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SEED-BORNE INFECTION COLLETOTRICHUM (SCHW.) ANDRUS AND MOORE IN SOYBEAN SEEDS AND ITS CONTROL

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By

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TABLE OF CONTENTS

PAGE

LIST OF TABLES

Table		Page

LIST OF FIGURES

Figure | Page

LIST OF PLATES

Plate Page

Plate		Page

Plate | Page

LIST OF ABBREVIATIONS

^{o}C	degree (s) Celsius
%	percent (percentage)
viz.	namely
g	gram
g/pt	gram per plant
h	hour
ha	hectare
cm	centimetre
m	metre
m	square metre
mm	millimetre
ml	millilitre
mg	milligram
mt	metric ton
ma	milliampere
min	minute
nm	nanometre
NA	nutrient agar
PDA	potato dextrose agar
M	molar
N	normal
pH	hydrogen ion concentration
rpm	round per minute
i.e.	that is
kg	kilogram
μ	micrometre
μl	microlitre
μg	microgram
HgCl	magnesium chloride
H_2O_2	hydrogen peroxide
H_2SO_4	sulfuric acid
MW	molecular weight
NaOH	sodium hydroxide
NaOCl	sodium hypochloride
NUV	near ultraviolet
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis

Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirements for the Degree of Doctor of Philosohpy.

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Supervisor : Assoc. Prof. Dr Sariah Meon

Faculty : Agriculture

C. truncatum have been recognized as a soybean seed pathogen for over 40 years, causing pre-and post-emergence damping-off of seedlings and seedling blight. Although much is known of the effects of this pathogen on soybean seed quality and vigour, fundamental studies on the pathogen, its seed-borne nature, seed transmission and control are rather limited. Present studies are thus focused on their issues.

Detection and isolation of this pathogen was carried out in order to understand the nature of the seed-borne pathogen. In the examination of seed-borne microorganisms of soybean (Glvcine max (L.) Merrill cultivar Palmetto, SJ4 and SJ5 collected from Malaysia and Thailand, nine genera comprising of 13 species of fungi were found to be associated with the seeds. Among these fungi, Colltotrichum truncatum incidence

was recorded in the range of 2.5-6.5%. The blotter method proved more suitable for detecting C. truncatum than agar plate method.

It has been confirmed that C. truncatum infection reduced seed quality and seedling vigour. Infestation of soybean seeds with conidial suspension or culture filtrate and infestation of soil with culture supension significantly reduced percentage of seed germination, seedling emergence and seedling height in in vitro and glasshouse studies. The fungus caused seed rot, pre- and post-emergence damping-off and lesions on the hypocotyls and cotyledons of young seedlings. Seeds infected by C. truncatum contained more soluble protein but less soluble carbohydrate than that in the healthy seeds. Eventhough the electrophoretic patterns are identical qualitatively between infected and healthy seeds, the relative amount still varies quantitatively. This further supported the observation that C. truncatum resulted in seed deterioration and reduced germibility. In addition, infected seeds have lower activity of soluble isozymes: esterase, peroxidase, acid phosphatase and alkaline phosphatase than healthy seeds.

Soybean plants were found to be more susceptible to C. truncatum at or during the maturing stage. Inoculation at different stages (R1, R3, R5 and R7) of plant growth showed that seed-borne C. truncatum was recovered in the highest percentage at R7 (17.56%) followed by R5 (10.38%), R3 (9.19%), R1 (7.69%) and uninoculated control (3.44%), respectively. Component plating of individual seed parts confirmed that the fungus was internally-borne and well-established within the seed coat. Therefore, this study positively showed that *C. truncatum* can be transmitted from mother plant to developing seeds. Transmission of the pathogen also occurred from infected seeds to seedlings. *C. truncatum* produced reddish brown to light brown lesions on infected cotyledonary leaves, hypocotyl and epicotyl regions of germinating seeds. Lesions became dark brown to black as they progressed producing numerous black acervuli in the centre. Severe infection resulted in pre- and post-emergence mortality and stunting of young seedlings. Seed transmission was assessed to be 83% and 59% by the seedling symptom tests on soil and sand, respectively.

Light and scanning electron microscopy confirmed that the fungus was internally-borne within infected soybean seed. Abundant inter- and intra-cellular hyphae were observed in all the three layers of the seed coat (palisade cell, hourglass cell and parenchyma cell layers) and in the hilar tracheids of the seed. Acervuli primordia were produced in the hourglass cell layer and endodermis cell. Fruiting structures consisting of mature setose acervuli were later produced on the seed surface. Parenchyma cells appeared collapsed and macerated.

Six of the 12 isolates of fungi and bacteria which were detected and isolated from soybean seeds were found promising as biocontrol agents against C. truncatum in dual culture. These were Chaetomium globosum, Curvularia lunata, Fusarium moniliforme. F. oxysporum. F. semitectum, and Bacillus sp.. Chaetomium globosum, F. oxysporum and Bacillus sp. were selected for further study as seed treatment in comparison with six other commonly used fungicides. All seed treatments significantly reduced the incidence of C. truncatum in the infected soybean seeds in in vitro test. However, in glasshouse studies, results showed that only the fungicidal seed treatment: Delsene, Benlate, Captan and Thiram improved the rate of seed germination and seedling emergence compared to the untreated control.

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JANGKITAN BAWAAN BIJI BENIH OLEH COLLETOTRICHUM TRUNCATUM (SCHW.) ANDRUS AND MOORE PADA BIJI BENIH KAJANG SOYA DAN KAWALANNYA.

oleh

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C. truncatum (Schw.) Andrus and Moore telah dikenali sebagai patogen bawaan biji benih kacang soya selama lebih daripada 40 tahun, menyebabkan pelecuhan pra- dan pos- cambah dan hawar anak benih. Walaupun banyak telah diketahui berkenaan kaitan patogen ini dengan kualiti dan kebernasan biji kacang soya, kajian-kajian asas tentang patogen dan cara bawaan biji benih, penyebaran melalui biji benih dan pengawalan masih lagi terhad. Oleh itu, kajian-kajian semasa ini ditumpukan kepada isu-isu tersebut.

Pengesanan dan pemencilan patogen dijalankan untuk memberi kefahaman tentang keadaan bawaan penyakit biji. Dalam pencerapan mikroorganisma-mikroorganisma bawaan biji benih., kacang soya (Glycine max (L.) Merrill), var Palmetto, SJ4 dan SJ5, yang dikutip dari Malaysia dan Thailand, 9 genera kulat

terdiri daripada 13 spesies didapati bersekutu dengan biji benih. Di antara kulat-kulat ini, kejadian C. truncatum dicatatkan dalam julat 2.5-6.5%. Kaedah kertas serap ditunjukkan lebih sesuai untuk pengesanan C. truncatum daripada kaedah piring agar.

Telah dibuktikan bahawa jangkitan C. truncatum mengurangkan kualiti biji benih dan kecergasan anak benih. Penjangkitan biji benih kacang soya dengan ampaian konidia atau filtrat kultur dan penjangkitan tanah dengan ampaian kultur mengurangkan dengan signifikannya peratus percambahan biji benih, penjelmaan dan ketinggian anak benih dalam kajian in vitro dan rumah kaca. Kulat menyebabkan pereputan biji benih, pelecuhan pra- dan pos- penjelmaan dan lesion pada hipokotil dan kotilidon anak benih muda. Biji benih yang dijangkiti oleh C. truncatum mengandungi lebih banyak protein terlarut tetapi kurang karbohidrat terlarut dibandingkan dengan biji benih yang sihat. Walaupun corak elektroforesis protein bersamaan secara kualitatif di antara biji benih yang dijangkiti dan biji benih yang sihat, namun relatifnya masih berbeza secara kuantitatif. Ini menyokong pencerapan yang menunjukkan bahawa C. truncatum menyebabkan kemerosotan mutu biji benih dan mengurangkan kadar percambahan. Di samping itu, biji benih yang dijangkiti mempunyai aktiviti isozim terlarut yang rendah; esterase, peroksidase, fosfatase asid dan fosfatase beralkali daripada biji benih yang sihat.

Tanaman kacang soya didapati lebih rentan kepada jangkitan C. truncatum pada atau semasa peringkat kematangan. Penginokulatan pada peringkat pertumbuhan yang berbeza (R1 , R3 R5 dan R7) menunjukkan C. truncatum bawaan biji benih dapat dikesan pada peratus yang tinggi pada peringkat pertumbuhan R7 (17.56%) diikuti oleh R5 (10.38%), R3 (9.19%), R1 (7.69%) dan kawalan (3.44%) berturutan. Pengasingan daripada bahagian-bahagian individu biji benih membuktikan yang kulat ini didapati dan menetap di dalam lapisan kulit biji benih. Oleh itu kajian ini mempastikan secara positif yang C. truncatum boleh disebarkan daripada tanaman induk kepada biji benih yang sedang terbentuk. Penyebaran patogen juga berlaku daripada biji benih yang dijangkiti kepada anak benih. Jangkitan C. truncatum menghasilkan lesion berwarna perang kemerahan kepada perang muda pada daun-daun kotilidon yang dijangkiti, hipokotil dan bahagian-bahagian epikotil biji benih yang bercambah. Lesion kemudian bertukar kepada perang pekat hingga hitam dengan pengeluaran aservulus berwarna hitam dengan banyaknya di tengah-tengah lesion. Jangkitan serius mengakibatkan kematian pra- dan pos- cambah dan kebantutan anak-anak benih yang muda. Penyebaran melalui biji benih telah dinilai sehingga 83% dan 59% melalui ujian simptom anak benih dalam tanah dan pasir berturutan.

Kajian-kajian mikroskop cahaya dan SEM membuktikan bahawa kulat bertempat di dalam biji kacang soya yang dijangkiti. Hifa inter- dan intra- sel didapati dengan banyaknya dalam ketiga-tiga lapisan kulit biji benih (lapisan sel palisad, sel hourglass dan sel parenkima) dan dalam tracheid hilum biji benih. Primordia acervulus terbentuk dalam lapisan "hourglass" dan endodermis. Struktur-struktur berbuah kemudian dikeluarkan di atas permukaan biji benih. Sel-sel parenkima kelihatan runtuh dan maserat.

Enam daripada 12 pencilan kulat dan bakteria yang dikesan dan dipencilkan daripada biji benih kacang soya didapati berpotensi sebagai agen biologi terhadap C. truncatum dalam kultur saingan. Mereka adalah Chaetomium globosum. Curvularia lunata, Fusarium moniliforme. F. oxysporum. F. semitectum dan Bacillus sp.. C. globosum. F. oxysporum dan Bacillus sp. dipilih untuk kajian seterusnya sebagai rawatan biji benih dibandingkan dengan 6 racun kulat yang biasa digunakan. Kesemua rawatan biji benih mengurangkan kejadian C. truncatum dengan signifikan dalam biji benih secara in vitro. Walau bagaimanapun, ujian di rumah kaca, menunjukkan hanya rawatan racun kulat, Delsene, Benlate, Captan dan Thiram membaikelokan kadar percambahan dan penjelmaan anak benih dibandingkan dengan kawalan.

CHAPTER I

INTRODUCTION

Soybean, Glvcine *max* (L.) Merrill is a subtropical annual short day legume. It originated in the northeastern provinces of China and Manchuria (Probst and Judd, 1973; Hymowitz, 1976). The crop is now the most important grain legume and is widely cultivated throughout the world due to the strong demand for edible oils and protein feed supplements. Table 1 shows the production trend around the world during the past 20 year period (between 1960-1980). Major increased in world production were in the United States followed by several countries of the America's, mostly Brazil and Argentina, whereas production in Asia remained nearly static (Hinson and Hartwig, 1982). Increased production in most countries have been contributed by an increased in areas planted and the adoption of improved cultivars and management practices. However, yield was relatively quite low in developing countries especially in Asia. At present, United States is the biggest producer and exporter of soybean, producing about 51.8 million mt, followed by Brazil, China and Argentina, producing 18.5, 11.8, and 8.5 million mt, respectively (Sinclair and Backman, 1989).

Soybean is an important field crop as a major source of protein and edible oil. The approximate composition of soybean