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OPTIMIZATION OF SEED AND SEEDLING PRODUCTION IN HEMPEDU BUMI (Andrographis paniculata (Burm. F.) Wall. Ex Nees)

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By

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

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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

OPTIMIZATION OF SEED AND SEEDLING PRODUCTION IN HEMPEDU BUMI (*Andrographis paniculata* (Burm. F.) Wall. Ex Nees)

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March 2016

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

The propagation of *Andrographis paniculata* is generally through seed but the germination is poor due to seed dormancy. Therefore, seven seed dormancy breaking treatments namely water soaking for 24 hours, sand paper scarification, hot water soaking at 40°C for 10 minutes, hot water soaking at 50°C for 10 minutes, hot water soaking at 60°C for 5 minutes and combination of sand paper scarification, hot water soaking (60°C for 5 minutes) and a control were investigated to determine the best seed dormancy breaking treatments. The percentage of seed germination scarified using sand paper was 91%. Seeds took the shortest time of 3.5 days to reach 50 % germination after scarified and followed with hot water soaking at 60°C for 5 minutes.

Maturity at harvest and storage are important that will affect the seed quality. Currently no study has been carried out to study the effect of maturity and storage on seed quality dan germination. The effects of two maturity stages (grey purple capsule and grey brown capsule) and four storage periods (0, 3, 6 and 9 months) with and without sand paper scarification were investigated to determine the seed germinability. Freshly harvested seeds from grey brown capsule had lower 1000 seed weight, lower seed moisture and higher seed dry matter of 1 gram seed compared to seeds from grey purple capsules. However, maturity stage and storage period did not affect 1000 seed weight, seed moisture and seed dry matter of 1 gram seed after the capsules were slow dried in desiccator. Germination percentage was recorded higher for seeds from grey brown capsule rather than grey purple capsule. Germination improved after 6 months of storage without scarification. None of the untreated seeds from different maturity stages and storage periods had more than 50 % germination. Seeds from grey brown capsule reached 50 % germination faster than seeds from grey purple capsule. Time to 50 % germination was significantly reduced after 6 months of storage.

The effects of three seedling tray cell volume (43 cm^3, 30 cm^3 and 25 cm^3) and seven growing media (mixed soil, cocopeat, 4 cocopeat : 1 rice husk biochar, 3 cocopeat : 2 rice husk biochar, 4 cocopeat : 1 EFB compost, 3 cocopeat : 2 EFB compost and 4 cocopeat : 3 rice husk biochar : 3 EFB compost) were investigated to determine a suitable growing media and seedling tray for the production of organically grown

seedlings *A. paniculata*. Seedling height, fresh weight, total leaf area and dry weight were increased when the cell volume increased from 43 cm^3 to 30 cm^3 then reduced at cell with 25 cm^3. Root shoot ratio did not differ significantly between seedling tray cell volumes. Root length and root volume increased significantly when the number of cell in tray increased. Seedlings grown in media cocopeat : biochar : EFB compost in ratio 4:3:3 (CBE) had the highest seedling height, fresh weight, total leaf area, dry weight, root length and root volume. There were significant interaction effects between seedling tray cell volume and growing media on seedling height, fresh weight, total leaf area, root length and root volume. Quality seedlings can be obtained in cell with 30 cm^3 with media CBE.

Light duration is one of the environmental factors important that can influence flowering, seed fertility and seed production. However, no photoperiodic treatments has been studied for this plant. Therefore, the effects of four short day treatments (12 hours, 10 hours, 8 hours and 6 hours) was investigated to determine the suitable short day treatment for seed production. Plant height increased significantly when the day length provided becomes shorter. Short day treatments did not affect secondary branches and dry weight of plants. The heaviest plants of 172.70 g in fresh weight were grown in 6 hours day length. Prolonged days to flowering and days to mature capsule formation were observed when day length was reduced from 12 hours to 6 hours. *A. paniculata* grown in 12 hours (control) recorded the highest number of flower of 459.50, number of capsule of 359.67 and harvest index of 9.78 % respectively. The highest 1000 seed weight of 0.146 g was recorded from the plants grown in 12 hours. However, short day treatments did not influence the seed moisture content, seed dry matter of 1 gram seed and seed germinability.

It is recommended that the seedling and seed production of *A. paniculata* can optimized by planting sand paper scarified seeds from grey brown capsule on combination media of cocopeat:biochar:EFB compost in 30 cm^3 seedling tray. *A. paniculata* is not a short day plants.

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PENAMBAHBAIKKAN BIJI BENIH DAN PENGELUARAN ANAK BENIH (*Andrographis paniculata* (Burm. F.) Wall. Ex Nees)

Oleh

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Mac 2016

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Pembiakan *Andrographis paniculata* biasanya melalui biji benih tetapi percambahan tidak memuaskan disebabkan oleh kedormanan biji benih. Oleh itu, tujuh kaedah rawatan pemecahan kedormanan biji benih iaitu rendaman air selama 24 jam, pelelasan dengan kertas pasir, rendaman air panas bersuhu 40°C selama 10 minit, rendaman air panas bersuhu 50°C selama 10 minit, rendaman air panas bersuhu 60°C selama 5 minit dan kombinasi pelelasan kertas pasir dan rendaman air panas bersuhu 60°C selama 5 minit dan satu kawalan tanpa rawatan telah dikaji. Peratusan percambahan biji benih hempedu bumi selepas dilelaskan dengan kertas pasir adalah 91 %. Biji benih menggunakan masa yang paling singkat iaitu 3.5 hari untuk mencapai 50 % percambahan selepas menggunakan kaedah pelelasan kertas pasir lalu direndam dalam air panas bersuhu 60°C selama 5 minit.

Kematang semasa penuaian dan penyimpanan adalah penting dan akan menjejaskan kualiti biji benih. Tiada kajian dilakukan untuk kesan kematangan dan penyimpanan ke atas kualiti percambahan biji benih. Kesan dua peringkat kematangan (kapsul ungu kelabu dan kapsul perang kelabu) dan empat tempoh penyimpanan (0, 3, 6 dan 9 bulan) dengan dan tanpa pelelasan kertas pasir telah dikaji untuk menentukan kemampuan percambahan biji benih. Biji benih dari kapsul perang kelabu yang baru dituai telah merekod berat 1000 biji benih, kelembapan biji benih yang lebih rendah dan isi kering biji benih untuk 1 gram biji benih yang lebih tinggi berbanding dengan biji benih dari kapsul perang ungu. Manakala, peringkat kematangan dan tempoh penyimpanan tidak menjejaskan berat 1000 biji benih, kelembapan biji benih dan isi kering biji benih untuk 1 gram biji benih selepas dikeringkan dalam dessicator. Peratus percambahan adalah tinggi bagi biji benih dari kapsul perang kelabu berbanding dengan biji benih dari kapsul perang ungu. Percambahan bertambah selapas penyimpanan 6 bulan. Biji benih yang tidak dilelaskan daripada peringkat kematangan dan tempoh penyimpanan yang berbeza tidak bercambah lebih daripada 50 % percambahan. Masa yang lebih singkat untuk 50 % percambahan telah diperhatikan dalam biji benih kapsul perang kelabu selepas pelelasan. Masa untuk 50 % percambahan telah dikurangkan dengan ketara selepas 6 bulan penyimpanan.

Untuk tujuan penanaman organic bagi menghasilkan ubat-ubatan, kesan tiga isipadu sel dulang (berisipadu 43 cm^3, 30 cm^3 dan 25 cm^3) dan tujuh media pertumbuhan (tanah campuran, habuk sabut kelapa, 4 habuk sabut kelapa : 1 biochar sekam padi, 3 habuk sabut kelapa : 2 biochar sekam padi, 4 habuk sabut kelapa : 1 kompos tandan kosong kelapa sawit, 3 habuk sabut kelapa : 2 kompos tandan kosong kelapa sawit dan 4 habuk sabut kelapa : 3 biochar sekam padi : 3 kompos tandan kosong kelapa sawit) telah dikaji untuk menentukan media pertumbuhan dan dulang anak benih yang sesuai bagi pertumbuhan anak benih hempedu bumi. Ketinggian anak benih, berat basah, jumlah luas permukaan daun dan berat kering meningkat dari dulang bersel 43 cm^3 ke dulang bersel 30 cm^3 tetapi menurun pada dulang bersel 25 cm^3. Nisbah akar kepada pucuk tidak mempunyai perbezaan ketara antara saiz dulang. Pajang akar dan isi padu akar menunjukkan peningkatan ketara apabila nombor sel dalam dulang bertambah. Biji benih ditanam dalam media habuk sabut kelapa : biochar : kompos tandan kosong kelapa sawit dalam nisbah 4:3:3 (CBE) menpunyai ketinggian anak benih, berat basah, jumlah luas permukaan daun, berat kering, panjang akar dan isi padu akar yang paling tinggi. Terdapat kesan interaksi yang ketara antara saiz dulang dan media pertumbuhan terhadap ketinggian anak benih, berat basah, jumlah luas permukaan daun, panjang akar dan isi padu akar. Anak benih yang ditanam dalam dulang bersel 30 cm^3 dengan media CBE menghasilkan anak benih yang lebih berkualiti.

Tempoh cahaya adalah salah satu factor persekitaran yang penting untuk berbunga, kesuburan biji benih dan pengeluaran benih. Walau bagaimanapun, tiada kajian dilakukan ke atas tumbuhan ini. Empat kesan rawatan hari siang singkat (12 jam, 10 jam, 8 jam dan 6 jam) telah dikajikan untuk menentukan rawatan hari siang singkat yang sesuai untuk pengeluaran biji benih. Ketinggian tumbuhan meningkat dengan ketara apabila kepanjangan hari siang yang diberikan menjadi pendek. Rawatan hari siang singkat tidak menjejaskan dahan sekunder dan berat kering tumbuhan. Berat basah paling tinggi direkodkan daripada hempedu bumi yang telah ditanam dalam 6 jam panjang hari siang, iaitu 172.70 g. Pemanjangan hari untuk pembungaan dan hari untuk pembentukan kapsul yang matang didapati apabila panjang hari siang dipendekan daripada 12 jam kepada 6 jam. Hempedu bumi ditanam dalam 12 jam (kawalan) mencatatkan bilangan bunga, bilangan kapsul dan indeks menuai yang paling tinggi dengan masing-masing 459.50, 359.67 dan 9.78 %. Berat 1000 biji benih yang paling tinggi, 0.146 g telah direkodkan daripada tumbuhan yang ditanam dalam 12 jam. Rawatan hari siang singkat tidak menjejaskan kandungan kelembapan, isi keing biji benih daripada 1 gram biji benih dan kemampuan percambahan biji benih.

Ia adalah disyorkan bahawa pengeluaran anak benih dan benih *A. paniculata* boleh dioptimumkan dengan menanam benih dari kapsul perang kerabu yang telah dilelaskan dengan kertas pasir dalam dulang bersaiz 30 cm^3 dengan gabungan media habuk sabut kelapa:biochar:kompos tandan kosong kelapa sawit. *A. paniculata* bukan tanaman hari pendek.

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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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%	Percentage
˚C	Degree Celsius
ABA	Abscisic Acid
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
C	Carbon
Ca	Calcium
CEC	Cation Exchange Capacity
cm	Centimeter
cm^2	Square Centimeter
cm^3	Cubic Centimeter
CRD	Completely Randomized Design
DN	Day Neutral
EC	Electrical Conductivity
EFB	Empty Fruit Bunches
et al.	And friends
g	Gram
g^{-1}	Per Gram
GA_3	Gibberellic Acid
g cm^{-3}	Gram per Cubic Centimeter
GP	Germination Percentage
h	hour
H_2O	Water
IAA	Indole-3-acetic Acid
IBA	Indole-3-butyric Acid
ISTA	International Seed Testing Association
K	Potassium
KCl	Potassium Chloride
kg	Kilogram
KNO_3	Potassium Nitrate
LD	Long Day
LSD	Least Significant Difference
N	Nitrogen
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
M	Molar
meq	Milliequivalents
Mg	Magnesium
MGT	Mean Germination Time

min	Minute
ml	Milliliter
mm	Millimeter
mM	Millimolar
mS/cm	Millisiemens per Centimeter
n.d.	No date
$NaHClO_3$	Sodium Hypochlorite
NI	Night Interruption
No.	Number
P	Phosphorus
pH	Measurement of Acidity/Alkalinity
ppm	Part Per Million
RCBD	Randomized Complete Block Design
rpm	Revolutions per Minute
SAS	Statistical Analysis System
SD	Short Day
T_{50}	Time to 50% Germination
US$	United States dollar

CHAPTER 1

INTRODUCTION

Malaysia has a suitable climate for the growth of various herbal plants. There are more than 2000 plant species with medicinal value found in Malaysia with commercialization potential. Malaysia Agricultural Research and Development Institute (MARDI) has reported that an annual gross profit from herbal related products was more than RM 5.4 billion (Ahmad and Othman, 2013). However, Malaysian herbal and medicinal plants industry is still lagging behind in terms of Research and Development (R&D) when compared to China and also other ASEAN countries. Furthermore, the knowledge on plant part uses, preparation methods and other traditional knowledge is important for the development of herbal industry in Malaysia (Ahmad and Othman, 2013).

In Malaysia, some popular medicinal plants like male aphrodisiac *Eurycoma longifolia* Jack. (tongkat ali), *Labisia pumila* (kacip fatima), *Orthosiphon stamineus* (misai kucing), *Andrographis paniculata* (hempedu bumi), *Phyllanthus niruri* (dukung anak), *Centella asiatica* (pegaga) and *Momordica charantia* (peria) are under extensive research (Jamal, 2006). Tongkat ali, kacip ftima, misai kucing, hempedu bumi and dukung anak were listed in the agriculture sector under National Key Economic Areas (NKEAs) as main herbal plants to focus on (Ganesan, 2011). The Malaysian Economic Transformation Programmed through NKEA Agriculture sector has identified *A. paniculata* as one of the high value herbal crops that need to be commercially exploited for a new source of economic growth in the herbal industry. There is extensive knowledge of herbs for treatment of various ailments and the recognization of biomedical values in the global scenario (Noorhanin *et al.*, 2013).

A. paniculata (Family: Acanthaceae) commonly known as hempedu bumi (bile of earth) in Malaysia or 'King of Bitters' due to its extremely bitter taste in all parts of the plant. The bitter taste of this plant comes from chemical substances called diterpene lactones which have medicinal benefits. This medicinal plant grows well in tropical Asian countries (Parashar *et al*., 2011; Valdiani *et al.* 2012). This plant is widely used for treating fever, liver diseases, diabetes, snake bite, bronchitis and a variety of ailments (Parashar *et al.*, 2011). A kilogram of good quality *A. paniculata* dried leaves can be priced for US$5, while one kilogram of purified active compounds, andrographolide and its derivatives, are sold for US$100,000 from specialist chemical suppliers (Chulabhorn, 2005). Shamar *et al.* (2008) reported that the estimated consumption of this plant to be 250 tons annually. Since this plant is propagated through seed, high seed quality is needed to ensure crop stand and yield.

The propagation of *A. paniculata* is generally through seeds. However, the main issue of *A. paniculata* is due to its low germination. Generally, this indicates the presence of seed dormancy. It is a survival strategy for plants to disperse the seeds without germination under unfavorable condition. Seed dormancy is an internal condition that

prevents seeds from germination under favorable condition (appropriate temperature regime, adequate moisture, normal atmosphere and light requirement in some cases) (Schmidt, 2007; Batlla & Benech-Arnold, 2010). Some plant species have developed regulatory mechanisms to delay germination under the conditions where seedlings can hardly survive. Depending on species of plants, seed dormancy can be imposed by the envelopes (seed coat, endosperm, etc.), embryo, or both factors together (Debeaujon *et al*., 2000). Talei *et al.,* (2012) mentioned that the *A. paniculata* seeds seriously stagnated during dormant stage after imbibition. Therefore, there is a need to study the seed dormancy of *A. paniculata* to improve the germination.

Seed germination and emergence depends on the seed properties such as seed quality. High quality seed lots can improve crop yield. One of the main factor to determine the seed quality is the seed maturation. Generally, seed quality parameters are associated with the harvesting stage of the seed crop. High quality seed with maximum dry weight, high viability and vigor can be obtained if the seed crop harvested at physiological maturity. For storage purposes, fully matured seeds are desirable to be collected, rather than the immature seeds (Eskandari, 2012; Kamotho *et al.,* 2014). Therefore, it is important to know the relationship between seed harvesting stage and seed quality. However, there is little information of seed maturity on relation to seed quality for *A. paniculata*.

Seed storage as *ex situ* germplasm is important for long term conservation of plant genetic resources. Depending on the storage method and duration, drying and long term storage may reduce the seed germination or eventually kill the seeds (Kamotho *et al*., 2014). On the other hand, the biochemical processes in the seeds after dispersed from the mother plant and experienced dry conditions also have a pronounced effect on seed dormancy and germination depend on seed crop species. During dry storage, the seed dormancy is gradually lost is due to the process called after-ripening. Hence, it is important to study the storage effect on *A. paniculata* seeds since the seeds are normally stored after harvest the matured plant by the farmer (Donohue, 2015).

For large scale plantation, direct sowing in the field is commonly practice. But the production of seedlings in containers is also gaining popularity recently compared to field production due to easy marketing, transportation and rapid product rotation (Aklibasinda *et al.*, 2011). Choosing the most suitable container size and growing media is important for plant growth as it will influence the transplant performance and production (Ingram, *et al*., 1993). With the changes in container size, plants may undergo morphological and physiological changes in the response on root growth. A proper growing media should provide biological, chemical and physical requirements for plants.

Besides the container size and growing media, some other factors like photoperiod also important which will physiologically influence the plant growth. However, there are few studies on the factors affecting production of the seeds of *A. paniculata.* Seed production can be affected by different photoperiods. The plant is widely found and well-adapted to tropical and subtropical Asia, south-east Asia and India, suggesting it is a short-day plant (Chang, 1986). To produce seeds successfully in Malaysia, there is a

need to determine the photoperiod effects on the seed production of *A. paniculata.* The objectives of this study were:

1. To determine the best seed dormancy breaking treatments for germinating *A. paniculata*.

2. To determine the seed maturity stages and storage periods of *A. paniculata* for highest seed quality production.

3. To determine the germination tray cell volume and growing media types for the best seedling growth of *A. paniculata*.

4. To determine the best photoperiod for the highest growth and seed production of *A. paniculata*.

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