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PRIMING OF DELAYED HARVEST SOYBEAN (Glycine max L. Merr.) SEEDS FOR QUALITY IMPROVEMENT

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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 Master of Science

May 2015

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 Master of Science.

PRIMING OF DELAYED HARVEST SOYBEAN (*Glycine max* L. Merr.) SEEDS FOR QUALITY IMPROVEMENT

By

PHYU SIN THANT

May 2015

Chairman: Associate Professor Adam Puteh, PhD
Faculty: Agriculture

Unfavorable weather condition causes delayed in harvesting of soybean seeds and consequently deteriorates seed quality. A study was undertaken at Universiti Putra Malaysia to investigate the potential seed priming treatments for seed quality improvement of delayed harvest soybean ***(****Glycine max* L. Merr.) seeds. AGS190, Cikurai, Willis and Deing cultivars were grown in the field for two consecutive years from 2013 to 2014. In year I, seeds were harvested at harvest maturity (HM), one week after HM (H1) and two weeks after HM (H2). In year II, the seeds were harvested at HM, H1 and three weeks after HM (H3). Polyethylene glycol (PEG) of 0, -0.8, -1.1 and -1.8 MPa and chitosan of 0, 0.25, 0.5 and 0.75% were used to prime the seeds. For seedling emergence in the field, seed priming treatments using PEG and chitosan with similar concentrations were used. Changes in catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) enzyme activities and malondialdehyde (MDA) level in delayed harvest seeds were detected during priming.

Seed quality of AGS-190 in year I was lower than in year II. The weather condition of high precipitation prior and during harvesting in year I was unfavorable to seed quality of AGS-190. The percent germination of Cikurai, Willis and Deing were more than 90% in year I. However, it significantly reduced when seeds were harvested three weeks after harvest maturity in year II due to longer exposure to field condition beyond HM.

Increase in percent germination, germination index, speed of germination and seedling vigor index occurred when the seeds were primed with -0.8 MPa PEG and 0.5% chitosan. Chitosan priming reduced mean germination time for all harvest dates. The 0.5% chitosan needed 5.28-5.7days while unprimed seeds took more time to germinate (5.71- 5.97days). Low concentration of chitosan (< 0.5%) improved seedling emergence percentage and emergence speed of soybean seeds. Seed priming with water also improved percent seedling emergence of seeds as well as chitosan and PEG priming.

Antioxidant enzyme activities decreased with the time of harvest in both AGS-190 and Willis cultivars. Seed primed with 0.5% chitosan enhanced CAT and POD in all harvested seeds of AGS-190 in year I. Moreover, the 0.5% chitosan enhanced POD and SOD in both HM and H1 seeds of AGS-190 cultivar in year II. For Willis cultivar,

CAT activity of all three harvested seeds were improved by 0.5% chitosan priming while -0.8MPa PEG enhanced CAT activity for HM and H1 seeds. The accumulation of MDA was reduced in primed seeds compared with unprimed seeds. The seed priming with water was also effective to reduce lipid peroxidation like 0.5% chitosan and -0.8MPa PEG.

The study suggests that soybean seed quality has considerably declined when the seeds are harvested at three weeks after harvest maturity in wet and warm tropical environment. Large seeded cultivars are more susceptible to reduction in seed quality than small seeded cultivars and delaying one week after harvest maturity is enough to decline seed quality. Chitosan priming improves seed vigor of delayed harvest soybean seeds by reducing mean time needed for germination. Hydropriming with water also can improve seed quality as well as priming with chitosan and PEG.

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

PRIMING BIJI BENIH KACANG SOYA (*Glycine max* L. Merr.) TERTUNDA PENUAIAN UNTUK PENINGKATAN KUALITI

Oleh

PHYU SIN THANT

Mei 2015

Pengerusi : Profesor Madya Adam Puteh, Ph.D
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Keadaan cuaca tidak menetu menyebabkan proses penuaian biji benih kacang soya tertunda dan menyebabkan penurunan kualiti biji benih. Kajian telah dijalankan di Universiti Putra Malaysia untuk menyelidik potensi proses rawatan priming biji benih untuk tujuan meningkatkan kualiti biji benih kacang soya (*Glycine max* L. Merr.) yang tertunda penuaiannya. Kultivar AGS190, Cikurai, Willis dan Deing telah ditanam di ladang selama dua tahun berturut-turut bermula dari 2013 hingga 2014. Pada tahun I, biji benih telah dituai ketika harvest maturity (HM), seminggu selepas HM (H 1) dan dua minggu selepas HM (H2). Pada tahun II, biji benih dituai ketika HM, H1 dan tiga minggu selepas HM (H3). Polyethylene glycol (PEG) pada kadar 0, -0.8, -1.1 dan -1.8 MPa serta chitosan 0, 0.25, 0.5 dan 0.75% telah digunakan bagi tujuan priming. Bagi percambahan anak benih di ladang, rawatan priming biji benih menggunakan PEG dan chitosan pada kadar yang sama turut digunakan. Perubahan bagi aktiviti enzim catalase (CAT), peroxidase (POD) dan superoxide dismutase (SOD) serta kadar malondialdehyde (MDA) dalam biji benih tertunda penuaian telah direkodkan semasa proses priming.

Kualiti biji benih AGS-190 pada tahun I adalah lebih rendah dari tahun II. Keadaan cuaca yang mengalami banyak hujan semasa tahun I adalah tidak sesuai dan telah mempengaruhi kualiti AGS-190. Peratusan percambahan bagi Cikurai, Willis dan Deing adalah lebih dari 90% semasa tahun I. Walaubagaimanapun, nilai ini menurun dengan signifikan apabila biji benih dituai tiga minggu pada H3 semasa tahun II kerana telah terdedah lebih lama pada keadaan ladang sebelum HM.

Peningkatan peratusan percambahan biji benih, index percambakan, kelajuan percambahan dan index kecergasan anak benih berlaku semasa biji benih dirawat dengan 0.8 MPa PEG dan 0.5% chitosan. Priming menggunakan chitosan mengurangkan masa percambahan untuk semua tempoh masa penuaian. Chitosan 0.5% memerlukan 5.28-5.7 hari manakala biji benih tanpa rawatan memerlukan lebih masa untuk bercambah (5.71-5.97 hari). Chitosan pada berkepekatan rendah (<0.5%) meningkatkan kadar percambahan anak benih dan kelajuan percambahan benih kacang soya. Menggunakan air meningkatkan kadar percambahan anak benih dan kelajuan percambahan serta menggunakan chitosan dan PEG.

Enzim antioksidan semakin berkurang apabila masa menuai di lewatkan bagi kultivar AGS-190 dan Willis. Biji benih yang dirawat dengan 0.5% hitosan meningkatkan CAT

dan POD dalam kesemua biji benih bagi tahun I. Chitosan pada kepekatan 0.5 % turut meningkatkan POD dan SOD bagi biji benih HM dan H1 semasa tahun II. Bagi kultivar Willis, aktiviti CAT bagi ketiga-tiga masa penuaian adalah ditingkatkan dengan menggunakan 0.5% chitosan manakala -0.8 MPa PEG meningkatkan aktiviti CAT bagi biji benih HM dan H1. Pengumpulan MDA adalah berkurangan dalam biji benih yang telah dirawat secara priming berbanding tanpa rawatan. Priming menggunakan air juga berkesan untuk mengurangkan proses lipid peroxidation seperti 0.5% chitosan dan -0.8 MPa PEG.

Kajian ini membuktikan bahawa kualiti biji benih kacang soya berkurang secara signifikan apabila dituai tiga minggu selepas harvest maturity dalam keadaan panas dan lembap cuaca tropika. Kultivar yang mempunyai biji benih yang besar adalah lebih mudah dipengaruhi kualiti berbanding biji benih yang kecil, dan menangguhkan penuaian selama seminggu sudah cukup untuk menurunkan kualiti biji benih. Rawatan priming chitosan meningkatkan kecergasan biji benih tertunda penuaian dengan mengurangkan masa diperlukan untuk percambahan. Hydropriming menggunakan air juga bolah meningkatkan kualiti biji benih seperti priming menggunakan chitosan dan PEG.

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I certify that a Thesis Examination Committee has met on……………….to conduct the final examination of PHYU SIN THANT on her thesis entitled "Priming of delayed harvest soybean (*Glycine max* L. Merr.) seeds for quality improvement" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

AOSA	Association of Official Seed Analysts
CAT	Catalase
cm	Centimetre
EC	Electrical conductivity of seed leachates
g	Gram
GI	Germination index
HM	Harvest maturity
ISTA	International Seed Testing Association
M	Molar
MDA	Malondialdehyde
mg	Milligram
MGT	Mean germination time
mM	Millimolar
mmol	Millimole
mol	Mole
nmol	Nanomole
PEG	polyethylene glycol
PM	Physiological maturity
POD	Peroxidase
RH	Relative humidity
SOD	Superoxide Dismutase
SVI	Seedling vigor Index
µM	Micromolar
µmol	Micromole

CHAPTER 1

INTRODUCTION

Soybean (*Glycine max* L., Merr.) is a globally important legume crop providing high protein and oil. The seeds of soybean contain up to 40% protein and 20% oil along with calcium, iron, carotene, thiamine, and ascorbic acid (Probst and Judd, 1973). Soybean is one of the main vegetable crops in China, Japan, Korea and Malaysia (Oerke et al., 1994). Several countries are importing soybean to supplement their domestic requirement for human and animal consumption.

As soybean is regarded as a major source of protein and oil, the production of soybean has been increasing globally. Although soybean originated from China, United States of America is the leading producer in the world today producing about 89.48 million matric tonnes in 2013. Brazil and Argentina rank second and third in terms of production of 81.70 and 49.31 milliom matric tonnes, respectively. In Asia, the major soybean producing countries are China (12.50 million matric tonnes) and India (11.95 million matric tonnes) in 2013 (FAOSTAT, 2014).

About 90% of the world's soybean is produced in the tropical and semi-arid tropical regions, which are characterized by high temperature and low or erratic rainfall (Sanadhya and Dubeya, 2014). Production of high quality soybean seeds under tropical conditions is commonly a challenge due to unexpected weather events. Soybean is sensitive to environmental conditions and the qualities of soybean seeds are influenced by field weather conditions during harvesting time (Pádua et al., 2009). Phillbrook and Oplinger (1989) indicated that soybean should be harvested within 14 days after harvest maturity. Further delays in harvest reduce seed quality and potential yield. In tropical region like Malaysia where the climate is categorized as equatorial, being hot and humid throughout the year, seed quality losses due to delayed harvest in soybean are unavoidable. Continuous rainfall can cause delay in harvesting of soybean and consequently cause seed quality deterioration. Delaying harvest beyond optimum maturity extends field exposure and intensifies seed deterioration. Reduction in seed quality due to delayed harvest is reported by many workers (Tecrony et al., 1980; Marcos-Filho et al., 1994; Philbrook and Oplinger, 2004; Mengistu et al., 2007). However, little information is available about the effect of delayed harvest on soybean seed quality loss under Malaysian growing conditions.

The use of high quality seed is critical to establish a suitable plant population in a soybean field for better returns. Vigorous seeds of high quality, produce normal seedlings germinate rapidly, uniformly and are able to withstand environmental adversity after sowing (Ajouri et al., 2004). Delayed harvest subjects the seed to natural aging condition in the field, especially under humid environment, that will subsequently decrease the quality of the seed. Seed aging is usually estimated by a decrease in germination percentage measured as an average value for a seed lot. Seed deterioration exhibits itself in the appearance of weak seeds producing seedlings with some morphological abnormalities.

Seed aging is associated with degradation and inactivation of enzymes due to changes in their macromolecular structures (Lehner et al., 2008). The general decrease in enzyme activity in the seed retards the respiratory capacity, which in turn depresses both energy (ATP) and assimilates supply of the germinating seed and hence, several changes in the enzyme activities may contribute to their lowered germination efficiency (Shaban, 2013). Moreover, three non-enzymatic processes that are relevant to seed deterioration are lipid peroxidation, hydrolysis of carbohydrates and amino-carbonyl reactions (Murthy et al., 2003). Several studies for the relationship between seed aging and seed quality deterioration are mainly performed under artificial aging conditions like accelerated aging testing (Lekić, 2003; Tatić et al., 2008; Mosavi et al., 2011). Some workers reported seed longevity associated with natural aging condition under longer storage conditions (Fabrizius et al., 1999; Balešević-Tubić et al., 2011). The study on seed quality evaluation for naturally aged seeds due to delayed harvest is still largely unknown.

Seed priming is a presowing seed treatment that increases seed performance by increasing germination rate and uniformity. This promising technique has been used in biotic and abiotic stress management for improvement of germination speed, germination vigor and seedling establishment (van Hulten et al., 2006). The beneficial effects of seed priming on soybean seed quality have been reported by osmopriming (Arif et al., 2008; Sadeghi et al., 2011), hydropriming (Maroufi et al., 2011), chemical priming (Ahmadvand et al., 2012), hydropriming and chemical priming (Ghassemi-Golezani et al., 2011).

The biological mechanism of priming can be the response of antioxidant systems. Seed priming improves the antioxidant enzymes activities which decrease the adverse effects of reactive oxygen species (ROS) (Chen and Arora, 2011; Umair et al., 2011). Improving germination performance and changes in antioxidant enzyme activity in deteriorated seeds under storage conditions have been reported in sesame (Tabatabaei, 2013), rice (Zhu et al., 2010), sorghum (Azadi et al., 2013). However, no study till date has attempted such comprehensive investigation of antioxidant responses of delayed harvest seeds to seed priming.

Therefore, there is a research need to identify the consequence of delayed harvest on seed quality in Malaysian growing conditions and the effect of seed priming on seed quality and changes of enzyme activities. Although numerous studies have investigated seed priming effect on seed quality under abiotic stress conditions, very little work has been done on delayed harvest soybean seeds or escaped attention. Most of the studies on cell membrane deterioration and antioxidant enzyme system have been performed under artificial aging conditions. However, the study on activities of antioxidant enzymes, accumulation of reactive oxygen species (ROS) and lipid peroxidation for naturally aged seeds due to delayed harvest is still largely unknown. Therefore, the present study was undertaken with the following objectives.

Objectives

1. To evaluate seed quality of four soybean cultivars harvested at three different harvest times.

2. To determine the effect of priming on seed quality, seedling performance and seedling emergence in the field of delayed harvest seeds.

3. To investigate changes in antioxidant enzyme activities and lipid peroxidation of delayed harvest seeds during priming.

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LIST OF PUBLICATION

Thant, P.S., Puteh, A. and Sinniah, U.R. (2014). Changes in antioxidant enzyme activities and lipid peroxidation of delayed harvest soybean seeds during priming. 8th National Seed Symposium. 28-30 April. Putrajaya. Malaysia.

Thant, P.S., Puteh, A. and Sinniah, U.R. (2014). Improvement of seed quality and seedling performance of delayed harvest soybean seeds using priming. International Agriculture Congress. 25-27 November. Putrajaya. Malaysia.

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