PLANT BRANCHING HABITS, SEED MATURITY STAGES AND DRYING EFFECTS ON SEED QUALITY OF DUKUNG ANAK (Phyllanthus amarus Schum. & Thonn. AND Phyllanthus debilis Klein ex Willd)

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By

SHARIF AZMI BIN ABDURAHMAN

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

November 2018

Per Angusta Ad Augusta

To a great father, who taught me, by hard work and difficulties you reach great things

Allahyarham Abdurahman @ Sharif Abdul Rahman bin Sharif Yasin
(1943-2009)

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

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November 2018

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Dukung Anak is a well-known herb due to its medicinal value. Realizing the potential, Malaysian government through National Key Economic Areas (NKEA) has extensively promoted the cultivation of this herb to boost the economy. *Phyllanthus amarus* and *Phyllanthus debilis* are two of the commercially grown species in Malaysia. However, the availability of good quality seeds as planting material is limited resulting in poor quality (< 50% germination) and erratic germination. Current method of collecting dehisced seeds may be improved by harvesting matured seeds prior to dehiscence and subjecting them to desiccation. Therefore, three studies were carried out; plant branching habits in relation to fruit production, differences in seed quality at different maturity stages and drying effects on seed quality of *P. amarus* and *P. debilis*. In the first study, plants were grown using standard cultural practises, in which plant growth and development event was observed and studied and plant architecture model was developed. Both *P. amarus* and *P. debilis* had main stem with monopodial while branches being plagiotropic with sympodial growth. Fruits were formed on branchlets from main stem as well as primary branches. Substantial number of fruits were obtained from 56 to 98 days after sowing (DAS) for *P. amarus* and 63 to 105 DAS for *P. debilis*. In the second study, fruits and seeds were collected at different maturity stages (7, 9, 11, 13, 15 days after anthesis (DAA) and dehisced seeds for *P. amarus* and 7, 9, 11, 13 DAA and dehisced seeds for *P. debilis*) and from different branchlets on main stem and primary branches. Seed moisture content and seed germination test was carried out to know the viability and vigour of the seeds. Fruits and seeds collected from different position showed no effects on seed quality but it was significantly affected by the level of maturity stages. Maximum dry weight was observed 13 DAA for *P. amarus* (43.74 mg/100 seeds) and 11 DAA for *P. debilis* (73.56 mg/ 100 seeds) indicating PM with germination at PM of 74%, and 69% respectively. However, moisture content was high at 35% for *P. amarus* and 33% for *P. debilis*. However, best quality seed was obtained during dehiscence with a moisture content of 15% and

germination of 95% (*P. amarus*) and 92% (*P. debilis*). In the third study, fresh seeds of different maturity stages were immediately dried using two different methods, under rain shelter and in air-conditioned room for three days before seed moisture content and germination test. Both methods managed to reduced seed moisture content to less than 15% for all maturity stages. Seed quality during PM was improved to 89% (*P. amarus*) and 79% (*P. debilis*) after drying under rain shelter condition for three days. In conclusion, seeds can be collected prior to dehiscence as early as 13 DAA and 11 DAA for *P. amarus* and *P. debilis* and dried under rain shelter for three days to obtain seed quality similar to that at dehiscence. For seed collection, fruits can be harvested from 56 to 98 DAS for *P. amarus* and 63 to 105 DAS for *P. debilis*

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

PEMBENTUKAN CABANG POKOK, PERINGKAT KEMATANGAN BIJI BENIH DAN KESAN PENGERINGAN TERHADAP KUALITI BIJI BENIH DUKUNG ANAK *Phyllanthus amarus* Schum. & Thonn. AND *Phyllanthus debilis* Klein ex Willd)

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Dukung Anak merupakan herba terkenal kerana khasiat yang tinggi. Menyedari perkara ini, kerajaan Malaysia melalui NKEA telah menggalakkan penanaman herba ini untuk tujuan meningkatkan ekonomi negara. *Phyllanthus amarus* dan *Phyllanthus debilis* merupakan antara spesies Dukung Anak yang ditanam secara komersial di Malaysia. Walau bagaimanapun, biji benih berkualiti adalah terhad menyebabkan kadar percambahan rendah (<50%) dan percambahan yang tidak seragam. Kaedah pengumpulan bij benih setelah ianya merekah boleh diperbaiki dengan menuai biji benih yang telah matang sebelum ianya merekah dan megeringkannya. Oleh itu, tiga kajian telah dilakukan dengan tujuan untuk mengkaji arkitektur pokok dan kaitannya dengan perkembangan buah dan biji benih, perbezaan antara kualiti biji benih dari tahap kematangan yang berbeza dan kesan pengeringan terhadap kualiti biji benih *P. amarus* dan *P. debilis*. Dalam kajian pertama, pokok ditanam mengikut amalan penanaman piawai dimana pertumbuhan, perkembangan dan cara pembentukan cabang pokok diteliti. Kedua-dua spesies mempunyai batang utama yang berciri mopodial manakala ranting yang tumbuh pada batang utama bersifat simpodial. Buah dihasilkan pada branclet di batang utama dan cabang primer. Jumlah buah yang tinggi diperoleh diantara 56 hingga 98 hari selepas penyemaian untuk *P. amarus* dan 63 hingga 105 hari selepas penyemaian untuk *P. debilis.* Dalam kajian kedua, buah dan biji benih dituai pada peringkat kematangan yang bebeza (7, 9, 11, 13, 15 hari selepas antesis (DAA) dan biji benih yang gugur untuk *P. amarus*) dan 7, 9, 11, 13 DAA dan biji benih yang gugur untuk *P. debilis*) dan dari branclet yang berbeza iaitu pada batang utama dan cabang primer. Ujian kelembapan dan percambahan biji benih dilakukan untuk mengetahui kualiti biji benih. Tiada perbezaan signifikan antara kualiti biji benih berdasarkan lokasi buah diambil, tetapi terdapat perbezaan signikan antara kualiti biji benih berdasarkan peringkat kematangan. Berat kering maksimum diperoleh pada 13 hari selepas antesis untuk *P. amarus* (43.74 mg/100 biji benih) dan 11 hari selepas antesis untuk *P. debilis* ((73.56 mg/ 100 biji benih)

dengan peratus percambahan, 74%, and 69% masing-masing. Walau bagaimanapun, kandungan kelembapan adalah tinggi iaitu 35% untuk *P. amarus* dan 33% untuk *P. debilis*, manakala buah yang gugur mempunyai percambahan maksimum 95% (*P. amarus*) and 92% (*P. debilis*) pada kandungan kelembapan 15%. Dalam kajian ketiga, biji benih mengikut peringkat kematangan yang berbeza dikeringkan menggunakan dua kaedah berbeza, dibawah bangunan berbumbung dan di dalam bilik berhawa dingin selama tiga hari berturut-turut sebelum ujian kelembapan dan percambahan biji benih dilakukan. Kedua-dua kaedah berjaya mengurangkan kandungan kelembapan biji benih kurang dari 15% untuk semua peringkat kematangan. Kualiti biji benih semasa PM dengan percambahan 89% (*P. amarus*) dan 79% (*P. debilis*) selepas pengeringan di bawah bangunan berbumbung. Kesimpulannya, biji benih boleh dituai sebelum ianya gugur seawal 13 hari selepas antesis untuk *P. amarus* dan 11 hari selepas antesis untuk *P. debilis*. Biji benih perlu dikeringkan dibawah bangunan berbumbung selama tiga hari untuk meningkatkan kualiti biji benih. Bagi pengumpulan biji benih, buah boleh dituai dari 56 hingga 98 hari selepas penyemaian untuk *P. amarus* dan 63 hingga 105 hari selepas penyemaian untuk *P. debilis.*

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

°C	Degree Celcius
ANOVA	Analysis of Variance
cm	centimetre
CVG	Coefficient Velocity of Germination
DAA	Days After Anthesis
DAS	Days After Sowing
DOA	Department of Agriculture
ETP	Economic Transformation Programme
FGP	Final Germination Percentage
GI	Germination Index
GNI	Gross National Income
HM	Harvest Maturity
MARDI	Malaysia Agriculture Research and Development Institute
kg	Kilogram
MDG	Mean Daily Germination
mg	milligram
MGT	Mean Germination Time
NKEA	National Key Economic Area
PM	Physiological Maturity
RH	Relative Humidity
SVI	Seedling Vigour Index

CHAPTER 1

INTRODUCTION

The ETP, Economic Transformation Program was initiated by the Malaysian Government to attain the high-income economy status by 2020. National Key Economic Areas (NKEA) were identified of which, agriculture is one out of the 12 NKEA's with 17 Entry Point Projects to transform agricultural sector. High value herbal products were listed as one of the core focus under EPP1 (Entry Point Project), which is expected to generate a Gross National Income (GNI) of RM 2.2 billion in the year 2020. Five targeted herbs received very high attention of which Dukung Anak (*Phyllanthus* spp.) is one (PEMANDU, 2010).

Dukung Anak is a popular herb that can be found growing wild in Malaysia and is considered as a weed because it is able to grow in adverse growing condition (Wan Zaki and Musa, 2007). The plant itself is unique where the row of bead-like fruits can be seen on the stem underneath the leaves, hence the name Dukung Anak. There are a few species of Dukung Anak commercially grown in Malaysia namely *Phyllanthus amarus*, *Phyllanthus niruri*, *Phyllanthus debilis* and *Phyllanthus urinaria* (Zaharah, 2012; Department of Agriculture, 2014).

Phyllanthus species has been traditionally used worldwide to treat medical condition for example stomach and genitourinary problems, liver, kidney, spleen, jaundice and diabetes (Ali, et al., 2006; Sarin et al., 2014; Sekar et al., 2014). Many of the species have been phytochemically and pharmacologically studied leading to many therapeutic findings of various compounds such as alkaloids, flavonoids, coumarins, saponins, steroids, lactones, tannins and lignans (Calixto et al., 1998; Poh-Hwa et al., 2012; Tang et al., 2014; Kumara, 2014). Among the four species commercially grown in Malaysia. *P. debilis* possess the highest antioxidant activity while *P. amarus* produced higher phenolic compound compared to *P. niruri* and *P. urinaria* (Kumaran & Karunakaran, 2007; Poh-Hwa et al., 2012). To date, Nova® Laboratories Sdn. Bhd, a Malaysian company with many products for liver related diseases such as Hepatitis B is a major user of *Phyllanthus* spp. Realizing the therapeutic importance of this herb, government is extensively promoting the cultivation of *Phyllanthus* spp. in large scale to boost the economy.

Despite the potential, the availability of seed as planting material is currently limited, thus hindering large scale production of *Phyllanthus* spp. The seed production of commercial *Phyllanthus* spp. especially *P. amarus* and *P. debilis* is not systematically carried out resulting in seeds of low quality with less than 50% germination and erratic germination ranging from ten days to three weeks (Unander et al., 1995). It was also reported that germination of freshly collected seeds was slower than older seeds (Unander et al., 1995, Rao, 2012), implicating that desiccation may play a role in enhancing seed quality. Hence, there is a need to

understand seed production of *Phyllanthus* spp. and to improve germination related issues.

Phyllanthus species has indeterminate growth and it produces branchlet on its main stem instead of leaf. The branchlet carry both leaves as well as fruits. In addition, it produces primary branches which then produce its own branchlet. Hence, at any point of time, there will be seeds of different maturity stages depending on the location of the fruit on the branchlet. Seeds that attain maturity will dehisce and this process of dehiscence begin as early as fifth week and continues till the end of plant cycle.

Current method used in Malaysia is to mulch the soil with silver shine and dehisced seeds are vacuumed every week until foliage harvesting (Musa et al., 2006). This practice, though practical results in poor quality seeds and require extensive cleaning process.

The plant growth and their pattern of fruit and seed production has received considerably less attention. In a study conducted by Lee (1988), *Lycopersicon esculentum* (tomato) showed that fruit and seed maturation were affected by their position on the plant. Proximal fruits showed an early maturation compared to later fruits and seeds. Therefore, depending on which part of the plant seeds are taken, it will affect the time taken by the seeds to reach maturity. The age of plant is correlated to the plant architecture especially branching habits as it grows bigger, it becomes more complex and it will affect seed production. A good understanding on plant architecture towards seed production of *P. amarus* and *P. debilis* will provide sound understanding on the best way to harvest the seeds.

Other than plant branching habits and its influence on seed quality development, seed maturity stages also play a great role in determining seed quality as immature seed will lead to poor quality and late harvesting will lead to loss of seeds due to shattering (Bewley et al., 2013). Harvesting seeds after physiological maturity (PM) prior to dehiscence may provide a solution to loss of seeds due to shattering. This study will determine physiological maturity in seeds using various indicators of seed produced in various location of the plant and asses the germination of seeds harvested at different maturity stages before and after physiological maturity. This is based on the understanding that seeds have highest potential at PM (Copeland & McDonald, 2001; Muasya et al., 2002; Bewley et al, 2013). Thus, early harvesting followed by artificial desiccation may become an option for seed collection.

Desiccation is an important process in attaining maturity in seeds. It has been reported to act as switch from development to germination (Bewley et al., 2013). The ability to tolerate desiccation is a trait that is acquired during seed development with seeds of different species responding differently. The method of drying has also been reported to play an important role in seed quality, for example in *Arachis hypogaea* (Krzyzanowski et al., 2006) and *Sorghum bicolor* (Babiker et al., 2010). Hence, the

ability of seeds harvested prior to dehiscence to tolerate desiccation using different drying methods must be studied.

Therefore, the objectives of this study were;

1. To understand plant growth, development and branching habits of *P. amarus* and *P. debilis* in relation to fruit and seed production,
2. To identify differences in seed quality of *P. amarus* and *P. debilis* harvested at different maturity stages on different position of the plant,
3. To determine the effect of different drying method on desiccation tolerance and seed quality of seeds harvested at different maturity stages.

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