EFFECT OF ACCELERATED AGEING ON RICE (*Oryza sativa* L.) SEED QUALITY

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF BACHELOR OF
AGRICULTURAL SCIENCE WITH HONOURS

HORTICULTURE AND LANDSCAPING PROGRAMME
FACULTY OF SUSTAINABLE AGRICULTURE
UNIVERSITI MALAYSIA SABAH
2018

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN TESIS

JUDUL: EFFECT OF ACCELERATED AGEING ON RICE (Oryza sativa L.) ~~Seed Quality~~ SEED QUALITY

IJAZAH: BACHELOR OF AGRICULTURE SCIENCE (HONS.) (HORTICULTURE AND LANDSCAPING) SARJANA MUDA DENGAN KEPUJIAN (HORTIKULTUR DAN LANDSKAP)

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ACKNOWLEDGEMENT

I would like to express my utmost gratitude and appreciation to my supervisor, Professor Dr. Mohammad bin Mohd. Lassim, for guidance given throughout this project. I am truly grateful for his patience and advice. I have gained a lot throughout this research.

I would like to record my appreciation to Madam Nurul Syakina Binti Marli and Madam Arjah Jekan for assistance in my laboratory work; Miss Izyan Ayuni binti Mohamad Selamat and Miss Shahida Mohd Sharif for arranging our milestones in dissertation.

I would also like to thank my beloved family, especially my parents, Neo Tong Guan and Leow Chee Wee for supporting me and always believing in me. My siblings who motivate me to strive harder.

Last but not least, my precious friends, lecturers, housemates and juniors who helped me throughout this research. A million thanks to everyone who helped me to accomplish this research.

ABSTRACT

A study was conducted to investigate the effect of accelerated ageing on aerobic rice (*Oryza sativa* L.) seed quality. The experiment was carried out at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah. The study aimed to determine the most reliable method in assessing seed deterioration. Completely Randomized Design (CRD) with four replicates for each treatment was used. Aerobic rice seeds were artificially aged by exposing them to temperatures of 42 °C and 45 °C for six different durations: 12, 24, 36, 48, 72 and 96 hours. Parameters evaluated in this study were viability, germination percentage, abnormal seedlings, dead seeds, seedling vigour index, length of root & shoot on the seventh and fourteenth days, fresh & dry weight of seedlings and seed moisture content. Initial seed viability and germination percentages were 67.50 % and 67.50 % respectively. These values increased to 93.50% and 86.00 % when exposed to 42 °C for 12 hours ($P=0.004$ and $P=0.052$), suggesting some breaking of dormancy had occurred during the early period of artificial ageing. Seed vigour index indicated the robustness of seeds. Non-aged seeds (control) showed the best performance, with high values for seed vigour index (1057.7), shoot (4.42 cm) and root length (11.17 cm). Seed quality were greatly reduced beyond 48 hours of exposure to high temperatures and relative humidity conditions. The study concluded that accelerated ageing (by exposing seeds to different temperatures for a variable period of time) adversely affected rice seed vigour.

KESAN PENUAAN BUATAN TERHADAP KUALITI BIJI BENIH PADI (***Oryza sativa*** **L.)**

ABSTRAK

Satu kajian telah dijalankan untuk mengkaji kesan penuaan buatan terhadap kualiti biji benih padi aerobik (*Oryza sativa* L.). Semua eksperimen telah dijalankan di Fakulti Pertanian Lestari, Universiti Malaysia Sabah. Kajian ini dijalankan untuk menentukan cara terbaik bagi penilaian kemerosotan biji benih. Kajian ini disusun dalam Reka Bentuk Rawak Lengkap dengan empat replikasi bagi setiap rawatan. Biji benih padi telah didedahkan kepada prosedur penuaan buatan pada suhu 42 ºC dan 45 ºC selama enam tempoh: 12, 24, 36, 48, 72 dan 96 jam. Parameter yang dinilai dalam kajian ini termasuklah peratusan kebolehidupan, peratusan percambahan, peratusan biji benih tidak normal, peratusan biji benih mati, indeks kecergasan biji benih, panjang tunas, panjang akar tujuh dan empat belas hari lepas semai, berat basah anak benih, berat kering anak benih dan kandungan kelembapan biji benih. Kualiti benih padi sebelum rawatan adalah 67.50 % dan 67.50 % untuk peratusan kebolehidupan dan peratusan percambahan. Peratusan ini menunjukkan peningkatan kepada 93.50 % dan 86.00 % selepas didedahkan kepada suhu 42 ºC selama 12 jam ($P=0.004$ and $P=0.052$). Ini menunjukkan berlakunya pemecahan dormansi diperingkat awal proses menuaan buatan. Indeks kecergasan menunjukkan ketegapan biji benih. Biji benih tanpa penuaan buatan (Kontrol) menunjukkan prestasi yang terbaik keseluruhan dengan nilai tinggi dalam indeks kecergasan (1057.7), tunas (4.42 cm) dan akar (11.17 cm). Kualiti biji benih menurun selepas terdedah kepada suhu dan kelembapan persekitaran yang tinggi. Kajian ini telah menunjukkan penuaan buatan (mendedahkan biji benih kepada suhu dan masa yang berbeza) akan memberi kesan negatif terhadap kualiti biji benih padi.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Per cent
ºC	Degree Celcius
AA	Accelerated ageing
ANOVA	Analysis of variance
AOSA	Association of Official Seed Analysts
cm	Centimetre
CRD	Completely randomized design
EPP	Entry Point Project
g	Gram
IRRI	International Rice Research Institute
ISTA	International Seed Testing Association
KADA	Kemubu Agricultural Development Authority
MADA	Muda Agricultural Development Authority
MARDI	Malaysia Agricultural Research and Development Institute
MC	Moisture Content
NKEA	National Key Economics Area
TZ	Tetrazolium Test

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3.1 Percentage of Viable Seeds 15

$$\text{Viability percentage (\%)} = \frac{\text{Number of *viable seeds}}{\text{number of seeds sown}} \times 100$$

*viable seeds = germinated seeds + hard seeds

3.2 Percentage of Germination 15

$$\text{Percentage of Germination (\%)} = \frac{\text{Number of seed germinated}}{\text{number of seeds sown}} \times 100$$

3.3 Number of Abnormal Seedlings 16

$$\text{Abnormal seedlings (\%)} = \frac{\text{Number of abnormal seedlings}}{\text{number of seeds sown}} \times 100$$

3.4 Seed Vigour Index 16

Seed vigour index

= Seedling height at day 7 after sowing × Percentage of Germination

3.5 Seed Moisture Content 16

$$\%\,\text{MC} = \frac{\text{M1-M2}}{\text{M1}} \times 100$$

M1 = weight of seed materials before drying (g)
M2 = weight of seed materials after drying (g)
M1 - M2 = Moisture loss (%)

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Rice (*Oryza sativa* L.) of family Poaceae originated in India, Thailand and southern China. According to International Rice Research Institute (IRRI), rice is the most important food crop in the developing world. More than 3.5 billion people gain their daily calories from rice. Asia has the largest production of rice as it is the staple crop for the local consumption (Matthews *et al.*, 1995). In future, it is believed that the demand for rice will increase and the supply will decline (Rajamoorthy *et al.*, 2015; Papademetriou, 2000). The demand of rice in Malaysia is high as it is the staple food. The self-sufficiency of rice production in Malaysia is about 65 to 70 % (Arshad *et al.*, 2011). The supply of rice in Malaysia comes from either local supply or imports. Food security in Malaysia is largely about rice production and availability. In Malaysia, rice production is protected through price control, subsidies, tariffs and buffer stock (Paul, 2010). Farmers are given subsidy in order to produce quality and ample amount of rice (Bala *et al.*, 2014).

There are two major rice-growing areas in West Malaysia which are Muda Agricultural Development Authority (MADA) and Kemubu Agricultural Development Authority (KADA) (Akinbile *et al.*, 2011). KADA stated that paddy land that are available for rice cultivation in Malaysia is 28072 hectares (KADA, 2016). The Entry Point Project (EPP) under National Key Economic Areas (NKEA) has included production of rice crop in Malaysia. The aim of EPP 11 is to increase rice productivity and boost self-sufficiency of Malaysia in rice to 85 percent (Bakar *et al.*, 2012). In Malaysia, there are two types of rice planted: lowland rice and upland rice. Three-quarters of the total fresh water supply are used in irrigated agriculture in Malaysia and 90 percent of that is devoted to lowland rice cultivation. The heavy consumption of water may cause water scarcity in the near future. However, upland rice is grown aerobically in upland environments as in Sabah and Sarawak (Chan *et al.,* 2012). Upland rice have the stability of growth under adverse environmental conditions but the yield are subsequently low (IRRI, n.d.).

As the declining of water availability combined with erratic climatic conditions (El Nino), aerobic rice system is developed and promoted (Dimaano *et al.*, 2017). Aerobic rice is a production system for specially developed "aerobic rice" varieties. Aerobic rice can grow well in well-drained, non-puddled and non-saturated soils (IRRI, n.d.). According to IRRI, aerobic rice are suitable for uplands, slopes and water-short irrigated lowlands. Different from upland rice, aerobic rice has combined both characteristics of upland and high yielding low land rice varieties (Tuong, 1999).

1.2 Justification

Seed quality is an important factor in agriculture production. Poor seed quality will lead to the loss for farmers. Seed quality includes physical appearance such as size of the seeds, colour of the seeds, seed viability and seed vigour. There are various tests used to determine seed quality. For example, seed germination test and tetrazolium test are used to determine the viability of seeds. While accelerated ageing test, cold test and conductivity test can be used to determine seed vigour.

Seed vigour evaluates how fast seeds germinate and develop in the early stage of growth. Different seed species are evaluated with different seed vigour tests. Additional research and interpretation need to be carried out in order to determine a reliable test for seed vigour. Accelerated ageing test (AA) is commonly used to predict the storability of seeds and to determine the seed vigour. The process of seed deterioration under accelerated ageing test is similar under normal conditions (Vijay *et al.*, 2015). Seeds are hygroscopic in nature. Under high humidity environment, the seeds will absorb water.

Accelerated ageing test had been used to test several species of seeds. Bean seeds had been successfully aged with satisfactory results at 41 °C at 48 hours of exposure and 43 °C at 24 hours (Danila *et al.*, 2011). The best periods of accelerated ageing test for lettuce seeds was 48 hours while 72 hours for endive seeds (Franciele *et al.*, 2011). Based on these researches, different species of seeds respond differently to exposure to different temperatures with different exposure periods. Hence, this research was carried out to determine a reliable test for seed vigour of aerobic rice seeds.

1.3 Objective

The objective of this study was to determine the effect of accelerated ageing test on the quality of aerobic rice (*Oryza sativa* L.) seeds.

1.4 Hypothesis

H_0: There is no effect of accelerated ageing on the quality of aerobic rice (*Oryza sativa* L.) seeds.

H_A: There is effect of accelerated ageing on the quality of aerobic rice (*Oryza sativa* L.) seeds.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy and Biology of Rice

Table 2.1 Taxonomy of *Oryza sativa* L.

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Cyperales
Family	Poaceae
Genus	*Oryza*
Species	*Oryza sativa*

Source: Adapted from Ashfaq *et al.*, 2015

According to Ashfaq *et al.,* the taxonomy of rice is as presented in Table 2.1 above. Cultivated rice (*Oryza sativa* L.) belongs to the Poaceae family. It is a monocot commonly considered as a semiaquatic annual grass. At maturity, each rice plant will produce a main stem with several tillers. Plant height of rice normally varies according to variety and environmental conditions, ranging from approximately 0.4 meter to more than 5 meters. A rice seed consists of the true fruit and hull. The embryo and endosperm are enclosed in several thin layer of differentiated tissues (GRiSP, 2013). The seeds of rice are orthodox. The seeds can be dried and stored at low temperatures to prolong viability (Krishnan *et al.,* 2011).

CHAPTER 2

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2.2 Rice Production in Malaysia

Rice is the second most important crop in the world after wheat. The increase in rice production to meet the country's demand in future was highlighted in the National Agrofood Policy of Malaysia, 2011-2020 (Dasar Agromakanan Negara). The current target of self-sufficiency level (SSL) of rice is 75 % in Malaysia (Vaghefi *et al.*, 2011). As the production of rice is lower than market demand, Malaysia still imports rice from other countries such as Thailand and China (Rajamoorthy *et al.*, 2015). Irrigated rice production supplies more than 75 % of world's rice in Asia (Cabangon *et al.*, 2002). In Malaysia, irrigated lowland rice is the most important rice ecosystem. Approximately 85 % of the rice production are of irrigated lowland rice. Ninety percent of the water is utilized in lowland rice cultivation (Chan *et al.*, 2012).

Upland rice is grown in rain-fed, low fertility, strongly weathered soils with little or no-fertilizers applied. In Malaysia, the cultivation of upland rice are mostly in Sabah and Sarawak. There are around 165,888 hectares of land cultivated with upland rice (Hanafi *et al.*, 2009). Some upland rice have desirable characteristics especially the fragrance, colour and sizes. However, upland rice is not commercialized due to the low production.

2.2.1 Aerobic Rice

There are many challenges faced in rice production. One of them is adverse change in microclimate (Siwar *et al.*, 2014). Climate change has shown a huge negative impact on rice production (Fitriani, 2017). Water scarcity has been one of the major problems due to climate change (Juraimi *et al.*, 2012). Aerobic rice cultivation is a production system where rice can grow under non-puddled and non-saturated soil under non-flooded conditions (Chan *et al.*, 2012). It was introduced in order to solve the problem of water scarcity. Aerobic rice can reduce the total water usage 50 to 60 % compared to lowland flood-irrigated rice (Bouman *et al.*, 2002). As the water inputs are lower, the gross return of aerobic rice will relatively increase. Aerobic rice can also be planted at water-deficient areas.

Upland rice varieties have been reported to give a stable but low yield in adverse environments. (Tuong and Bouman, 2003). While lowland rice has high yield but it does not survive under adverse environmental conditions. According to Tuong *et al.* (2005), the combination of the characteristics of upland and lowland rice has become components of aerobic rice. The genotypes can be achieved by crossing between upland adapted, weed-competitive and drought tolerant varieties and high yielding lowland varieties (Zhao *et al.*, 2010). Aerobic rice has shorter maturation period compared to lowland rice. It is believed that aerobic rice can improve the quality and quantity of rice production (Suria *et al.*, 2011).

2.3 Seed Quality

Seed quality is the potential performance of a seed lot. It is a measure of seeds' ability to establish the desired field stand at low planting rates and grow into healthy plants with high yields (Gregg and Billups, 2010). Seed moisture content, temperature and relative humidity are the most important factors influencing seed longevity (Ellis and Roberts, 1980). Seeds with high moisture content tend to lose their quality faster due to the high respiration rate, fungal activity, heating and loss of physical quality. Seed quality is not a permanent condition. The decrease in quality starts as early as during the process of seed production. The loss in seed quality are contributed by several environmental factors (Jyoti and Malik, 2013). The inherent longevity of seeds and the conditions which they are exposed prior to storage can also influence the storability of seeds (Teng, 1980). Seed quality can be reduced rapidly if it suffers from mechanical injuries and exposed to high drying temperatures during seed production. Seed quality included seed size, seed colour, seed viability, seed germination and seed vigour. A good quality seed can produce healthy seedlings with high vigour.

2.3.1 Seed Viability

Seed viability is the potential of a seed to germinate and produce a seedling. Seed viability is affected by environmental conditions, genetics of the plants, pests and diseases, and age of seeds (Copeland and McDonald, 2001). Failure of seed to germinate under optimum conditions does not indicate that the seed is not viable (Nasreen *et al.*, 2002). The failure of a seed to germinate under favourable environmental conditions can attributed to seed dormancy.

2.3.2 Seed Germination

Seed germination is the active growth of the embryo that results in the rupture of the seed coat. There are some environmental factors that influence the percentage of germination. Water is one of the important environmental factors that affect seed germination. Seeds are hydrophilic. During seed imbibition, water will be absorbed through the seed coat and increase seed turgidity (Woodstock, 1988). The restriction of water will constraint the expansion of the embryo and reduce the seed quality (Haigh and Barlow, 1987). Temperature is another factor that affects the rate of seed germination (Guan, 2009). Temperature affects the rate of water absorption, rate of respiration and chemical reactions. At low temperature, germination rates are low; while at high-temperature proteins got denatured and seeds get killed. Hence, optimum temperature must be obtain during seed germination. For most seeds, the optimum temperature for seed germination is 25-30 °C (Melado *et al.*, 2014; Simao and Takaki, 2008).

2.3.3 Seed Vigour

Seed vigour is the properties that determine the potential growth and performance of a seed during germination and seedling emergence (ISTA, 1997). Seed vigour is important to determine the good health and robustness in seeds. High vigour seeds are seeds with good potential performance while low vigour seeds are seeds with poor potential performance. The vigour of seeds can be influenced by several factors. Genetic constitution of the seeds, conditions of mother plants, maturity of seeds, seed size and deterioration of seeds will influence the vigour of seeds. Poor seed vigour will result in significant loss of grain yield (Lazarova *et al.*, 2016). McDonald (1980) showed that seed vigour tests should have several important criteria such as inexpensive, rapid, simple, reproducible and correlated to field performance (McDonald, 1995).

2.4 Seed Deterioration

Seed deterioration is the loss of seed quality, vigour and viability due to ageing caused by adverse environmental factors. It is an irreversible and inevitable physiological condition. Seed deterioration occurred over time when the seeds are exposed to adverse external conditions, thus reducing their ability to survive (Jyoti and Malik, 2013).

Seed deterioration is a natural phenomenon influenced by two environmental factors, relative humidity and temperature. Higher temperature and relative humidity will result in rapid seed deterioration. However, the rate of seed deterioration are also dependent on the ability of seeds to resist degradation and protection mechanism of seeds (Shaban, 2013). It is a serious problem in the developing countries as seeds are normally stored without proper temperature and humidity control (Kapoor *et al.*, 2010; Shivasharanappa *et al.*, 2017).

Oxidative reactions have been reportedly to be the largest factor of ageing in dry seeds but they are dependent on the seed water content (Hendry, 1993). Seed deterioration will reduce the biosynthetic activities and respiration of the seeds. Besides, it will also make the membranes less selective, sensitive to temperature and reduce the seed vigour (Santos and Barbedo, 2017). The extent of seed deterioration vary among seed populations.

2.5 Seed Moisture Content

Seed moisture content is the most important factor affecting the quality of seeds. Every year, large amounts of seeds are lost due to excess moisture in seeds. High moisture content in seeds not only will reduce the quality of seeds, it will also attract microorganisms and pests infestation (Siddique and Wright, 2003). The seed conditions are varied with different levels of moisture content. Rice seeds are orthodox seed and they can be dried to low moisture content, 5 % or less (Roberts, 1999; Rao and Jackson, 1996). Normally, seeds reach physiological maturity when the moisture content are around 18-40 %. Seeds are harvested and then dried to moisture content of around 13 to 18 %. The safe seed moisture content for storage is around 4-8 % (Gregg and Billups, 1970). Seeds are hygroscopic, in humid areas; the seed moisture content might be altered with the fluctuation in the relative humidity of the surrounding air.

2.6 Seed Dormancy

Seed dormancy is a state at which seeds do not germinate even though provided with favourable environmental conditions. In nature, seed dormancy can be an advantage as dormancy can prevent seeds from germinating under unfavourable period (Waheed *et al.*, 2012). Seed under dormancy are also named as hard seeds. Dormant seeds are viable seeds that do not imbibe water and failed in germination (Rolston, 1978).

Exogenous dormancy is a condition where essential germination components are not available to seeds. For example, water, light and temperature. Indurate lemma, palea and dormant embryo in seeds are main factors of dormancy (Zemetra *et al.*, 1983). The palea and lemma tightly wrapped on the endosperm will cause impermeable seed coat. The exogenous dormancy is genetically controlled. The degree of dormancy is affected by genetic factors, degree of maturation and environments (Mutinda *et al.*, 2017). Rice seed dormancy can happen due to the thick seed coat or embryonic factors (Seshu and Dadlani, 1991). The thick seed coat will then limit the entry of oxygen into the embryo, hence, limit the germination of seeds.

Endogenous dormancy is a most prevalent dormancy found in seeds. The endogenous dormancy is normally due to the inherent properties of the seed. Endogenous dormancy is normally caused by the environmental conditions during seed development. Endogenous inhibitors play an important role in seed dormancy. For example, abscisic acid (ABA) and gibberellins (GAs). ABA is hormone which regulate the physiological processes of seed embryo development (Si *et al.*, 2016; Takahashi *et al.*, 1976). ABA helps in the maintenance of seed dormancy whereas gibberellic acids help to break the dormancy.

Rice seed dormancy has been reported in several studies. Seed dormancy normally happens in wild rice species (Halimathul Saadiah, 1992). The dormancy in rice can be absent or can last as long as 4 months. The seed dormancy will consequently lead to reduction of yields and crop stand. Rice seed dormancy is reportedly broken by preheated at 50 °C a day prior to seed germination (ISTA, 1997).

2.7 Seed Quality Test

A successful cultivation of rice should start with establishing the right plant population in the field. In this case, seeds are of utmost importance. Seed quality cannot be determined simply from seedling growth test. As seed lot with similar germination rates frequently exhibit different physiological performances in field (Leao *et al.*, 2016). Hence, it is important to determine which test are best suited for different species and varieties of crops. There are many tests used to determine seed vigour. For example, cold test, electrical conductivity test (EC) and accelerated ageing test. EC test are best estimate of seed vigour for legume while controlled ageing test (CD) are reported to be best vigour test for forage legumes and grasses (Wang *et al.*, 2002). Speed of germination test has been used to detect the vigour of seeds. Nevertheless, it has low sensitivity and not able to detect small difference between seed lots (Silva Almeida *et al.,* 2014).

2.7.1 Cold Test

Cold test is one of the oldest tests for seed vigour. According to the Association of Official Seed Analysts (AOSA), cold test is generally used for soybean, maize, cotton and sorghum (AOSA, 1983). Suitable seed vigour tests are different for different seed species. The cold test was subjected to a combination of low temperature and high water content to the seeds. The accelerated ageing test are as effective as cold test to access the seed vigour for maize (Lovato *et al.*, 2005).

2.7.2 Electrical Conductivity Test

Electrical conductivity test is a measurement of electrolytes leaking from plant tissues (AOSA, 1983). It determines indirectly the integrity of seed membrane systems. The test is valid to assess pea and soybean seed vigour. The EC test assesses seed vigour by measuring the electrolytes leaking from the plant tissue. EC test are affected by several factors. For example, seed size, soaking temperature and soaking period (Ramos *et al.*, 2012). Nevertheless, accelerated ageing tests are more sensitive to decline of seed physiological quality than EC (Fessel *et al.*, 2006). Accelerated ageing test has been reportedly to be the most reliable test for seed vigour.

2.8 Accelerated Ageing Test

Accelerated ageing test is commonly used for seeds storability determination (Delouche and Baskin, 1973). In accelerated ageing test, seeds are exposed to two environmental variables known to cause deterioration, temperature and relative humidity. The degree of deterioration are influenced by other factors such as exposure periods and seed chemical composition (Mersal, 2011). Same as germination test, accelerated ageing test (AA) is a physiological test for seed vigour (Shaban, 2013).

High temperatures with high relative humidity will reduce seed quality (Wassmann *et al.*, 2009). Temperature and relative humidity are interdependent in

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