UNIVERSITI PUTRA MALAYSIA

CHARACTERISATION OF CAUSATIVE AGENTS AND PATHOLOGICAL ASSESSMENT OF SELECTED CORN INBRED LINES AGAINST SOUTHERN LEAF BLIGHT DISEASE IN MALAYSIA

ABDULAZIZ BASHIR KUTAWA

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By

ABDULAZIZ BASHIR KUTAWA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

May 2016

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DEDICATION

This research work is dedicated to my beloved Father and Mother, for their excellent encouragement, caring, advise and constant prayers during the course of my study. To the memory of my late grandfather, and my beloved grandmothers. The words could have been silent without you.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

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Chairman : Assoc. Prof. Kamaruzaman Sijam, PhD
Faculty : Agriculture

In Malaysia, corn is produced in small scale due to many diseases affecting this crop as a result of poor cultural practices or by planting susceptible hybrids to diseases. Leaf blight diseases have been identified as one of the main constraint for corn production in Malaysia. Yield loss could be up to 20-90%, if necessary disease management strategies were not employed, and this may leads to low income generation to the farmers. This research was carried out to characterize the causative agents of northern and southern leaf blight diseases in corn, and to assess resistance in selected inbred lines against southern leaf blight disease, as well as to determine the plant secondary metabolites produced during the interaction. Infected leaf samples were collected from infected farms of five major corn growing areas in Peninsular Malaysia from 2014 to 2015. A total of 15 isolates, were studied for their morphology. Based on morphological characteristics, the isolates were identified as *Exserohilum turcicum* and *Cochliobolus heterostrophus*. The conidial shapes for most of these pathogens were spindle, elongated and curved. Results of cultural characteristics showed that, the isolates varied in colony growth and colour. Colony growth rate of 15 isolates was significantly different at $P \leq 0.05$ after being grown on three different media viz., potato dextrose agar (PDA), corn meal agar (CMA) and potato sucrose agar (PSA), where PSA showed the highest growth rate for southern leaf blight pathogen, while CMA was the highest for northern leaf blight pathogen and PDA media was the least for all the pathogens. Based on the colony colour, all the 15 isolates were grouped into five categories i.e. dark gray, light gray, gray, light to gray and gray to green. Based on colony growth, the 15 isolates were categorized into 3 groups' viz., poor restricted growth, moderate growth and profused growth. Result of conidial measurement indicated that, the number of septa ranged from 4-6 and 8-10 for isolates CH006 and CH004, respectively. The highest conidial length was 89.44 µm for isolates ET003 and the least isolate was CH006 with 44.12 µm. Likewise, the highest conidial width was 17.43 µm for isolates CH004 and the least were isolates ET002 and CH009 having 11.34 µm. Based on the pathogenicity test, isolate CH001 and CH009 shows the highest level of aggressiveness with disease severity index of 80% each, at the fourth week after inoculation. Isolate ET005 was found to be the least aggressive among the isolates tested, by having disease severity index of 22%. Molecular characterisation confirmed the identification of species, 10 of

the isolates were *C. heterostophus* and 5 of the isolates were *E. turcicum*. Both the morphological and molecular identification have showed the same results. Results from assessment of resistance of selected inbred lines showed that, line SLBR5 was the most resistant line with mean disease severity index of 17.35%, while line SLBS3 was the most susceptible line by having mean disease severity 51.65%. The concentration of peroxidase (PO), polyphenols oxidase (PPO) and total phenolic content (TPC) were determined. In PO, a resistant inbred line, SLBR5 produced higher compounds with 6320, 7600 and 5800 mgGAE/g at 1st, 2nd and 3rd week after inoculation, respectively. Susceptible line, SLBS3 was found to produce less with 1640, 1800 and 1920 mgGAE/g at the same assessment periods. For PPO, inbred line SLBR5 was also found to produce higher PPO with 2440, 2560, and 2760 mgGAE/g at the 1st, 2nd and 3rd week after inoculation, respectively. While SLBS2 produced less PPO with 1080, 1240 and 880 mgGAE/g at the 1st, 2nd and 3rd week after inoculation, respectively. Similarly, in TPC, inbred line SLBR5 produced the highest TPC with 15720, 15960 and 17720 mgGAE/g at the 1st, 2nd and 3rd week after inoculation, respectively. Line SLBS3 was found to produce less TPC with 11960, 10240 and 10840 mgGAE/g at the 1st, 2nd and 3rd week after inoculation, respectively.

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

PENCIRIAN AGEN PENYEBAB DAN PENILAIAN PATOLOGI BARISAN INBRED JAGUNG TERPILIH TERHADAP PENYAKIT HAWAR DAUN SELATAN DI MALAYSIA

Oleh

ABDULAZIZ BASHIR KUTAWA

Mei 2016

Pengerusi : Profesor Madya Kamaruzaman Sijam, PhD
Fakulti : Pertanian

Di Malaysia, jagung dihasilkan dalam skala yang kecil kerana diserang dengan banyak penyakit yang berpunca daripada amalan kultura yang buruk atau menaman hibrit yang rentan penyakit. Penyakit hawar daun telah dikenalpasti sebagai salah satu darpada halangan utama pengeluaran jagung di Malaysia. Kehilangan hasil boleh mencecah 20-90%, sekiranya tiada langkah pengurusan penyakit dilaksanakan, dan ini akan menyebabkan penjanaan pendapatan yang rendah kepada petani. Kajian ini telah dijalankan untuk mencirikan agen penyebab penyakit hawar daun utara dan selatan pada jagung dan menilai kerintangan barisan inbred terpilih terhadap penyakit hawar selatan seterusnya menentukan metabolit sekunder tumbuhan yang terhasil daripada interaksi tersebut. Sampel daun berpenyakit telah dikumpul daripada lima buah ladang jagung di kawasan penanaman utama di Malaysia dari tahun 2014-2015. Sebanyak 15 isolat kulat telah disisih dan dikaji morfologinya. Berdasarkan pencirian morfologi, isolat tersebut dikenalpasti sebagai *Exserohilum turcicum* dan *Cochiliobolus heterostrophus*. Bentuk konidia bagi kebanyakan patogen adalah berbentuk gelendong, memanjang dan melengkung. Keputusan bagi pencirian morfologi kultur menunjukkan bahawa variasi pada pertumbuhan koloni dan warna isolat. Kadar pertumbuhan koloni bagi 15 isolat adalah sangat berbeza, $P \leq 0.05$ selepas dikultur pada tiga jenis media iaitu 'potato dextrose agar' (PDA), 'corn meal agar' (CMA) dan 'potato sucrose agar' (PSA), yang mana PSA menunjukkan kadar pertumbuhan yang paling tinggi bagi patogen hawar daun selatan, sementara CMA adalah tertinggi bagi patogen hawar daun utara dan PDA adalah yang terendah bagi semua patogen. Berdasarkan warna koloni pula, kesemua 15 isolat telah dikelaskan kepada lima kategori iaitu kelabu gelap, kelabu terang, kelabu, kelabu terang kepada kelabu, kelabu kepada hijau. Berdasarkan pertumbuhan koloni, 15 isolat telah dikategorikan kepada tiga kumpulan iaitu pertumbuhan rendah, pertumbuhan sederhana dan pertumbuhan tinggi. Keputusan ukuran konidia menunjukkan bahawa bilangan septa di antara 4-6 dan 8-10 bagi isolat CH006 dan CH004. Konidia terpanjang adalah 89.44 µm bagi ET003 dan terpendek adalah 44.12 µm bagi CH006. Begitu juga, saiz lebar konidia terpanjang adalah 17.43 µm bagi isolat CH004 dan terpendek adalah isolat ET002 dan CH009 dengan 11.34 µm. Berdasarkan ujian patogenisiti, isolat CH001 dan CH009 menunjukkan paras aggrasif yang tinggi dengan indek keparahan penyakit 80% pada minggu ke-4 selepas diinokulasi. Isolat ET005 didapati kurang

aggresif di kalangan isolat yang diuji, dengan indek keparahan penyakit, 22%. Pencirian molekular mengesahkan pengecaman spesis, 10 daripada 15 isolat adalah *C. heterostrophus* dan lima daripada bakinya adalah *E. turcicum*. Kedua-dua pencirian molekular dan morfologi menunjukkan keputusan yang sama. Keputusan daripada penilaian kerintangan barisan inbred menunjukkan bahawa, baris SLBR5 didapati paling rintang dengan purata indek keparahan penyakit, 17.35% manakala baris SLBS3 adalah paling rentan dengan purata indek keparahan penyakit, 51.65%. Kepekatan peroksida (PO), polifenol oksida (PPO) dan jumlah kandungan fenol (TPC) telah ditentukan. Bagi PO, baris rintang, SLBR5 menghasilkan kompoun tertinggi iaitu 6320, 7600 dan 5800 mgGAE/g pada minggu ke-1, ke-2 dan ke-3 selepas diinokulasi. Baris rentan, SLBS3 didapati menghasilkan lebih rendah iaitu 1640, 1800 dan 1920 mgGAE/g pada masa penilaian yang sama. Bagi PPO, baris rintang, SLBR5 didapati menghasilkan PPO yang tinggi iaitu 2440, 2560 dan 2760 mgGAE/g pada minggu ke-1, ke-2 dan ke-3 selepas diinokulasi. Manakala SLBS2 menghasilkan PPO yang terendah iaitu 1080, 1240 dan 880 mgGAE/g pada minggu ke-1, ke-2 dan ke-3 selepas diinokulasi. Begitu juga dengan TPC, baris rintang SLBR5 menghasilkan TPC tertinggi iaitu 15720, 15960 dan 17720 mgGAE/g pada minggu ke-1, ke-2 dan ke-3 selepas diinokulasi. Baris SLBS3 didapati menghasilkan TPC lebih rendah iaitu 11960, 10240 dan 10840 pada minggu ke-1, ke-2 dan ke-3 selepas diinokulasi.

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I certify that a Thesis Examination Committee has met on 05 May 2016 to conduct the final examination of Abdulaziz Bashir Kutawa on his thesis entitled "Characterisation of Causative Agents and Pathological Assessment of Selected Corn Inbred Lines Against Southern Leaf Blight Disease in Malaysia" in accordance with the Universities and University Colleges 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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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Signature: _____
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AL	Aluminium
ANOVA	Analysis of variance
AUDPC	Area under disease progressive curve
AVR	Avirulence
BP	Base pairs
BXS	Benzoxazinoids
CMA	Corn meal agar
DI	Disease incidence
DNA	Deoxyribonucleic acid
DSI	Disease severity index
EU	European union
FAO	Food and Agricultural Organization
GPDH	Glycerol-3-phosphate dehydrogenase
GWG	Green world genetics
HT	High-throughput
ITS	Internal transcribed spacer
KB	Kilo base
LSD	Least significant differences
MM	Millimeter
MEA	Malt extract agar
MLB	Maydis leaf blight
MR	Moderately resistant
MS	Moderately susceptible
NCLB	Northern corn leaf blight
OECD	Organization for economic Development and cooperation
OPC	Open photoacoustic cell technique
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PO	Peroxidase
PPO	Polyphenols oxidase
PSA	Potato sucrose agar
PVP	polyvinyl pyrrolidone
QTL	Quantitative trait loci
R	Resistant
RP	*Retinitis pigmentosa*
S	Susceptible
SCLB	Southern corn leaf blight
TCMS	Texas cytoplasmic male sterility
TLB	Turcicum leaf blight
TPC	Total phenolic content
USDA	United States Department of Agriculture
WAI	Week after inoculation
WT	Wild type

CHAPTER 1

INTRODUCTION

1.1 Background

Cereals are considered as the most vital staple crops in the world including Asia. These crops have the most established record of cultivation by humanity, and have taken after difficult and interrelated course of evolution (Harlan *et al.,* 1998). Human civilisation has been, and is still, firmly connected with cereals and has moved over the world conveying cereals culture amid humankind relocations. This is reflected through the journey of rice, wheat, millets and barley from old to the new world and of corn on the other hand. Numerous different crops are cultivated with, before or after this crop, to fulfill the taste and address the issue of people and also their necessity for dress, animal feed and shelter (Sadras *et al.,* 2009). Wheat, rice and corn contribute about 60% of the human food globally (Tilman *et al.,* 2002). These three crops represent over 90% of Asia's cereal production and cover more than 284 million ha of prime agricultural area (FAO, 2008). The minor crops incorporate sorghum, barley and other several kinds of millets. General production of cereals has been always expanding in Asia regardless of declining in cumulative region compared in production in 1980s (FAO, 2008). Corn is the second most imperative cereal crop after rice in Asia. It is the substitute staple for individuals in mountainous and rural areas, particularly amid times of rice deficiency. It is cultivated purposely for human utilization as either fresh or processes form. In addition, corn is produced for industrial purposes and animal feed, the industrial uses of corn includes: flour, ethanol, cooking syrup and starch (Kolawole, 2009).

Every year, different types of pathogens i.e. fungi, viruses, bacteria and nematodes caused significant loss by affecting corn plant worldwide. Various diseases such as southern rust, common rust, sting, brown spots, seed rots, stalk rots, corn dwarf mosaic virus, northern corn leaf blight and southern corn leaf bight were found to affect corn, with individual fields regularly enduring serious losses. All parts of corn are vulnerable to attack (the leaves, stalks, roots and ears) at different phases of growth. Consequently, these diseases reduce the quality and value of the grain and might influence the operational costs. In corn ear, stalk and leaf disease for the most part are favoured by wet and warm climate (Harry *et al.,* 2014).

In Malaysia, northern and southern corn leaf blight, leaf rust and spots still remain the main foliar disease of corn in relatively most areas where corn is grown. This research will therefore be directed towards investigating two main diseases of corn southern and northern corn leaf blight diseases in Malaysia. So far, not many investigations have been put in place to study these diseases, therefore it might be quite challenging task to know the background as well as the current status of corn leaf blight diseases in Malaysia due to the inadequacy of published materials, since there are only few reports, if any on the prevalence of corn in corn growing areas in Malaysia. There is high requirement for an investigation on the portrayal of the pathogen and management strategies of leaf blight diseases in corn producing zones. Despite the fact that, many researchers have worked to

find out the actual pathogens responsible for causing southern and northern corn leaf blight diseases, up to now, a lot of work needs to be done with regards to morphological variability of these pathogens, for better and easy identification and to put more emphasis on production of hybrids that are resistant to the diseases. Based on this, the research work is focused to find out more about the molecular and morphological variability of northern and southern corn leaf blight pathogens, as well as to determine the aggressiveness of the fungal pathogens. Chapter 3 will focus on isolation and characterisation of corn leaf blight pathogens, while the assessment of resistance among selected inbred lines of corn, and determination of secondary metabolites produced during the interaction will be discuss in Chapter 4.

1.2 Objectives of the study

The objectives of this research are to:

i. Isolate and characterize the causative agents of northern and southern corn leaf blight diseases in Malaysia.
ii. Assess disease resistance of selected inbred lines against southern corn leaf blight disease.
iii. Quantitate selected secondary metabolites, produced by tested corn inbred lines during the interaction.

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