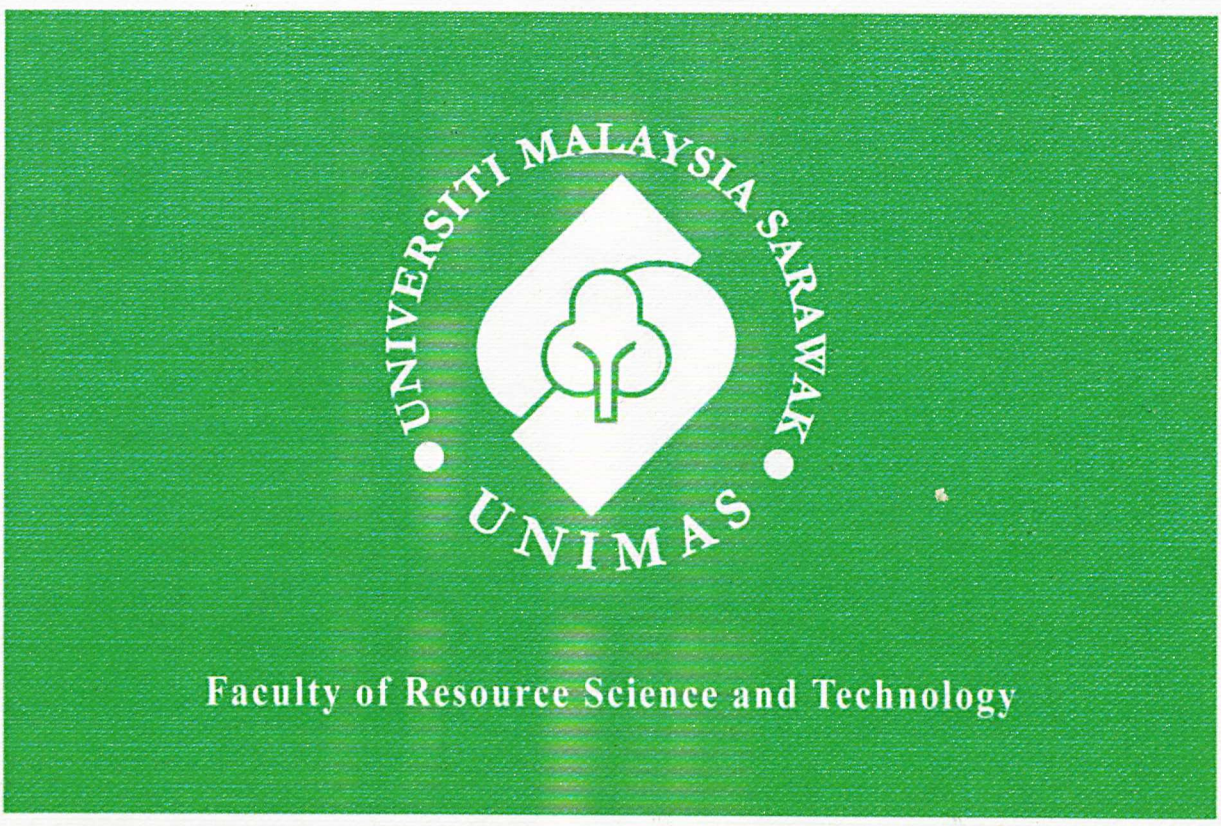

Ex vitro Acclimatization of Tissue Cultured MD2 Pineapple

Lesley Juilih (47330)

Bachelor of Science with Honours
Resource Biotechnology
2018

Ex vitro **acclimatization of tissue cultured MD2 Pineapple**

Lesley Juilih
47330

A report submitted in partial fulfilment of the Final Year Project (STF 3013)

Supervisor: Assoc. Prof. Dr. Ho Wei Seng

Resource Biotechnology
Faculty of Resource Science and Technology
University Malaysia Sarawak
2018

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ACKNOWLEDGEMENTS

3 years in WS47 Resource Biotechnology has come into its peak with the completion of this Final Year Project (FYP) successfully. I am really grateful that I manage to complete this project within the time given. First of all, this work cannot be completed without guidance from my supervisor, Associate Professor Dr. Ho Wei Seng on his guidance and supervision on my project. Without his support and encouragement, this project will only remain my dream.

I also sincerely thank Associate Professor Dr Hairul Azman @ Amir Hamzah bin Roslan for his comments, advice and constructive recommendation in revising and rectifying my project. Besides, I wish to acknowledge the help provided by Forest Genomic and Informatics Laboratory assistance, Miss Karmaliawati and staff, for their assistance throughout this study.

Next, I would like to thank the PhD students, Mr Eng Wee Hiang and all the master students in the Forest Genomics and Informatics Laboratory, Mr Sew Jun Wu, Miss Mok Pei Kieng, and Mr Delvino Disonneo anak Dalin for giving me the valuable ideas and comments to improve my study. I also really thankful to Anthanesius Samad, Alven Liu, Khairul Iradat and all my friends in the Forest Genomics and Informatics Laboratory, especially Lee Tian Wee and Ong Li Ying for their help and support along the completion of this project.

Last but not least, I would like to offer my special thanks to my family and Nervina for their encouragement and support, no matter financially or emotionally upon completion of this project. Not forgotten to those involved directly or indirectly, I am really grateful for the help in completing this project.

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LIST OF ABBREVIATION

ANOVA	Analysis of variances
CO_2	Carbon dioxide
DMRT	Duncan's multiple range tests
Ha	Hectare
kPa	Kilo Pascal (pressure)
MD2 Pineapple	A type (variety) of pineapple
MPIB	Malaysian Pineapple Industry Board
MS medium	Murashige and Skoog medium (for micropropagation)
MT	Metric Tons (weight)
RH	Relative Humidity
SD	Standard deviation

Ex vitro acclimatization of tissue cultured MD2 Pineapple

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ABSTRACT

MD2 Pineapple (*Ananas comosus)* is one of a commercially grown variety of pineapple categorized under the family Bromeliaceae (sub-family: Bromelioideae). It is favoured compared to other varieties due to the good qualities such as physical appearance, texture, taste, and others. Due to these qualities, the market demand for MD2 Pineapple is increasing over time. However, the conventional method of planting is not able to fulfil the market demand. As an alternative, tissue culture is used to open opportunities to increase the production of MD2 Pineapple. Thus, this experiment was conducted to improve the acclimatization step, which is one of the limiting factors towards the success of tissue cultured plants. The *in vitro* cultured plantlets were tested in a different environment which is left outside lab (control), inside the bottle and, using mini-greenhouse for 5 weeks of acclimatization period to find out if there is a significant difference in each environment. The result shows there is a significant difference for plantlets inside the mini-greenhouse compared to the plantlets left outside lab (control) and inside the bottle. However, there is no significant difference between plantlets covered with bottle compared with the control. So, it can be concluded that the mini-greenhouse is the most suitable environment for the plantlets to be acclimatized.

Keywords:MD2 Pineapple, tissue culture, acclimatization, plantlets.

Ex vitro acclimatization of tissue cultured MD2 Pineapple

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ABSTRAK

Nanas MD2 (Ananas comosus) merupakan salah satu nanas yang ditanam secara komersial dan dikategorikan di bawah keluarga Bromeliaceae (sub-keluarga: Bromelioideae). Nanas MD2 disukai berbanding varieti lain kerana kualiti yang baik seperti penampilan fizikal, tekstur, rasa, dan lain-lain. Oleh kerana kualiti ini, permintaan pasaran untuk nanas MD2 semakin meningkat dari masa ke semasa. Walau bagaimanapun, kaedah penanaman konvensional tidak dapat memenuhi permintaan pasaran. Sebagai alternatif, kultur tisu digunakan untuk membuka peluang untuk meningkatkan pengeluaran nanas MD2. Oleh itu, eksperimen ini dijalankan untuk meningkatkan langkah penyesuaian diri, yang merupakan salah satu faktor yang membataskan kejayaan tisu kultur tisu. Anak pokok daripada kultur tisu diuji dalam persekitaran yang berbeza yang ditinggalkan di luar makmal (kawalan), di dalam botol dan, menggunakan rumah hijau mini selama 5 minggu tempoh penyesuaian untuk mengetahui sekiranya terdapat perbezaan yang signifikan dalam setiap pemboleh ubah manipulasi. Hasilnya menunjukkan terdapat perbezaan yang signifikan untuk tanaman di dalam rumah hijau mini berbanding dengan tanaman di luar makmal (kawalan) dan di dalam botol. Walau bagaimanapun, tidak ada perbezaan yang ketara antara anak pokok yang dilindungi dengan botol berbanding dengan kawalan. Oleh itu, dapat disimpulkan bahawa rumah hijau mini adalah persekitaran yang paling sesuai untuk Nanas MD2 untuk menyesuaikan diri kepada persekitaran luar.

Kata kunci: Nanas MD2, kultur tisu, penyesuaian, anak pokok.

CHAPTER 1

INTRODUCTION

Due to the high market demand for pineapple, the conventionally planted MD2 Pineapple cannot fulfil the market demands. MD2 Pineapple is popular with great physical appearance, taste, and smell. However, the value of MD2 Pineapple decreases greatly due to the limitations including transmission of diseases, less uniformity and inadequacy for commercial production as stated by Mengesha *et al.* (2013) and Ayelign *et al.* (2013). To fulfil the demands, micropropagation is the best way to increase the crop production (Akin-Idowu *et al.*, 2009). In addition, it is uniform since the plantlets derived from a single plant can be alternatively selected by choosing favored traits from the best quality of MD2 Pineapple.

Despite having the various problems such as browning of cultured tissues, vitrification of tissues, bacterial and fungal contaminations in micropropagation (Abdullatif Ali Alkhateeb, 2008), many problems also arise when transferring *in vitro* plantlets into the field. Tissue cultured plant is restricted by abnormal growth or death after the *ex vitro* transfer, which leads to the research of this topic. In the process of the successful micropropagation, acclimatization is the bottleneck for the success rate of the plants to survive (Hazarika, 2006). Most plantlets require adapting to the new non-sterile environment before it can be transferred to the field. This is due to the difference between *in vitro* and *ex vitro* environment, which affects the survival rate of transplanted plantlets (Tesfa *et al.*, 2016).

This research is conducted to identify the effect of plantlets acclimatized in the mini-greenhouse, covered with the plastic bottle and left in open area (control). Different

treatment of plantlets will result in different survival rate in each growing environment. Besides, it is also conducted to understand the correlation between MD2 Pineapples which are planted in the mini-greenhouse, covered with the plastic bottle, and left in open area towards the growth of MD2 Pineapple survived. Parameters including the height of the plants and number of leaves are recorded throughout experiment every week to determine the growth of MD2 plantlets.

In this current study, there are many types of research conducted regarding *ex vitro* acclimatization. However, the previous research was focused on either generally or on specific plants only. As known, different plants may require special treatment such as different types of soil, specific pH value, humidity, temperature and others to survive. The acclimatization protocols are still new and a lot of information regarding MD2 Pineapple growth still undiscovered. In addition to that, the equipment available also limited and some factors such as specific humidity and light intensity are difficult to control without proper equipment. As for the significance of this research, the production of MD2 Pineapple can be increased and the quality can be maintained simultaneously by optimizing acclimatization of plantlets. Thus, this protocol can be used as the reference for large-scale production of MD2 Pineapple, especially commercial purposes. As for the objectives, this research is conducted with purpose:-

i. To understand the correlation between MD2 Pineapple which planted in the mini-greenhouse, covered with the plastic bottle, and left in open area towards the success rate of MD2 Pineapple survived.

ii. To identify effect of plantlets acclimatized in the mini-greenhouse, covered with the plastic bottle and left in open area (control)

CHAPTER 2

LITERATURE REVIEW

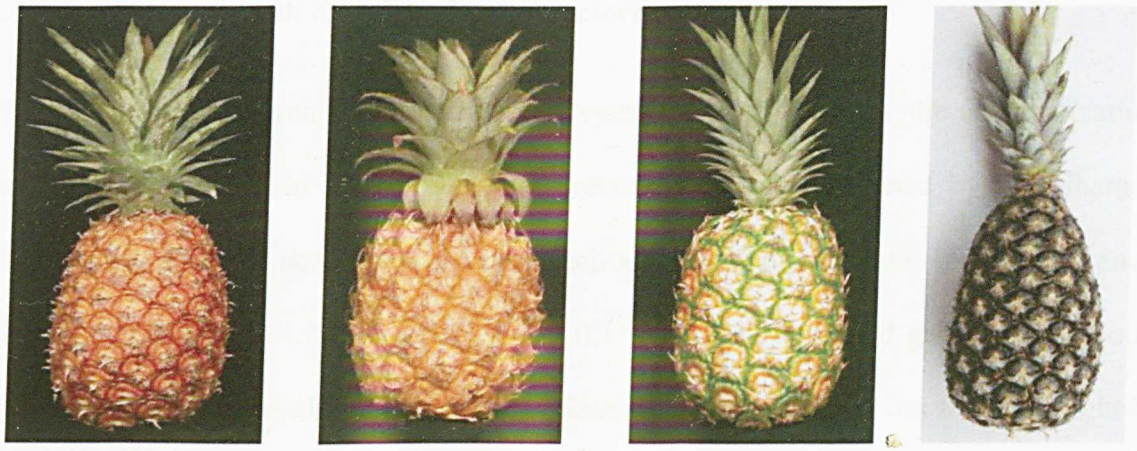
2.1 MD2 Pineapple

2.1.1 History of MD2 Pineapple

MD2 Pineapple *(Ananas comosus)* discovered by Pineapple Research Institute in Hawaii before the 1980s when they hybridize two seedlings numbered 73 – 114. Bartholomew *et al.*(2012) stated that this hybrid resulted from the cross between two pri hybrids, 59-443 and 58-1184 which were complex mixtures of several varieties, each more than 50% Smooth Cayenne (another pineapple types). The testing of the selections was made in Maui between 1973 and 1980; also in Oahu between 1978 and 1980. For further evaluations, the lines were released to Del Monte and Maui Pineapple Company. In 1981, 73-114 was named MD2. According to Thalip (2015), it is named after Millie, the wife of Frank Dillard, the General Manager of the pineapple company. MD2 started to be shipped in the mid 80's first to Costa Rica. Now, MD2 production surpasses the Smooth Cayenne and it is grown in many countries including Mexico, Ecuador, Honduras, Australia, Guatemala, and Panama with Costa Rica the leading producing country.

2.1.2 The MD2 Pineapple

By referring to taxonomy, MD2 Pineapple belongs to the family Bromeliaceae, sub-family Bromelioideae, under the genus *Ananas* with the species name *comosus*. Listed under the kingdom Plantae, pineapple generally grouped under angiosperm; is a monocot and a herbaceous perennial. Pineapple has sessile leaves which enclose the stem on two-thirds of its circumference and tend to be sword-like sharp edges. Besides, pineapple can grow up to 1-2 m high and wide.



Smooth Cayenne Queen Victoria MD2 Sugar Loaf

Figure 2.1 Common commercially grown varieties of pineapple. Adapted from https://humannutritionresearch.wordpress.com/2013/10/14/lananas-e-realmente-un-alimento-magico/

The MD2 Pineapple is known as "Super Sweet", "Golden Ripe", "Gold" or "Rompine" as its trade names. In contrast to the other type pineapples (refer to Figure 2.1), MD2 variety is favored by the farmers to be cultivated due to the good qualities as an advantage (Kleemann, 2013). The advantages include uniform bright gold colour and sweeter taste. Besides, it also enriched with Vitamin C, four times more than the other pineapples. Due to these qualities, MD2 Pineapple can have value three times more than the other types of pineapples. Meanwhile, it has lower fibre, lower acidity and thinner skin. In addition, it also weigh on average 1.5 kg per fruit and had a longer shelf life. Consequently, these drive the planters to plant MD2 and Hamid *et al.* (2013) stated that MD2 Pineapple has a longer shelf life (about 30 days) compared to the other varieties of pineapples (average 25 days). Therefore, it is very advantageous for long-distance shipping.

2.1.3 Recent research on soil and other factors

There is various research conducted on the types of soil suitable for the acclimatization period of tissue cultured MD2 Pineapple. Previous research conducted by Abdelhamid (2014) about effect of peat moss and sand each alone in 8 mixing ratio (peat moss:sand; 1:0, 5:1, 5:2, 5:3, 5:4, 1:1, 1:2, 1:3, 1:4 and 0:1) on the survival and growth of Smooth cayaenne pineapple plantlets during acclimatization stage. He found out that the highest plantlets survival (100 %) obtained in pots mix of 1 part of peat moss and 3 parts of sand (1: 3), and pots filled with sand alone (0 : 1). In the meantime, Mengesha *et al.*(2013) stated that using soil mix which made up from the soil, coffee husk and sand (1:2:1) is a good substrate for primary acclimatization. Plus, it is cheap and easily available. Mengesha also investigates the best medium suitable for the acclimatization of Smooth cayaenne pineapple plantlets. According to Mengesha *et al.* (2013), polybag had higher root number than poly sleeves and saved approximately 27% of substrates per plant. They concluded that the soil mix and polybags were preferable over substrates and pots for subsequent *in vitro* pineapple acclimatization. Atawia (2016) also emphasize the use of polybags for the plantlets transferred to the soil.

Pineapple cannot withstand waterlogging, which makes them different from most of the tissue cultured plantlets (Morton, 1987). In addition, drainage must be improved if there is impervious subsoil. Some of the soils such as pure sand, red loam, clay loam and gravelly soils usually need organic enrichment to enhance the growth of pineapple. For the pH of the soil, Morton (1987) stated that pH of soil ranged from 4.5 to 6.5 is suitable for growing pineapple. Temperature suitable for acclimatization is at 25°C with 60 % relative humidity (RH)(Mahanom *et al.*, 2015). Newly acclimatized plantlets only need little exposure to sunlight but require high humidity since Villalobo *et al.* (2012) mentioned that

gradually increase light intensity and reducing humidity after 30 days does not form inhibitory factors.

2.1.4 Current issue

Pineapple is one of the third most important tropical fruit in the world (Hossain, 2015). Including MD2, there are nine varieties which are mostly planted including Moris, Moris Gajah, N36, Masapine, Yankee, Sarawak, Gandul, and Josapine, Some of this cultivar are only grown locally for the fresh local fruit market. MD2 Pineapple consists of three grades which are Grade A, B and C based on their weight. According to statistics by Ahmadi *et al.*(2015), grade A pineapple is mainly for export purpose with weight more than 1.7 kg per fruit worth RM 3.20 (0.74 USD) or more. The grade B pineapples range between 1.3 to 2.5 kg each, and grade C weigh below 1.3 kg with price RM 2.40 and RM 1.50 respectively. In 2012, 15, 649 hectares in Malaysia have been planted with total production estimated to be 335 000 MT (metric tons) production per yield. Malaysian Pineapple Industry Board (MPIB) has projected that Malaysian pineapple supply will increase from 350,000 MT (2013) to 700,000 MT by the year 2020. To achieve this, around 23, 000 ha of plantation area are expected to support the production volume. MD2 are chosen by MPIB to be used for the industrial planting.

2.2 *Ex vitro* acclimatization

Since micropropagation has been discovered in the late 1950s, it has been used for rapid multiplication of various plant species. However, as stated in the problem statement, the use of this method is often restricted by the low surviving rate after transferred to *ex vitro* condition such as field or greenhouse, exactly as what Mengesha *et al.*(2013) said. The success of micropropagation depends on the explant establishment, initial growth *in vitro* followed by transplanting into the field or greenhouse.

2.2.1 Before *ex vitro*

Generally known, the plantlets grow under *in vitro* have relatively air-tight cultivation vessels and unlike conventional culture, air humidity is higher and irradiance is lower during *in vitro* condition. The closed vessel will decrease air turbulence and thus microbial contamination can be prevented. Hence, it increases leaf boundary layers which limit carbon dioxide (CO_2) inflow and outflow of gaseous plant products proportional to the photosynthetic response (Figueira & Janick, 1994).

As the energy sources, plantlets are often supplemented with saccharides as energy sources. But, the addition of saccharide decreases water potential of medium considerably and indirectly increases the fungal and bacterial contamination risk (Pospíšilová *et al.*, 1999). In addition, plantlets also usually supplied with large doses of growth regulator. As a consequence, it may result in the.formation of abnormal anatomy, physiology and morphology of plantlets. However, Pospíšilová *et al.* (1999) also stated that abnormalities of plantlets cultivated *in vitro* can be repaired after transfer to *ex vitro* conditions.

2.2.2 During *ex vitro*

As stated by Pospóšilová *et al.* (1999), the tissue cultured plant with the abnormalities stated above needs to be acclimatized to the *ex vitro* environment. The condition of *ex vitro* especially in the field, the air humidity is much lower and the irradiance is much higher compared to *in vitro* condition. In addition, the plantlets also may quickly wilt due to the water loss through their leaves are not restricted (Chandra *et al.*, 2010). Moreover, the low hydraulic conductivity of roots and stem-root connection can limit the water supply. As a result, many plantlets die during this period.

Apparently, plantlets need periods of acclimatization with gradual lowering in air humidity before *ex vitro* transplantation. Acclimatization factor such as photosynthetic activity, humidity, amount of water supplied and others are controlled. The leaves formed during *in vitro* are mostly failed to develop further under *ex vitro* conditions and replaced by the newly formed leaves in most of the tissue cultured plants (Pospíšilová *et al.*, 2009)

According to Pospóšilová *et al.* (1999), during the acclimatization period, 2 different stages will be observed which are an adaptation period with slow root formation and shoot growth, followed by a period of fast growth of shoots and roots. The growth of the plantlets may increase excessively if the *ex vitro* transplantation successful. It may be higher, taller, larger leaf area, and leaf thickness.

CHAPTER 3

MATERIALS AND METHODS

3.1 Source and preparation of plant material

The source of plantlets was from Forest Genomics and Informatics Laboratory in University Malaysia Sarawak by previous researcher that have accomplished *in vitro* propagation process. Selection of plantlets was done to pick healthy plantlets with roots as in Figure 3.2 and Figure 3.3. Figure 3.1 shows few of *in vitro* plantlets which was evaluated for suitability for this experiment in term of physical appearance.

Figure 3.1 *In vitro* MD2 Pineapple plantlets

Figure 3.2 Tissue cultured MD2 Pineapple plantlet (matured) cultured since January 2017

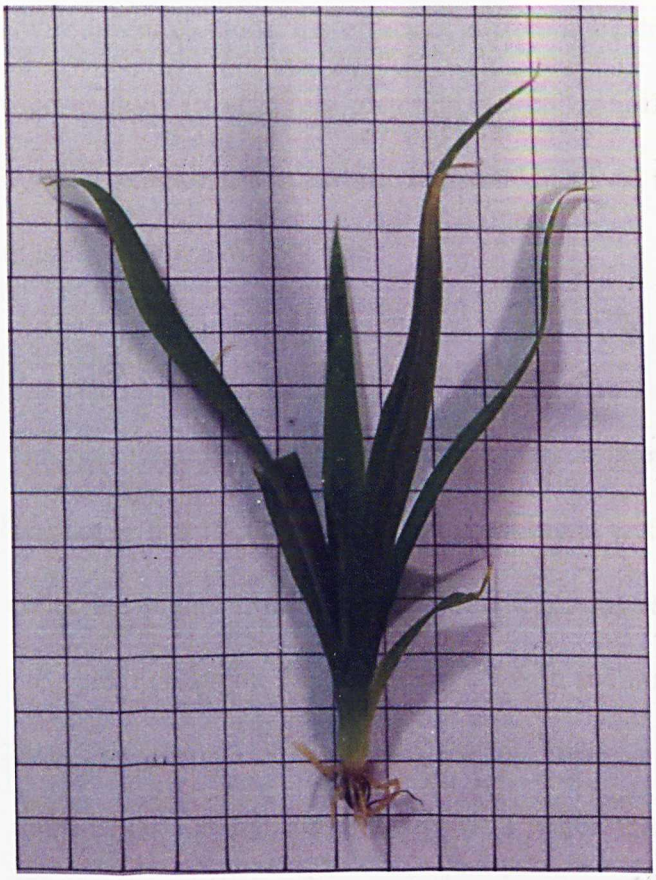


Figure 3.3 Sample of MD2 Pineapple plantlets which are removed from bottle jar (grid: 1cm × 1cm)

3.2 Soil mixtures and polybag preparation during hardening stage

The soil mixtures used in all variables were combination of peat moss and sand. In this experiment, 3:1 soil mixture was used for the acclimatization. The sand was obtained from Batu 13, Kota Samarahan near river. The peat moss (RomaTech Agro, Holland) was purchased at Giant Supermarket, Batu Kawah. Meanwhile in this study, polybag was used instead of pots.

3.3 Variables design

There are a few things considered in selecting the material used and its suitability during conducting this experiment. The purpose is to provide different acclimatization environment to study the effect of different treatments. The bottle and greenhouse were used to study its effect on plantlets that cultured in single different environment provided by each treatment. Meanwhile, it also helps to cut the cost, and to reuse or recycle material to reduce source of pollution.

3.3.1 Bottle

The first treatment was using bottle to assist plantlets to acclimatize to the environment. The bottle is a 1500mL of transparent mineral water bottle which has it bottom removed to cover the plants from top. The bottle cap was removed (refer to Figure 3.4) to provide little aeration for air exchange and helps in acclimatize faster due to the exposure to the *ex vitro* condition. The difference for this treatment and treatment 2 is the plantlets were treated individually unlike treatment 2 which the plantlets was treated in group. Besides,