



UNIVERSITI PUTRA MALAYSIA

***IMPROVED PROPAGATION, EARLY ESTABLISHMENT
AND FRUIT DEVELOPMENT OF THE MIRACLE FRUIT,
(SYNSEPALUM DULCIFICUM DANIELL.)***

CHEN XINGWEI

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UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI

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**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

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DANIELL.)**

By

CHEN XINGWEI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Master of Science**

October 2013

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

IMPROVED PROPAGATION, EARLY ESTABLISHMENT AND FRUIT DEVELOPMENT OF THE MIRACLE FRUIT, (*SYNSEPALUM DULCIFICUM* DANIELL.)

By
CHEN XINGWEI
October 2013

Chairman: Associate Professor Thohirah Lee Abdullah, PhD
Faculty: Agriculture

Propagation of miracle fruit, *Synsepalum dulcificum* using cuttings has been investigated for early fruiting planting materials. However, there is very little information on the nutrient requirements during early establishment and growth of the species. Studies on its reproductive biology, fruit set and development are equally important to understand the flowering and fruiting behaviour. The objectives for this study are: (i) to improve the propagation of *Synsepalum dulcificum* through stem cutting using Indole-3-butyric acid (IBA); (ii) to establish seedlings under different shade levels and nitrogen (N) rates; (iii) to describe the flower and fruit development of *Synsepalum dulcificum*. Three experiments were conducted to achieve the objectives. In Experiment 1, rooting responses of softwood cuttings of *Synsepalum dulcificum* as affected by IBA concentrations were conducted. Soft wood stem cuttings were treated with 0 mg/L, 200 mg/L, 400 mg/L, 600 mg/L and 800 mg/L IBA concentrations and arranged in Randomized Complete Block Design (RCBD) with 5 replications. Callus formation started three weeks after sowing and adventitious roots emerged from the basal ends eight weeks after planting. High concentration of IBA (800 mgL⁻¹) increased rooting percentage to 23.64 ± 0.19%. The anatomy of stem cutting at day 0 showed the phloem being surrounded by 2-3 layers of fibers. Root primordial developed from secondary phloems and penetrated the fiber layers 5 weeks after planting. In Experiment 2, growth and physiological responses of miracle fruit seedlings to different shade levels and nitrogen (N) rates were recorded. Six months old seedlings were subjected to 0%, 45% and 75% shade and received 0, 300, 600 and 900 mg N plant⁻¹ year⁻¹. Treatments were arranged in Nested RCBD with 4 replications. Different levels of shades significantly affected plant height, dry weight, leaf area, specific leaf area, number of branches, root:shoot ratio, chlorophyll fluorescence and chlorophyll contents throughout the growing season. Miracle fruits seedlings under 75% shade were taller, had higher photosynthetic rate (1.82 ± 1.113 μ mol m⁻² s⁻¹) than other treatments and had high relative growth rate (0.37 g g⁻¹ month⁻¹) in the early establishment stage which suggest that this shade level is more suitable for establishment of young miracle fruit plants. Nitrogen fertilization was found to prevent photoinhibition in miracle fruit seedlings in the early establishment stages. Nitrogen rate at 300 mg N plant⁻¹ year⁻¹ was adequate for growth and development since the plant was more efficient in utilizing the available N in low N input (N utilization efficiency = 36.56 mg mg⁻¹). In Experiment 3, flower

ontogenesis and fruit development of *Synsepalum dulcificum* was observed. Flowers and flower buds were processed and observed under light microscope and scanning electron microscope. Miracle fruit flower takes 100 days to develop from reproductive meristem to full anthesis. The flower development can be divided into six stages based on the size and appearance of the flower bud. The fruit with persistence style developed and ripened 90 days after anthesis. Heavy fruit drop was observed at 40 – 60 days after anthesis which contributed to the final fruit set average of 5.06% per plant.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

MENAMBAHBAIKKAN PEMBIAKAN, PERTUMBUHAN AWAL DAN PERKEMBANGAN BUAH PADA BUAH AJAIB, (*SYNSEPALUM DULCIFICUM* DANIELL.)

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Pembiakan buah ajaib, *Synsepalum dulcificum* melalui keratan telah dikaji untuk mendapatkan bahan tanaman yang boleh berbuah awal. Maklumat mengenai nutrisi yang diperlukan oleh spesis ini pada peringkat awal dan semasa pertumbuhan amat kurang. Kajian pada biologi pembiakan, pembentukan buah dan perkembangan buah pokok ini adalah sama penting untuk memahami tabiat pembungaan dan pembentukan buah. Objektif kajian ini adalah: (i) untuk menambahbaikkan pembiakan *Synsepalum dulcificum* melalui keratan batang dengan menggunakan Indole-3-butyric acid (IBA); (ii) untuk menanam anak benih di bawah tahap naungan dan kadar nitrogen yang berbeza; (iii) untuk mengetahui perkembangan pembentukan bunga dan buah *Synsepalum dulcificum*. Tiga eksperimen telah dijalankan untuk mencapai objektif tersebut. Dalam Eksperimen 1, tindak balas pembentukan akar pada keratan batang lembut *Synsepalum dulcificum* dipengaruhi oleh kepekatan IBA telah dijalankan. Keratan batang lembut telah dirawat dengan kepekatan IBA pada 0 mg/L, 200 mg/L, 400 mg/L, 600 mg/L and 800 mg/L dan disusun dalam Rekabentuk Rawak Berblok Lengkap (RCBD) dengan 5 replikasi. Kalus mula terbentuk tiga minggu selepas keratan disemai dan akar baharu muncul dari pangkal keratan lapan minggu selepas semaian dilakukan. IBA yang berkepekatan tinggi (800 mgL^{-1}) meningkatkan peratus pengakaran kepada $23.64 \pm 0.19\%$ apabila dibandingkan dengan rawatan lain. Anatomi keratan batang pada hari 0 menunjukkan phloem dikelilingi oleh 2-3 lapisan serat. Akar primordia berkembang dari phloem sekunder dan menembusi lapisan serat 5 minggu selepas semaian dilakukan. Dalam Eksperimen 2, pertumbuhan dan tindak balas fisiologi anak benih pokok buah ajaib terhadap tahap naungan dan kadar nitrogen (N) yang berbeza telah dicatat. Anak benih berumur enam bulan didedahkan kepada 0%, 45% and 75% naungan dan diberi 0, 300, 600 and 900 mg N pokok⁻¹ tahun⁻¹. Rawatan disusun dalam 'Nested RCBD' dengan 4 replikasi. Tahap naungan yang berbeza memberi kesan kepada ketinggian pokok, berat kering, luas permukaan daun, permukaan daun spesifik, bilangan dahan, ratio akar dan batang, klorofil floresen dan kandungan klorofil sepanjang musim pertumbuhan. Anak benih pokok buah ajaib di bawah naungan 75% adalah lebih tinggi, mempunyai kadar fotosintesis yang tinggi ($1.82 \pm 1.113 \mu \text{ mol m}^{-2} \text{ s}^{-1}$) berbanding rawatan lain dan mempunyai kadar pertumbuhan relatif yang tinggi ($0.37 \text{ g g}^{-1} \text{ month}^{-1}$) pada peringkat awal pertumbuhan. Perkara ini mencadangkan tahap naungan ini lebih sesuai digunakan pada anak benih pokok buah ajaib. Baja nitrogen didapati dapat mengurangkan

'photoinhibition' pada anak benih pokok buah ajaib pada peringkat awal penanaman. Kadar nitrogen pada 300 mg N pokok⁻¹ tahun⁻¹ adalah cukup untuk pertumbuhan dan perkembangan memandangkan pokok ini menggunakan N lebih cekap pada pemberian N berkadar rendah (kecekapan penggunaan N = 36.56 mg mg⁻¹). Dalam Eksperimen 3, pembungaan dan perkembangan buah pada *Synsepalum dulcificum* telah diperhatikan. Bunga dan kudup bunga diproses dan diperhatikan di bawah mikroskop cahaya dan imbasan mikroskop electron. Bunga buah ajaib mengambil masa 100 hari untuk berkembang dari meristem reproduktif ke antesis. Proses perkembangan bunga boleh dibahagikan kepada enam peringkat mengikut saiz dan rupa bentuk kudup bunga. Buah yang mempunyai stil berkembang dan masak pada hari 90 selepas antesis. Keguguran buah yang lebat didapati berlaku pada hari ke40 – 60 selepas antesis. Perkara ini menyumbangkan peratusan buah yang berjaya berkembang adalah kira-kira 5.06% sepokok.

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I certify that a Thesis Examination Committee has met on 7 October 2013 to conduct the final examination of Chen Xingwei on his thesis entitled “Improved Propagation, Early Establishment and Fruit Development of The Miracle Fruit, (*Synsepalum dulcificum* Daniell.)” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



CHEN XINGWEI

Date: 7 October 2013



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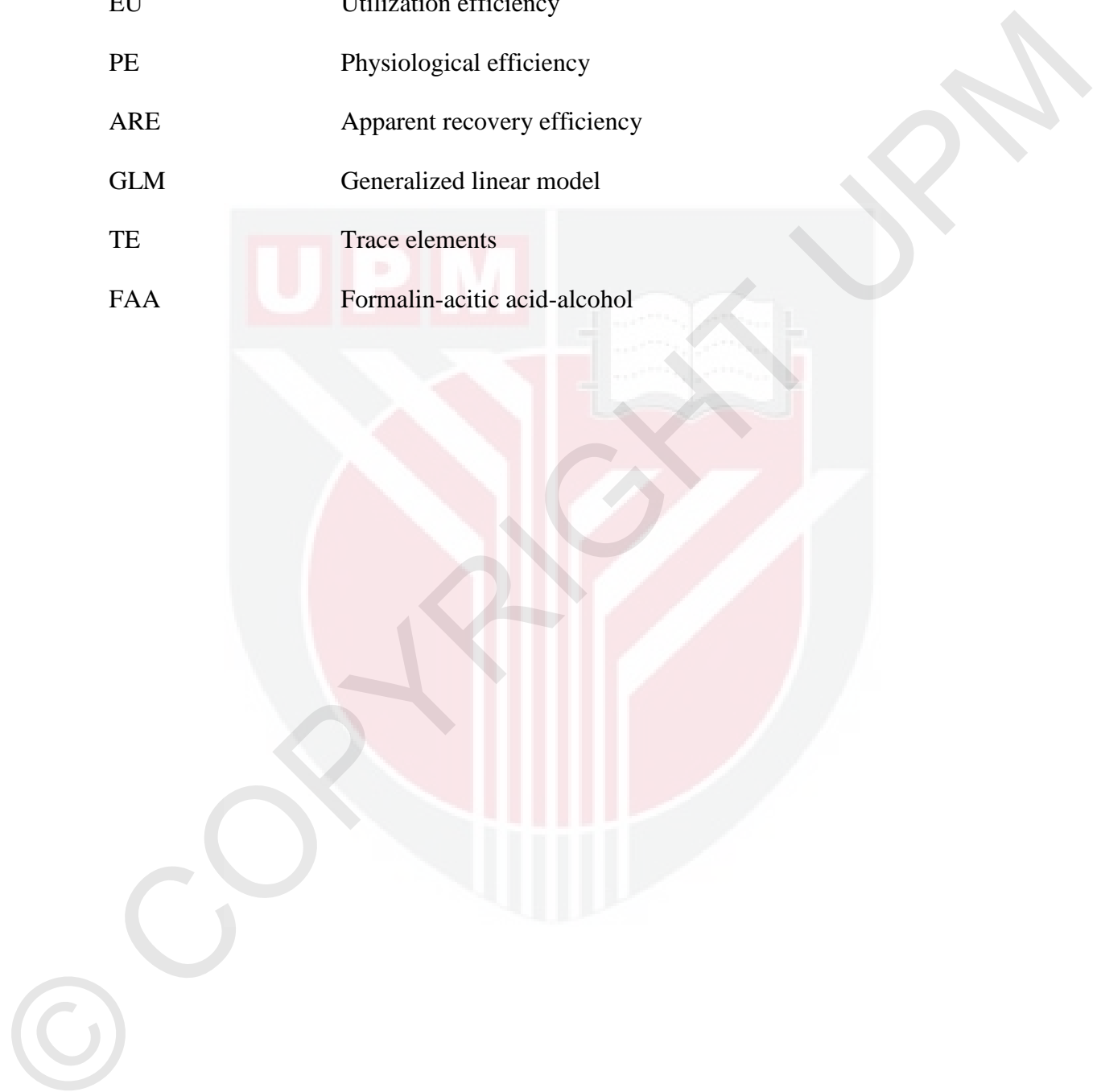
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LIST OF ABBREVIATIONS

IBA	Indole-3-butyric acid
N	Nitrogen
IAA	Indole-3-acetic acid
NAA	α -naphthalene acetic acid
PAR	Photosynthetically active radiation
CO ₂	Carbon dioxide
O ₂	Oxygen
ATP	Adenosine triphosphate
NADPH	Nicotinamide adenine dinucleotide phosphate
PSI	Photosystem I
PSII	photosystem II
Rubisco	Ribulose 1,5-bisphosphate carboxylase-oxygenase
RuBP	Ribulose 1,5-bisphosphate
PGA	3-phosphoglyceric acid
P _{max}	Light saturated rate of photosynthesis
LHC I	Light harvesting complexes I
LHC II	light harvesting complexes II
Q _A	Plastoquinone A
Q _B	plastoquinone B
NH ₄ ⁺	Ammonium
NO ₃ ⁻	Nitrate

DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
CO(NH ₂) ₂	Urea
NH ₃	Ammonia
NO ₂ ⁻	nitrite
SEM	Scanning electron microscopy
LM	Light microscope
CPD	Critical point drying
UPM	Universiti Putra Malaysia
RH	Relative humidity
FAA	Formalin-acetic acid-alcohol
TBA	Tertiary Butyl-Alcohol
RCBD	Randomized Complete Block Design
ANOVA	Analysis of variance
SAS	Statistical Analysis System
DMRT	Duncan Multiple Range Test
P	Phosphorous
K	Potassium
Ca	Calcium
Mg	Magnesium
SLA	Specific leaf area
RGR	Relative growth rate

F_v	Fluorescence
F_m	Maximum fluorescence
EU	Utilization efficiency
PE	Physiological efficiency
ARE	Apparent recovery efficiency
GLM	Generalized linear model
TE	Trace elements
FAA	Formalin-acetic acid-alcohol



CHAPTER 1

INTRODUCTION

Miracle fruit or miracle berry plant (*Synsepalum dulcificum* Daniell.) from the family Sapotaceae is a tropical evergreen shrub or small tree growing 2 m – 5 m tall (Keay, 1992; Duke and duCellier, 1993). This plant is indigenous to tropical West Africa (Opeke, 1982) and commonly found growing in the wild in virgin forest, costal area and in mountainous forest (Duke and duCellier, 1993). Miracle fruit plant produces small creamy white flowers which measured around 0.5 cm on the axillary buds throughout the year. The plant produces small ellipsoid red berries (2 to 3 cm long) with a single seed. After eaten the fruit and allowed the fleshy pulp to coat the taste buds of the tongue, every sour or acidic food eaten or drunk afterwards will taste very sweet. The taste modifying effect will last for 30 minutes. Daniell was the first to describe scientifically the unusual quality of this fruit, which he called it “miraculous berry” (Daniell, 1852). The sweetening property is due to the presence of glycoprotein in the pulp of the berry called miraculin (Kant, 2005). The fruit could possibly help diabetic people eating less calorie food without taking in sugar. Miracle fruit has been investigated as a food additive and a possible source for a natural food sweetener (Kant, 2005).

Miracle fruit has been cultivated not only for its fruit but also as an ornamental plant. The tree form of *Synsepalum dulcificum* is cone or pyramid shape. The moderate growth rate and free branching characteristic of this plant make it a potential ornamental small tree or shrub, hedges and potted plant for urban planting. Pruning is not necessary for this plant since it is bushy and forms a beautiful tree form naturally. Besides that, the miracle fruit plant also can be trained into a bonsai or topiary. *Synsepalum dulcificum* has red to orange colour young leaves which adds to the esthetic value of the plant. The plant produces fragrant flowers and edible fruits all year round; thus it is a good candidate for a fragrance garden or edible garden. The miracle berries turn bright red when ripen and makes the tree a stunning and attractive ornamental plant. The plant also attracts birds to feed on its berries.

Despite the need of large-scale production of miracle berry to exploit its potential and enhance its improvement, commercial production of the plant has been a constraint. The plant is recalcitrant to propagation both by seeds and cuttings (Ogunsola and Ilori, 2008). The seeds dry very quickly after harvest and loses viability after drying while rooting of the cuttings have been very difficult and it takes long time to establish (Joyner, 2006). Large scale production of miracle fruit plants is still rely on seeds (Joyner, 2006). The growth of the seedlings is slow in the nursery stage (Duke and duCellier, 1993). Plants begin to bear fruit 3 to 4 years after seeding in the nursery. Fully regeneration of miracle berry through tissue culture using nodal explants from mature plants and young seedlings was not very successful (Ogunsola and Ilori, 2008). A technique for propagation by cuttings needs to be devised so that plants will begin to bear fruits earlier

(Duke, 1993). There is lack of scientific literature on propagation miracle fruit through stem cuttings and the origin of adventitious root development is unknown.

For the miracle fruit production practices, shading will be given to the young seedlings and will be thinned out as the plant begins bearing (Duke and duCellier, 1993). Miracle fruit is a shade plant, adapted to the under storey of forests in its native habitat and is able to tolerate full sunshine when the plant grew bigger (Duke and duCellier, 1993). However, there is very little information on the growth of miracle fruit seedlings under different shade levels and N rates for successfully produce the planting materials. The effects of different shade level on miracle fruit seedlings during early establishment have not been reported. There is also lack of information on the nutrient requirement for miracle fruit during early growth. Despite the economical importance of the berry of *Synsepalum dulcificum*, interest has been drawn to investigate the development of the flower and fruit of this plant. There is still lack of study on fruit set and development of *Synsepalum dulcificum*. Hence, three studies had been carried out to investigate the problems stated with the general objectives:

1. To improve the propagation of *Synsepalum dulcificum* through stem cutting using Indole-3-butyric acid (IBA)
2. To successfully establish the miracle fruit seedlings under different shade levels and nitrogen (N) rates
3. To describe the flower and fruit development of *Synsepalum dulcificum*

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