EFFECT OF SUCROSE AND CYTOKININ ON *IN VITRO* MIRCRORHIZOME INDUCTION OF GINGER (*Zingiber officinale* Roscoe cv. Tambunan)

LING EE CHUWAN

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

CROP PRODUCTION PROGRAMME
FACULTY OF SUSTAINABLE AGRICULTURE
UNIVERSITI MALAYSIA SABAH
2017

PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN TESIS

JUDUL: EFFECT OF SUCROSE AND CYTOKININ ON IN VITRO MICRORHIZOME INDUCTION OF GINGER (Zingiber officinale Roscoe cv. Tambunan)

IJAZAH: Degree of bachelor of Agriculture Science with Honours (Crop Production Programme)

SAYA: LING EE CHUWAN **SESI PENGAJIAN:** 2013 - 2017

(HURUF BESAR)

Mengaku membenarkan tesis *(LPSM/Sarjana/Doktor Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

[ ]	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di AKTA RAHSIA RASMI 1972)
[ ]	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
[✓]	TIDAK TERHAD	

Disahkan oleh:

(TANDATANGAN PENULIS)

Alamat Tetap: 12, LINTANG UNTA, TAMAN BERKELEY, 41150, KLANG, SELANGOR.

TARIKH: 13/1/2017

(TANDATANGAN PUSTAKAWAN)

Dr. Devina David

(NAMA PENYELIA)

TARIKH: 13/1/17

Catatan:
*Potong yang tidak berkenaan.
*Jika tesis ini SULIT dan TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
*Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana Secara Penyelidikan atau disertai bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I hereby declare that this dissertation is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that no part of this dissertation has been previously or concurrently submitted for a degree at this or any other university.

LING EE CHUWAN
BR13110085
13 JANUARY 2017

VERIFIED BY

1. Pn. Devina David
   SUPERVISOR

ACKNOWLEDGMENT

Firstly, I would like to express my deepest gratitude to my supervisor, Madam Devina David, for her excellent guidance, advice, caring and patience throughout the research. I would also like to thank her for providing me with an excellent atmosphere for doing research. Apart from that, she also financially, physically and mentally supported my research. This thesis would not be the same as presented here without her guidance and supports. I, therefore, appreciated her consultation very much.

In my daily work, I have been blessed with a friendly and cheerful group of fellow friends. I would like to express my best gratitude to them for their help, moral support and friendly advice for the research especially Teh Ning Wei and Huang Yen Zen. Besides, I would like to thank my seniors, who were always willing to help and give their best suggestions to me. Without them, I would not able to finish my thesis.

Last but not least, I would like to express my sincere appreciation to my beloved family members who were always giving spiritual support and unfailing love to me. In addition, I would like to express deepest gratitude to my beloved parents, who always be there for me to count on when times are rough and never gives up on me.

ABSTRACT

Ginger is a spice plant which had been proven to bring various uses and benefits to people. Although ginger has a very high demand in the market, but the production of ginger especially Tambunan ginger (*Zingiber officinale* Roscoe cv. Tambunan) rarely satisfy the market demand. This study was carried out at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah from March 2016 until October 2016 to investigate the effect of sucrose (30, 60, and 90 g/L) and cytokinin (6 and 9 mg/L) on *in vitro* microrhizome induction in ginger (*Zingiber officinale* Roscoe cv. Tambunan). The treatments were arranged in completely randomized design (CRD) with ten replications. Data collected was analysed using two-way Analysis of Variance (ANOVA) at 5% significance level. The significance between means were compared by Tukey at 5% significant level. All the vegetative growth parameters such as leaf number, root number, shoot number and shoot height showed no significant differences as affected by the interaction of sucrose and cytokinin. As for the microrhizome parameters, it was found out that the interaction of sucrose and cytokinin had significant differences on the number microrhizome formation, total microrhizome weight, total number of buds and number of buds per microrhizome except for the average weight of microrhizome. The treatment 60 g/L sucrose concentration and 6 mg/L BAP produced the highest number of microzhome (7.1), the heaviest total microrhizome weight (2.897g) and microrhizome with the highest total number of buds (35). As for the highest number of buds per microrhizome, treatment 30 g/L sucrose with 9 mg/L BAP had the highest record of 8.3. The production of microrhizome in ginger was found to be influenced by several factors such as sucrose concentration and BAP rate. The usage of treatment 60 g/L sucrose and 6 mg/L BAP can provide solution for the lack of planting materials in ginger. The future study is recommended to include range between 60 g/L to 80 g/L sucrose concentration in order to know the precise treatment that cultured the most number of microrhizome in ginger.

KESAN SUKROSA DAN CYTOKININ TERHADAP INDUKSI IN VITRO MICRORHIZOME DALAM HALIA (Zingiber officinale* Roscoe*)

ABSTRAK

Halia ialah sejenis tumbuhan rempah yang telah dibuktikan bahawa ia dapat membawa pelbagai kegunaan dan faedah kepada kesihatan manusia. Walaupun halia mempunyai permintaan yang sangat tinggi dalam pasaran, tetapi pengeluaran halia terutamanya halia Tambunan (Zingiber officinale Roscoe cv. Tambunan*) jarang memenuhi permintaan pasaran. Kajian ini dijalankan di Fakulti Pertanian Lestari, Universiti Malaysia Sabah bermula Mac 2016 sehingga Oktober 2016 untuk mengkaji kesan sukrosa (30, 60 dan 90 g/L) dan cytokinin (6 dan 9 mg/L) terhadap induksi in vitro microrhizome dalam halia. Rawatan-rawatan tersebut diatur sebagai reka bentuk rawak secara keseluruhan (CRD) dengan sepuluh replikasi. Data yang diperoleh dianalisis dengan menggunakan ANOVA dua-hala atas keertian 5%. Rawatan-rawatan telah dibandingkan dengan pengunnaan Tukey atas keertian 5%. Semua parameter pertumbuhan vegetative seperti bilangan daun, bilangan akar, bilangan pucuk dan ketinggian pucuk telah didapati tiada perbezaan yang signifikan di antara interaksi sukrosa dan cytokinin. Bagi parameter microrhizome, didapati bahawa interaksi sukrosa dan cytokinin mempunyai perbezaan yang signifikan terhadap pembentukan microrhizome jumlah, jumlah berat microrhizome, jumlah tunas dan purata tunas per microrhizome kecuali purata keberatan microrhizome. Rawatan dengan kepekatan sukrosa pada 60 g / L dan 6 mg / L BAP telah menghasilkan bilangan tertinggi microzhome (7.1), yang paling berat jumlah berat microrhizome (2.897g) dan microrhizome dengan bilangan tertinggi jumlah tunas (35). Bagi bilangan tertinggi tunas per microrhizome, rawatan 30 g/L Sukrosa dengan 9 mg/L BAP mencatatkan rekod tertinggi iaitu 8.3. Pengeluaran microrhizome halia didapati dipengaruhi oleh beberapa faktor seperti kepekatan sukrosa dan kadar BAP. Pengunaan rawatan 60 g/L sukrosa dan 6 mg/L BAP boleh menyelesaikan masalah kekurangan bahan tanaman halia. Kajian selepas ini adalah disyorkan untuk memasukkan rawatan antara 60 g/L sehingga 80 g/L kepekatan sukrosa untuk memastikan rawatan yang paling sesuai untuk menghasilkan microrhizome halia.*

TABLE OF CONTENTS

Content **Page**

DECLARATION		ii
VERIFICATION		iii
ACKNOWLEDGEMENT		iv
ABTRACT		v
ABSTRAK		vi
TABLE OF CONTENT		vii
LIST OF TABLE		ix
LIST OF FIGURES		x
LIST OF SYMBOLS, UNITS AND ABBREVIATIONS		xii
CHAPTER 1	**INTRODUCTION**	1
1.1	Introduction	1
1.2	Justification	2
1.3	Significance of the Study	3
1.4	Objectives	3
1.5	Hypothesis	3
CHAPTER 2	**LITERATURE REVIEW**	4
2.1	Ginger	4
	2.1.1 Tambunan ginger	4
2.2	Botanical description of ginger	5
2.3	Cultivation of ginger	8
	2.3.1 Production of ginger	8
	2.3.2 Propagation of ginger	8
	2.3.3 Problems in the cultivation of Tambunan ginger	11
2.4	Tissue culture in ginger	12
	2.4.1 Microrhizome culture	13
	2.4.2 Factors affecting microrhizome induction in ginger	15
	2.4.3 Acclimatization	17
CHAPTER 3	**METHODOLOGY**	19
3.1	Site of study	19
3.2	Experimental period	19
3.3	Materials and Methods	19
	3.3.1 Origin of explants	19
	3.3.2 Preparation of explants	20
	3.3.3 Preparation of stock solutions	20
	3.3.4 Preparation of hormone	20
	3.3.5 Media preparation	20
	3.3.6 Treatments	21
	3.3.7 Acclimatization	21
3.4	Experimental design and layout	21
3.5	Data collection	22
	3.5.1 Determination of shoots number and shoot length	22
	3.5.2 Determination of leaf number	22
	3.5.3 Determination of root number	23
	3.5.4 Determination of the number of microrhizome	23
	3.5.5 Determination of microrhizome weight	23
	3.5.6 Determination of average weight of microrhizome	23

3.5.7 Determination of the total number of buds 23
3.5.8 Determination of the average number of buds per microrhizome 23
3.5.9 Determination of survival rate in nursery 24
3.6 Statistical analysis 24

CHAPTER 4 RESULTS 25
4.1 Effect of Sucrose and Cytokinin on the Development Stages and Vegetative Growth of *In vitro Zingiber officinale* Roscoe cv. Tambunan 25
4.1.1 Effect of sucrose and cytokinin on the vegetative growth of *in vitro Zingiber officinale* Roscoe cv. Tambunan 27
4.1.2 Effect of sucrose and cytokinin on the leaf number of *in vitro Zingiber officinale* Roscoe cv. Tambunan 30
4.1.3 Effect of sucrose and cytokinin on the root number of *in vitro Zingiber officinale* Roscoe cv. Tambunan 31
4.1.4 Effect of sucrose and cytokinin on the shoot number of *in vitro Zingiber officinale* Roscoe cv. Tambunan 32
4.1.5 Effect of sucrose and cytokinin on the shoot height of *in vitro Zingiber officinale* Roscoe cv. Tambunan 33
4.2 Effect of Sucrose and Cytokinin on the Microrhizome Induction of *In vitro Zingiber officinale* Roscoe cv. Tambunan 34
4.2.1 Effect of sucrose and cytokinin on the microrhizome of *in vitro Zingiber officinale* Roscoe cv. Tambunan 36
4.2.2 Effect of sucrose and cytokinin on the number of microrhizome of *in vitro Zingiber officinale* Roscoe cv. Tambunan 38
4.2.3 Effect of sucrose and cytokinin on the total weight of microrhizome of *in vitro Zingiber officinale* Roscoe cv. Tambunan 39
4.2.4 Effect of sucrose and cytokinin on the average weight of microrhizome of *in vitro Zingiber officinale* Roscoe cv. Tambunan 39
4.2.5 Effect of sucrose and cytokinin on the total number of buds of *in vitro Zingiber officinale* Roscoe cv. Tambunan 40
4.2.6 Effect of sucrose and cytokinin on the number of buds per microrhizome of *in vitro Zingiber officinale* Roscoe cv. Tambunan 41
4.3 Acclimatization of Microrhizomes and Plantlets 42

CHAPTER 5 DISCUSSION 43
5.1 Effect of Sucrose and Cytokinin on the Vegetative Growth of *In vitro Zingiber officinale* Roscoe cv. Tambunan 43
5.2 Effect of Sucrose and Cytokinin on the Microrhizome Induction of *In vitro Zingiber officinale* Roscoe cv. Tambunan 44
5.3 Acclimatization of Microrhizomes and Plantlets 47

CHAPTER 6 CONCLUSION AND RECOMMENDATION 48

REFERENCES 49
APPENDICES 52

LIST OF TABLES

Table		Page
3..1	The experimental layout using Complete Randomized Design (CRD)	22
4.1	Vegetative growth of *in vitro Zingiber officinale* Roscoe cv. Tambunan harvested after 12 weeks of cultivation as influenced by sucrose concentrations and cytokinin rates.	28
4.2	The percentage of plantlet producing microrhizome (%) influenced by different concentration of sucrose and cytokinin.	36
4.3	Microrhizome yield of *in vitro Zingiber officinale* Roscoe cv. Tambunan harvested after 12 weeks of cultivation as influenced by sucrose concentrations and cytokinin rates.	37
4.4	The survival rate of different planting materials after 40 days of acclimatization	42

LIST OF FIGURES

Figure		Page
2.1	Sketch of the ginger showing the origin of shoots, inflorescence and flower	6
4.1	The vegetative growth and development stages of *in vitro Zingiber officinale* Roscoe cv. Tambunan.	26
4.2	*In vitro* plantlet of *Zingiber officinale* Roscoe cv. Tambunan after 12 weeks of culture	29
4.3	Effect of sucrose and cytokinin on the leaf number of *in vitro Zingiber officinale* Roscoe cv. Tambunan over 12 weeks of culture	30
4.4	Effect of sucrose and cytokinin on the root number of *in vitro Zingiber officinale* Roscoe cv. Tambunan over 12 weeks of culture	31
4.5	Effect of sucrose and cytokinin on shoot number of *in vitro Zingiber officinale* Roscoe cv. Tambunan over 12 weeks of culture	32
4.6	Effect of sucrose and cytokinin on shoot height of *in vitro Zingiber officinale* Roscoe cv. Tambunan	33
4.7	Sign of microrhizome	34
4.8	The different types of microrhizome produced by *in vitro Zingiber officinale* Roscoe cv. Tambunan	35
4.9	The microrhizomes produced by *in vitro Zingiber officinale* Roscoe cv. Tambunan according to different treatments applied	35
4.10	Effect of sucrose and cytokinin on the number of microrhizome produced in *in vitro Zingiber officinale* Roscoe cv. Tambunan over 12 weeks of culture	38
4.11	Effect of sucrose and cytokinin on the weight of microrhizome of *in vitro Zingiber officinale* Roscoe cv. Tambunan over 12 weeks of culture.	39
4.12	Effect of sucrose and cytokinin on Total number of buds of *in vitro Zingiber officinale* Roscoe cv. Tambunan over 12 weeks of culture	40

4.13 Effect of sucrose and cytokinin on number of buds per microrhizome of *in vitro Zingiber officinale* Roscoe cv. Tambunan over 12 weeks of culture 41
4.14 The acclimatization of the two planting materials: plantlets and microrhizome. 42

LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Percentage
ANOVA	Analysis of Variance
BAP	6-Benzylaminopurine
CRD	Completely Randomized Design
FAOSTAT	Food and Agricultural Organization Corporate Statistical Database
g	Gram
H_0	Null Hypothesis
H_A	Alternative Hypothesis
ha	Hectare
HCl	Hydrochloric acid
hrs	hours
M	Molarity
mg	Miligram
MT	Metric Tonnes
NaOH	Sodium Hydroxide
SPSS	Statistical Package for Social Science

CHAPTER 1

INTRODUCTION

1.1 Introduction

Ginger (*Zingiber officinale* Roscoe) is a rhizomatous plant that belongs to the family of Zingiberaceae. It has an essential role in human's daily life by acting as a source of primary health care to maintain people's health and also an important spice for culinary uses. Today, the needs of ginger increased drastically due to the increase of health awareness in the society. Tambunan Ginger (*Zingiber officinale* Roscoe cv. Tambunan) is a variety of ginger which planted in the area of Tambunan, Sabah, Malaysia. This ginger has a pungent aromatic flavour yet less spiciness compared to other varieties found in Malaysia. Therefore, it has a very high demand in the market. However, a steady supply of proper and disease-free seedlings as planting material is the challenge faced by the farmers in Tambunan.

Over the years, ginger has proven to face some problems in production especially when big scale production was required. Ginger has a low proliferation rate by rhizome propagation and the soil-borne pathogens such as bacterial wilt (*Pseudomonas solanacearum*), soft rot (*Pythium aphanidermatum*), and nematodes (*Meloidogyne spp.*) has attacked the growth of ginger which cause the drastic decrease in ginger yield as well as the plantation area of ginger (Rout *et al.*, 2001; Guo and Zhang, 2005; Guo *et al.*, 2007; Guan *et al.* , 2008 and Zheng *et al.*, 2008).

Microrhizome is a miniature rhizome which able to be induce in an *in vitro* condition and possess similar characteristics as a normal ginger rhizome. *In vitro* microrhizomes production of ginger is able to solve these constraints faced by the famers by acting as a planting material which is disease free to prevent the transfer of soil-borne pathogens. Besides that, it has a steady supply since it can be produce during any season *in vitro* in a sterile conditions. It also has a potential commercial value where it is easier to transport, store and can used in germplasm conservation (Abbas *et al.*,2014). Researches about the induction of microrhizhome had been done years ago. First, George (1993) found out that miniature version of storage organ can be produced by tuber plant in medium with high cytokinin levels. After that, Sharma & Singh (1995) concluded that MS medium supplemented with 8 mg/L BAP produced the microrhizomes. Roh *et al.* (1996) reported that young plantlets of ginger formed rhizomes when cultured on MS medium containing sucrose 90 g/L. The recent paper conducted by Abbas *et al.* (2014) shown the effect of sucrose concentration, 6-Benzylaminopurine (BAP) rates and various photoperiodism on the microrhizome induction. Therefore, this research will be carried out to study these factors and treatments that can be used to induce microrhizome in ginger.

1.2 Justification

This study aims to study the effect of sucrose and cytokinin on *in vitro* microrhizome induction in ginger (*Zingiber officinale* Roscoe cv. Tambunan). *Zingiber officinale* Roscoe cv. Tambunan was chosen for my study because of its high demand among the local and even overseas market yet low production to cover the market consumption. This ginger is recognised for its unique flavour and aroma.

Zingiber officinale Roscoe cv. Tambunan is a specialty in Tambunan, Sabah. It deserves more attention from the people in the whole Malaysia or even the whole world. However, the production of this ginger was retarded by the attack of pathogen to the planting soil dan seed rhizomes. This caused a huge impact to the future exportation and development of this product due to its inability to provide a stable supply.

Microrhizome can be an effective solution to these problems. This *in vitro* induced rhizome worked and function like a normal rhizome but it is a seed material which is safe from soil-borne diseases that usually imbedded in the rhizome after harvest, easier to handle, easier to grow (do not required hardening) and supply a steady planting materials. Thus this research is aimed to find out the method for the induction of microrhizome. The previous researches found out that the usage of high sucrose and cytokinin concentration will effectively induce the production of microrhizome in ginger. Therefore, this research will be use to understand the effect of sucrose and cytokinin on the microrhizome induction of ginger (*Zingiber officinale* Roscoe cv. Tambunan).

1.3 Significance of Study

This study may contribute to the scientists or researchers in tissue culturist, plant biotechnologist, as well as those practicing farmers. Especially to those who are currently facing problems in planting *Zingiber officinale* Roscoe cv. Tambunan. Besides that, this research could also show the effect of different sucrose and cytokinin combination in the quality of microrhizome produced.

1.4 Objectives

The objective of this study is to identify the effect of sucrose and cytokinin on *in vitro* microrhizome induction in ginger.

1.5 Hypothesis

H_0: There is no significance difference between the effect of sucrose and cytokinin on *in vitro* microrhizome induction in ginger.

H_1: There is a significant difference between the effect of sucrose and cytokinin on *in vitro* microrhizome induction in ginger.

CHAPTER 2

LITERATURE REVIEW

2.1 Ginger

Ginger, also botanically known as *Zingiber officinale* Roscoe, belongs to the family Zingiberaceae and in the order of Zingiberales. Ginger is one of the most popular spice that widely used around the world. Ginger mainly spread and cultivated in the tropical and subtropical countries covering China, India and Southern East Asia region. The country that produced the largest amount of ginger is India which contributing about 30 to 40% of the world production (Ravindran and Nirmal Babu, 2005). In the ancient era, ginger was highly valuable as the lack of proper medicine developed in the days back then. The medicinal properties of ginger was essential by acting as the mild carminatives in primary health care for many countries such as India, China and England. Nowadays, the method to use or consume ginger has been developed and more diversified. Now, ginger rhizome can be consumed as a fresh paste, dried powder, pickles, slices preserved in syrup, crystallized ginger and even for flavouring tea. Fresh ginger is normally used in food preparation such as vegetable dishes and meat dishes and act as a flavouring agent in beverages and many other food preparations in many countries, especially in India and China. Ginger brings many benefits as a natural dietary component which has antioxidant and anti-carcinogenic properties (Manju and Nalini, 2005).

2.1.1 Tambunan Ginger

Tambunan ginger also botanically known as *Zingiber officinale* Roscoe cv. Tambunan. The cultivars of ginger usually will get named after the location of its cultivation. Therefore, this Tambunan ginger is cultivated in the district of Tambunan, Sabah, Malaysia. Tambunan ginger differ from other ginger types in Malaysia such as Bentong ginger cultivated in Bukit Tinggi, Malaysia by their size and taste. Tambunan ginger has a larger rhizome, less spiciness and contains much juice and flavour than them. Tambunan ginger grown in the area about 750 metres above sea level. The ginger farmers in Tambunan still practice the traditional methods of ginger cultivation which is the manual planting method. The soil and temperature in Tambunan is suitable for ginger cultivation as the pH of the soil is between 5.5-6.5, hot and humid climate and suitable temperature between 25 °C and 30 °C (Intellectual Property Corporation of Malaysia, 2009).

2.2 Botanical Description of Ginger

Ginger plant is herbaceous perennial plant. However, it had been grown and harvested annually like an annual crop. The rhizomes that produced by the ginger plant was the target plant part harvested for consumption and use. Whenever the rhizome was not harvested, it was left in the soil for regenerate again. Ginger is actually sterile and does not set seeds, thus the rhizome act as the planting material for vegetative propagation of ginger. Ginger plant is erect consisting aerial shoots with leaves, purple flowers, many fibrous roots and the underground stem which is known as rhizome (Ravindran and Nirmal Babu, 2005).

Unlike the others crop, the aerial shoots of ginger consist of many narrow leaves attach to a very short petioles. These petioles have narrow and long sheaths that overlap to produce the aerial shoot. The joint between the leaves and sheath forms a pair of ligules. Ginger plant's leaves are organized into distichous manner. After the appearance of approximately 3 to 5 sheath leaves and 6 to 12 scale leaves, the foliage leaves that consists of a leaf sheath, a ligule, and an elliptical—lanceolate blade are produced. The leaf sheath is about 15 to 18 cm and lamina about 12 to 15 cm long (Shah and Raju, 1975). Volatile oil cells can be found in the root, leaves and shoot apex, same goes to the rhizome (Remashree *et al.*, 1999). In Malaysia, the leaves can be pounded to be used as a poultice to relieve muscular pains and are boiled and consumed for stomach ache relieve.

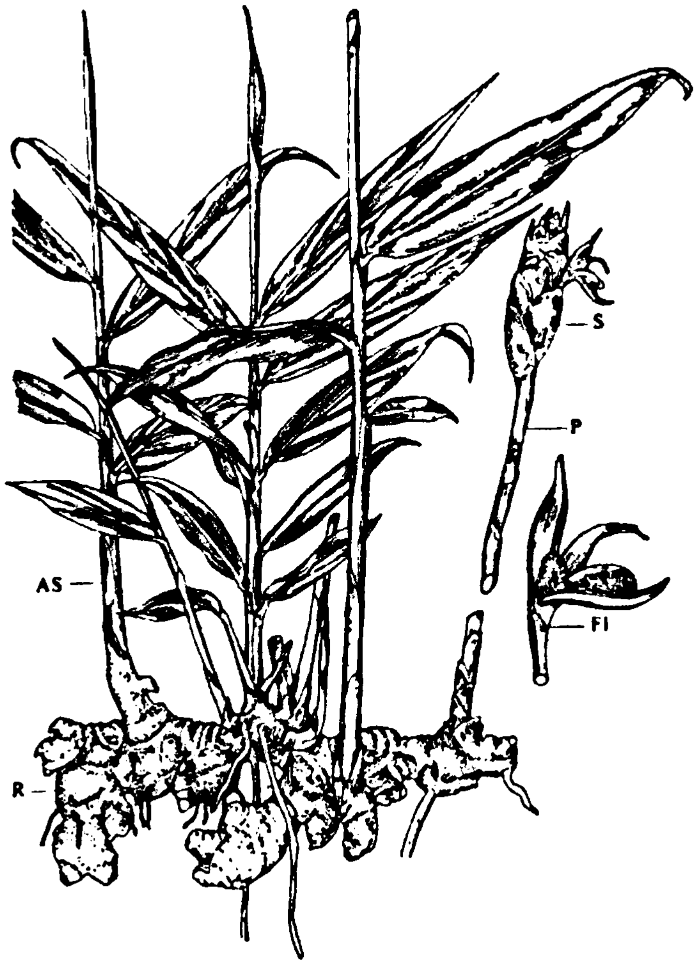

Figure 2.1 Sketch of the ginger plant showing the origin of shoots, inflorescence, and flower. AS: Aerial shoot, R: Rhizome, Fl: Flower, P: Peduncle (scape), S: Spike.

Source Ravindran and Nirmal Babu, 2005

The stem are slender, erect and able to reach up to 100 cm with a diameter of 50 cm x 5-10 mm. In most case, the stem of ginger is pale green with some reddish tint at the base of it. The stem consist of prominent parallel veins, glabrous but there are some short hair existed at the base of its leaf blades that bearing 8 – 12 distichous leaves.

Ginger's inflorescence generally arise directly from the rootstock. The inflorescence's upper sheaths consist of short leafy tips, the scape is slender with length 10-20 cm, it is carried on a 15-30 cm tall slim leafless stem. This short-lived flowers are single and axillary in each bract. The stamen is short and broad and the stigma shown below the apex by the slender dark purple connective grown to appendage containing the style's upper section. The ovary is inferior and trilocular with several ovules per loculus.

The roots of ginger can be divided into fibrous and fleshy roots. After establishment on the field, the ginger's roots have indefinite growth at base of the sprouts. These new thin roots are known as fibrous roots which function in absorption of water and nutrients with their root hairs, they grow along with the growth of tillers. As it continues to grow, several thicker, milky white coloured, with fewer root hairs, and no lateral roots fleshy roots of indefinite growth are produced from the lower nodes of the primary fingers and mother ginger (Ravindran and Nirmal Babu, 2005).

Generally, this thickened root is a sympodial rhizome which spread out near the soil surface. The rhizome has a rigid flesh, however it has various skin colour depending on its cultivars from buff to very dark brown and flesh colour from pale yellow to dark reddish-orange depending on its cultivars. The rhizome is a modified subterranean stem, its function as the storage of food materials and raw material for vegetative propagation in ginger. Rhizome consists of internodes and nodes with scales leaves. When the rhizome is meant for seed setting, one or more apical buds could be found on it. However usually only one is active. The apical bud is very limited because all the nodes will consist of axillary buds except the first few nodes that will be consist of apical buds. Despite of that, more than one bud can develop simultaneously when a large rhizome piece is used. In this condition, the branches of the mature rhizome will lie in same plane when the adult rhizome is grown and developed from more than one branch from the parent rhizome (Shah and Raju, 1975).

There are numerous active ingredients in ginger. They are terpenes and non-volatile pungent components oleoresin which also called the ginger oil. Ginger consist of volatile oil that comprise around 1 - 3% of its weight. The major active ingredients in ginger oil are the sesquiterpenes: bisapolene, zingiberene, and zingiberol. The concentrations of active ingredients vary with growing conditions. Ginger's active ingredients have a variety of physiologic effects. For example, the gingerols have analgesic, sedative, antipyretic and antibacterial effects *in vitro* and in animals (Mascolo *et al.*, 1989)The characteristic odor and flavor of ginger is caused by a mixture of zingerone, shogaols and gingerols, volatile oils.

2.3 Cultivation of Ginger

Ginger is a very popular and widely used spices around the world. It has different species and cultivars spread out in each countries. India is the largest producer out of all the countries that cultivate ginger. The cultivation of ginger can be produce in very small scales for family use to smallholder crop for local area supply and to even large scale mechanized ginger farm for country's use and exportation. Each species of ginger might have their own specific cultivation method, however most of them has the similar general requirements to grow healthy and high in quality.

2.3.1 Production of Ginger

In the 1980s, 100,000 tonnes of ginger world production was estimated but this amount is not confirmed due to the possibility of the ginger get traded or high proportion of it is consumed after growth or been used as a seed for the next season production. The world production of ginger shown a stable increase as the time goes by from 300,000 tonnes in 1980, to 500,000 tonnes in 1990, to 600,000 tonnes in 1998. The International Trade Centre data show that China and Thailand were the major exporting countries in 1998-2000. China seems to be the preferred provider for Korea, while Pakistan buys most of its ginger from India. Indonesia also provides ginger to Malaysia, and Hong Kong before it became part the People's Republic of China. Brazil is the third exporter of dried ginger. The three leading exporting countries in 2000 were China, Thailand, and Brazil.

In the year of 2001 to 2007, the world production of ginger continues to show a steady growth and increase. This shown that the demand of ginger has gone up in the world's market with the increasing health conscious of society. The top three countries that produce the most ginger are India, China and Nepal (FAOSTAT, 2013).

2.3.2 Propagation of Ginger

Ginger mainly propagated vegetatively due to the poor seed settings, the rhizome is the main planting material for vegetative production. However, the totipotency natural of ginger plant make it possible to be micropropagated using either rhizome sections or meristems or tissue culture (Ayenew *et al.*, 2012)

a) Climate

Ginger is native to the countries that are humid tropical region, mainly in the South-east Asia. The warm and sunny weather is important for the ginger to grow and mature in the specific time frame. The temperature of ground in 25 to 30 °C is the optimum temperature for good rhizome growth in ginger. The rainfall of 2500 – 3000mm over the year is optimum to produce high quality rhizome. The ginger will be cultivated successfully by having moderate rainfall for sprouting stage, heavy rainfall for growing stage and lastly dry weather a month before harvesting (Weiss, 2002).

b) Soil

The most suitable soil for ginger cultivation is a friable fertile soil with an adequate depth for the growth of rhizome under the ground. The soil should not be compacted as this could cause the limitation on the space for rhizome growth. Ginger should be planted in well-drained soil with ideal pH within the range of pH 6.0 to 6.5.

c) Land Preparation

The land is ploughed and tilled throughout the soil for 4 to 5 times in order to make the soil to fine tilth. Raised beds with 1 m width, 30 cm height with the desired length are made to plant ginger. Then between the beds can leave an inter-space of 50 cm for enough space for the growth of rhizome underground and prevent overcrowded of the rhizomes. The area that previously attacked by the nematode and rhizome rot disease, the beds should be solarized for 40 days under the cover of transparent polythene sheets.

d) Planting

The commercial propagation method is by seed rhizome. There preserved rhizomes are cut into pieces of 2.5-5.0 cm length and 20-25g weigh each containing one or two active buds. The seed rate differs according to different planting factor but usually 1500 to 1800 kg / ha is an optimum seed rate for most land. While higher latitudes the seed rate could be range from 2000 to 2500 kg/ha. Before planting, the seed rhizomes will usually be treated with mancozeb 0.3% diluted in 3 g / L water for 30 minutes. These newly planted seed should be shade dried for 3-4 hours then planted in spacing of 20-25 cm along the rows and between the rows.

e) Fertilizing

Since the land of Malaysia is usually acidic, magnesium limestone of 4 – 5 t ha^{-1} is added with an NPK mixture and 17 kg copper sulphate during land preparation to yield 18 t ha^{-1} from the acid peat soil (Weiss, 2002). At the time of planting, well decomposed cattle manure or compost at 25-30 t ha^{-1} has to be applied either by broadcasting over the beds prior to planting or applied in the pits at the time of planting.

f) Irrigation

Most of the smallholder ginger farm is rain-grown thus the weather plays a big role to yield good ginger. Whereas the large scale mechanized ginger farm is usually partially or fully irrigated. The ginger plant can use mulching to conserve the moisture of the soil and reduce soil temperature when the irrigation water is unavailable. Adequate water need to be given to the ginger plant when it is in the growing phase to increase rhizome yield and quality (Weiss, 2002).

g) Intercropping and crop rotation

Crop rotation is generally followed in ginger. Ginger always in a rotation for it to grow once every 3 to 4 years to prevent the build-up of nematode and disease in the soil. The irrigated ginger is recommended to have a rotation of 6 years with banana, sugarcane, rice and other vegetable crops. The crops frequently underplanted in coconut, young coffee and orange and those which can provide high light shade. However, crop rotation using tomato, potato, chillies, brinjal and peanut should be avoided, as these plants are hosts for the wilt causing organism, *Ralstonia solanacearum*. Although ginger plant also usually intercropped but this action is not recommended as the yield might reduce due to the crowded space limited for the rhizome growth even though they intercropping provide shade and soil cover to the ginger.

h) Pest and Disease Management

Currently, there are many pathogens that could infest ginger plants. These serious diseases are caused by bacterial, viral, fungal, and nematode infections. Control of rhizome infection with antibiotics may be possible (Singh *et al.*, 2000). In general, diseases of rhizome are more devastating than insect infestation. Diseases are capable in widespread and thus destroy large scale of cultivation.

The common diseases that attack the ginger are rhizome soft rot, bacterial wilt and bacterial soft rot. The common pests that could be found in ginger are *Meloidogyne incognita* and *Radopholus.* Rhizome soft rot that caused by *Pythuim spp.* can be identify when the plant appears to be pale with tips of leaves turning yellow and lastly the rhizome reduced to soft decaying mass. *Ralstonia solanacearum* (*Pseudomonas solanacearum*) is the organism that caused bacterial wilt in ginger. The symptoms are wilting and yellowing of the lower leaves while white milky exudates easily gush out when the stem and shoots are pressed between the fingers. Next the bacterial soft rot that caused by *Erwinia carotorora* shows symptoms include soft rotting and an unpleasant odour of stem tubers. Lastly, nematodes such as *Meloidogyne incognita* and *Radopholus* attack ginger by showing symptoms of galls formation on feeder roots. There may be necrotic spots on the outer layer of the rhizome while small light-brown water-soaked spots appear inside.

Most of the diseases and nematodes are soil-borne, thus crop rotation with other crops should be done for few years before planting another season of ginger. Generally, pre-planting treatment of setts should be done before planting the ginger to minimise the possibility of the disease attack. For instant, treating of setts with ceresin in 0.25% solution before planting for rhizome soft rot. As for the nematodes, pre-planting seed treatment in warm water in 48 °C for 20 minutes helps. Elimination of nematodes could be done by tissue culture. Besides that, ensure the clean disease free rhizome seed has been use for plantation. Lastly, minimal bruising or wounding of timely harvested rhizomes is a step to ensure a disease-free product. Rhizomes should be allowed to cure then store them in a cool dry well-ventilated shed (Ravindran and Nirmal Babu, 2004).

2.3.3 Problems in the Cultivation of *Zingiber officinale* cv. Tambunan

The demand of Tambunan ginger is very high even in the local community. It is a highly potential crops to be planted in order to increase the income of the farmer. However, a low total production has been recorded in the early month of year 2015, which the production is only 10 metric tonnes per month when the minimum demand in the market is predicted to be 300 metric tonnes per week. This cause the phenomenon of higher price recorded for the ginger which is around RM 1,500 per metric tonnes.

REFERENCES

Abbas, M. A. U., Taha, H., and Gaber, E. S. 2014. *In vitro* Production of Microrhizomes in Ginger (*Zingiber officinale* Roscoe). *Journal of Microbiology, Biotechnology and Food Sciences*, **04(02)**, 142–148. http://doi.org/10.15414/jmbfs.2014.4.2.142-148

Anisuzzaman, M., Sharmin, S. A., Mondal, S. C., Sulana, R., Khalekuzzaman, M. and Alam, M.F. 2008. *In-vitro* microrhizome induction in *Curcuma zedoaria* (Christm.) Rsoscoe - A conservation prioritized medicinal plant. *Journal of Biological Sciences.* 1-5

Archana, C. P., Geetha, S. P., and Balachandran, I. 2013. Microrhizome and minirhizome production in three high yielding cultivars of ginger (*Zingiber officinale* Rosc .), **2(10)**, 477–484.

Ayenew, B., Tefera, W. and Kassahun, B. 2012. *In vitro* propagation of Ethiopian ginger (*Zingiber officinale* Rosc.) cultivars: Evaluation of explant types and hormone combinations. *African Journal of Biotechnology* **11 (16)**: 3911-3918

Bhat, S. R., Chandel, K. P. S., & Kackar, A. 1994. *In vitro* induction of rhizomes in ginger *Zingiber officinale* Roscoe. *Indian Journal of Experimental Biology*, **32(5)**, 340–344.

Bizet, F., Hummel, I., and Bogeat-Triboulot, M. B. 2015. Length and activity of the root apical meristem revealed in vivo by infrared imaging. *Journal of Experimental Botany*, **66(5)**, 1387–1395. http://doi.org/10.1093/jxb/eru488

David, D., Teoh, Y. J., and Gansau, J. A. 2016. *In vitro* Propagation of *Zingiber officinale* Rosc . " Tambunan ," **3(3)**, 162–167.

Dun, E. A., Ferguson, B. J., and Beveridge, C. A. (2006). Apical dominance and shoot branching. Divergent opinions or divergent mechanisms? *Plant Physiology*, **142(3)**, 812–819. http://doi.org/10.1104/pp.106.086868

FAOSTAT. 2013. Annual report on production for World Ginger production. Statistic Division.

Geetha, S. P., 2002. *In vitro* technology for genetic conservation of some genera of zingiberaceae. Ph. D. Thesis. Calicut. University. Kerala. India.

George, E. F. 1993. Plant propagation by tissue culture: Part 1, the technology, 2nd edition. Exegetics Ltd, England. Pp. **3**-468.

Guan, Q. Z., Guo, Y. H., Sui, X. L., Li, W., and Zhang, Z. X. 2008. Changes in photosynthetic capacity and antioxidant enzymatic systems in micropropagated *Zingiber officinale* plantlets during their acclimation. *Photosynthetica*, **46(2)**, 193–201. http://doi.org/10.1007/s11099-008-0031-y

Guo, Y., Bai, J., & Zhang, Z. 2007. Plant regeneration from embryogenic suspension-derived protoplasts of ginger (*Zingiber officinale* Rosc.). *Plant Cell, Tissue and Organ Culture*, **89(2-3)**, 151–157. http://doi.org/10.1007/s11240-007-9223-6

Guo, Y., and Zhang, Z. 2005. Establishment and plant regeneration of somatic embryogenic cell suspension cultures of the *Zingiber officinale* Rosc. *Scientia Horticulturae*, **107(1)**, 90–96. http://doi.org/10.1016/j.scienta.2005.07.003

Intellectual Property Corporation of Malaysia. 2009. Tambunan Ginger. Ministry of Domestic Trade, Co-operatives and Consumerism.

Islam, M.A., Klopstech, K. and Jacobsan, H.J. 2004. Efficient procedure for *In-vitro* microrhizome induction in *Curcuma longa* L. (Zingiberaceae) – a medicinal plant of tropical Asia. Plant Tissue Cult. **14(2)**:123-134.

Kapoor, P. and Rao, L. U. 2006. In Vitro rhizome induction and plantlets formation from multiple shoots in B*ambusa bambos* var. gigantea Bennet and Gaur by using growth regulators and sucrose. *Plant Cell, Tissue and Organ Culture*, **85, 2**: 211–217. http://dx.doi.org/10.1007/s11240-005-9074-y

Miler, D., and Leyser, O. 2011. Auxin, cytokinin and the control of shoot branching. *Annals of Botany*. http://doi.org/10.1093/aob/mcr069

Manju, V., and Nalini, N. 2005. Chemopreventive efficacy of ginger, a naturally occurring anticarcinogen during the initiation, post-initiation stages of 1,2 dimethylhydrazine-induced colon cancer. *Clinica Chimica Acta*, **358(1-2)**, 60–67. http://doi.org/10.1016/j.cccn.2005.02.018

Mascolo, N., Jain, R., Jain, S. C., and Capasso, F. 1989. Ethnopharmacologic investigation of ginger (*Zingiber officinale*). *Journal of Ethnopharmacology*, **27(1-2)**, 129–140. http://doi.org/10.1016/0378-8741(89)90085-8

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Plant Physiol.* **15**: 473-497

Nayak, S., and Naik, P. K. 2000. Factors effecting *in vitro* microrhizome formation and growth in Curcuma longa L. and improved field performance of micropropagated plants. *ScienceAsia*, **32(1)**, 31–37. http://doi.org/10.2306/scienceasia1513-1874.2006.32.031

Nirmal Babu, K., Samsudeen, K., and Ravindran, P.N. 1996. Biotechnological approaches for crop improvement in ginger, *Zingiber officinale* Rosc. In: Ravisankar, G.A and Venkataraman, L.V (eds.), *Recent Advances in Biotechnological Applications on Plant Tissue and Cell Culture*, Oxford Pub., New Delhi, pp. 321-332.

Ravindran, P. N., K.N. Babu and Shivaraman, K. N. 2005. Botany and crop improvement of ginger. In: Ravindran, P. N. and Babu, K. N., (Eds.) *Ginger the genus Zingiber.* CRC Press, London.pp. 15-85.

Remashree, A.B., Unnikrishnan, K., and Ravindran, P.N. 1999. Development of oil cells, and ducts in ginger (*Zingiber officinale* Rosc.). J. Spices and Aromatic Crops, 8, 163-170.

Roh, K. H., Ium, T. S., Lee, J. H., Choi, I. L., Choi, Y. H. and Jang, Y. S. 1996. *In vitro* propagation and tuberization of plantlet regenerated from shoot-tip culture in ginger. *Korean J. Plant Tissue Culture,* **23,** 3: 129-134.

Rout, G. R., Palai, S. K., Samantaray, S., & Das, P. 2001. Effect of growth regulator and culture conditions on shoot multiplication and rhizome formation in ginger (*Zingiber officinale* Rosc.) *in vitro. In vitro Cellular & Developmental Biology - Plant.* http://doi.org/10.1007/s11627-001-0135-6

Shah, J.J., and Raju, E.C. 1975 General morphology, growth and branching behavior of the rhizomes of ginger, turmeric and mango ginger. *New Botanist*, **11**, 59-69.

Sharma, T. R., and Singh, B. M. 1995. *In vitro* microrhizome production in *Zingiber officinale* Rosc. *Plant Cell Reports*, **15(3-4)**, 274–277. http://doi.org/10.1007/BF00193735

Sharma, T. R., and Singh, B. M. 1997. High-frequency *in vitro* multiplication of disease-free *Zingiber officinale* Rosc. *Plant Cell Reports*, **17(1)**, 68–72. http://doi.org/10.1007/s002990050354

Shirgurkar, M.V., John, C.K. and Nadgauda, R.S. 2001. Factors affecting *in-vitro* microrhizome production in turmeric. *Plant Cell Tiss. Org. Cult.* 64:5-11

Silip, J. J. 2015. Ginger resource in Tambunan District: potential and challenges as source of income. Conference.

Singh, P.P., Singh, V.B., Singh, A., and Singh, H.B. 2000. Evaluation of different ginger cultivars for growth, yield and quality character under Nagaland condition. *J. Medicinal and Aromatic Plant Sci,* **21**, 716-718.

Singh, T.D., Chakpram, L. and Devi, H.S. 2014. Induction of Microrhizome using silver nitrate in *Zingiber officinale* Rosc. var Baishey and Nadia. *Indian Journal of Biotechnology. 13*:256-262

Swarnathilaka, D.B.R., Kottearachchi, N.S. and Weerakkody, J.S.K. 2016. Factors affecting on induction of Microrhizomes in ginger (*Zingiber officinale* Rosc.), cultivar local from Sri Lanka. *British Biotechnology Journal* **12 (2)**: 1-7

Thayamini, H. S. 2014. In vitro Propagation of Ginger (*Zingiber officinale* Rosc.) through Direct Organogenesis: A Review. *Parkistan Journal of Biological Sciences,* 2014.

Thorpe, T. A. 2007. History of plant tissue culture. *Molecular Biotechnology.* http://doi.org/10.1007/s12033-007-0031-3

Weiss, E.A. 2002. Zingiberaceae. In Weiss, E.A. (Eds.). Spice crops. CABI Publishing

Zheng, Y. G., Y. Liu, M. Ma and Xu, K. 2008. Increasing *in vitro* microrhizome production of ginger (*Zingiber officinale* Roscoe). *Acta Physiol. Planta.* **30(4)**: 513 519.