



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 menanam hibrid yang rentan penyakit. Penyakit hawar daun telah dikenalpasti sebagai salah satu daripada halangan utama pengeluaran jagung di Malaysia. Kehilangan hasil boleh mencecah 20-90%, sekiranya tiada langkah pengurusan penyakit dilaksanakan, dan ini akan menyebabkan penjaan 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 *Cochliobolus 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

agresif di kalangan isolat yang diuji, dengan indeks keparahan penyakit, 22%. Pencirian molekular mengesahkan pengesanan spesies, 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 indeks keparahan penyakit, 17.35% manakala baris SLBS3 adalah paling rentan dengan purata indeks keparahan penyakit, 51.65%. Kepekatan peroksida (PO), polifenol oksida (PPO) dan jumlah kandungan fenol (TPC) telah ditentukan. Bagi PO, baris rintang, SLBR5 menghasilkan kompaun 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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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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LIST OF ABBREVIATIONS

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	<i>Retinitis pigmentosa</i>
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.

REFERENCES

- Agrios, G. N. 2005. *Plant Pathology* (5th ed.). USA: Elsevier Academic Press, 952 p.
- Ait Barka, E. and Nowak, J. C. 2006. Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia phytofirmans* strain PSJN. *Applied Environmental Microbiology*, **72**: 724–725.
- Alcorn, J. L. 1983. A Generic concepts of *Drechslera*, *Bipolaris* and *Exserohilum*. *Mycotaxon* **17**: 1–86.
- Alcorn, J. L. 1988. The taxonomy of “*Helminthosporium*” species. *Annual Review of Phytopathology* **26**: 37–56.
- Almaguer, M., Rojas, T. I., Dobal, V. and Batista, A. M. 2012. Effect of temperature on growth and germination of conidia in *Curvularia* and *Bipolaris* species isolated from the air. *Aerobiologia* **53**: 29–57.
- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, Z., Miller, W. and Lipman, D. J. 1997. Blast and Psi-Blast: Protein database search programs. *Nucleic Acid Research*, **25**(17): 2289–4402.
- Amin, N., Nasruddin, A. and Daha, L. 2012. Isolation, Identification and *in-vitro* screening of fungal endophytes against pathogen of maize leaf blight, *Helminthosporium Maydis*. *Indonesian Phytopathology* **20**: 37–92.
- Andreasen, M. F., Kroon, P. A., Williamson, G. and Garcia-Conesa, M. T. 2001. Esterase activity able to hydrolyze dietary antioxidant hydroxycinnamates is distributed along the intestine of mammals. *Journal of Agricultural Food Chemistry* **49**: 5679–5684.
- Anselmi, C., Centini, M., Granata, P., Sega, A., Buonocore, A., Bernini, A. and Facino, R. M. 2004. Antioxidant activity of ferulic acid alkyl esters in a heterophasic system: a mechanistic insight. *Journal of Agricultural Food Chemistry* **52**: 6425–6432.
- Ates, E., Godula, M., Stroka, J. and Senyuva, H. 2014. Screening of plant and fungal metabolites in wheat, maize and animal feed using automated online clean-up coupled to high resolution mass spectrometry. *Food Chemistry* **142**: 276–284.
- Ayers, J. E., Nelson, R. R., Berger, R. D., Koons, C. and Scheifele, G. L. 1970. Plant Disease and Reproduction. *Phytopathology* **54**: 277–281.
- Bajaj, H. K. and Bhatti, D. S. 1985. New and known species of *Pratylenchus filipjev*, 1936 (Nematoda: Pratylenchidae) from Haryana, India, with remarks on intraspecific variations. *Journal of Nematology* **16**: 360–367.

- Balint-Kurti, P. J., Zwonitzer, J. C., Wisser, R. J., Carson, M. L., Oropeza-Rosas, M. a., Holland, J. B. and Szalma, S. J. 2007. Precise mapping of quantitative trait loci for resistance to southern leaf blight, caused by *Cochliobolus heterostrophus* race O, and flowering time using advanced intercross maize lines. *Genetics* **176**(1): 645–657.
- Begoude, B. A. D., Slippers, B., Wingfield, M. J. and Roux, J. 2010. *Botryosphaeriaceae* associated with *Terminalia catappa* in Cameroon, South Africa and Madagascar. *Mycological Progress* **9**(1): 101–123.
- Bennett, R. N. and Wallsgrave, R. M. 1994. Secondary metabolites in plant defence mechanisms. *Phytopathology* **127**: 617–633.
- Berbee, M. L. and Pirseyedi, M. H. S. 1999. *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia* **91**: 964–977.
- Blandino, M., Galeazzi, M., Savoia, W. and Reyneri, A. 2012. Timing of azoxystrobin+propiconazole application on maize to control northern corn leaf blight and maximize grain yield. *Field Crops Research* **139**: 20–29.
- Boedijn K. B. and Einige, U. 1933. Phragmosporen Dematiaceen. *Annales Du Jardin Botanique de Buitenzorg* **13**: 123-155.
- Boote, K. J., Jones, J. W., Mishoe, J. W. and Berger, R. D. 1983. Coupling pests to growth simulators to predict yield reductions. *The American Phytopathological Society* **21**: 371- 400.
- Boyer, C. D. and Len, C. C. H. 1994. *Kernel Mutants Corn*. USA: CRC Press Inc. Boca Raton, 26 p.
- Bravo, H. R. and Lazo, W. 1996. Antialgal and antifungal activity of natural hydroxamic acids and related compounds. *Journal of Agriculture and Food Chemistry* **44**: 1569–1571.
- Buckler, E. S., Thornsberry, J. and San, K. 2001. Molecular diversity, structure and domestication of grasses. *Genetical Research* **77**: 213 – 218.
- Campbell, C. L. and Madden, L. V. 1990. *Introduction to Plant Disease Epidemiology*. USA: Wiley-Interscience Press, 532 p.
- Cerović, S., Radosav B. and Pajić, Z. 2014. Pollen germination and pollen tube growth in ZP maize lines. *Genetika* **46**(3): 935–948.
- Choudhary, D. K., Prakash, A. and Johri, B. N. 2007. Induced systemic resistance (ISR) in Plants: Mechanism of action. *Indian Journal of Microbiology* **47**(4): 289–297.
- Chizzali, A., Cornelia, S. and Beerhues, L. 2012. "Phytoalexins of the Pyrinae: Biphenyls and dibenzofurans. *Journal of Organic Chemistry* **8**: 613–620.

- Daniel Abebe, N. S. 2006. Morphological , cultural and pathogenicity variation of *Exserohilum turcicum* (Pass) Leonard and Suggs isolates in maize (*Zea mays* L.). *Kasetsart Journal of Natural Science* **40**: 341–352.
- Degani, O. 2014. Pathogenicity assay for *Cochliobolus heterostrophus* G-Protein and MAPK signaling deficiency strains. *American Journal of Plant Sciences* **5**(9): 1318–1328.
- Del Pozo, D., Brenes, C. H., Serna, S. O. and Talcott, S. T. 2006. Polyphenolic and antioxidant content of white and blue corn (*Zea mays* L.) products. *Food Research International* **39**(6): 696–703.
- Delp, B. R., Stowell, L. J. and Marois, J. J. 1986. Field runner: A disease incidence, severity, and spatial pattern assessment system. *Plant Disease* **70**:954-957.
- Devegowda, A. S. 2004. Rice and corn quality workshop,. Retrieved from <http://pr.hec.gov.pk/Chapters/128S-2.pdf> *Review of Literature*, 22–24.
- Dhugga, K.S. and Grünwald, N. J. 2007. Maize biomass yield and composition for biofuels. *Crop Science* **47**: 2211–2227.
- Didvania, S., Shah, R. and John, K. S. 2012. A new disease of bell pepper (*Capsicum annum* var.grossum) caused by *Drechslera bicolor*, its pathophysiology, efficacy of fungicides and botanicals. *Journal of Plant Pathology* **70**: 332-341.
- Drechsler, C. 1934. Leaf spot of maize caused by *Ophiobolus heterostrophus*, the asigerous stage of *Helminthosporium* exhibiting bipolar germination. *Journal of Agricultural Resources* **31**: 701–26.
- Duvick, D. N. 2005. The contribution of breeding to yield advances in maize (*Zea mays* L.). *Advances in Agronomy* **86**: 21-33.
- Emami, K. and Hack, E. 2002. Conservation of XYN11A and XYN11B Xylanase Genes in *Bipolaris sorghicola*, *Cochliobolus sativus*, *Cochliobolus heterostrophus*, and *Cochliobolus spicifer*. *Journal of Microbiology* **7**(45): 303–306.
- FAO. 2008. Statisticaldatabase.<http://www.fao.org> (Verified on 4 September 2008).
- FAO. 2015. Statisticaldatabase.<http://www.fao.org>.
- Farfan, I. D. B., Murray, S. C., Labar, S. and Pietsch, D. 2013. A multi-environment trial analysis shows slight grain yield improvement in Texas commercial maize. *Field Crops Research* **149**: 167–176.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783-791
- Guarro, J., Soler, L. and Reu, M. G. 1999. Pathogenicity and antifungal susceptibility of *Chaetomium* species. *European Journal of Clinical Microbiology Infected Diseases* **14**: 613–618.

- Hallauer, A. R. 2000. Potential for outcrossing and weediness of genetically modified insect protected corn. *APHIS-USDA* **10**: 97–110.
- Hari Kumar Prasai, S., Sharma, U. K. and Jen. S. 2015. Evaluation of quality protein maize and drought tolerant maize in far western hills of Nepal. *Int J. Appl Sci Biotechnol* **3**(3): 387–391.
- Harlan, J.R., James, M. J., De, W. and Eden, G. P. 1998. *Comparative Evolution of Cereals*. USA: Evolution Press, 1–17 p.
- Harlapur, S. I., Kulkarni, M. S., Hedge, Y. and Kaikarni, S. 2007. Variability in *Exserohilum turcicum* (Pass) Leonard Suggs, causal agent of *turcicum* leaf blight of maize. *Karnatak Journal of Agricultural Research* **20**(3): 665–666.
- Harry, E. Duncan, J. and Gary, K. J. 2014. Major corn diseases in north Carolina. *Plant Pathology* **20**: 453–455.
- Hesseltine, C. W., Ellis, J. J. and Shotwell, O. L. 1971. Secondary Metabolites, southern leaf blight of corn, and Biology. *Agricultural and Food Chemistry* **19**(4): 707–717.
- Ho, W. C. and Ko, W. H. 2007. A simple method for obtaining single spore isolates of fungi. *Botanical Bulletin of Academia sinica* **38**: 41–44.
- Ito, S. 1930. On some new ascigerous stages of the species of *Helminthosporium* parasitic on cereals. *Proceedings of the Imperial Academy Japan* **6**(8): 352–355.
- Jenkins, C. E. and M. T. 1946. *Helminthosporium turcicum* leaf blight of corn. *Phytopathology* **36**: 660–666.
- Johnson, R. and Moy, P. H. 1994. Corn pollination under moisture and high temperature stress. Proceedings of the corn and sorghum industry research. *American Seed* **21**: 66–67.
- Kachapur, M. R. and Hegde, R. K. 1988. Studies on *turcicum* leaf blight of maize caused by *Exserohilum turcicum*. *Plant Pathology* **6**: 33–35.
- Kim, H. S., Sneller, C. H., Diers, B. W. 1999. Evaluation of soybean cultivars for resistance to sclerotinia stem rot in field environments. *Crop Science* **39**: 64–68
- Kinraide, L., Peter, R., Ryan, T. and Ban, V. K. 1991. Al³⁺-Ca²⁺ interactions in aluminum rhizotoxicity. *Journal of Phytopathology* **192** (1): 98–103.
- Kokkinakis, D. M. and Brook, J. L. 1979. Tomato peroxidase: purification, characterization and catalytic properties. *Plant Physiology* **63**: 93–99.
- Kolawole, L. A. 2009. Growth and yield of maize as influenced by sowing date and poultry manure application. *Notulae Botanicae Horti Agrobotanici* **37**: 221–232.

- Kroon, L. P. N. M., Bakker, F. T., Van den Bosch, G. B. M., Bonants, P. J. M., and Flier, W. G. 2004. Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. *Fungal Genetics and Biology* **41**(8): 766–782.
- Kumar, A., Guta, M. and Mahmood, A. 1977. Studies on leaf blight of maize caused by *Helminthosporium turcicum* Pass. and *Helminthosporium maydis* Nisk and Miyake,. *Science and Culture* **42**: 533–535.
- Kump, K. I., Bradbury, P. J., Wisser, R. J., Buckler, E. S., Belcher, A. R., Rosas, M. O. A., Zwonitzer, J. C., Kresovich, S., McMullen, M. D., Ware, D., Balint-Kurti, P. J. and Holland, J. B. 2011. Genome-wide association study of quantitative resistance to southern leaf blight in maize nested association mapping population. *Nature Genetics* **43**:163-68.
- Leonard, K. J. and Suggs, E. G. 1974. *Setosphaeria prolata*, the ascigerous state of *Exserohilum prolatum*. *Mycologia* **66**: 281–297.
- Levic J. and Viktorija P. 1993. Morphology of a New pathotype of *Bipolaris zeicola* (Stout) Shoemaker. *Journal of Phytopathology* **139**(4): 339–346.
- Levy, Y. 1991. Variation in fitness among field isolates of *Exserohilum turcicum* in Israel. *Plant Disease* **75**: 163–166.
- Lim, S. M., Hooker, A. L. and Paxton, J. D. 1971. Disease Determination of *Helminthosporium maydis* Race T. *Journal of Phytopathology* **62**(1): 968–971.
- Luttrell, L. S. 1958. Taxonomic criteria in *Helminthosporium*. *Mycologia* **55**: 643-674.
- Madden, L. V. and Hughes, G. 1995. Plant disease incidence: distributions, heterogeneity, and temporal analysis. *Annual Review of Phytopathology* **33**: 529-564.
- Madden, L. V., Hughes, G. and Van den Bosch, F. 2007. *The Study of Plant Diseases Epidemics*. USA: APS Press, 621 P.
- Manamgoda, D. S., Cai, L. and Bahkali, A. H. 2011. *Cochliobolus*: an overview and current status of species. *Fungal Diversity* **51**: 3–42.
- Manamgoda, D. S., Cai, L. and McKenzie, E. H. C. 2012. A phylogenetic and taxonomic re-evaluation of the *Bipolaris* – *Cochliobolus* – *Curvularia* complex. *Fungal Diversity* **56**: 131–144.
- Manamgoda, D. S., Rossman, A. Y., Castlebury, L. A., Crous, P. W., Madrid, H., Chukeatirote, E. and Hyde, K. D. 2015. The genus *Bipolaris*. *Studies in Mycology* **79**: 221–288.
- Margaret, T. M. 2013. Effectively managing northern and southern corn leaf blight. *Phytopathology* **21**(8): 221-234.

- Marocco, A, Lorenzoni, C., and Fracheboud, Y. 2005. Maize disease resistance. *Maydica* **50**: 571–580.
- Mateille, T. and Folkertsma, S. 1994b. A survey of nematodes and fungi in roots of banana CV Poyo in the Ivory Coast. *Review of Nematology* **14**(1): 3-8.
- Miller, P. R. 1970. Southern corn leaf blight. *Plant Disease Reporter* **54**: 1099–1136.
- Mitchell, J. and John, F. P. 1988. Heat stress effects on isolated reproductive organs of maize. *Plant Physiology* **133**: 2625–68.
- Mohali, S., Slippers, B. and Wingfield, M. J. 2006. Two new *Fusicoccum* Species from *Acacia* and *Eucalyptus* in Venezuela based on morphology and DNA sequence data. *Mycological Research* **110**(4): 405–415.
- Morris, M. L. 1998. *Maize Seed Industries in Developing Countries*. Publishers,. Inc. and Lynne Rienn, Int. Retrieved from <http://link.springer.com/10.1007/s10327-014-0519-1>.
- Muiru, W. M., Mutitu, E. W., and Ken, J. W. 2008. Distribution of *turcicum* leaf blight of maize in Kenya and cultural variability of its causal agent, *Exserohilum turcicum*. *Journal of Tropical Microbiology and Biotechnology* **4**(1): 32–39.
- Muiru W. M., Koopmann, B., Tiedemann, A. V. and Mutitu, K. J. 2010. Race typing and evaluation of aggressiveness of *Exserohilum turcicum* isolates of Kenyan, German and Austrian origin. *Journal of Agricultural Sciences* **6**(3): 277–284.
- Mungoma, C. and Pollak, L. M. 1998. Heterotic patterns among ten Corn Belt and exotic maize population. *Crop Science* **28**:500-504.
- Naz, I., Sehar, S., Perveen, I., Rehman, A., Hameed, A., Ahmad, Y. and Ahmed, S. 2012. Optimization of cultural conditions for *Cochliobolus heterostrophus* isolates from infected maize plants from different Agricultural Zones of Pakistan. *British Microbiology Research Journal* **2**(4): 233–242.
- Nelson, R. R., Mek, D. R. and schiefefe, G. L. 1970. Interaction of genes for phytopathogenicity and virulence in *Trichometaspaeria turcica* with different numbers of gene for vertical resistance in *Zea mays*. *Phytopathology* **60**: 1250–1254.
- Niemeyer, H. M. 1988. Hydroxamic acids (4-hydroxy-1,4 benzoxazin-3-ones), defence chemicals in the *gramineae*. *Phytochemistry* **27**: 3349–3358.
- Nisikado, Y. 1929. Studies on the *Helminthosporium* diseases of *Gramineae* in Japan. *Berichte Des Ohara Institute Landwirtschaftliche Forschungen* **4**: 111–126.
- Odjakova, M. and Hadjiivaova, C. 2001. The complexity of pathogen defense in Plants. *Bulgarian Journal of Plant Physiology* **27**(1): 101-109.

- Organization for Economic Cooperation and Development (OECD). 2005. Biology of *Zea mays* subsp. *mays* (Maize). *ENV/JM/MONO*, 34p.
- Pan, X., Niu, G. and Liu, H. 2003. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chemical Engineering and Processing* **42**(2): 129–133.
- Pandurangegowda, K. T., Shetty, H. S., Gowda, B. J. and Prakash, S. 1993. Comparison of two methods for assessment of yield losses due to *turcicum* leaf blight of maize. *Indian Phytopathology* **45**: 316–320.
- Pataky, J., Williams, M., Meyer, M., Warsaw, B. and Moody, J. 2008. Sweet corn hybrid disease nursery. *Phytopathology* **7**: 73-97.
- Peipp, H., Maier, W., Schmidt, J. and Wray, V. S. D. 1997. Arbuscular mycorrhizal fungus-induced changes in the accumulation of secondary compounds in barley roots. *Phytochemistry* **44**: 581–587.
- Praveen Kumar, M., Narayan Reddy, P. R. and Ranga Reddy, A. S. 2010. Management of *turcicum* leaf blight caused by *Exserohilum turcicum* in maize. *Indian Journal of Plant Protection* **38**(1): 63–66.
- Rahul, K. and Singh, I. S. 2002. Inheritance of resistance to banded leaf and sheath blight (*Rhizoctonia solani* f. sp. *Sasakii*) of maize. *Phytopathology* **11**: 356-65.
- Rajeshwar, R. P., Narayan, R., Ranga, R. S. and Sefa, R. 2014. Cultural and morphological variability among *Exserohilum turcicum* Isolates. *Indian Journal of Agriculture* **34**: 37-52.
- Ramakrishna, W. J., Emberton, M., Ogden, P., SanMiguel, and Joh. L. 2002. Structural analysis of the maize Rpl complex reveals numerous sites and unexpected mechanisms of local rearrangement. *Plant Cell* **14**: 213–223.
- Raymundo, A. and Hooker, A. 1981. Measuring the relationship between northern corn leaf blight and yield losses. *Plant Disease* **81**: 541 620.
- Reddy, T. R., Pan, N., Reddy, R. R. and Pradesh, A. 2013. Review article *Turcicum* leaf blight of maize incited by *Exserohilum turcicum* : A review. *IJABPT* **5**(1): 376–455.
- Richardson, A., Barea, J. M. and McNeill, A. P. C. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil* **321**: 305–339.
- Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P. and Glover, W. 1999. Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry* **66**(4): 401–436.
- Robert, A. L. and Sprague, G. F. 1960. Adaption of the corn leaf blight fungus to a resistant and susceptible corn host. *Phytopathology* **50**: 261–263.

- Sadras, V., Calderini, D., and Sharma, R. C. 2009. *Crop Physiology*. USA: Elsevier Academic Press, 10 p.
- Salati, M. A. I. 2010. First report of *Pseudoperonospora cubensis* causing downy mildew of *Trichosanthes cucumerina* in Malaysia. *Plant Disease* **94**: 642-643.
- Samatha, T., Shyamsundarachary, R., Srinivas, P. and Swamy, N. R. 2012. Quantification of total phenolic and total flavonoid contents in extracts of *Oroxylum indicum*. *Asian Journal of Pharmaceutical and Clinical Research* **5**: 177-179.
- Savory, E. A. 2012. Analysis of the *Pseudoperonospora cubensis* transcriptome during cucumber (*Cucumis sativus* L.) Infection. *Mycologia* **7**(4): 351-360.
- Scheifele, G. L., Whitehead, W. and Rowe, C. 1970. *Journal of Plant Disease* **4**: 315-317.
- Sharma, J. P. and Mishra, B. 1988. Effect of spray schedule of mancozeb (dithane M-45) on *Turcicum* leaf blight & impact on grain yield in maize. *Indian Journal of Plant Protection* **16**: 189-193.
- Shimizu, K., Tanaka, C., and Peng Y. L. . 1998. Phylogeny of *Bipolaris* inferred from nucleotide sequences of Brn1, a reductase gene involved in melanin biosynthesis. *Journal of General and Applied Microbiology* **44**: 251-258.
- Shoemaker, S. 1959. Nomenclature of *Drechslera* and *Bipolaris*, grass parasites segregated from *Helminthosporium*. *Canadian Journal of Botany* **37** 879-887.
- Sicker, D., Frey, M., Schulz, M. and Gierl, A. 2000. Role of natural benzoxazinones in the survival strategy of plants. *Int. Rev. Cytol.* **198**: 319-346.
- Singh, G. P. 1966. A leaf spot disease of maize caused by *Bipolaris maydis* (Nisikado) Shoemaker. *Natural Academy of Science* **36**: 303-305.
- Singh, N. P., and Norong, N. N. 2005. *Maize in India : Production Systems, Constraints , and Research Priorities*. India: Mexa Press, 21-27 p.
- Singh, R. and Srivastava, R. P. 2012. Southern corn leaf blight- An important disease of maize : An extension fact sheet. *Indian Research Journal of Extension Education* **I**(I): 334-337.
- Singleton, V. L., Orthofer, R. and Lord, R. R. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymology* **299**: 152-178.
- Sivanesan, A. 1987. Graminicolous species of *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* and their teleomorphs. *Mycological Papers* **158**: 1-261.
- Sleper, D. A. and Poehlman, J. M. 2006. *Breeding Field Crops* (5th ed.). *Blackwell Publishing*, 200 p.

- Slinkard, K. S. V. 1977. Total phenol analyses: automation and comparison with manual methods. *American Journal of Entomology* **28**: 49–55.
- Smith, D. R., Hooker, A. L. and Lim, S. M. 1970. Physiologic races of *Helminthosporium maydis*. *Plant Disease Report* **54**: 819–22.
- Sprague, G. F., Dudley, J. W., Hallauer, A. R., Russell, W. A. and Lamkey, K. 1988. *Breeding in Corn*. U.S.A: WI Press, 463–564 p.
- Tefferi, A., Mengestu, H. and Welz, H. G. 1996. Assessment of damage and grain yield loss in maize caused by northern leaf blight in western Ethiopia. *Journal of Plant Disease and Protection* **103**: 353–363.
- Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., and Polasky, S. 2002. Agricultural sustainability and intensive production practices. *Nature*. **418** (68): 671–7.
- Tsaftaris, A. S. 1995. *The biology of maize (Zea mays, L.) Document XI/754/95 European Commission*.
- Tsuda, K., Nozoe, S., i'llorisaki, M., Hirai, K., Itai, A., Okuda, S., Canonica, L., Fiecchi, A., Galli Kienle, M. and Scala, A. 1977. Tetra- Izedron for controlling plant diseases. *Journal of Botany* **12**: 33–69.
- Turgeon, B. G., Garber, R. C. and Yoder, O. C. 1985. Transformation of the fungal maize pathogen *Cochliobolus heterostrophus* using the *Aspergillus nidulans* *ds* Gene. *Molecular and General Genetics* **201**: 450–453.
- Ullstrup, A. J. 1966. The impacts of southern corn leaf blight epidemics of 1970–1971. *Phytopathology* **10**: 37–50.
- United States Department of Agriculture (USDA). 2016. *Corn: World Markets and Trade*. USA: Foreign Agricultural Service, USDA, 37 p.
- Vieira, R. A., Mesquini, R. M., Silva, C. N., Hata, F. T., Tessmann, D. J. and Scapim, C. A. 2014. A new diagrammatic scale for the assessment of northern corn leaf blight. *Crop Protection* **56**: 55–57.
- Walker, V., Couillerot, O., Von Felten, A., Bellvert, F., Jansa, J., Maurhofer, M. and Comte, G. 2012. Variation of secondary metabolite levels in maize seedling roots induced by inoculation with *Azospirillum*, *Pseudomonas* and *Glomus* consortium under field conditions. *Plant and Soil* **356**: 151–163.
- Wallin, J. R. and Loonan, D. 1977. Temperature and humidity associated with sporulation of *Helminthosporium maydis* race T. *Phytopathology* **67**: 13: 70–72.
- Wei, J., Lui, K., Chen, J., Luo, P. and Lin, S. O. 1988. Pathological and physiological identification of race C of *Bipolaris maydis* in China. *Phytopathology* **78**: 550–54.

- Welz, H. G. and Geiger, H. H. 2000. Genes for resistance to northern corn leaf blight in diverse maize populations. *Plant Breeding* **119**: 1 – 14.
- White, T. J., Bruns, T., Lee, S. and Talor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics In: PCR protocols, a guide to methods and applications. *Journal of Molecular Biology* **44**: 315–322.
- Wise, K. 2015. Fungicide efficacy for control of corn diseases. *Purdue Extension Publication* **6**: 160-161.
- Ye, Y., Li, Q., Fu, G., Yuan, G., Miao, J. and Lin, W. 2005. Identification of antifungal substance (Iturin A2) Produced by *Bacillus subtilis* B47 and Its effect on southern corn leaf blight. *Journal of Integrative Agriculture* **11**(1): 90–99.
- Zaidi, P. H., Rashid, Z., Vinayan, M. T. and Anil Babu, T. 2012. Pre-germination anaerobic stress tolerance in tropical maize (*Zea mays* L.). *American Journal of Crop Science* **6**(12): 170–171.