

UNIVERSITI PUTRA MALAYSIA

THE USE OF ENDOMYCORRHIZA IN REHABILITATING MATURED COCOA STANDS

MARIA VIVA RINI

FP 1996 12



THE USE OF ENDOMYCORRHIZA IN REHABILITATING MATURED COCOA STANDS

Ву

MARIA VIVA RINI

Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Agricultural Science in the Faculty of Agriculture, Universiti Pertanian Malaysia

July 1996



"Dedicated to my beloved husband IING LUKMAN, and children: Vanukh Rabil Al-Faraby, Haifa Farmaz Atmaya and Dutri Indialoka, whose sacrifice and understanding has enable me to complete this study successfully"



ACKNOWLEDGEMENTS

In the name of Allah The Beneficient and The Compassionate.

Praise be to Allah SWT, upon His permission I could complete this thesis smoothly. Contributions of individuals and institution for the successful completion of this thesis are also deeply acknowledged.

I wish to extend my most sincere gratitude to my chairperson, Asso. Prof. Dr. Azizah Hashim, for her patience, discussion, constant and invaluable guidance, supervision, motivation and encouragement extended throughout the period of this study and in the preparation of this thesis.

I am also indebted to my committee members Asso. Prof. Dr. Mohd. Idris Zainal Abidin and Dr. Jamal Talib for their kind advice, criticism and invaluable discussion. Sincere appreciation is also extended to Dr. Anuar Abdul Rahim and Dr. Mohd. Ridzwan for their useful assistance, suggestions and discussion in preparation of the experimental designs and statistical analysis of this study.

I would also like to take this opportunity to thank the technical staff of the Department of Soil Science UPM, particularly Mr. Halimi Mustafar, Mr. Rahim Utar, Mr. Jamil, Mr. Ariffin, Mr. Hanif, Mr. Mukhtar, Mrs. Sharimah



and Mrs. Fauziah for their help in the laboratory and Mr. Johari Sepet, Mr. Suzairy, Mr. Linggam and Mr. Samad for their help in the field work.

To my friends especially Mrs. Sriani, Ibrahim Saleh, Junaini, Ali Aman and Juriah, thank you for your assistance.

Sincere appreciation is also extended to the SEAMEO-SEARCA for financial support throughout the period of this study, for without which this research could not be carried out successfully.



TABLE OF CONTENTS

		Page
ACKNO	WLEDGEMENTS	iii
	TABLES	
	FIGURES	
	FPLATES	
	ACT	
	AK	
CHAPT	ER	
I	INTRODUCTION	. 1
	Objectives of the Study	
II	LITERATURE REVIEW	. 6
	Cocoa: A General View	. 6
	Cocoa Root System	. 6
	Cocoa Propagation	. 7
	Grafting	
	Advantages of Vegetative Propagation	. 9
	Maintenance of Clones	
	Avoidance of Long Juvenile Periods	. 9
	Control of Growth Form	. 10
	Combining of Clones	. 10
	Economics	. 10
	Rehabilitation of Cocoa Farm	. 11
	Side-Cleft Grafting	. 11
	Mycorrhiza	
	Vesicular-Arbuscular Mycorrhiza (VAM)	. 13
	VAM Formation	. 14
	Factors Affecting VAM	. 16
	Host Plant	. 16
	Soil pH	
	Soil Moisture	
	Soil Temperature	
	Soil Fertility	
	Other Factors Affecting VAM	
	The Reneficial Effects of VAM	21



		Page
	Enhancing Nutrient Uptake	21
	Increase Water Uptake	24
	Interaction with Plant Pathogen	25
	Improving Aggregation of Soil	26
	VAM Interaction with Phosphate-Solubilizing	
	Bacteria (PSB)	28
III	THE EFFECTIVENESS OF TWO VAM SPECIES IN	
	ENHANCING GROWTH OF COCOA SEEDLINGS	30
	Introduction	30
	Objective of the Study	32
	Materials and Methods	32
	Location of Experiment	32
	Experimental Design	33
	Inoculum Preparation	
	Soils	34
	Preparation of Cocoa Seedlings	34
	Planting and Inoculum Placement	
	Data Collection	
	Statistical Analysis	
	Results	37
	Plant Growth	
	Nutrient Uptake	
	Mycorrhizal Infection	
	Discussion	42
	Discussion	72
IV	THE ENDOMYCORRHIZA FUNGI FOR REHABILITA	
1 4	TION OF MATURED COCOA TREES WITH FIVE	
	SELECTED COCOA CLONES	48
	Introduction	
	Objectives of the Study	
	Materials and Methods	51
	Site and Duration of Experiment	
	Experimental Design	
	Climatic Condition of the Site	53
	Plant History	
	Soil Type	. 54 54
	Scion Preparation	
	Preparation of the Stock Tree	56



		Page
	Insertion of Scion into the Stock Tree	56
	Post-Grafting Operation	57
	VAM Inoculation	60
	Data Collection	61
	Results	70
	Growth Response	70
	Pod Formation	71
	Physiological Responses	72
	Nutrient Concentrations in Leaf	75
	Soil Nutrient Contents	77
	Microbiological Studies	78
	Soil Physical Properties	81
	Discussion	103
	Mycorrhiza Development	104
	Plant Growth Response	105
	Nutrient Contents	107
	VAM-Rhizosphere Microbe Interaction	109
	Pod Formation	110
	Soil Physical Properties	111
V	GENERAL DISCUSSION AND CONCLUSION	114
	Greenhouse Experiment	114
	Field Experiment	
BIBLIOG	RAPHY	118
APPENDI	CES	. 132
A	Additional Methodologies	133
В	Additional Tables	138
C	Additional Figures	144
VITA		. 146



LIST OF TABLES

able		Page
1	N, P, K, Ca and Mg concentrations in shoot of cocoa seed- lings inoculated with G. mosseae (Gm), S. calospora (Sc), GmSc or uninoculated control plant	41
2	Characteristics of cocoa clones KKM 3, KKM 4, KKM 5, PBC 137 and PBC 178	55
3	Relative water content, stomatal resistance and chlorophyll content in leaf of five cocoa clones as affected by VAM 3, 5 and 7 months after inoculation	92
4	Tissue N, P and K concentrations in the five cocoa clones as affected by VAM 3, 5 and 7 months after inoculation	93
5	Tissue Ca and Mg concentrations in the five cocoa clones as affected by VAM 3, 5 and 7 months after inoculation	94
6	Nutrient contents in the soil as affected by VAM 3, 5 and 7 months after inoculation	95
7	Nutrient contents in the soil as affected by clone 3, 5 and 7 months after VAM inoculation	96
8	Total bacteria and fungi population in the soil as affected by VAM 3, 5 and 7 months after inoculation	97
9	Total bacteria and fungi population in the soil as affected by clone and VAM 5 and 7 months after inoculation	97
10	Bulk desity (BD), total porosity (TPS), % aggregate, stability index (SI) and instability index (II) of the soil as affected by VAM 7 months after inoculation	98
11	Summary of Anova list for the effectiveness of two VAM species in enhancing growth of cocoa seedlings Experiment.	138



		Page
12	Summary of Anova table for diameter and nutrient contents in leaf tissue of matured cocoa trees before treatment	139
13	Hoagland Solution	140
14	Fisher and Yates tables	141
15	Summary of Anova list for field experiment	142
16	Summary of Anova list for the soil physical properties	143



LIST OF FIGURES

Figure		Page
1	Effect of G. mosseae and S. calospora as a single and mixed inoculum on height of cocoa seedlings	39
2	Effect of G. mosseae and S. calospora as a single and mixed inoculum on total leaf area per plant	40
3	Effect of G. mosseae and S. calospora as a single and mixed inoculum on root dry weight of plant	40
4	Percentage root colonization of plants inoculated with G . mosseae and S . calospora as a single and mixed inoculum	42
5	The Layout of the Experimental Plots in the Field	52
6	Length of scion (A), diameter of scion (B) and leaf area index (C) of cocoa as affected by VAM 3, 5 and 7 months after inoculation	83
7	Diameter of scion (A), length of scion (B) and leafarea index (C) of five cocoa clones as affected by VAM 3, 5 and 7 months after inoculation	84
8	Stomatal resistance (A), chlorophyll content (B) and relative water content (C) of cocoa as affected by VAM 3, 5 and 7 months after inoculation	85
9	Nutrient concentrations (N, P and K) in leaf of cocoa as affected by VAM 3, 5 and 7 months after inoculation	86
10	Nutrient concentrations (Ca and Mg) in leaf of cocoa as affected by VAM 3, 5 and 7 months after inoculation	87
11	Number of spores in the soil (A) and percent root colonization (B) of cocoa as affected by VAM 3, 5 and 7 months after inoculation	88



		Page
12	Number of spores in the rhizosphere soil of five cocoa clones as affected by VAM 3, 5 and 7 months after inoculation	89
13	Percent root colonization of five cocoa clones as affected by VAM 5 and 7 months after inoculation	90
14	Soil moisture characteristic curves as affected by VAM treatment at 7 months after inoculation	91
15	Rainfall distribution pattern at field site in 1995	144
16	Map of the State of Negeri Sembilan showing the field study area	145



LIST OF PLATES

Plate		Page
1	Plants inoculated with G. mosseae (Gm), S. calospora (Sc), mixed G. mosseae and S. calospora (GmSc) and control	39
2	Budsticks taken from UPM farm and used as the scion	58
3	Scion preparation, side A is placed adjacent to the stock tree	58
4	Incision made on the stock tree	59
5	Insertion of scion into the stock tree	59
6	Securing and protecting the graft	60
7	One month old KKM 3 scion	99
8	One month old PBC 137 scion	99
9	Twelve month old mycorrhizal KKM 3 scion	100
10	Twelve month old uninoculated KKM 5 scion	100
11	Eleven month old mycorrhizal PBC 137 scion	101
12	Eleven month old uninoculated PBC 137 scion	101
13	Ten month old uninoculated PBC 178 scion	102
14	Ten month old mycorrhizal PBC 178 scion	102
15	Pod formation in eleven month old mycorrhizal PBC 137	103



Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirements for the degree of Master of Agricultural Science.

THE USE OF ENDOMYCORRHIZA IN REHABILITATING MATURED COCOA STANDS

By

MARIA VIVA RINI

JULY 1996

Chairperson: ASSO. PROF. DR. AZIZAH HASHIM

Faculty: Agriculture

A pot experiment was conducted to study the effectiveness of two VAM species in enhancing growth of cocoa seedlings. Cocoa seedlings of hybrid UIT1xNa32 inoculated with *Glomus mosseae* and *Scutellospora calospora* either as a single inoculum or as a mixed inoculum and uninoculated control were grown in 2 kg sterilized Tai Tak series soil. The experiment was a single factor experiment arranged in a completely randomized design, with type of inoculum as a factor with four replications. The trial was done in greenhouse No. 11C of Universiti Pertanian Malaysia.

Of the four treatments used, plants inoculated with mixed inoculum gave more pronounced and significant vegetative growth (as measured by

plant height, total leaf area, root dry weight and nutrient content in leaf tissue) compared to the other treatments.

The effect of mixed inoculum G. mosseae and S. calospora was further evaluated in the field using a split-plot design, with VAM treatment as the main plot and cocoa clone as the subplot. Five selected cocoa clones (KKM3, KKM4, KKM5, PBC137 and PBC 178) were grafted onto nine year old cocoa trees from hybrid UIT1xNa32. All data were collected at 3, 5 and 7 months after VAM inoculation. Inoculated clones showed better and significant plant growth, with longer scion lengths and larger leaf area index than uninoculated ones. The crop physiology was also significantly affected by VAM, with higher relative water and chlorophyll content in the leaf and lower stomatal resistance. Results obtained also indicated that the mixed inoculum significantly improved VAM development in the soil, subsequently enhancing P, K and Mg uptake by the plants. This subsequently shortened the plant vegetative phase enabling them to flower earlier. This is true for the PBC clones. In fact, between the two clones tested, the PBC clones-especially PBC 137 proved to be far superior than the KKM clones. Soil physical properties such as soil moisture, percent aggregate and aggregate stability were also improved in the presence of the mycorrhiza fungi. The difference between treatments however was not significant.



Abstrak tesis dikemukakan kepada Senat Universiti Pertanian Malaysia untuk memenuhi keperluan Ijazah Master Sains Pertanian.

PENGGUNAAN ENDOMIKORIZA DALAM PEMULIHAN TANAMAN KOKO DEWASA

Oleh

MARIA VIVA RINI

JULAI 1996

Pengerusi: PROF. MADYA DR. AZIZAH HASHIM

Fakulti: Pertanian

Satu kajian berpasu telah dijalankan untuk mengkaji keberkesanan dua spesies kulat mikoriza vesikul-arbuskul (MVA) ke atas pertumbuhan anak benih koko. Anak benih koko dari hibrid UIT1xNa32 yang diinokulasi dengan Glomus mosseae dan Scutellospora calospora sama ada sebagai inokulum tunggal, atau campuran dan tanpa inokulum (kawalan), ditanam pada 2 kg tanah daripada siri Tai Tak yang telah disucihama. Kajian menggunakan rekabentuk rawak lengkap (CRD) iaitu jenis inokulum sebagai rawatan, dengan empat replikasi. Percubaan dijalankan di rumah hijau No. 11C, Universiti Pertanian Malaysia.

Di antara empat rawatan yang digunakan, pokok yang diinokulasi dengan inokulum campuran Glomus mosseae dengan Scutellospora



calospora memberi tumbesaran yang lebih baik dan bererti (berdasarkan tinggi pokok, jumlah luas daun, berat kering akar dan kandungan nutrien dalam tisu daun) berbanding rawatan-rawatan yang lain.

Kesan inokulum campuran Glomus mosseae dengan Scutellospora calospora seterusnya telah diuji di ladang dengan menggunakan rekabentuk kajian belahan plot (Split-plot), dengan rawatan MVA sebagai petak utama dan klon koko sebagai anak petak. Sebanyak lima klon koko terpilih (KKM3, KKM4, KKM5, PBC137 dan PBC178) telah dicantumkan kepada pokok koko hibrid UIT1xNa32 berumur sembilan tahun. Pengumpulan data dilakukan 3, 5 dan 7 bulan setelah pemberian rawatan MVA. Klon-klon yang diberi rawatan mikoriza menunjukkan tumbesaran tanaman yang lebih baik dan bererti, menghasilkan sion yang lebih panjang dan indeks luas daun yang lebih besar berbanding kawalan. Fisiologi pokok juga secara bererti dipengaruhi oleh kulat MVA. Sion yang dirawat dengan MVA menghasilkan kandungan air relatif dan klorofil yang lebih tinggi dan rintangan stomata yang rendah berbanding kawalan. Hasil kajian yang diperolehi juga menunjukkan inokulum campuran ini secara bererti telah menggalakkan perkembangan kulat MVA di dalam tanah, selanjutnya meningkatkan pengambilan P, K dan Mg oleh pokok perumah. Keadaan ini seterusnya memendekkan fasa vegetatif dan membolehkan tanaman ini mengeluarkan



bunga lebih awal, terutamanya bagi klon-klon PBC. Di antara dua jenis klon yang diuji, klon PBC, khasnya PBC 137 secara bererti terbukti jauh lebih baik berbanding klon-klon KKM. Sifat-sifat fizik tanah seperti lengasan tanah, peratus agregat dan kestabilan agregat juga turut diperbaiki dengan kehadiran kulat MVA di dalam tanah, walaupun perbezaan antara rawatan secara statistik tidak bererti.



CHAPTER I

INTRODUCTION

Malaysia has an annual rainfall averaging 2030 mm and an average annual temperature of 27°C. The high temperature and heavy rainfall coupled with high and continous humidity, provide a favourable ecological zone for the cultivation of cocoa (MARDI, 1978).

The first cocoa in Malaysia was grown on a half-acre plot at the Government's Agricultural Research Station at Serdang. Some of this cocoa came into bearing in 1937. However, the first cocoa-planting exercise on a commercial scale was only launched in 1950 at Jerangau in Terengganu (MARDI, 1978).

Cocoa cultivation in Malaysia expanded at a rapid rate in the early seventies and late eighties as a result of a good price of cocoa beans. Since then the Malaysian cocoa industry has expanded by leaps and bounds to the extent of almost tripling its output from 86.000 tonnes in 1984 to 240.000 tonnes in 1989. By 1991, the area under the crop was about 430.323 ha of which 30% is in Peninsular Malaysia, 58% in Sabah and 12% in Sarawak (Dept. of Statistics Malaysia, 1993). In Peninsular Malaysia more than half



of the area is under smallholders and could only produce 500 kg of dry cocoa beans/ha/year in comparison to 1,300 kg/ha/year from the estates (Yusof, 1981).

Earlier planting of cocoa by smallholders using low quality planting materials from seeds and F2 generation seedlings resulted in variation in the productivity of many cocoa plantations in Malaysia. Subsequently, this often resulted in poor yield and poor quality products. In addition, owing to the current fluctuating price of Malaysian cocoa beans, a more effective cost benefit programme has to be sought to reduce input cost and to restore or improve the plant's yield to a profitable position. One of the ways is to rehabilitate these cocoa areas with more productive and high resistant clones.

Several techniques have been developed to rehabilitate unproductive matured cocoa trees. These include: complete or step by step replanting, chupon regeneration, underplanting, mature budding or grafting (Jelani and Maulud, 1984). The choice of a certain technique is dependent on the problem that must be solved, taking into consideration other factors such as plant age and the environmental conditions.

The cocoa industry in Malaysia is still young, averaging between eight to fifteen years. The earlier practice of rehabilitating unproductive cocoa areas is through replanting and underplanting of these areas with better high



yielding clones. This automatically removes the existing young cocoa trees. However, the technique of budding or grafting onto the existing matured cocoa trees will help speed up the scion's establishment process as the stock plants are already matured and established. As such, large areas can be successfully rehabilitated through clonal planting which can become productive in a relatively short period (Jelani, 1985). In addition, this technique allows the existing stock trees to provide temporary shade for the developing scion. The growth of the scion is also faster enabling them to produce yield earlier in comparison to complete replanting. This technique is also more cost effective.

The vesicular-arbuscular mycorrhiza (VAM) is a symbiotic association between soil fungi and plant roots. Infection of plants by VAM is widespread and has been shown to occur in the majority of economically important plants. Tropical crop plants, such as cassava, sweet potato, soybean, maize, cotton, tobacco, rubber, oil palm, tea, cocoa and legumes are often heavily colonized by VAM fungi under natural conditions (Sieverding, 1991).

Benefits from VAM symbiosis occur because VAM hyphae extend beyond the root hair zone, thus increasing the absorptive surface areas of the root. Presence of the mycorrhizal hyphae has also been shown to enhance the formation of soil aggregates, while altering the chemical and microbiological composition of the rhizosphere soils (Millner, 1991).



Cocoa has been shown to give a positive response to mycorrhizal inoculation, with the growth of inoculated seedlings significantly increased compared to uninoculated seedlings (Jamaluddin and Azizah, 1984). These plants have also been found to be mycorrhizal dependent plant (Azizah and Ragu, 1986). The degree of mycorrhizal dependence as defined by Gerdemann (1975) is the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield, at a given level of soil fertility. Azizah and Martin (1992) further demonstrated that preinoculation of vegetatively propagated cocoa materials through budding, stem cutting and marcotting resulted in an increase in plant growth with time. Observations made on budded mycorrhizal plants in the field showed higher number of pods from these plants as compared to the controls (Azizah, pers. comm., 1995).

Objectives of the Study

Although several reports have been published on VAM interactions with cocoa under the Malaysian conditions (Azizah et al., 1985; Jamaluddin and Azizah, 1984; Azizah, 1991; Azizah and Martin, 1992), no report is available on the rehabilitation of matured cocoa trees through the mycorrhiza symbiosis. The present project carried out therefore aimed to evaluate: i) the role of the VAM endophyte in enhancing growth and nutrient uptake of hybrid cocoa seedlings under controlled greenhouse conditions, and ii) to



evaluate the response of different cocoa clones grafted onto matured cocoa trees under field conditions with or without the mycorrhiza fungi.



CHAPTER II

LITERATURE REVIEW

Cocoa: A General View

Cocoa, *Theobroma cacao* L., one of some twenty-two species that constitute the genus **Theobroma** belongs to the family Sterculiaceaea, a group of small trees which occurs in the wild in the Amazon basin and other tropical areas of South and Central America (Wood and Lass, 1985).

Cultivation of cocoa requires rainfall between 1500-2000 mm per annum, with the dry season for no more than three months, a mean maximum temperature of 30°-32 °C and a mean minimum temperature of 18°-20 °C with no persistent strong winds. A hot moist climate is favourable for growth of cocoa (Urquhart, 1961).

Cocoa Root System

The tap root of young cocoa seedling grows straight down into the ground with the lateral roots arising in a collar just below the soil surface (Wood, 1975). The root system of mature cocoa tree consists of 50-120 cm long tap root, with an extensive system of lateral feeder roots, most of which



lie in the top 15-20 cm of the soil. The tip of the main lateral roots are covered by bunches of fine rootlets which become abundant in the presence of decomposing plant residues (McCreary et al. 1943). Root development is strongly influenced by soil structure. In cocoa, absorption of water and mineral nutrients is actively done by the feeder roots in the top 10 to 20 cm soil layer (Urquhart, 1961).

Cocoa Propagation

Cocoa can be propagated either by generative propagation through seedlings or by vegetative propagation. Under normal conditions, cocoa is usually planted from seed because seeds are cheap and easy to obtain. This situation is simplified further by planting uniform seeds from the Amazon hybrids, although there exist some genetic variation within these seeds (Wood and Lass, 1985).

Asexual or vegetative propagation is reproduction from the vegetative parts of the original plant whereby every cell of the plant contains the genetic informations necessary to regenerate the entire plant (Hartmann and Kester, 1983). There are several types of vegetative propagations. These include cuttings of all types - stem, leaf or root cuttings, layering, budding and various types of grafting. Several methods of vegetative propagation of cocoa have

