SEED QUALITY OF HYACINTH BEANS (*Lablab purpureus* L.) INFLUENCED BY DRYING METHODS AND STORAGE TEMPERATURES

By

ALIYAH BT MOHD YAHAYA

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Chairman : Professor Uma Rani a/p Sinniah, PhD
Faculty : Agriculture

Lablab purpureus or known as Hyacinth bean is a high-value legume that has potential as an important vegetable crop due to its dietary value which is high in protein content. It also has anti-cancer properties but the crop has remained unexploited. To promote the cultivation of this legume good quality seeds would be a prerequisite. Apart from the field related production factors, problems that often occur in seed production are the post-harvest handling which includes drying and storage. Therefore, this study was conducted to determine suitable drying method and storage temperature for Hyacinth bean seed. Seeds were harvested at 30 days after anthesis (DAA) and subjected to different drying methods namely sun, shade, oven (35±2**°C**) and drying beads to obtain target moisture content (14, 12, 10%). The experiment adopted the Completely Randomized Design (CRD) with three replicates. Dried samples were taken randomly for seed physical characteristics, moisture content and germination. Results showed that, Hyacinth bean seeds dried using oven and drying beads attained 10% moisture content within 24 hours while those dried under the sun and shade required up to 72 hours to reach 10% MC. The most rapid rate of drying was by using drying beads and seeds dried with drying beads recorded the highest germination percentage (71%) and had high seed vigour index (20.46). Both shade and drying beads gave good results with drying beads being more economical for adoption by farmers. In contrast, sun and oven drying had large number of dead seeds. Bead- dried seeds were then stored at two storage temperature which are ambient (27.7-34.2°C; 74±5% RH) and fridge (2.0-5.0°C; 25±5% RH) for six months. Experiment was arranged in CRD with three replicates. The results revealed that after six months of storage, seeds stored under refrigerated temperature at ≤5.0℃ recorded higher germination (≥70%) compared with (≤ 50%) in ambient temperature. Seed vigour index was also higher (17.49) when stored under refrigerated temperature compared to ambient (8.67). In addition, under ambient declining in antioxidant enzymes activities, germination performance and seedling growth were recorded after six months of storage. Therefore, it is best stored refrigerated (≤ 5℃) in order to retain

storability. It is concluded that Hyacinth seeds were best to bead-dried to 10% MC and further stored under refrigerated temperature in which the germination and defence mechanism of the Hyacinth were maintained.

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

KUALITI BIJI BENIH KACANG KARA (*Lablab purpureus* L.) DIPENGARUHI KAEDAH PENGERINGAN DAN SUHU PENYIMPANAN

Oleh

ALIYAH BT MOHD YAHAYA

November 2020

Pengerusi : Professor Uma Rani a/p Sinniah, PhD
Fakulti : Pertanian

Lablab purpureus atau Kacang Kara merupakan kekacang bernilai tinggi yang mempunyai potensi untuk menjadi sayuran penting berikutan kandungan protein yang tinggi. Kacang ini juga mempunyai sifat anti-kanser. Walaupun mempunyai pelbagai ciri-ciri yang baik, kacang ini masih tidak dieksploitasi. Masalah yang sering berlaku dalam penghasilan biji benih adalah pengendalian pasca-tuai termasuk pengeringan dan penyimpanan biji benih. Oleh itu, kajian ini telah dijalankan untuk mengenalpasti kaedah pengeringan dan suhu simpanan terbaik bagi Kacang Kara. Biji benih dituai 30 hari selepas antesis dan dikeringkan menggunakan kaedah pengeringan yang berbeza iaitu matahari, teduh, ketuhar (35±2**°C**) dan manik zeolit. Kajian ini menerima pakai reka bentuk rawak sepenuhnya dengan tiga replikasi untuk penilaian fizikal, kelembapan dan percambahan biji benih. Kaedah yang berlainan telah mempengaruhi masa yang diambil untuk mencapai nilai sasaran kelembapan (14%, 12%, 10%) dan juga peratus percambahan. Keputusan menunjukkan bahawa biji benih Kacang Kara yang dikeringkan menggunakan ketuhar dan manik zeolit mencapai nilai sasaran kelembapan (10%) dalam tempoh 24 jam manakala, kaedah pengeringan di bawah matahari dan teduh, memerlukan sehingga 72 jam untuk mencapai 10%. Kaedah pengeringan terpantas adalah menggunakan manik zeolit dan juga tertinggi bagi peratus percambahan (71%) dan kecergasan biji benih (20.46). Manik zeolit dan pengeringan di bawah teduh berjaya mengekalkan kualiti percambahan biji benih Kacang Kara. Namun, manik zeolit lebih menjimatkan bagi petani dan pengeluar biji benih berikutan penggunaan berulang kali. Sebaliknya, pengeringan di bawah matahari dan ketuhar mencatatkan bilangan biji benih mati yang tinggi. Biji benih dikeringkan oleh zeolit telah disimpan pada dua suhu berlainan iaitu ambien (27.7-34.2°C; 74±5% RH) dan peti sejuk (2.0-5.0°C; 25±5% RH) untuk tempoh 6 bulan. Kajian disusun dalam reka bentuk rawak dengan tiga replikasi. Sepanjang tempoh penyimpanan, peratus percambahan adalah ketara dipengaruhi oleh suhu penyimpanan. Hasil kajian mendedahkan bahawa setelah disimpan 6 bulan, biji benih yang disimpan pada suhu peti sejuk mencatatkan percambahan yang lebih

tinggi ($\geq 70\%$) berbanding dengan ($\leq 50\%$) suhu ambien. Kecergasan biji benih juga lebih tinggi (17.49) apabila disimpan pada suhu peti sejuk berbanding suhu ambien (8.67). Suhu ambien mencatatkan penurunan bagi aktiviti enzim antioksidan, prestasi percambahan dan pertumbuhan anak benih. Dengan itu, biji benih Kacang Kara adalah sesuai untuk disimpan pada suhu peti sejuk untuk memastikan keboleh simpanan benih. Secara kesimpulan, biji benih Kacang Kara disyorkan untuk dikeringkan menggunakan manik zeolit ke 10% dan seterusnya disimpan pada suhu peti sejuk bagi mengekalkan kualiti percambahan dan mekanisme pertahanan biji benih.

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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:

Uma Rani a/p Sinniah, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Azizah binti Misran, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

_____

ZALILAH BINTI MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 09 March 2022

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Signature: _____
Name of Chairman of Supervisory Committee: Uma Rani a/p Sinniah

Signature: _____
Name of Chairman of Supervisory Committee: Azizah binti Misran

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LIST OF ABBREVIATIONS

μm	Micromolar
μmol	Micromole
AA	Accelerated Aging
AOSA	Association of Official Seed Analyst
ANOVA	Analysis of Variance
CAT	Catalase
cm	Centimetre
CRD	Completely Randomized Design
df	Degree of Freedom
EC	Electrical Conductivity of Seed Leachates
GRI	Germination Rate Index
HM	Harvest Maturity
hr	hour
ISTA	International Seed Testing Association
LSD	Least Significant Difference
M	Molarity
MD	Maturity Desiccation
min	minutes
mm	Millimolar
mmol	Millimole
mol	Mole
nmol	Nanomole
PM	Physiological Maturity

POD	Peroxidase
RH	Relative Humidity
ROS	Reactive Oxygen Species
rpm	Revolution per Minute
SVI	Seedling Vigour Index
WRR	Water Rate Removal

CHAPTER 1

INTRODUCTION

Legumes belonging to the family Fabaceae are major components in human diet and it is often consumed as vegetables. Generally, legumes are rich in protein, fibre, iron, B-vitamins and low in fat (Graham and Vance, 2003). Legume seeds are the major source of food second only to cereals (Poaceae) and highly nutritious. There are a number of popular legumes in Malaysia which is used as vegetable such as long beans, winged beans, French beans and green peas. Some legumes have been used commonly while others have remained underutilized. One such example of an underutilized bean in Malaysia is *Lablab purpureus* or commonly known as Hyacinth bean. Hyacinth bean originated from Africa, but today, Bangladesh is the leading producer in the world with a total production area of around 48,000 ha. This bean is widely distributed in many tropical countries such as Malaysia, Indonesia and Philippines (Gowda, 2013).

In Malaysia, Hyacinth bean is known as 'Kacang Kara' and according to Cook et al. (2005) it is a high-value legume that has the potential of being an important vegetable crop because of its high protein content (20-28%) nearly three times compared with most beans. In addition it is also known to have numerous medicinal advantages such as anti-cancer, anti-diabetic, anti-inflammatory, helps in eczema and also used for the treatment of anaemia (Al-snafi, 2017). In Asia, Hyacinth bean is mainly grown for its pods and consumed as vegetable, while dry seeds are used in making tofu and tempeh (Subagio and Morita, 2008).

Due to the goodness of this bean, Malaysia is importing it from Thailand and Bangladesh (UC Davis, 2017). Despite the suitable environmental condition to grow this bean, the crop has remained unexploited locally. In view of its high protein content, other medicinal uses as well as the growing popularity among Malaysian to consume this bean, initiatives should be taken to recommend this crop as future legume in fulfilling food security demand. This is in line with the agenda mentioned in the National Agricultural Policy 4 (NAP4) whereby vegetables are prioritized for development in Malaysia for food security. It is important to know that Hyacinth bean is grown using seeds as the planting material and low supply of good quality seeds will be a major constraint for Hyacinth bean crop production.

Good quality seeds are crucial in any crop production system, and determined both by pre-harvest management and post-harvest handling. In many instances the post-harvest component is underestimated and less attention is given. Shelar et al, (2008) stated the drop in seed quality under tropical conditions is much more extreme because environmental conditions in these areas are challenging to retain seed viability. As a leading producer of Hyacinth bean

seeds, Bangladesh recorded large postharvest losses due to traditional method of drying seeds (UC Davis, 2017). Bangladeshi seed companies estimate a loss of 5-10% of their seeds worth millions of dollars when dried on tarpaulin under the sun due to poor drying techniques.

Method of drying has been reported to effect seed quality. A number of drying techniques including natural and artificial drying used in legumes have been reported by many researches (Scariot et al., 2017; Ellis and Roberts 1991). Drying is mostly done naturally by exposing seeds to direct sunlight or under shade in open fields. Although it is a cost-saving method, sun-drying has been reported to be slow and weather dependent (Muckle and Stirling, 1971). For example, a study by Ali et al., (2015) suggested soybean seeds can be sun dried to $\leq$ 8% MC without affecting seed quality. However, groundnut deteriorated rapidly when sun-dried and resulted in lower germination (Hon, 1977). Groundnut was best dried using oven method. Larger companies and seed producers preferred oven drying where constant temperature can be maintained (Babiker et al., 2010).

It has been shown that a wide gradient of desiccation sensitivity or tolerance exists among legume species (Berjak and Pammenter, 2008). Ellis and Roberts (1991) have proposed other methods of drying such as sun, shade, freeze and refrigeration drying with low relative humidity but concluded that response to drying can be species specific which depends on the ability to tolerate desiccation. Hence, there is a need to determine proper drying method for Hyacinth which is still unknown. A simple, low-cost but efficient method for farmers and seed producers to adopt would be useful.

Recently, drying using zeolite drying beads have been utilized particularly in small-scale drying such as for gene bank. Drying beads, a zeolite-based desiccant was developed by Rhino Tech Thailand which potentially enables seedsman to dry seeds to safe moisture content. It is said to be effective, economical and can be used repeatedly over time. Drying using the beads ensured better quality seeds and was able to prolong storage. A study by Nassari et al., (2014) on tomato seeds confirmed that rapid drying took place when drying beads were used compared with sun-drying with maintained viability and vigour. Another study by Atilia and Zaitialia (2016) in chilly, seeds dried using bead drying had better quality compared with sun dried seeds. Furthermore, the application of drying beads in drying 200 tons of vegetable seed in Bangladesh proved to provide an estimated 100,000 farmers with superior seed (Anon, 2018). Thus, this study attempts to use zeolite beads on Hyacinth bean seed to see its drying efficacy.

According to Gupta et al., (2005) when seeds are in dry state, it is necessary to store them appropriately. Seed quality can remain at its initial level or drop to an unacceptable level for planting during storage. Surki et al., (2012) stated that unfavourable storage temperature contributes to acceleration of seed deterioration. Deterioration is related to reactive oxygen species (ROS) and

yearly losses due to deterioration can amount to 25% of harvested crop. ROS are usually produced from stress conditions such as high temperature during storage and could be demolished by scavenger enzymes such as catalase and peroxides (Jyoti and Malik, 2013). Peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD), and are main enzymatic antioxidants that function as a detoxifying mechanism that stops the degrading radicals produced during stress and thus prevents seed from deteriorating. Few studies have revealed connection concerning seed deterioration and deprivation of specific antioxidant enzyme activities in legumes seed such as pea (Yao et al., 2012) and soybean (Tian et al., 2008). However, currently no specific research has been conducted to understand the connection between storage temperature and deterioration related with antioxidant enzyme activities in Hyacinth bean seeds.

Hyacinth bean has a potential to be commercialized widely in Malaysia however, a good protocol on how to dry and store the seeds is yet to be established and is very much needed. Therefore, this study was conducted with the following objectives:

1. To determine the influence of drying using different methods on seed quality of *Lablab purpureus*

2. To evaluate the effects of temperature on seed viability of *Lablab purpureus* during storage

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