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State of the art on southern corn leaf blight disease incited by *Cochliobolus heterostrophus*: detection, pathogenic variability and novel control measures

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Abstract

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Corn is grown mainly for human utilization either in its fresh or processed form. However, the decline in the average corn yield has been attributed to the poor cultural practices, and this essentially involves failure to employ effective control measures. Southern corn leaf blight (SCLB) disease has been identified as one of the main challenges hampering corn yield. In this regards, the main focus of this paper is to critically review the recent studies on the SCLB pathogen detection, pathogenic variability and control methods. This includes a concise overview of the taxonomy, morphological and molecular characteristics as well as pathogenicity. More so, the recent advances in the control measures were diligently reviewed and reported in this paper. Southern corn leaf blight is a foliar disease caused by a fungal pathogen *Bipolaris maydis*, anamorph or *Cochliobolus heterostrophus*, teleomorph. Based on morphological characteristics, the colony colour of the pathogen was found to be grey, grey to green, dark grey and light grey. The conidial shape was elongated and it length and width ranges from 42-133 µm and 6-21 µm respectively. Both universal and specific primers were used to identify the pathogen using molecular method. The best method of controlling the disease is by using resistant varieties like brocade TSW and gang won, some new inbred lines (SLBR3 and SLBR5) were recently assessed to SCLB in Malaysia and found to be resistant to the disease. Moreover, *Trichoderma harzianum* (SH2303) and *T. atroviride* (SG3403) have shown a strong biocontrol activity against SCLB pathogen. Cultural practices like crop rotation and tillage helps immensely in minimizing the crop residues in corn growing farms.

Keywords: Cochliobolus heterostrophus; control measures; detection; pathogenic variability; southern corn leaf blight

Abbreviations: cms-T (Texas Male-Sterile Cytoplasm); GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase); ITS (Internal Transcribed Spacer); PDA (Potato Dextrose Agar); SCLB (Southern Corn Leaf Blight); SLBR (Southern Leaf Blight Resistant); TSS (Thai Super Sweet); *TSW* (Thousand *Seed* Weight)

Introduction

Corn is grown mainly for human utilization as fresh or processed form. It is cultivated for industrial purposes and for feeding animals, the industrial uses of corn include: ethanol, flour, starch and cooking syrup (Kalawole, 2009). In developed nations corn is used for two purposes: 1) as raw material for extractive commercial ventures; and 2) as feed for animals, specifically in forage and grain or sold forms to the industry where feed is produced. In U.S, corn has little consideration as human food (Moris, 1998). In the European Union (EU), corn is utilized for consumption and also as raw material to the industries (Bashir et al., 2018). Being an insensitive and photoperiod crop, corn can be cultivated consistently in different periods of the year. It is developed in two seasons viz., autumn and spring. Notwithstanding, the potential of yield during spring crop is much higher than that of autumn sown corn yet high temperature at anthesis is an extreme imperative to accomplish the potential of the yield (Devegowda, 2004).

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 different fields regularly enduring serious losses (Johnson & Herrera, 1981). 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 grain and might influence the operational costs. Stalk and leaf diseases are mostly favored by wet and warm climate (Harry et al., 2009).

Southern corn leaf blight disease was first discovered in the United States in 1923. It attacked the green tissue of the leaves, by reducing the photosynthetic source area in corn plant. With the exception of some outbreaks, before the 1970 epidemic, SCLB was not regarded as a major disease of corn in the United States (Carson, 2016; Bruns, 2017). *Cochliobolus heterostrophus,* telemorph (Nisikado) *Helminthosporium maydis* is a necrotrophic fungal pathogen and the causal agent of SCLB disease. The disease in most cases is found amid the summer period in humid corn growing areas (Lim et al., 1971; Balint-Kurti et al., 2007; Tijjani et al., 2018). Ye et al. (2012) stated that, SCLB represents 20-30% significant yield losses of corn. Southern corn leaf blight disease cycle is a polycyclic disease, both asexual spore and sexual ascospores can infect the corn plant. Asexual spore is of great concern and known to occur naturally. During hot period and favorable moist conditions, the conidia are released from the infected corn lesions and spread to the adjacent seedlings by wind or rain splashing (Naz et al., 2012; Singh & Srivastava, 2012). After conidial arrival on sheath (leaf of a healthy corn plant), these conidia grow on the tissue of leaf by the process of germ tubes production. The produced germ tubes may penetrate through the tissue of the leaf or enter through natural openings such as the stomata or hyderthode in order to cause infection (Figure 1).

There is need for an investigation on the portrayal of SCLB pathogen and management strategies of leaf blight diseases in corn producing regions. Although, some researchers have worked to discover the actual pathogen causing SCLB disease. Even now, a lot of work is required to study the morphological and molecular characteristics as well as the aggressiveness of the pathogen, for easy identification and management of SCLB disease. This research work is aimed to study the morphological, molecular and pathogenic variability of the SCLB fungal pathogen, as well as to come up with novel management strategies of SCLB disease.

Distribution and Host Range

Cochliobolus heterostrophus race T pathogen was found to be pervasive in the U.S Corn growing zones in 1970. Race T was pathogenic on cytoplasm of Texas male-ster-

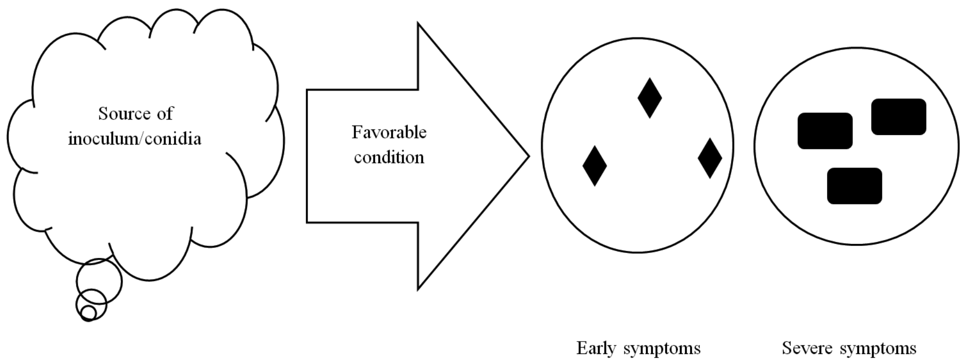


Fig. 1. Condition and processes of southern corn leaf blight disease infection on the host plant (corn)

ile (cms-T), causing significant epidemic of SCLB disease around 1970 to 1971 (Ullstrup, 1972; Ye et al., 2012). This epidemic of disease resulted in huge yield losses in U.S. Another epidemic was reported by Ye et al. (2012) in Hubei Province (China) in the year 1968 causing more than 400 million kg of corn yield losses. Similarly, there were SCLB reports in countries like Malaysia, Nepal, Australia, Nicaragua, Bolivia, New Zealand, Nigeria, Malawi, Egypt, Brunei Darussalam, Bhutan, Brazil, India, Myanmar, Ghana, (Sivanesan, 1987; Balint-Kurti et al., 2007; Manamgoda et al., 2014).

The most economically natural hosts of *C. heterostrophus* includes: guinea corn (*Sorghum bicolor*) and corn (*Zea mays*). Other host plant includes *Bothriochloa insculpta*, *Antirrhinum majus*, *Chloris gayana*, *C. virgate, Brachiaria foliosa*, *Cymbopogon citratus*, *Coix lacryma*-jobi*,* *C. martini*, *Dactyloctenium aegyptium*, *Cynodon dactylon*, *Oryza sativa*, *Dianthus caryophyllus*, *Echinochloa colonum*, *E. crus-galli*, *Digitaria ciliaris*, *Eleusine indica*, *Euchlaena mexicana*, and *Eriochloa procera* (Ullstrup, 1972; Naz et al., 2012).

Symptomatology of Southern Corn Leaf Blight Disease

Early symptoms of "O" strain of SCLB fungus show up as small diamond shaped lesions. When the lesions mature, it became elongated (Singh & Srivastava, 2012). The growth is restricted by nearby veins; finally the shape of the lesion is rectangular, 2 to 3 cm in length. These lesions might combine, creating a complete blight of vast zones on the leaves surfaces, while "T" strain of the fungus caused extreme damage to corn hybrids. The types of lesions formed by T strain are bigger than those of O strain and are oval shape (Singh & Srivastava, 2012). The necrotic lesions of SCLB are brown, and at times having purplish tinge or brownish red edge, sometimes zonate, coalesce and turn to grey, 2.5-3 cm long, elliptic in the beginning and after that prolong longitudinally to become rectangular when spots are confined by veins as presented in Figure 2 (Manamgoda et al., 2014).

Taxonomy of Southern Corn Leaf Blight Pathogen

Cochliobolus (Sivanesan, 1987), *Bipolaris* and *Curvularia* are plant pathogens having more than 60 hosts (plants) (Drachsler, 1934; Shoemaker, 1959; Manamgoda et al., 2011). A couple of species in these genera are infrequently included in the infection of vertebrates. Phylogenetic investigations of *Curvularia* and *Bipolaris* have demonstrated that the genus *Cochliobolus* is not monophyletic and some *Cochliobolus* species with short straight conidia cluster with *Curvularia* (Barbee et al., 1999; Emami & Hack, 2002; Agrios, 2005). *Pseudocochliobolus* was principally separated from

Fig. 2. A brownish red necrotic lesion of southern corn leaf blight disease on thai super sweet (TSS) variety of corn, forming blighted zones on the surface of the leaves

Cochliobolus on the premise of sexual spore (Shah et al., 2006), although it is considered as a synonym. In *Pseudocochliobolus*, the level of ascospore curling is less than *Bipolaris*, and stromatic tissue is underneath the ascomata. High intraspecific diversity of the ascospore and ascomata was found by Alcorn (1988), who questioned their use in generic delineation. Both *Curvularia* and *Bipolaris* produce asexual states, which was the premise to synonymize *Cochliobolus* with *Pseudocochliobolus*. In his monograph by Sivanesan (1987) *Cochliobolus* is regarded as a synonym of *Pseudocochliobolus.*

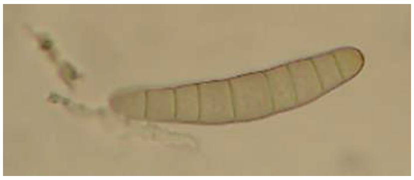
Morphological Variability of Southern Corn Leaf Blight Pathogen

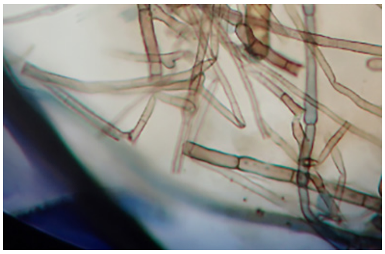
Drechslera, (Ito, 1930) was placed previously in subgenus Cylindro-*Helminthosporium*. *Drechslera* differ from other different graminicolous *Helminthosporoid* by its capacity to develop a germ tube from any of the cells in the distoseptate conidia (Sivanesan, 1987). Hilum morphology can be considered in separating *Drechslera* and *Bipolaris*. In *Drechslera* a flat scar exists inside the lowest portion of the basal cell, though in *Bipolaris* it is unnoticeable or marginally protuberant. The conidial shapes of *C. heterostrophus* were curved and elongated and the conidial length and width ranges from 42-133 µm and 6-21 µm respectively, while mycelial colour was brown and found to grow faster as presented in Table 1 and Figure 3 (A-D).

The colony color of *C. heterostrophus* were grey, dark grey, light grey and grey to green, while reversed colony appearance of *C. heterostrophus* were dark grey and black as

Table 1. Cultural and morphological characteristics of southern corn leaf blight pathogen

Fungal Strains	Colony Characterization	Average Conidial Length, µm	Average Conidial Width, µm	Host	Variety	Source
Cochliobolus heterostrophus 680+	dark grey to black appearance	93.5 ± 4.0 (52–126)	13.9 ± 0.35 (10–17)	corn	Zhenghong 505	(Xiaofang et al., 2020)
C. heterostrophus 499	blackish grey, entire or irrengular margin	64.9 ± 3.1 (42–92)	14.4 ± 0.57 (9–21)	corn	Zhenghong 505	(Xiaofang et al., 2020)
C. heterostrophus MASHIKI2-2	Grey	60.5±2.2 (50-70)	14.0 ± 0.52 (8–20)	corn	Super	(Tanaka et al., 1991)
C. heterostrophus HITO7711	Light grey	67.5±3.5 (54-73)	12.4 ± 0.29 (7–19)	corn	Super	(Tanaka et al., 1991)
C. heterostrophus SL-1	blackish grey	51.38±2.0 (54-73)	13.0 ± 0.3 (8–16)	corn	C-7 one	(Sivanesan, 1987; Abdul Gafur & Titik, 2002)
C. heterostrophus SM-1	dark grey to black appearance	97.13±4.3 (53-133)	12.3 ± 0.28 (8–17)	corn	Bisma	(Abdul Gafur & Titik, 2002)
C. heterostrophus F169	dark grey	92.11±3.9 (51-130)	11.4 ± 0.19 (7–16)	corn	Gangwon	(Injeong et al., 2018)
C. heterostrophus F217	dark grey to black appearance	70.13±3.7 (49-88)	10.4 ± 0.1 (7–15)	corn	Gangwon	(Injeong et al., 2018)
C. heterostrophus F218	Grey to green	60.15±2.3 (53-133)	10.5 ± 0.1 (8–16)	corn	Gangwon	(Sivanesan, 1987)
C. heterostrophus ET001	dark grey	65.38±3.02 (50-80)	13.74± 0.34 (11–16)	corn	Thai super sweet (TSS)	(Bashir et al., 2017a)
C. heterostrophus ET002	Light grey	56.70±2.1 (48-83)	11.34± 0.18 (6–15)	corn	Thai super sweet (TSS)	(Bashir et al., 2017a)
C. heteros-trophus001	dark grey	55.2±2.1 (47-77)	13.9 ± 0.35 (11–15)	corn	Maiz-1	(Manamgoda et al., 2015)
C. heteros-trophus002	dark grey	77±3.8 (55-99)	10.4 ± 0.1 (6–17)	corn	Maiz-1	(Manamgoda et al., 2015)


A)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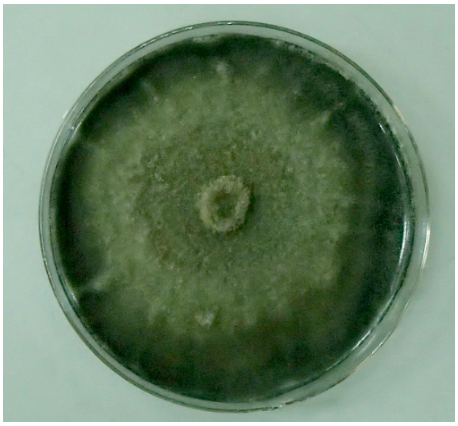
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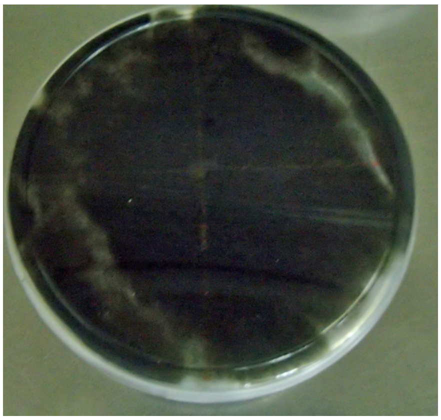
D)

Fig. 3. A – Elongated spore of *C. heterostrophus* with a clear simple septal arranged in a linear order; B – Brownish colour mycelia of *C. heterostrophus* grown on PDA media after incubation for a period of two weeks viewed under compound light microscope (magnification 40X); C – Culture of *C. heterostrophus* showing dark grey to green colour on PDA after incubation period of two weeks; D – Reversed view of *C. heterostrophus* culture showing dark grey coloration after incubation for two weeks

presented in Table 1 (Sivanesan, 1987). The sexual stage of *Bipolaris* is called *Cochliobolus*, *Exserohilum* can be separated from different graminicolous *Helminthosporoid* by having truncate in most cases with enveloping bubble and protruding hilum (Alcorn, 1988).

Molecular Identification of Southern Corn Leaf Blight Pathogen

Molecular analysis using rDNA sequence of internal transcribed spacer (ITS) region, have supplemented classical method of identification and yielded an accurate and quick identification of several species from distinctive hosts ((Begoude et al., 2010; Mohali et al., 2010; Bashir et al., 2017b). Barbee et al. (1999) worked on phylogenetic analysis of 32 *Cochliobolus* and *Curvularia* by using ITS sequences. Their phylogenies showed two distinctive groups. Group one includes the most aggressive fungal pathogens, for instance, *C. heterostrophus*, while group two comprises of fungal species that were transferred from *Cochliobolus* to *Pseudocochliobolus*. Different sets of primers were used in identification of *C. heterostrophus* fungus; these primers were either universal or specific as presented in Table 2.

Pathogenicity Test

Inoculation Protocol

There are numerous techniques for inoculating plants with *C. heterostrophus* in a glasshouse. Attempts are being made to alter them with the aim of developing a more precise and effective method (Savory et al., 2012). To reduce cost, energy and time in a pathogenicity test, an efficient method of inoculation is required. Pathogenicity test is critical and of vital role in assessing the resistant hybrids, in this manner the spraying method of inoculation is essential as it influences the disease severity and disease incidence (Savory et al., 2012).

Spraying Method

Spraying method is the basic technique that has been used on cotyledon as well as in early stage of seedlings development when they reach around one to three stage of true leaf (Savory et al., 2012). The seedlings were splashed with the spore suspension of *C. heterostrophus* using a hand-pumped spray bottle. The concentration of spore suspension use varies from 10^6 to 10^5 spore/ml as proposed by (Salati, 2010). The spore suspension was splashed into the upper and lower leaf surfaces continuously until runoff.

Pathogenic Variability of the Fungus

Three races of *C. heterostrophus* called O, T and C are known so far. Race O is considered as the most widely recognized race in many regions like India, Brazil, Egypt, Nigeria and Myanmar, which is controlled by genes (Degani, 2014). Race T, caused the 1970's epidemic of SCLB disease in North America, this epidemic was particularly associated with corn containing Texas male-sterile cytoplasm (cms-T), which is controlled for the most part by cytoplasmic components (Degani, 2014). The most conspicuous distinction

Table 2. Different types of primers used to identify southern corn leaf blight pathogen

Primer	Primer Sequences (5'-3')	Gene Name	Size (bp)	Source
TS1/ITS5	(5'-TCCGTAGGTGAACCTGCGG-3') and (5'-GGAAGTAAAAGTCGTAACAAGG)-3')	Internal transcribed spacer (ITS)	250	(Pataky et al., 2008; Manamgoda et al., 2014; Xiaofang et al., 2020)
GPD1/GPD2	(5'-CAACGGCTTCGGTCGCATTG-3') and (5'-GCCAAGCAGTTGGTTGTGC-3')	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	1182	(Marin-Felix et al., 2017; Xiaofang et al., 2020;)
Bm-F /Bm-R	(5'-TCTCGACAAGCAAATCAAAC-3') and 5'-(AGATGATTGCAGTGGTGTTG-3')	Specific primer for *Bipolaris maydis*	539	(Injeong et al., 2018)
EF1_F /EF1_R	(5'-GGCTTTCACCGACTACCCTCCTCT-3') and (5'-ACTTCTCGACGGCCTTGATGACAC-3')	Eukaryotic translation elongation factor 1 alpha	91	(Lysoe et al., 2009)
TUBUF2/TUBUR1	(5'-CGGTAACAACTGGGCCA AGG-3') and (5'-CCTGGTACTGCT GGTACTCAG-3')	β-tubulin	1000	(Kroon et al., 2004; Bashir et al., 2017)
ITS1/ITS4	(5'-TCCGTAGGTGAACCTGCGG-3') and (5'-TCCTCCGCTTATTGATATGC-3')	Internal transcribed spacer (ITS)	600	(White et al., 1990)
ITS4/ ITS5	(5'-TCCTCCGCTTATTGTATGC-3') and (5'-GGAAGTAAAAGTCGTAACAAGG-3')	Internal transcribed spacer (ITS)	586	(White et al., 1990; Injeong et al., 2018)
ITS4/ITS6	(5'-TCCTCCGCTTATTGTATGC-3') and (5'-GAAGGTGAAGTCGTAACAAGG-3')	Internal transcribed spacer (ITS)	862	(Kwok et al., 1990; Cooke et al., 2000; Pataky et al., 2008)

Table 3. Different varieties of corn and their reactions to southern corn leaf blight pathogen

Fungal Isolate	Disease Incidence (DI), %	Disease Severity Index (DSI), %	Host	Status/ Reaction	Variety	Source
C. heterostrophus JW-1	100	80	Corn	Susceptible	Bisma	(Abdula Gafur & Titik, 2002)
Cochliobolus heterostrophus JW-3	100	60	Corn	Susceptible	C-7 one	(Abdula Gafur & Titik, 2002)
Cochliobolus heterostrophus 680+	100	54.3	Corn	Susceptible	Zhenghong 505	(Xiaofang et al., 2020)
Cochliobolus heterostrophus 680+	100	77.8	Corn	Susceptible	Zhenghong 505	(Xiaofang et al., 2020)
Cochliobolus heterostrophus F169	100	25.1	corn	Resistant	Gangwon	(Injeong et al., 2018)
Cochliobolus heterostrophus F217	100	16.4	corn	Resistant	Gangwon	(Injeong et al., 2018)
Cochliobolus heterostrophus F218	100	10.3	corn	Resistant	Gangwon	(Injeong et al., 2018)
C. heterostrophus ET004	100	65.3	Corn	Susceptible t	TSS	(Bashir et al., 2017a)
C. heterostrophus ET005	100	70.5	corn	Susceptible	TSS	(Manamgoda et al., 2015)
C. heterostrophus CH001	100	29.10	Corn	Resistant	SLBR3	(Bashir et al., 2017a)
C. heterostrophus CH001	82	20.30	corn	Resistant	SLBR5	(Manamgoda et al., 2015)
C. heterostrophus TSW	60	15.00	corn	Resistant	brocade TSW	(Margaret et al., 2013)

between race T and O is, race O generally infect leaves while race T infects both stalks, ear husks, ears leaf sheaths, cobs and leaves. Race C is a cms-C cytoplasm particular race reported only in China (Degani, 2014).

Disease Management

Frequent and early scouting for diseases is a routine and the most suitable control practice to manage pest before it leads to economic damage. For southern corn leaf blight disease, mainly observe for symptoms in the lower leaves of the corn plant which become nutrient stressed with the leaf blight disease (Calvert & Marcus, 2012). The symptoms are not found at the ear leaf or above. In corn, the most suitable time for scouting is at the tasseling through the stages of R4 growth, earlier in seed production fields (Calvert & Marcus, 2012). Generally, the control measures of SCLB disease comprises of the following methods:

Hybrid Selection

Genetic disease resistance in choosing corn hybrids offers the best effective defense against SCLB and other diseases (Margaret et al., 2013; Bashir et al., 2017b). Even though, there is no hybrid that provides resistance to all the kind of diseases, even resistance that is partial goes a long way in protecting yield of corn (Margaret et al., 2013). The most reliable method of controlling SCLB disease is by developing resistant host through breeding. The inbreeds and hybrids are accessible with both polygenic and monogenic resistance, and ought to be utilized at whatever point possible. A typical cytoplasm hybrid of corn could resist both Race C and Race T. Corn varieties such as brocade TSW, and precious gem were found to be resistant to SCLB disease. Recently new inbred lines (SLBR3 and SLBR5) of corn were assessed to southern corn leaf blight disease in Malaysia and found to be resistant to the disease (Bashir et al., 2017b). There are several varieties of corn, and these varieties could either be resistant or susceptible to SCLB disease (Table 3).

Biological Control Method

Trichoderma belongs to a genus of fungi of the family called ''Hypocreaceae", it is found in all the different soil types, they served as the most commonly fungi that grow in culture. Several species of the fungi of this genus are considered as non-virulent plant symbionts that are opportunistic (Harman et al., 2004). Many *Trichoderma* species have the ability to form mutualistic endophytic associations together with different plant species (Druzhinina & Kubicek, 2005; Bae et al., 2011).

Trichoderma atroviride (SG3403) and *Trichoderma harzianum* (SH2303) possessed a high biological control effect against SCLB pathogen (*Cochliobolus heterostrophus*). *T. atroviride* could leads to the death of *C. heterostrophus* race O hypha (Meng et al., 2015). The spray of *T. atroviride* and *T. harzianum* conidial suspension on corn seedling leaves has protected the corn plant from SCLB disease infection. The effect of biocontrol could last for one month in the field. The strain of *Trichoderma* was able to activate resistance response in corn plant leaves against the *C. heterostrophus* (Meng et al., 2015; Shao-qing et al., 2019).

Cultural Practices

Crop rotation is still a solid strategy to help in diminishing the threats of the disease. Crop rotation from corn to non-host crop helps in reducing suitable conditions of the environment for SCLB pathogen, disease levels and risk of infection (Calvert & Marcus, 2012). Any kind of tillage that helps in reducing crop residues from a previous corn will help in controlling SCLB and other diseases. The combination of integrated control practice is highly recommended in area where corn is continuously grown with no-till (Calvert & Marcus, 2012).

Chemical Method

The method of seed treatment using mercury dusts in Sudan grass has been successful in minimizing leaf blight disease (Nisikado, 1929; Miller, 1970; Kumar et al., 1977). A different type of fungicides like chlorothalonil is utilized, for protecting corn from SCLB that tends to be the limiting factor for quality corn production (Rahul & Singh, 2002; Singh, 2005). The application of foliar fungicides such as zineb, mancozeb and propiconazole was reported to be successful in controlling *C. heterpstrophus* infection (Turgeon & Baker, 2007; Amin et al., 2012; Didvania et al., 2012). Another effective control method is by seed dressing using carboxin + thiram, maneb, benomyl + thiram and captan which enhanced germination of the seed (Kump et al., 2011; Iffat et al., 2012; Manamgoda et al., 2012). Kumar et al. (1977) Also found that the used of fungicides can reduce it severity by at least 55%.

Conclusion

Morphological, molecular and pathogenic characteristics of *C. heterostrophus* pathogen help immensely in detecting the pathogen. Based on morphological characteristics, the colony color of the pathogen was found to be grey, grey to green, dark grey and light grey. The conidial shape was elongated and it length and width ranges from 42-133 µm and 6-21 µm respectively. Different universal and specific primers were used to identify the pathogen using molecular method. The use of foliar fungicides like propiconazole, mancozeb and Zineb was reported to reduce the epidemic of SCLB disease, even though these chemicals were not friendly to the environment. However, the best method of controlling the disease is by using resistant varieties such as Gangwon, Brocade TSW, SLBR3 and SLBR5. Moreover, *Trichoderma harzianum* (SH2303) and *T. atroviride* (SG3403) have shown a strong biocontrol activity against SCLB pathogen. Cultural practices like crop rotation and tillage help in minimizing the crop residues in corn growing farms. Early and frequent scouting also helps in reducing yield loss as well as effect of the disease on corn plant. Future researches should focus on the development and assessment of more varieties that are resistant to the disease as well as to using more advance molecular tool to detect the SCLB pathogen.

Author Contributions

All authors in this manuscript have read and agree to the published version of the manuscript. Writing original draft preparation, A.B.K.; writing a review and editing, K. S., K. A., M. Z. H., A. A. W and M. A. A; supervision, K. S., K.A.; funding acquisition, K. S., K.A. All the authors in this manuscript have read and agreed to the published version of the manuscript.

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References

Abdul Gafur, S. M. & Titik, N. A. (2002). Morphological and pathological variation in the Indonesian *Cochliobolus heterostrophus* (Pleosporaceae, Pleosporales, Euascomycetes*). Pakistan Journal Biological Science, 5*, 1195-1198.

Agrios, G. N. (2005). Plant pathology. USA: Elsevier Academic Press.

Alcorn, J. L. (1988). The taxonomy of "*Helminthosporium*" species. *Annual Review of Phytopathology*, *26*, 37–56.

Amin, N., Nasruddin, A. & Dalha, L. (2012). Isolation, Identification and *in-vitro* screening of fungal endophytes against pathogen of maize leaf blight, *Helminthosporium Maydis. Yhe 21st National Congress of the Indonesian Phytopathology Society***.**

Bae, H., Roberts, D. P., Lim, H. S. Strem, M. D., Park, S. C., Ryu, C. M., Melnick, R. L. & Bailey, B. A. *(2011). Endophytic Trichoderma isolates from tropical environments delay disease onset and induce resistance against Phytophthora capsici in hot pepper using multiple mechanisms Molecular Plant Microbe Interaction, 24(3), 336–51.*

Balint-Kurti, P. J., Zwonitzer, J. C., Wisser, R. J., Carson, M. L., Oropeza-Rosas, M., Holland, J. B. & Szalma, S. J. (2007). Precise mapping of quantitative trait loci for resstance to southern leaf blight, caused by *Cochliobolus heterostrophus* race O, and flowering time using advanced intercross maize lines. *Genetics, 176*, 645–657.

Bashir, K. A., Kamaruzamn, S. & Khairulmazmi, A. (2018). First report of northern corn leaf blight disease caused by *Exserohilum turcicum* on *Zea mays* in Malaysia. *Journal of Molecular Genetics Medicine*, *12*, 1-2.

Bashir, K. A., Kamaruzamn, S., Khairulmazmi, A., Zulkifli, A. S., Mohd Shahril, F. A. & Norzihan, A. (2017a). Characterization and pathological variability of *Exserohilum turcicum* responsible for causing northern corn leaf blight (NCLB) disease in Malaysia. *Malaysian Journal of Microbiology*, *13*, 41-49.

Bashir, K. A., Sijam, K. & Ahmad, K. (2017b). Evaluation of resistance in response to infection of southern corn leaf blight disease and determination of secondary metabolites produced during the interaction. *Journal of Applied Science & Environmental Sustainability, 3*, 1-17.

Begoude, B. A. D., Slippers, B., Wingfield, M. J. & Roux, J. (2010). Botryosphaeriaceae associated with *Terminalia catappa* in Cameroon, South Africa and Madagascar. *Journal of Mycology Progress*, *9*, 101–123.

Berbee, M. L., Pirseyedi, M. & Hubbard, 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.

Bruns, H. A. (2017). Southern corn leaf blight: A story worth retelling. *Agronomy Journal*, *109*, 1218.

Calvert, O. H. & Marcus, S. Z. (2012). Ear-rotting potential of *Helminthosporium maydis* Race T in corn. *Phytopathology, 63,* 769-72.

Carson, M. L. (2016). Foliar diseases. In: G.P. Munkvold and D.G. White (eds), Compendium of corn diseases. 4th ed. The American Phytopathological Society St, Paul, MN. 29–31.

Cooke, D., Drenth, A., Duncan, J. M., Wagels, G. & Brasier, C. M. (2000). A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics & Biology, 30*, 17–32.

Degani, O. (2014). Pathogenicity assay for *Cochliobolus heterostrophus* G-Protein and MAPK signaling deficiency strains. *American Journal Plant Science, 5*, 1318–1328.

Devegowda, A. S. (2004). Rice and corn quality workshop. *Review of Literature*, 22–24. http://prr.hec.gov.pk/Chapters/128S-2.pdf

Didvania, S., Shah, R. & John, K. S. A. (2012). New disease of bell pepper (*Capsicum annuum* var. grossum) caused by *Drechslera bicolor*, its pathophysiology, efficacy of fungicides and botanicals. *Plant Pathology Journal*, *11*, 68-72.

Drechsler, C. (1934). Leaf spot of maize caused by *Ophiobolus heterostrophus*, the asigerous stage of *Helminthosporium* exhibiting bipolar germination. *Journal of Agricultural Research, 31*, 701–26.

Druzhinina, I. & Kubicek, C. P. *(2005). Species concepts and biodiversity in Trichoderma and Hypocrea: from aggregate species to species clusters? Journal of Zhejiang University Science, 6(2), 100–112.*

Emami, K. & Hack, E. (2002). Conservation of XYN11A and XYN11B Xylanase Genes in *Bipolaris sorghicola, Cochliobolus sativus, Cochliobolus heterostrophus,* and *Cochliobolus spicifer. Journal of Microbiology, 7*, 303–306.

Harman, G. E., Howell, C.R., Viterbo, A., Chet, I. & Lorito, M. *(2004). Trichoderma species—opportunistic avirulent plant symbionts. Nature Reviews Microbiology. 2 (1), 43–56.*

Harry, E., Duncan, J. & Gary, K. J. (2014). Major corn diseases in north Carolina. *Plant Pathology, 20*, 453–455.

Iffat, N., Shama, S., Irum, P., Abdul, R., Amber, H., Yasmin, A. & Safia, A. (2012). Optimization of cultural conditions for *Cochliobolus heterostrophus* isolates from infected maize plants from different Agricultural Zones of Pakistan. *British Microbiology Research Journal*, *2*, 233–242.

In Jeong, K., Hyeong, K. S., Jae, H. R., Sunggi, H. & Dong, B. S. (2018). Simple detection of *Cochliobolus* fungal pathogens in maize. *Plant Pathology Journal, 34*, 327-334.

Johnson, R. & Herrera, M. P. (1981). Corn pollination under moisture and high temperature stress. *Proceedings of the Corn and Sorghum Industry Research Conference, Chicago, II*. American Seed Trade Association, Washington, DC. 66-77.

Kolawole, L. A. (2009). Growth and yield of maize as influenced by sowing date and poultry manure application. *Agrobotanical, 37*, 221-232.

Kroon, L. P., Bakker, F. T., Van den Bosch, G. B., Bonants, P. J. & Flier, W. G. (2004). Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. *Fungal Genetics and Biology, 41*, 766-782.

Kumar, A., Guta, M. & 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., Zwonitser J. C. Kresovich, S., McMullen, M. D., Ware, D., Balint-Kurti, P. J. & Holland J. B. (2011). Genome-wide association study of quantitative resistance to southern leaf blight in maize nested association mapping population. *Nature Genetics,43*, 163–168.

Kwok, S., Kellogg, D. E., McKinney, N., Spasic, D., Godal, L., Levenson, C. & Sninsky, J. J. (1990). Effects of primer-template mismatches on the polymerase chain reaction: human immunodeficiency virus type 1 model studies. *Nucleic Acids Research 18*, 999–1005.

Lim, S. M., Hooker, A. L. & Smith, D. R. (1971). Use of *Helminthosporium maydis* Race T pathotoxin to determine disease reaction of germinating. *Phytopathology, 11*, 2–773.

Lysøe, E., Bone, K. R. & Klemsdal, S. S. (2009). Real-time quantitative expression studies of the zearalenone biosynthetic gene cluster in *Fusarium graminearum*. *Phytopathology*, *99*,176-184.

Manamgoda, D. S., Cai, L. & Bahkali, A. H. (2011). *Cochliobolus*: an overview and current status of species. *Fungal Diversity, 51*, 3–42.

Manamgoda, D. S., Cai, L. & 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. & Hyde, K. D. (2014). The genus *Bipolaris. Mycologia, 79*, 221–288.

Manamgoda, D. S., Rossman, A. Y., Castlebury, L. A., Crous, P. W., Madrid, H., Chukeatirote, E. & Hyde, K. D. (2015). The genus *Bipolaris*. *Studies in Mycology, 79*, 221–288.

Margaret, T. M. (2013). Effectively managing northern corn leaf blight. *Phytopathology 21*, 221-234.

Marin-Felix, Y. Groenewald, J. Z., Cai, L., Chen, Q., Marincowitz, S., Barnes, I., Bensch, K., Braun, U., Camporesi, E. & Damm, U. (2017). Genera of phytopathogenic fungi: GOPHY 1. *Studies in Mycologia*, *86*, 99–216.

Meng, W., Jia, M., Lili, F., Kehe, F., Chuanjin, Y., Jinxin, G., Yaqian, L. & Jie, C. (2015). Biological control of southern corn leaf blight by *Trichoderma atroviride* SG3403. *Biocontrol*

Science & Technoligy, 25(10), 1133-1146.

Miller, P. R. (1970). Southern corn leaf blight. *Plant Disease Report, 54*, 1099–1136.

Mohali, S., Slippers, B. & Wingfield, M. J. (2010). Two new *Fusicoccum* species from *Acacia* and *Eucalyptus* in Venezuela based on morphology and DNA sequence data. *Journal of Mycology Research, 110*, 405–413.

Morris, M. L. (1998). Maize seed industries in developing countries. Publishers, Inc. and Lynne Rienn, Int. http://link.springer.com/10.1007/s10327-014-0519-1

Naz, I., Sehar, S., Perveen, I., Rehman, A., Hameed, A., Ahmad, Y. & 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*, 233–242.

Nisikado, Y. (1929). Studies on the *Helminthosporium* diseases of Gramineae in Japan. *Berichte Des Ohara Institute Landwirtschaftliche Forschungen, 4*, 111–126.

Pataky, J., Williams, M., Meyer, M., Warsaw, B. & Moody, J. (2008). Sweet corn hybrid disease nursery. *Phytopathology*, *7,* 73-97.

Rahul, K. & Singh, I. S. (2002). Inheritance of resistance to banded leaf and sheath blight (*Rhizoctonia solani* f. sp. Sasakii) of maize. In: *Proceedings 8th Asian Regional Maize Works*. Bangkok, Thailand, *5*, 356–365.

Salati, M. A. l. (2010). First report of *Pseudoperonospora cubensis* causing downy mildew of *Trichosanthes cucumerina* in Malaysia. *Plant Dis*ease, *94*, 642-643.

Savory, E. A. (2012). Analysis of the *Pseudoperonospora cubensis* transcriptome during cucumber (*Cucumis sativus* L.) Infection. *Mycologia, 7*, 351-360.

Shah, S. S., Rahman, H., Khalil, I. H. & Rafi, A. (2006). Reaction of two maize synthetics to *maydis* leaf blight following recurrent selection for grain yield. *Agricultural Journal, 22,* 263-269.

Shao-qing, W., Jia, M., Meng, W., Xin-hua, W., Ya-qian, L. & Jie, C. (2019). Combined application of *Trichoderma harzianum* SH2303 and difenoconazole-propiconazolein controlling Southern corn leaf blight disease caused by *Cochliobolus heterostrophus* in maize. *Journal of Integrated Agriculture, 18*, 2063-2071.

Shoemaker, R. A. (1959). Nomenclature of *Drechslera* and *Bipolaris*, grass parasites segregated from *Helminthosporium*. *Canadian Journal of Botany, 37*, 879–887.

Singh, N. P. (2005). Maize in India: Production systems, constraints, and research priorities. *Indian Research Journal of Extension Education, 42*, 821-833.

Singh, R. & Srivastava, R. P. (2012). Southern corn leaf blight – An important disease of maize: An extension fact sheet. *Indian Research Journal of Extension Education, I*, 334-337.

Sivanesan, A. (1987). Graminicolous species of *Bipolaris, Curvularia, Drechslera, Exserohilum* and their teleomorphs. *Mycology Journal, 15*, 1-261.

Tanaka, C., Kubo, Y. & Tsuda, M. (1991). Genetic analysis and characterization of *Cochliobolus heterostrophus* colour mutants. *Mycology Research, 92,* 49-56.

Tijjani, A., Bashir K. A., Gurama, A. U. & Adebitan, S. A. (2018). Potential impact of some cultural practices on incidence and severity of *Ralstonia solanacearum*, causing Bacterial Wilt Disease in greenhouse tomatoes. *International Journal of Current Science Studies*, *2*, 1-7.

Turgeon, B. G. & Baker, S. E. (2007). Genetic and genomic dissection of the *Cochliobolus heterostrophus* Tox1 locus controlling biosynthesis of the polyketide virulence factor T-toxin. *Advances in Genetics*, *57*, 219-261.

Ullstrup, A. J. (1972). The impacts of the southern corn leaf blight epidemics of 1970-1971. *Annual Review of Phytopathology, 10*, 37-50.

White, T. J., Bruns, T., Lee, S. & 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.

Xiaofang, S. Xiaobo, Q. Wei, W. Xuan, L. Henan, Z. Cuiping, W. Xiaoli, C. Min Z. Huabao, C. & Guoshu, G. (2020). Etiology and Symptoms of Maize Leaf Spot Caused by *Bipolaris* spp. in Sichuan, China. *Pathology. 9*, 229.

Ye, Y. Li, Q. Fu, G. Yuan, G. Miao, J. & Lin, W. (2012). Identification of antifungal substance (Iturin A2) Produced by *Bacillus subtilis* B47 and Its effect on southern corn leaf blight. *Journal of Integrated Agriculture*, *11*, 90-99.

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