UNIVERSITI PUTRA MALAYSIA

PHYSIOLOGICAL AND CHROMOSOMAL CHANGES OF DELAYED HARVEST AND STORED SOYBEAN (Glycine max L. Merr.) SEEDS FOLLOWING PRIMING

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FP 2018 34

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By

PHYU SIN THANT

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

February 2018

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DEDICATION

Dedicated to:

(My Beloved Parents)

U Kyi Myint and Daw Kyin

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

PHYSIOLOGICAL AND CHROMOSOMAL CHANGES OF DELAYED HARVEST AND STORED SOYBEAN (*Glycine max* L. Merr.) SEEDS FOLLOWING PRIMING

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February 2018

Chairman: Professor Adam bin Puteh, PhD
Faculty: Agriculture

Seed deteriorations begin on the mother plant in the field and continue during storage. Basic factors influencing seed ageing are temperature, relative humidity, seed moisture content and duration of storage. Seed priming known as a pre-sowing seed treatment using natural or synthetic compounds is commonly practiced to improve seed germination and seedling emergence in a wide range of crop species. This study was undertaken to evaluate the effectiveness of seed priming treatments and post-storage priming treatments on changes in seed quality and mechanisms involved in deterioration process during field weathering and under controlled storage. For field weathering, seeds of AGS-190 and Cikurai were harvested at harvest maturity (HM), one week after HM (H1) and three weeks after HM (H3). At the time of harvest, the seeds from main stem and branches were differentiated to evaluate seed quality from different positions under field weather conditions. For storage study, the HM seeds of AGS-190 and Cikurai were stored in cold room (8±2ºC) or room temperature (25±2ºC) for 3, 6, 9, 12 and 15 months. Aged seeds of delayed harvest or stored seeds were primed with -0.8MPa PEG (Polyethylene glycol), 0.5% chitosan and water.

The delayed harvested seeds from main stem and branches showed no difference in seed quality and seedling performance. Soybean seeds harvested past the HM showed adverse effects on seed physiological assessments and consequently affected seed quality. Cultivar AGS-190 was more sensitive to adverse weather conditions as shown by deterioration of seed quality at one week after HM while cultivar Cikurai with black seed coat features could maintain better seed quality up to one week after HM. Soybean seed viability and vigor considerably declined in H3 seeds with decreased activities of catalase (CAT) and superoxide dismutase (SOD) and increased accumulation of malondialdehyde (MDA) content and chromosomal aberrations. Loss of soybean seed quality in delayed harvest seeds was influenced by seed moisture content during harvest and *Phomopsis* sp. infection. The reactive oxygen species (ROS) production in moist seeds are much higher than dry seeds. Higher accumulation of ROS with concomitant increase in MDA content not only injured cell membrane but also caused oxidative damage to DNA of delayed harvest seeds and chromosomal aberrations.

Seed priming with 0.5% chitosan and -0.8MPa PEG enhanced viability of H1 seeds in both cultivars with better germination percentage, germination index, better seedling vigour index and faster speed of germination time. Priming treatments decreased the contents of MDA and the accumulation of chromosomal aberrations in delayed harvest seeds. Recovery of germinability in field weathered seeds during priming is strongly associated with increased synthesis in CAT or SOD activities, inhibiting accumulation of lipid peroxidation and genetic damage.

Significant increases in MDA content and electrical conductivity (EC) of seed leachate with increasing storage periods indicated that ageing of the seeds in room temperature (25±2ºC) caused oxidative damage to cell membrane integrity. The activities of CAT and SOD in the seeds stored at room temperature decreased with longer storage time which was favorable for ROS accumulation. Oxidative damage caused by ROS accumulation during storage at room temperature not only oxidized lipid but also damaged the nucleic acid which led to chromosomal aberrations. Deteriorative effects of seed ageing inhibited to some extent of the metabolic processes for root and shoot growth resulting longer mean germination time (MGT) and slower speed of germination, lower performance of seedling growth in aged seeds. Storing soybean seeds at 25±2ºC could maintain seed viability and vigour until 3 months. Soybean seeds stored at 8±2ºC could maintain seed viability up to 15 months and seed vigour up to 12 months.

Post-storage priming with -0.8MPaPEG in 6 months stored seeds of AGS-190 resulted in higher germination percentage, germination index and better seedling performance. Moreover, post-storage priming with 0.5% chitosan and -0.8MPa PEG of seeds stored for 6-9 months improved germination percentage, germination index, seedling vigour index of cultivar Cikurai. Osmopriming with -0.8MPa PEG improved the mechanisms involved in seed germination involving CAT and SOD activities, MDA accumulation and chromosomal changes of stored seeds of AGS-190 and Cikurai.

There were six different types of chromosomal aberrations observed in deteriorated seeds during field weathering and controlled storage. Under both conditions, the most abundant types of chromosomal aberrations are single bridge and sticky chromosomes. Priming with water, 0.5% chitosan and -0.8MPa PEG generally reduced single bridge and sticky types of chromosomal aberrations in both field deteriorated seeds and stored seeds. Priming not only repairs the chromosomal damage, but appears to slow down the ageing process. This study indicates that deterioration process of soybean seeds involves the production of reactive oxygen species (ROS) through depletion of antioxidant enzymes, and lipid peroxidation which ultimately interferes with cell mitotic activity. Priming improves seed quality through increase activities of antioxidant enzymes by repairing membrane damage and minimizing chromosomal damage.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PERUBAHAN FISIOLOGI DAN KROMOSOM BAGI BIJI BENIH KACANG SOYA (*Glycine max* L. Merr.) TERTANGGUH PENUAIAN DAN DISIMPAN SETELAH PRIMING

Oleh

PHYU SIN THANT

Februari 2018

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Kemerosotan biji benih bermula dari pokok induk di ladang dan berterusan sehingga penyimpanan. Faktor asas yang mempengaruhi kemerosotan biji benih adalah suhu, kandungan kelembapan benih dan tempoh penyimpanan. Priming biji benih yang dikenali sebagai rawatan benih pra-menanam menggunakan bahan semulajadi atau sintetik biasanya diamalkan untuk meningkatkan percambahan biji benih dan kemunculan anak benih untuk pelbagai spesies tanaman. Kajian ini dijalankan untuk menilai keberkesanan rawatan pembenihan benih dan rawatan penyembuhan pasca penyimpanan mengenai perubahan dalam kualiti benih dan mekanisma yang terlibat dalam proses kemerosotan semasa diladang dan semasa simpanan terkawal. Untuk kajian kemerosotan di ladang, benih AGS-190 dan Cikurai dituai pada peningkat matang penuaian (HM), satu minggu selepas HM (H1) dan tiga minggu selepas HM (H3). Pada masa penuaian, benih dari batang utama dan dahan dibezakan untuk menilai kualiti benih dari kedudukan yang berbeza di bawah keadaan cuaca lapangan. Untuk kajian penyimpanan, benih HM AGS-190 dan Cikurai disimpan di bilik sejuk (8 ± 2ºC) atau suhu bilik (25 ± 2ºC) selama 3, 6, 9, 12 dan 15 bulan. Biji benih yang mengalami kemerosotan di ladang dan biji benih yang disimpan dirawat dengan -0.8MPa PEG, 0.5% kitosan dan air.

Biji yang ditangguhkan penuaian dari batang utama dan dahan tidak menunjukkan perbezaan dalam kualiti benih dan prestasi anak benih. Biji benih kacang soya yang dituai melepasi HM menunjukkan kesan buruk terhadap penilaian fisiologi benih dan akibatnya kualiti benih terjejas. Kultivar kacang soya bersaiz besar lebih sensitif terhadap keadaan cuaca yang buruk seperti yang ditunjukkan oleh kemerosotan kualiti benih pada satu minggu selepas HM manakala kultivar bersaiz kecil dengan ciri-ciri benih hitam boleh mengekalkan kualiti biji benih yang lebih baik sehingga satu minggu selepas HM. Kebernasan dan kecergasan biji benih kacang soya agak menurun bagi benih H3 dengan penurunan aktiviti catalase (CAT) dan superoxide dismutase (SOD) dan peningkatan pengumpulan kandungan malondialdehid (MDA) dan perubahan kromosom. Kehilangan kualiti biji benih kacang soya yang tertangguh penuaian dipengaruhi oleh kandungan kelembapan benih semasa tuaian dan jangkitan *Phomopsis*

sp.. Kandungan ROS dalam benih lembab jauh lebih tinggi daripada benih kering. Pengumpulan lebih tinggi ROS dengan peningkatan bersamaan dengan kandungan MDA bukan sahaja mencederakan membran sel tetapi turut menyebabkan kerosakan oksidatif kepada DNA biji benih penuaian tertangguh dan kerosakan kromosom.

Priming biji benih dengan 0.5% chitosan dan -0.8MPa PEG meningkatkan kebernasan benih H1 untuk kedua-dua kultivar dengan meningkatkan peratusan percambahan, indeks percambahan, indeks perkembangan biji benih yang lebih baik serta percambahan yang lebih cepat. Rawatan priming menurunkan kandungan MDA dan pengumpulan perubahan kromosom dalam biji benih kacang soya yang ditangguhkan penuaian diladang. Pemulihan kebolehan bercambah bagi biji benih yang mengalami kemerosotan di ladang semasa priming dikaitkan dengan peningkatan sintesis dalam aktiviti CAT atau SOD, yang menghalang pengumpulan peroxidation lipid dan kerosakan genetik.

Peningkatan ketara dalam kandungan MDA dan EC leachate biji benih dengan peningkatan tempoh penyimpanan menunjukkan bahawa kemerosotan biji benih dalam suhu bilik (25 ± 2ºC) menyebabkan kerosakan oksidatif kepada integriti membran sel. Aktiviti CAT dan SOD dalam biji benih yang disimpan pada suhu bilik berkurangan dengan masa penyimpanan lebih lama yang menggalakkan pengumpulan ROS. Kerosakan oksidatif yang disebabkan oleh pengumpulan ROS semasa penyimpanan pada suhu bilik bukan sahaja menyebabkan lipid teroksida tetapi juga merosakkan asid nukleik yang menyebabkan kerosakan kepada kromosom. Kesan kemerosotan biji benih menghindar proses metabolik untuk pertumbuhan akar dan pucuk menyebabkan MGT yang lebih lama dan masa percambahan lebih perlahan, di samping prestasi benih yang lebih rendah dalam benih tua. Menyimpan biji benih kacang soya pada 25 ± 2ºC dapat mengekalkan kebernasan biji benih selama 3 bulan tetapi kecergasan biji benih menurun dengan peningkatan tempoh penyimpanan. Biji kacang soya yang disimpan pada 8 ± 2ºC dapat mengekalkan kecergasan benih hingga 15 bulan dan kebernasan benih hingga 12 bulan.

Priming pasca penyimpanan dengan -0.8Mpa PEG dalam biji benih AGS-190 disimpan 6 bulan menghasilkan peratusan percambahan yang lebih tinggi, indeks percambahan dan prestasi biji benih yang lebih baik. Selain itu, priming pasca penyimpanan dengan 0.5% kitosan dan -0.8MPa PEG bagi biji benih yang disimpan untuk 6-9 bulan meningkatkan peratusan percambahan, indeks percambahan, indeks kesegahan anak benih bagi kultivar Cikurai. Osmopriming dengan -0.8MPa PEG telah meningkatkan mekanisma yang terlibat dalam percambahan benih yang melibatkan aktiviti CAT dan SOD, pengumpulan MDA dan perubahan kromosom bagi biji benih AGS-190 dan Cikurai yang disimpan.

Terdapat enam jenis kerosakan kromosom dalam benih yang terdedah pada persekitaran diladang dan semasa simpanan terkawal. Pada kedua-dua keadaan, jenis kerosakan kromosom yang paling banyak adalah jambatan tunggal dan kromosom melekat. Priming dengan air, 0.5% chitosan dan -0.8MPa PEG secara amnya mengurangkan kerosakan kromosom jambatan tunggal dan kromosom melekit. Priming bukan sahaja membaiki kerosakan kromosom, juga memperlakankan proses kemesosotan. Kajian ini menunjukkan bahawa proses kemerosotan biji benih kacang soya melibatkan penghasilan spesies oksigen reaktif (ROS) melalui pengurangan enzim antioksidan, dan peroxidation lipid yang mengganggu aktiviti mitosis sel. Priming meningkatkan kualiti biji benih melalui peningkatan aktiviti enzim antioksidan dengan membaiki kerosakan membran dan mengurangkan perubahan genetik.

ACKNOWLEDGEMENTS

The completion of this thesis was a long process and there are many people for whose support I am grateful. I would like to thank first and foremost my supervisor, Prof. Dr. Adam Puteh for giving me the opportunity to study in the area of seed technology. He provided me enormous support and guidance during this period of study. My special appreciation is extended to my supervisory committee member, Prof Dr. Uma Rani Sinniah for her intellectual guidance and professional expertise throughout my research and the thesis preparation process. I would like to express my deep sincere gratitude to Dr. Mohd Firdaus Bin Ismail for his valuable comments and help throughout my study.

I am truly grateful to Organization for Women in Science for the Developing World (OWSDW) for financial support by awarding me the scholarships from MSc leading to PhD. My thanks also go to OWSDW secretariats for their helpful communications and suggestion during my study.

I very much appreciate Mr. Harris Ahmad from Seed Technology laboratory and all staff members from Physiology and Postharvest laboratories for their kind helps and also providing facilities and material for my research assistance in my experiments. Regarding to Mycology Lab 2, Department of Plant Protection, thank you to Mr. Mohamed Nazri Abdul Rahman for his friendly assistance in microscopic work.I would like to express my honest thanks to staff members of field 2, UPM for their kind assistance. I would also like to thank my friends working in the seed technology laboratory for your unconditional and genuine friendship and for the great times that we have shared together. My deep gratitude is dedicated to my family, who always support and encourage me under whatever circumstances.

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LIST OF ABBREVIATIONS

µM	Micromolar
µmol	Micromole
AOSA	Association of Official Seed Analysts
AVRDC	Asian Vegetable Research and Development Center
CAT	Catalase
DNA	Deoxyribose Nucleic Acid
EC	Electrical conductivity of seed leachates
GI	Germination index
H1	One week after HM
H3	Three weeks after HM
HM	Harvest maturity
ISTA	International Seed Testing Association
LSD	Least significant difference
M	Molarity
MDA	Malondialdehyde
MGT	Mean germination time
mM	Millimolar
mmol	Millimole
mol	Mole
nmol	Nanomole
OWSD	Organization of Women Scientists in Developing World
PEG	polyethylene glycol
PM	Physiological maturity
RCBD	Randomized complete block design
RH	Relative humidity
RNA	Ribose Nucleic Acid
ROS	Reactive oxygen species
Rpm	Revolution per minute
SOD	Superoxide Dismutase
SVI	Seedling vigor Index
TZ	Tetrazolium Test
UPM	University Putra Malaysia
UV	Ultra Voilet
w/v	Weight per volume
w/w	Weight per weight

CHAPTER 1

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is an economically important leguminous crop in the world. Soybean seeds consist of about 40% protein, 21 % oil and 34 % carbohydrates and 5% ash (Burton, 1997). Since soybean seed is a rich source of protein and oil, the production of soybean has been rapidly increasing as the world demand for protein and oil increase. Thus, soybean stands at the top in the world production of oil seed crops (Singh et al., 2011). Nowaday, soybean account for 35% of total harvested area devoted to annual and perennial oil crops (Prakash, 2008). Although soybean originated from China, United States of America is the leading producer in the world today producing about 117.21 million matric tonnes in 2016. Brazil and Argentina rank second and third in terms of production of 96.30 and 58.80 milliom matric tonnes, respectively. In Asia, the major soybean producing countries are China (11.97 million matric tonnes) and India (14.00 million matric tonnes) in 2016 (FAOSTAT, 2016).

High quality seeds which produce rapid and uniform field emergence of seedlings are essentially important for efficient production of soybean crop (Soltani et al., 2002). However, seed ageing which is described by reduction of seed viability with time is one of the major problems facing successful field establishment in agricultural production system. Deterioration of seed viability is an inevitable and irreversible process (Kapoor et al., 2011) and it is usually described by a decrease in germination percentage, reduced viability and vigour producing weak seedlings, and ultimately seed death (Tilebeni and Golpayegani, 2011). Seed ageing are influenced by a number of factors during field weathering, harvesting and storage.

Seed deterioration begins after harvest maturity. Unfavorable weather conditions which contribute to seed deterioration especially during post-maturation and pre-harvest period (Bhatia et al., 2010) are a challenge for production of high quality soybean seeds in the tropics and sub-tropics where the climate is characterized by high temperature and erratic rainfall (Pádua et al., 2009; Sanadhya and Dubeya, 2014). Delaying harvest past the harvest maturity stage causes longer exposure of seeds to adverse field conditions and consequently decreases the quality of the seeds (Eskandari, 2012). Losses of soybean seed viability and vigor due to delaying harvest have been reported by many researchers (Philbrook and Oplinger, 1989; Marcos-Filho et al., 1994; Dias et al. 2005; Diniz et al., 2013). However, the mechanisms involved in seed deterioration due to field weathering are unclear.

Seed aging after harvest and during storage is a major problem for maintaining high quality of planting materials. Major factors influencing seed longevity during storage are initial quality (Khatun et al., 2009), the storage conditions (Surki et al., 2012; Akter et al., 2014) and storage duration (Mbofung et al., 2013). Shelar et al. (2008) reported that decline in seed germination is much more serious under tropical conditions since environmental circumstances in these regions are challenging to maintain its viability during storage.

Seed deterioration is related to physiological and biochemical changes which include the decrease in enzyme activities, membrane integrity, protein synthesis and DNA degradation (Sun et al., 2007). Free radical reactions from reactive oxygen species (ROS) including superoxide radicals (O2-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet}OH$) are regarded as primary causes of seed deterioration during controlled storage (Pukacka and Ratajczak, 2007; Kaewnaree et al., 2011). A number of studies proved that lipid peroxidation increased with seed ageing during storage in sunflower (Kibinza et al., 2006), wheat (Lehner et al., 2008), Sal tree (Parkhey et al., 2012), soybean (Xin et al., 2014) and oat (Xia et al., 2015). Whether similar physiological mechanisms occurred in seed deterioration in the field is not known.

The damage to DNA is a fundamental reason for nonfunctional cells or an organ. Accumulation of chromosomal aberrations in the seeds through time in controlled storage may cause the embryos unable to germinate and leads to loss of seed viability and vigor (Villiers, 1974; Rao et al., 1987). The occurrence and accumulation of chromosomal aberrations are a function of environmental temperature and moisture content and time during seed storage (Villiers 1974; Khan et al., 2003). Association between seed quality loss and increased frequencies of chromosomal aberrations were reported in lettuce seeds (Rao, et al., 1987), barley, peas and wheat under artificial aging conditions (Menezes et al., 2014). However, the study related to accumulation of chromosomal aberrations during field weathering has escaped attention.

Although reactive oxygen species (ROS) buildup is a major contributor to seed deterioration, these ROS are scavenged by various antioxidant defense systems during ageing (Yao et al., 2012). The main function of antioxidant enzymes is to protect cells from oxidative damage during growth, development and desiccation. Balancing production of ROS and protection of antioxidants is important for the cell to counter oxidative challenges (Fuchs et al., 1997). Degradation and inactivation of enzymes during aging contribute to seed deterioration due to changes in their macromolecular structure (McDonald, 2004). Several studies have revealed a close relationship between seed deterioration and a reduction in the activity of various antioxidant enzyme systems in cotton (Goel et al., 2003), sunflower (Kibinza et al., 2006), wheat (Lehner et al., 2008), soybean (Tian et al., 2008) and pea (Yao et al., 2012) seeds.

Deteriorated seeds critically reduce seed germination, seedling strength and field emergence. It is needed to develop technologies to improve seed quality of aged seeds to ensure crop yield. Seed priming has been widely used to enhance seed quality and seedling establishment in a wide range of crops. This technique is a pre-sowing technique to control imbibition process by treating seeds with natural or synthetic compounds (Jisha et al. 2012). Using this technique, seeds are partly imbibed to a point to allow pre-germination metabolic processes without radical protrusion (Giri and Schilinger, 2003). Seed priming has been approved to be a beneficial technique to improve seed germination and seedling establishments in delayed harvest soybean seeds (Thant, 2015) and storability of soybean seeds (Assefa, 2008). Various studies reported that increase in seed quality of primed seeds is related to increase in activation of antioxidant enzymes in order to protect cell membrane damage from reactive oxygen species (Kibinza et al., 2011; De-Oliveria et al., 2012; Siri et al., 2013), repair in chromosomal changes (Rao et al., 1987; Sivritepe an Durado, 1995) in aged seeds.

Soybean is sensitive to environmental conditions and the qualities of soybean seeds are influenced by field weather conditions during harvesting time especially under adverse enviromental conditions. Moreover, these conditions make very difficult to maintain soybean seeds longevity during storage. It is important to know which mechanisms are involved in seed deterioration during field weathering and under controlled storage. On the other hand, it is critically important to produce rapid and uniform seedling emergence for good crop establishment mainly under adverse environmental conditions. Thus, the study needs to examine how seed priming treatments improve seed quality and reverse mechanisms involved in soybean seeds deterioration. Therefore, the present study was undertaken with the following objectives.

Objectives

1. To detect physiological mechanisms such as antioxidant enzyme activities, age-induced membrane deterioration and chromosomal aberrations in seed deterioration of delayed harvest and stored seeds.

2. To determine the beneficial effects of priming on changes in mechanisms involved in seed deterioration of delayed harvest and stored seeds.

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