determined. In this study, B5 media supplemented with different concentration of BAP (0.5, 1.0 and 1.5 mg/L) was used for *in vitro* shoot induction from nodal explants of *N. macrophylla.* The parameters used in this study were number of shoots, shoot regeneration percentage (%), multiple shoot induction percentage (%) and shoot length (cm). The data was analyzed by using One-way ANOVA and Duncan's Multiple Range Test (DMRT) in SPSSS version 22. Result showed that the B5 supplemented with 0.5 mg/L BAP had given the highest mean number of shoots with 3.80 shoots per explant. 100% of shoot regeneration was observed in B5 treated with 0.5 and 1.0 mg/L while highest shoot length was observed in B5 blank. One-way ANOVA showed significant effect of BAP in terms of number of shoots regenerated. In this study, B5 supplemented with 0.5 mg/L BAP was identified as optimum concentration on *in vitro* shoot induction of *N. macrophylla.*

Keywords: *Neolamarckia macrophylla,* BAP, number of shoots, shoot regeneration, multiple shoot induction.

Effect of BAP on *In Vitro* Shoot Induction of *Neolamarckia macrophylla* (Red Kelampayan)

Chia Chun Min

Resource Biotechnology
Faculty of Resource Science and Technology
University Malaysia Sarawak

ABSTRAK

Lebihan penduduk dan permintaan yang meningkat pemasa telah menyebabkan kekurangan sumber kayu. Oleh itu, *Neolamarckia macrophylla* (kelampayan merah) tergolong dalam keluarga Rubiaceae telah dipilih sebagai salah satu spesies untuk program perladangan hutan dan penanaman semula hutan di Sarawak kerana sifatnya yang cepat tumbuh. Walau bagaimanapun, pembiakan konvensional *N. macrophylla* sangat memakan masa dan mempunyai beberapa masalah yang disebutkan. Oleh itu, *in vitro* pucuk induksi *N. macrophylla* telah digalakkan untuk tujuan penyebaran besar-besaran untuk memenuhi permintaan global bagi sumber kayu. Kesan BAP in vitro pucuk induksi *N. macrophylla* telah dikaji. Kepekatan sesuai BAP in vitro pucuk induksi N. *macrophylla* telah ditentukan. Dalam kajian ini, media B5 ditambah dengan kepekatan BAP yang berbeza (0.5, 1.0 dan 1.5 mg/L) telah digunakan dalam vitro pucuk induksi dari eksplant nodus N. *macrophylla*. Parameter yang digunakan dalam kajian ini ialah bilangan pucuk, peratusan pertumbuhan semula pucuk (%) peratusan pelbagai pucuk induksi (%) dan panjang pucuk (cm). Data telah dianalisis dengan menggunakan One-way ANOVA dan Duncan's Multiple Range Test (DMRT) dalam SPSSS versi 22. Keputusan menunjukkan bahawa B5 ditambah dengan 0.5 mg/L BAP telah memberikan nombor min yang tertinggi iaitu bilangan pucuk dengan 3.80 pucuk pada setiap eksplan. 100% daripada pertumbuhan semula pucuk diperhatikan dalam B5 dirawatkan dengan 0.5 dan 1.0 mg/L manakala panjang pucuk tertinggi diperhatikan dalam B5 kosong. One-way ANOVA telah menunjukkan kesan BAP yang ketara BAP dari segi bilangan pucuk tertumbuh. Dalam kajian ini, B5 ditambah dengan 0.5 mg/L BAP telah dikenal pasti sebagai kepekatan optimum di in vitro pucuk induksi *N. macrophylla.*

Kata kunci: *Neolamarckia macrophylla*, BAP, bilangan pucuk, pertumbuhan pucuk semula, pelbagai pucuk induksi.

1.0 Introduction

Timber is a natural resource that is involved in the production of furniture, paper manufacture, building and construction. However, overpopulation and substantial increase in demand have caused the rapid depletion of timber resources. According to Malaysia Timber Industry Board (2009), it is estimated that the annual production of logs of Malaysia for the period 2016 – 2020 from Permanent Reserved Forest will be reduced to approximately 14 million m^3 from around 19.4 million m^3 during 2006 – 2010. Shortage of the timber resources indirectly increases the dependency of Malaysia to other ASEAN countries by importing the timber resources from them. Therefore, to ensure the sustainable supply of timber, large-scale plantation of fast growing species is one of the ways to solve this issue.

Neolamarckia macrophylla is one of the potential species to solve the issue due to its fast growing properties and self-pruning ability (Halawane *et al.*, 2011). According to Halawane *et al.* (2011), kelampayan tree has higher economic value compared to sengon tree with the same tree diameter. Therefore, this species had caught the attention of the government and private sectors due to its growth characteristic and economic profitability. Although large plantation of *N. macrophylla* provides good timber resources, the bottleneck of this species plantation lies with limited knowledge of propagation information and technology. Based on a research conducted by Irawan and Purwanto (2014), the community interest in kelampayan plantation for livelihood support and land rehabilitation were high. However, this situation did not balance with sufficient skill and knowledge of silviculture practices, particularly the proper seedling propagation methods. For example, the failure of kelampayan plantation in Southeast Sulawesi was due to the limited knowledge of seed germination and seedling propagation among the communities (Irawan and Purwanto, 2014). Besides, kelampayan propagation is also hampered

by its limited availability of seeds, low seeds germination and the seeds are not suitable for long term storage. This is because long term storage of *N. macrophylla* seeds will indirectly affect the germination rate and viability of the seeds due to the recalcitrant nature of the seeds (Da Rosa *et al.*, 2011; Yudohartono, 2013). Moreover, *N. macrophylla* seedlings are susceptible to diseases such as leaf curl, leaf spot, black mildew and damping off disease (Halawane *et al.*, 2011). Damping off disease is one of the diseases observed in seedling which cause the seedling to rot and fall over (Islam *et al.*, 2005).

According to FAO World Bank Development report, human population is projected to increase from 6.4 billion in 2005 to 7.5 billion in 2020 while the global demand for wood products is estimated to increase up to 6.4 billion m^3 in 2020. Therefore, tissue culture technology was encouraged to meet the expanding population and huge global demand for wood products. Since the conventional propagation methods of *N. macrophylla* are restricted by time consuming and several problems mentioned above, tissue culture of *in vitro* shoot induction of *N. macrophylla* for mass propagation should be explored. Application of 6-Benzylaminopurine (BAP) might induce the shoot regeneration of *N. macrophylla.* In this study, different concentrations of BAP was used to determine the suitable concentration for shoot regeneration of *N. macrophylla.*

The objectives of this study are:

I. To study the effect of BAP on *in vitro* shoot induction of *N. macrophylla.*
II. To determine the optimum concentration of BAP on *in vitro* shoot induction of *N. macrophylla*

2.0 Literature review

2.1 *Neolamarckia macrophylla*

Neolamarckia macrophylla is also known as red kelampayan or jabon merah is one of the fast growing species belong to family Rubiaceae which can be found in Indonesia, Malaysia, Vietnam, Sri Lanka, Filipina, Papua New Guinea, Thailand and China (Chang *et al.*, 2014). *N. macrophylla* has another scientific name known as *Anthocephalus macrophyllus*. Based on Pereira *et al.* (2015), the genus *Neolamarckia* is an accepted name for *Neolamarckia macrophylla* and *Neolamarckia cadamba* (white kelampayan). However, the genus name of *Anthocephalus* still frequently being used in Asia. The classification of red kelampayan is shown below:

Kingdom	: Plantae
Sub Kingdom	: Tracheobionta
Super Division	: Spermatophyta
Division	: Manoliophyta
Class	: Magnoliopsida
Sub Class	: Asteridae
Order	: Rubiales
Family	: Rubiaceae
Genus	: *Neolamarckia*
Species	: *Neolamarckia macrophylla*

In the early period, most of the Southeast Asia countries did not prioritize kelampayan species in tree domestication process due to lack of understanding of the characteristics of this species. At the moment, *N. macrophylla* has been identified as one of the promising timber species which extensively planted in Southeast Asia countries due to its fast growth characteristic and multiple uses (Supriyanto *et al.*, 2014). For example, around 18,851 hectares of kelampayan were planted in Sarawak in order to meet the aspiration of state government which had targeted to establish one million hectares of forest plantations by 2020 (Forest Department Sarawak, 2018). While in Indonesia, approximately 1200 hectares of red kelampayan have been planted in South Halmahera Regency and North Halmahera Regency in 2008 to 2009 through a program known as Hutan Tanaman Rakyat (HTR) (Cahyono *et al.*, 2012).

2.1.1 Morphology of *Neolamarckia macrophylla*

Neolamarckia macrophylla is able to grow up to 40 to 45 m with 1.5 m in diameter of straight and cylindrical bole without branching due to self-pruning ability of the species (Halawane *et al.*, 2011). The self-pruning ability of this species has reduced the needs and cost for tree management (Irawan and Purwanto, 2014). The height and diameter of *N. macrophylla* will increase 3 m and 7 cm respectively per year while it is ready for logging after 4 to 6 years of planting (Change, *et al.*, 2014). Besides, the stem is reddish black when young and become dark red when mature. The leaves are elliptic to ovate, deciduous and large with 20 to 60 cm long and 8 to 25 cm wide in opposite and decussate arrangement (Pereira *et al.*, 2015). The fruit of *N. macrophylla* is round shaped and reddish-brown when ripen while the seeds are very tiny with 0.5 mm in length. The morphological characteristic difference between *N. macrophylla* and *N. cadamba* are shown in Table 2.1.

Table 2.1 Morphological differences between *N. macrophylla* and *N. cadamba*

Characters	*N. macrophylla*	*N. cadamba*
Young leaf shoots color	Red	Light brown
Leaf base shape	Wedge-shaped	Rounded
Leaf midrib color	Red	Yellowish green
Leaf stalk	Absent	Present
Young stem color	Blackish red	Brownish green
Mature tree trunk color	Blackish	Brownish grey
Fruit color	Reddish brown when ripen	Yellow when ripen

Figure 2.1 Trunk of red kelampayan (left) and white kelampayan (right) (Halawane *et al.*, 2011)

Red kelampayan **White kelampayan**

Figure 2.2 Leaves, fruits and seeds of red kelampayan (A, C and E) and white kelampayan (B, D and F) (Bramasto *et al.*, 2015)

2.1.2 Uses of *Neolamarckia macrophylla*

The uses of *N. macrophylla* can be observed in ecological restoration, commercial and medicine value. Due to its fast growing characteristic, *N. macrophylla* can be used as nurse tree to provide shelters and nutrients for slow growing primary forest trees in ecological restoration of logged area. According to Pereira *et al.* (2015), *N. macrophylla* was able to improve the physical and chemical properties of soil through decomposition of its high turnover of leaf and woody matter which indirectly increase the carbon content and nutrients availability in soil. Besides, compared to other fast growing species such as *Paraserianthes falcataria* (sengon) and *Gmelina arborea* (gmelina), *N. macrophylla* are easier to grow and shows higher pests resistance (Irawan and Purwanto, 2014). Therefore, this species is suitable for reforestation program which is one of the concerns by the government.

Moreover, *N. macrophylla* has high commercial value due to its wood properties. The wood hardness is classified from Class I (the hardest) to Class V. Based on Palemba *et al.* (2013), the wood of *N. macrophylla* was harder than *N. cadamba.* For example, the hardness *N. macrophylla*'s wood was categorized as Class II-III which was better compared to *N. cadamba* and *P. falcataria* (sengon) (Halawane *et al.*, 2011) while it has a specific gravity of 0.48 and very low in shrinkage (Lempang, 2014). Furthermore, the natural durability of *N.* macrophylla's wood against the subterranean termite known as *Coptotermes curvignathu* was classified as Class II (Cahyono *et al.*, 2012). Due to its superior wood properties and durability, the wood of *N. macrophylla* was suitable for timber trade and provide good material for plywood making, boxes and crates manufacturing, furniture, paper and pulp manufacturing (Pereira *et al.*, 2015).

N. macrophylla also involved in traditional medicine. According to Pereira *et al.* (2015), the bark and leaves of *N. macrophylla* can be used to relieve fever, increase stamina, reduce cholesterol and increase womb fertility. Furthermore, the extracts of the flower exhibit nematicidal effects on nematode such as *Meloidogyne incognita* while the fruits are eaten by some of the local communities in Indonesia.

2.2 Conventional propagation of *Neolamarckia macrophylla*

The conventional propagation method of *N. macrophylla* implemented by the farmers are through seed sowing. The seeds of *N. macrophylla* are best sown in nursery instead of direct sowing in field as direct sowing will reduce the germination rate due to their small sizes and their susceptibility toward the extreme environmental conditions such as drought and flood. According to Pereira *et al.* (2015), the seeds are sown on sterile fine soil or 1:1 ratio of fine mineral soil and fine sand. Besides, the sowing media need to be sterilized before sowing to reduce the contamination rate and diseases which can affect the germination rate or the early germinants (Pereira *et al.* 2015). Optimal nursery media include the characteristics such as well drainage system, fertile soil, light texture and free from pests and diseases. After sowing, the seeds will be covered with a transparent plastic in order to maintain optimal nursery media (Irawan and Purwanto, 2014; Setyaji *et al.*, 2014). According to Halawane *et al.* (2011) and Setyaji *et al.*, (2014), the seeds will be germinated within 8 to 30 days after sowing.

According to Fern (2014) and Pereira *et al.* (2015), the 8 to 12 weeks old seedlings with 2 to 3 leaflets emerged and attained a height of around 2 to 3 cm can be transplanted to plastic bags or containers. Besides daily watering, fungicide should be applied once a week while the fertilizer is recommended to apply at least 2 weeks after the transplanting (Pereira *et al.*, 2015). Fertilizer is essential to improve the quality and growth of the seedling. For example, Palemba

et al. (2013) have identified that the application of 2 g/L of fertilizer Gandasil D was an optimum doses for good vegetative growth in *N. macrophylla.* Sometimes, liquid fertilizer is also used as alternative by the farmers to improve the seedling growth. Based on Supriyanto *et al.* (2014), application of liquid fertilizer of 150 ml/L cow urine was able to improve the seedling growth. However, the application of fertilizer was not necessary to enhance the seed germination due to the superior germination rate promoted by the pure soil and soil-sand media (Irawan and Purwanto, 2014). Therefore, farmers are encouraged not to wasting their time and effort in producing a special germination media. Besides, Irawan and Purwanto (2014) had identified that the best media for seedling growth was the mixed soil, compost of cow manure and husk charcoal in a ratio of 3:1:1. This media composition also shown to increase the diameter of the stem and total dry weight as reported by Irawan and Purwanto (2014). Fertilizer shows positive effects in seedling growth as the fertilizer contain the fundamental nutrient elements such as nitrogen, phosphorus and potassium which are important for plant development. When the seedlings reach a height of approximately 30 to 50 cm which is around 6 to 7 months after transplanting, the targeted seedlings can be outplanted to field.

2.3 *In vitro* micropropagation

In vitro micropropagation is one of the plant tissue culture techniques in which the cells, tissues or organs of desired plant was isolated and cultured in vessels under aseptic and controlled environment in order to produce identical plantlets (Altman, 2000). Many commercial plants were propagated by this method with the application of auxin and cytokinin on the culture medium (Rout and Jain, 2004). The first regeneration of Cyclamen shoots from tuber segment on MS medium supplemented with 10.7 μM NAA was conducted by Mayer in 1956. *In vitro* propagation technique has many advantages compared to the conventional vegetative method

such as large scale production plants in short period of time, production of genetically identical pathogen-free plants, the plants can be produced at any period of time and cheap labour cost (Kumar and Reddy, 2011). Therefore, this technique has been applied in plant tissue culture laboratories for commercial production of superior plants.

Many research of *in vitro* propagation of woody species has been conducted. According to Beck *et al.* (1998), multiple shoots were regenerated from nodal explants of *Acacia mernsii* by using 2.0mg/l of BAP. Based on a research conducted by Amin *et al.* (2002) on *Ixora fulgens* (Rubiaceae), the combination of 0.5 mg/l of BAP and 0.1mg/l of NAA showed the highest frequency of shoots regeneration from nodal and shoot tip explants. Dibax *el al.* (2005) had conducted *in vitro* plant regeneration of *Eucalyptus camaldulensis* from cotyledonary leaves. The best result obtained by this group of researcher was culturing the explants on the media supplemented with 2.7 µmol/L NAA and 4.44 µmol/L BAP. Furthermore, Kumar *et al.* (2010) had successfully regenerated *Jatropha curcas* from leaf tissues and petiole. Therefore, it is possible to *in vitro* micropropagate *N. macrophylla* by using plant growth regulators.

2.3.1 The stages of micropropagation

Generally, the standard procedures of micropropagation can be divided into five stages (Kumar and Reddy, 2011). The first step of micropropagation technique is the production of pathogen-free mother plants (stock plant) in greenhouse under hygienic condition. It is then followed by initiation stage which involves the selection of explants (shoot tip, node, leaf, root, internode and etc.) from pathogen-free mother plant before cultured on suitable media under aseptic and controlled environments (Kumar and Reddy, 2011). The next step is known as multiplication stage whereby the cultures are aseptically being subcultured into new media for propagule proliferation. At this stage, the growing explant can be induced to produce shoot by the

application of cytokinin. The subsequent phase is known as rooting phase in which the plantlets will be induced to produce roots by the application of auxin. After the plantlets have grown to desirable state, the plantlets are being transferred into *ex vitro* environment for acclimatization purposes (Zobayed *et al.,* 2000). Figure 2.3 shows the overall idea of micropropagation technique.

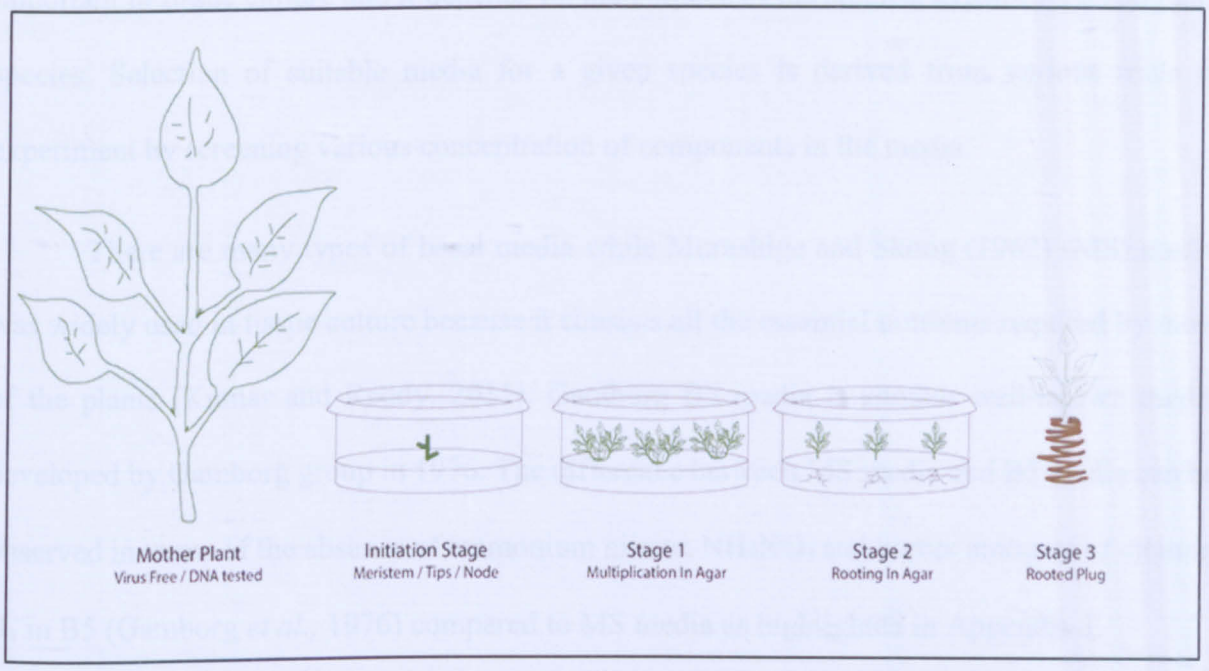

Figure 2.3 Stages of micropropagation (Hargreaves, 2018)

2.4 Factors affecting micropropagation

According to Kumar and Reddy (2011), there are many factors affecting *in vitro* micropropagation. This includes tissue culture media, carbon sources, type of explants and orientation of explants.