UNIVERSITI PUTRA MALAYSIA

PLANT DENSITY AND VARIETAL EFFECTS ON DIAPORTHE/PHOMOPSIS DISEASES INFECTION OF SOYBEAN AND ITS IMPACT ON SEED QUALITY

SAMIYEH RAEISI PARSAEI

FP 2011 42

PLANT DENSITY AND VARIETAL EFFECTS ON *DIAPORTHE/PHOMOPSIS* DISEASES INFECTION OF SOYBEAN AND ITS IMPACT ON SEED QUALITY

SAMIYEH RAEISI PARSAEI

DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

2011

PLANT DENSITY AND VARIETAL EFFECTS ON *DIAPORTHE/PHOMOPSIS* DISEASES INFECTION OF SOYBEAN AND ITS IMPACT ON SEED QUALITY

By

SAMIYEH RAEISI PARSAEI

Thesis Submitted to the School of Graduate Studies, University Putra Malsysia, In fulfillment of the Requirements for the Degree of Doctor of Philosophy

May 2011

DEDICATION

Dedicated to:

My dear and beloved family,

And

All workingman and toiler farmers

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

PLANT DENSITY AND VARIETAL EFFECTS ON *DIAPORTHE/PHOMOPSIS* DISEASES INFECTION OF SOYBEAN AND ITS IMPACT ON SEED QUALITY

By

SAMIYEH RAEISI PARSAEI

May 2011

Chairman: Associate Professor Adam Puteh, PhD.

Faculty: Agriculture

Soybean is one of the most important oilseed crops with high protein for human and animal consumptions. Environmental factors, field management and varieties affect on the quality of harvested seed. Storage environment and its duration are also known to influence pathogen survival in stored seed. A comprehensive study was undertaken at University Putra Malaysia to evaluate the effect of plant density and seasonal variations in relation to *Diaporthe / Phomopsis* complex (DPC) on seed yield components and quality. Three soybean varieties namely AGS190, Deing and Pershing at 300,000 and 600,000 plants per hectare were grown in the field for two seasons in 2009. Seed

germination and vigour were determined using standard germination (SG), tetrazolium (TZ) and electrical conductivity (EC) tests. Seed yield components were determined at harvest maturity growth stages (R8). Culture plate method and Scanning Electron Microscopy (SEM) were used to evaluate the incidence of Phomopsis seed decay (PSD) and the progression and colonization of DPC in different parts of soybean plant from R3 until R8 plant growth stages. Ten soybean seed lots which were stored at 0°C up to 13 years were used to evaluate the presence of DPC and to identify the isolates. Morphological and molecular methods were used to determine the longevity and frequency of DPC isolates on stored and freshly harvested soybean seeds.

Analysis of data showed significant differences for higher seed yield, 100 seed weight and seed viability in two seasons and they were higher in first planting season. Planting density affected on pod number per plant, height of plant, PSD infection and seed viability and vigour significantly. Higher pod number was recorded at lower plant density which was 30 pods per plant. The first node height increased at higher plant density. PSD infection was more at higher plant density, so seed viability and vigour were higher at lower plant density. The infection of PSD was 35% at higher plant density, whereas it was 27.5% at lower plant density. The varieties showed significant differences for all parameters. AGS190 with the highest 100 seed weight could produce 1839 kg seed yield per hectare. This variety also had the highest percentage of infection to DPC (41%), so it showed the lowest seed viability and vigour. The highest pod number was recorded for Deing variety, but it produced the lowest seed yield due to

very small size seed. PSD infection was negatively correlated with standard germination and tetrazolium test and showed a positive correlation with electrical conductivity.

Scanning electron microscopy micrograph revealed that fungi colonization and progression started at different growth stage in different parts of plant. The infection started from R4 growth stage as fungi hypha whereas alpha conidia of fungi were observed at R8 growth stage on the stem. Hypha was observed in the pod at growth stage R5 and mycelium invaded inside the pod through the stomata at growth stage R6, but alpha conidia invaded through the stomata at R7 growth stage. The seed was the last part that was infected. The mycelium penetrated inside the seed through the hilum and cracks on the surface of seed. Based on SEM observations, DPC infection can be predicted sooner than R6 growth stage, so controlling DPC presence and infection between R4-R5 can control the disease before damage being inflicted on the seed.

Seven isolates of DPC were detected, identified and characterized based on morphological and molecular methods in soybean seeds for the first time in Malaysia. The isolates which were identified molecularly were chosen from seeds that showed >10% infection based on culture plate method. Most of the isolates identified, belonged to *Phomopsis longicolla*, and all of them were registered in National Center for Biotechnology Information (NCBI). The longevity of isolates in storage was found to

be <9 years which suggested that *Diaporthe /Phomosis* sp. can survive up to 9 years in cold storage(0°C).

The results of this study showed that high quality soybean seed can be produced in wet and warm tropical environments like Malaysia, but it should be planted at a proper time in a production year and harvested on time, with no delay. Continuous rainfall during late plant growth stage can cause more infection of PSD which can severely affect seed quality and decrease the germinability of harvested seeds. Overall, 300,000 plant ha^{-1} is an acceptable planting density with proper plant managements to produce high quality soybean seeds in Malaysia.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falasafah

TANAMAN KEPADATAN DAN PENGARUH KELAINAN PADA INFEKSI PERYAKIT-PERYAKIT DIAPORTHE/PHOMOPSIS DARI KEDELAI DAN DAMPAK PADA KUALITAS BIJI

Oleh

SAMIYEH RAEISI PARSAEI

May 2011

Pengerusi: Profesor Madya Adam Puteh, PhD.

Fakulti: Pertanian

Soya merupakan salah satu tanaman biji minyak yang paling penting dengan protein tinggi untuk dimakan manusia dan haiwan. Faktor persekitaran, pengurusan lapangan dan kelainan berpengaruh terhadap high benih dituai. Simpanan persekitaran dan durasinya juga diketahui mempengaruhi kelangsungan hidup patogen pada biji yang disimpan. Sebuah kajian menyeluruh dilakukan di Universiti Putra Malaysia untuk menilai pengaruh kerapatan tanaman dan variasi bermusim dalam kaitannya dengan Diaporthe / kompleks Phomopsis (DPC) pada komponen hasil biji dan high. Tiga kelainan soya iaitu AGS190, Deing dan Pershing di 300.000 dan 600.000 tanaman per hektar ditanam di lapangan selama dua musim di 2009. perkecambahan benih dan vigor ditentukan menggunakan perkecambahan standard (SG), tetrazolium (TZ) dan

konduktiviti elektrik (EC) ujian. komponen hasil benih ditentukan pada tahap pertumbuhan tuai jatuh tempo (R8). Budaya pinggan kaedah dan Mikroskop Elektron (SEM) digunakan untuk menilai kejadian pembusukan benih Phomopsis (PSD) dan perkembangan dan penjajahan DPC di berbagai bahagian tanaman soya dari R3 sampai R8 tahap pertumbuhan tanaman. Sepuluh lot benih soya yang disimpan dalam 0 ° C hingga 13 tahun digunakan untuk menilai kewujudan DPC dan untuk mengenalpasti isolat. Morfologi dan kaedah molekul digunakan untuk menentukan umur panjang dan kekerapan DPC isolat disimpan dan baru dituai biji soya.

Analisis data menunjukkan perbezaan yang signifikan bagi hasil biji lebih tinggi, berat 100 biji dan viabilitas benih dalam dua musim dan mereka lebih tinggi pada musim tanam pertama. Penanaman kepadatan berpengaruh terhadap jumlah polong pada tanaman, tinggi tanaman, jangkitan PSD dan viabilitas benih dan kekuatan secara signifikan. jumlah polong lebih tinggi tercatat sebanyak kerapatan tanaman yang lebih rendah yang 30 polong pada tanaman. Ketinggi an node pertama meningkat pada kerapatan tanaman yang lebih tinggi. PSD jangkitan lebih pada kerapatan tanaman yang lebih tinggi, sehingga viabilitas benih dan vigor lebih tinggi pada kerapatan tanaman yang lebih rendah. Jangkitan PSD adalah 35% pada kerapatan tanaman yang lebih tinggi, sementara itu 27,5% pada kerapatan tanaman yang lebih rendah. Varietas menunjukkan perbezaan yang nyata untuk semua parameter. AGS190 dengan berat 100 biji tertinggi dapat menghasilkan 1.839 kg hasil biji per hektar. Kelainan ini juga mempunyai peratusan tertinggi jangkitan untuk DPC (41%), sehingga menunjukkan

viabilitas benih dan vigor terendah. Jumlah polong tertinggi tercatat untuk Deing, tapi ini menghasilkan benih terendah kerana saiz biji sangat kecil. PSD jangkitan berkorelasi negatif dengan standard dan uji perkecambahan tetrazolium dan menunjukkan korelasi positif dengan konduktiviti elektrik.

Scanning elektron mikrograf mikroskop mendedahkan bahawa cendawan penjajahan dan pembangunan bermula pada tahap pertumbuhan yang berbeza di pelbagai bahagian tanaman. Jangkitan bermula dari tahap pertumbuhan R4 sebagai hifa cendawan sedangkan alpha konidia cendawan diamati pada fasa R8 pertumbuhan pada batang. Hifa diamati di R5 polong pada tahap pertumbuhan dan miselium menyerang di dalam polong melalui stomata pada pertumbuhan tahap R6, tapi konidia alpha menyerbu melalui stomata pada R7 tahap pertumbuhan. Benih adalah bahagian terakhir yang dijangkiti. Miselium menembus di dalam benih melalui hilus dan retak pada permukaan benih. Berdasarkan pengamatan SEM, DPC jangkitan boleh diramal lebih cepat dari R6 tahap pertumbuhan, sehingga kewujudan DPC dan pengendalian jangkitan antara R4-R5 dapat mengawal penyakit sebelum kerosakan yang ditimbulkan pada benih.

Tujuh isolat DPC dikesan, dikenalpasti dan ditandakan berdasarkan kaedah morfologi dan molekul dalam biji kedelai untuk pertama kalinya di Malaysia. Isolat yang dikenalpasti molekul dipilih daripada biji yang menunjukkan jangkitan >10% berdasarkan kaedah plate budaya. Sebahagian besar isolat dikenalpasti, milik longicolla

Phomopsis, dan semua dari mereka yang berdaftar di Pusat Nasional untuk Biotechnology Information (NCBI). Umur panjang isolat dalam simpanan dijumpai <9 tahun yang menunjukkan bahawa Diaporthe / sp Phomosis. boleh bertahan hingga 9 tahun dalam cold storage (0°C).

Keputusan kajian menunjukkan bahawa biji soya high tinggi boleh dihasilkan dalam lingkungan tropika basah dan hangat seperti Malaysia, tetapi harus ditanam pada masa yang tepat dalam satu tahun pengeluaran dan tuai tepat waktu, dengan tidak ada penangguhan. curah hujan terus menerus selama tahap pertumbuhan tanaman akhir boleh menyebabkan jangkitan lebih dari PSD yang sangat boleh menjejaskan kualiti benih dan menurunkan perkecambahan biji dituai. Secara keseluruhan, tanaman 300.000 ha-1 adalah kerapatan tanam diterima dengan pengurusan kilang yang tepat untuk menghasilkan benih soya yang berkualiti tinggi di Malaysia.

ACKNOWLEDGEMENTS

At first my praises and endless thanks be to God, who gave me the strength and opportunity to complete my study. I hope and promise to use my knowledge for the people who need it. I would like to convey my deepest appreciation to my supervisor, Associate Professor Dr. Adam Puteh, for his supervision, valuable advices and helpful suggestions in my study, especially his skilful and kind guidance for my thesis writing that resulted in completion of my thesis. Thanks and sincere appreciation goes to my committee members, Associate Professor Dr Kamaruzaman Sijam and Dr Nur Ashikin Psyquay Abdullah, for their supports, help and kind permission to work in their laboratories and use all apparatus and facilities.

I would like to express my honest thanks to the staff members of Microbiology and Physiology laboratories, for their kind helps and also providing facilities and material for my research, especially, Mrs., Junaina and Mr. Davud. I am certainly grateful to Mr. Zol from seed technology laboratory for his assistance and all facilities that he supplied for my field and laboratory experiments, and also greatly thanks to Mr. Rahman for his valuable assistance in preparing and managing the field.

I know my duty, sincerely thank from all people in my dear country, Iran, for their support and create this opportunity for me to continue my supplementary study,

including, Agriculture Research Organization, Seed and Plant Improvement Institute, and especially Agricultural Research Center of Golestan.

My heartiest thanks and love to my beloved family for their constant emotional and physical supports, patients and endless helps and encouragements. Moreover, I deeply thank anybody who has helped me with continuing my study

I certify that a Thesis Examination Committee has met on 6 May 2011 to conduct the final examination of Samiyeh Raeisi Parsaei on her thesis entitled "Plant Density and Varietal Effects on *Diaporthe/Phomopsis* Diseases Infection of Soybean and its Impact on Seed Quality" in accordance with 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Abdul Shukor b Juraimi PhD
Associate Professor
Faculty of Agriculture
University Putra Malaysia
(Chairman)

Zainal Abdin b Mior Ahmad PhD
Associate Professor
Faculty of Agriculture
University Putra Malaysia
(Internal Examiner)

Mohd Ridzwan b Abd Halim PhD
Associate Professor
Faculty of Agriculture
University Putra Malaysia
(Internal Examiner)

Moshir Rahman PhD
Associate Professor
Department of agronomy
Bangladesh Agriculture University
(External Examiner)

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 27 June 2011

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of Supervisor Committee were as follows:

Adam Puteh, PhD
Associate Professor
Faculty of Agriculture
University Putra Malaysia
(Chairman)

Kamaruzaman Sijam, PhD
Associate Professor
Faculty of Agriculture
University Putra Malaysia
(Member)

Nur Ashikin Psyquay Abdullah, PhD
Senior Lecturer
Faculty of Agriculture
University Putra Malaysia
(Member)

HASANAH MOHD GHAZALI. PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: July 2011

DECLARATION

I declare that the thesis is my original work except for questions and citations, which have been duly acknowledged. I also declare that it has not been previously and it not currently submitted for any other degree at Universiti Putra Malaysia or any other institutions.

_____

SAMIYEH RAEISI PARSAEI

Date: 6 May 2011

TABLE OF CONTENTS