

Southern Corn Leaf Blight: Behavior of maize crop, Approaches and Future outlooks, and Research trends for disease control related to the effectiveness of new sources of resistance

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Abstract

In several corn-growing areas, the average production is relatively low as compared to normal potential productions in many countries around the world, although the area of cultivation is somehow increasing by every day in those areas with low yield of maize. The most cultivated cultivars don't resist to Southern Corn Leaf Blight where the disease is reported. Farmers try to develop a disease management method without success due to lack of efficacy from used techniques. Fungi and fungal-like organisms (FLOs) collectively cause more plant diseases than any other group of pests. Bipolaris maydis was reported to be the most important fungal plant pathogen to cause SCLB disease in maize crop. Acquisition of knowledge in identifying and controlling fungal diseases, especially SCLB to reduce its effects of toxicity, is needed nowadays. It is known that the plant pathogenic fungus, Cochliobolus heterostrophus race T, produces T-toxin (HMT-toxin), one of most dangerous mycotoxins affecting human life. Accordingly, it is estimated several millions of currencies are lost annually because of mycotoxin contamination of crops in many countries around the world. In maize, mycotoxin contamination often occurs in association with SCLB, which reduces quality and yield. This chapter gathered information on use of integrated SCLB disease management and revealed some effective techniques for control of the pathogen. General principles used in plant disease management and concepts of disease triangle were developed and adapted in case of the current disease for better understanding of its control. The information generated from this endeavor benefits plant breeders and other scientists, including plant pathologists and researchers in the public and private sectors interested in improving resistance to the infection of the fungal pathogen.

Keywords: Maize, *Bipolaris maydis*, Sources of resistance, integrated disease management

1. Introduction

Maize, also known as corn, is widely grown throughout the world. It is the most produced grain in the world and has the highest production of all cereals. It is an important food staple in many countries. There are many causes of low yields of maize and a major role is played by diseases.

SCLB is considered as one of the serious and major important diseases worldwide with its effects on yield crop. It is a pandemic and widespread disease. It is found everywhere the corn is grown. Obviously, humans have little or no control over the evolution of pathogenic organisms. Like any living thing, fungi along with all other plant-pathogenic microbes and pests, will find a way to survive and propagate (Bruns, 2017). Collectively, fungi and fungal-like organisms (FLOs) cause more plant diseases than any other group of pathogens (Bruns, 2017; Ahmar et al., 2020). Southern Corn Leaf Blight (SCLB), also called Maydis Leaf Blight (MLB) or Southern Leaf Blight of Maize (SLB *Zea mays*) is due to the infection of *Cochliobolus heterostrophus* (teleomorph), also known as *Bipolaris maydis* (Nisikado and Miyake) Shoem (anamorph), *Helminthosporium maydis* Nisikado (anamorph) or *Drechslera maydis* (Nisikado and Miyabe) (anamorph) or *Ophiobolus heterostrophus* Drechsler (Burton, 1968; Mubeen et al., 2015; Wang et al., 2017; Wang et al., 2019; Jeevan et al., 2020; Castellanos Gonzalez et al., 2020; Rehman et al., 2021; Singh et al., 2021; Meshram et al., 2022).

For its identification, signs and common symptoms of disease involved by the pathogen are diversified, including lesions on glumes found in inflorescence; abnormal colors, fungal growth, and wilting located on leaves; discolorations and rot found on seeds; and discoloration of bark observed on stems. Based on what's revealed by Agrios (2005) and Ali et al. (2011), SCLB symptoms depend on host germplasm and race of the pathogen (O, T and C). Generally, the methods used in management of SCLB include the utilization of chemicals including fungicides and botanical extracts, among which some of these practices are developed here.

The information gathered in this critical appraisal, is more interesting as it focuses on the disease which attracted more alerts in the world since its apparition. It was reported that, in the USA, which is the main origin of the disease, the epidemic of SCLB stimulated an immense amount of publicity. No plant disease in the United States has ever received so much attention from the press and other communications media. During the period from August to November 1970, the Chicago Tribune published 37 articles on the disease. The New York Times, the Wall Street Journal, and

many of the other influential daily newspapers and weekly news magazines kept the public aware of the gravity of the disease. Local papers serving small communities regarding disease development. Radio and television programs frequently gave descriptions of the SCLB situation, and how to cope with it. Many bulletins and circulars on the disease were issued by Agricultural Experiment Stations. Epidemiological studies were made in several states. Numerous conferences, meetings, symposiums, workshops, and seminars were held throughout the epidemic area. Ullstrup (1972) reported almost all these alerts which attracted more our interest, in his paper entitled “the impacts of the southern corn leaf blight epidemics of 1970-1971”.

Furthermore, this chapter intends to identify the new sources of resistance to SCLB under laboratory and field conditions. It carried on the purpose of gaining knowledge on the effectiveness of both SCLB disease control and adoption of new measures to eradicate the pathogen. It is mostly focused on integrated SCLB disease management including complete removal of the pathogen in the growing area of the maize crop by using of new techniques revealed here, such as creation of plant immunity and biological control. Empirical studies conducted in different areas in the world such as USA, India, China, Malaysia, and African countries were mostly considered in this chapter. More than hundred articles were selected, including some recently published papers in last five years, i.e., between 2017 to 2022. Around ten books served additionally as sources of information helping in achievement of objectives of this chapter. Leaf symptoms (**Fig. 1.**) were taken from Shukuru’s master’s dissertation (Shukuru et al., 2023).

2. Know the behavior of maize crop

2.1. Morphological and physiological description of corn

The word "corn" has many different meanings depending on what country you are in. Corn in the United States is also called Indian corn, or maize in many countries of Africa including DR Congo. Corn in England means wheat; in Scotland and Ireland, it refers to oats. Corn mentioned in the Bible probably refers to wheat or barley (Gibson and Benson, 2002). *Zea* means “sustaining life” derived from ancient Greek word and *mays* means “life giver” according to Taíno language. The word “maize” by the Spanish connotation “maiz” which is the most suitable way of presenting the plant. Different other names like muhindi (Africa) or makki (India) are useful to identify the plant.

The corn, *Zea mays*, is an annual herbaceous cereal, with low to no tillering, of the Poaceae family, native to Central America. Like most of the tropical poaceous, it presents a metabolism of C4 type

photosynthesis, which confers to the plant a higher efficiency than that of the temperate poaceous in the conversion of the light energy. It is a plant of short days whose tropical varieties are often photoperiodic. This oligogenic character, could be eliminated at the time of the adaptation of the species to the temperate latitudes (often from 58°N to 40°S). Maize is a monoecious plant, it bears two types of inflorescences: male flowers, grouped on the branched terminal panicle, and female flowers, associated on one or a few cobs inserted in the leaf axils. Although the corn is self-fertile, the allogamy is preponderant, and reaches 95%. It results from the monoecy and the protandry of the plant. The production of pollen by the flowers of the corn panicle is very abundant. The male flowering on a plant of corn is earlier than the female flowering (phenomenon of the protandry): that contributes to decrease very strongly the rate of self-pollination of the individuals which would not exceed 5%.

The spikelets of the male inflorescence are inserted in pairs. One of these spikelets is pedicellate and the other is sessile. Each biflorous spikelet, because it contains two flowers, is delimited by glumes, floral pieces with leaf structure. At maturity, the two flowers of each spikelet release their stamens. Each stamen is composed of a net and an anther, anther made of two pollen sacs. The female inflorescence of the corn is a ramification of the main stem. It is itself made up of a series of very short nodes. Each node carries a leaf organ called a spathe in the axil of which a bud remains non-functional. At the end of the branching develops the spike bearing spikelets, themselves composed of flowers and thus ovaries. These ovaries are surmounted by long silks or styles that receive pollen from the male flowers. The silks each surmount an ovary and escape from the "horn" of spathes to receive the pollen. The first setae that appear outside the spathe "cornet" are the setae that originate at the base of the spike. The spikelets are inserted in pairs on the central axis or stalk. Each pair of spikelets is surrounded on the stalk, by two tiny bracts barely visible on the screen (one at the extreme left and the other at the extreme right). Each spikelet is made of two glumes enclosing, on one hand, a sterile flower formed only by two glumellae, and, on the other hand, a fertile flower also composed of two glumellae embracing a gynoeceum, i.e., an ovary surmounted by a style.

2.2. History, origin, and geographic distribution of corn

The origin of maize (*Zea mays* L. subsp. *mays*) was clearly established, and its primary center of origin is in Mexico, the native of maize where fossil maize pollen with other archeological

evidence were discovered and the Central America. Maize was first domesticated in Tehuacán Valley (Gibson and Benson, 2002). Recent research has modified that it's the adjacent Balsas River Valley (Piperno, 2011) of south-central Mexico.

According to Gibson and Benson (2002), corn was only a garden curiosity in Europe, but it soon began to be recognized as a valuable food crop. Within a few years, it spread throughout France, Italy, and all southeastern Europe and northern Africa. By 1575, it was making its way into western China, and had become important in the Philippines and the East Indies. The principal role of the corn plant during the 19th century was closely tied to the development of the Midwest. In the movement westward, corn found its major home in the woodland clearings and grasslands of Ohio, Indiana, Illinois, Iowa, and adjacent states. These were places where it had not been grown widely in prehistoric times. Presently, maize is grown worldwide.

2.3. Corn diversity and environmental conditions

The genus *Zea* contains annual and perennial species native to Mexico and Central America. It includes wild forms, “**teosinte**”, and a cultivated form, “**corn**”. The genus *Tripsacum* includes many wild species whose center of diversity is in Mexico and Guatemala. It is a distant relative of corn. Corn is often classified as dent corn or field corn (*Zea mays* var. *indentata*), flint corn or Indian corn (*Zea mays* var. *indurata*), flour corn (*Zea mays* var. *amylacea*), popcorn (*Zea mays* var. *everta*), sweet corn (*Zea mays* var. *saccharata*), waxy corn or glutinous corn (*Zea mays* var. *ceratina*), and pod corn (*Zea mays* var. *tunicata*). Gibson and Benson (2002) reported that the great variability of the corn plant led to the selection of numerous widely adapted varieties which hardly resembled one another. The plant may have ranged from no more than a couple of feet tall to over 20 feet. It was not like the uniform sized plant that most people know today. For the Aztecs, Mayas, Incas and various Pueblo dwellers of the southwestern United States, corn growing took precedence over all other activities. The corn crop requires warm and sunny weather, between 23.9-30 °C, with intermittent moderate rains or artificial watering (around 50 cm) during growing season. Accordingly, Rehman et al. (2021) found that many of fungal diseases are mostly favored by humid and warm environmental conditions, but some others also prevalent in humid and cool conditions. This constitutes a suitable interaction between the maize crop and the fungal pathogen.

2.4. Constraints linked to maize production

Despite yield potential of maize and its economic advantages procured, its susceptibility to several biotic and abiotic stresses, including especially climate change and diseases, that constitute a major threat to its production. Most important crops next to wheat and rice in the world, and first crop in Africa, maize is often threatened by a variety of pathogens as well as poor crop management. The crop is prone to several biotic stresses like ear rot, and several foliar diseases caused by fungi, bacteria, and viruses. Nematodes and caterpillars are also still causing damage to maize crop. Under favorable environmental conditions, these pathogens can cause huge yield losses and deteriorate the quality of the produce. Based on what said Rahul and Singh (2002), it's about 65 pathogens causing disease to maize crop. Accordingly in Nepal, Subedi (2015) reported a total of 78 species (75 fungal and 3 bacterial species).

2.5. Maize genotypes sensitivity

Most of cultivated clones of maize, whether local and improved, hybrids, inbred lines, or pure lines, don't resist to SCLB where the disease is reported. Cultivars used in rural areas are susceptible to varying degrees to this disease given the intrinsic traits of each, and for reason that each genotype has its genetic heritage.

3. Symptomatology, pathogenesis, etiology and spread of the disease

SCLB is considered as one of the serious and major important diseases worldwide. It is found in all continents of the world. As pointed out previously, SCLB symptoms depend on host germplasm and race of the pathogen. For Pavan and Shete (2021), the fungus produces lengthy, cigar structured ovoid and greyish lesions on lower leaf parts. The ear husks, leaves, cobs, ears, sheaths, stalks, and shanks may be contaminated by *B. Maydis*. If the enough infection occurs earlier on the shank, the ear may be killed prematurely, causing the ear to fall. A felty, black mould that can cause cob rot, can cover the SCLB infected kernels. Ear rot is more prominent on cms-T cytoplasm corn with Race T (Calvert and Zuber, 1973). Infected seedlings can show the symptoms of wilting and the infected plant will die after planting in a few days (Agrios, 2005). The crop being infected with Race O exhibit symptoms like small minute lesions and later converted to triangle shape and become rectangular on maturing (Pal and Kaiser, 2005; Ali et al., 2011). Damage of leaf photosynthetic area so it could lead poor grain filling.



Fig. 1. Symptomatology of *B. maydis*

Cochliobolus heterostrophus Drechsler is an accurately member of taxonomic group of *Cochliobolus* genus, Pleosporaceae family, order of Pleosporales, subclass of Pleosporomycetidae, class of Dothideomycetes, subphylum of Pezizomycotina, phylum of Ascomycota, kingdom of Fungi and domain of Eukaryota. The genus *Bipolaris* includes important phytopathogen species with worldwide distribution (Alcorn, 1988; Manamgoda et al., 2014; Sun et al., 2020). *C. heterostrophus* is heterothallic. It is either asexual conidia which is primary source of inoculum or sexual ascospores. It produces perithecia (Tinline and Dickson, 1958), or pseudothecia in some conditions. The stages in *B. maydis* are flowering stage, fruiting stage, seedling stage, and vegetative growing stage. In good conditions, spores can germinate and penetrate the plant in just 6 hours. *B. maydis* overwinters in plant debris as spores until favorable conditions return. Pointed out above, this fungus is also capable of following a sexual disease cycle, but this still only being found during manipulation in laboratory conditions.

Based on what is proved in the Plant pathology book of Agrios (2005), the *C. heterostrophus* release the spores to infect maize plants. In nature, mostly asexual cycle occurs and is of primary concern. Conidia are mostly released from the lesions present on infected maize under favorable moist and warm conditions and transported to the healthy plants through wind or rain. The

germination of the pathogen by means of polar germ tubes can easily occur on the tissue once conidia have landed on the surface of leaf or leaf sheath of healthy plant. The germ tubes, like the stomata, either pass through the leaves or reach via natural opening. The fungal mycelium invades parenchymatous leaf tissue. leaf tissues begin to become brown in color and subsequently will collapse. Such lesions result into conidiophores formation that will either further invade the main host plants (husks, kernels, leaves, stalk) or release conidial spores to invade nearby plants under favorable conditions.

According to Robert (1953), the southern corn (*Zea mays* L.) leaf blight (SCLB) (no race designation) was first discovered in the United States in 1923. It killed the green tissue of the leaves, effectively reducing the photosynthetic source area of the plant. Except for isolated outbreaks, prior to the 1970 epidemic, SCLB was not considered a major pest of corn in the United States (Carson, 2016). For many Scientists, among them Reddy et al. (1973), and Nelson and Hill (1976), SCLB epidemic occurred in southern USA between 1970 and 1971 and had spread north to Maine and Minnesota by mid-August.

For having favorable conditions (**Fig. 2.**) of the pathogen means the water is present on the surface of the leaf (Rehman et al., 2021). For the survival and spread of the disease, favorable conditions mainly depend on rainfall amount, relative humidity, and temperature conditions of the area (Sumner and Littrell, 1974). Schenck et al. (1974) showed that prolonged sunny days with dry weathers are not suitable for disease progress. Causal organism survives in diseased maize debris on the surface of soil or inside seed. Based on research results of Aylor (1975), the temperature range of 20-28 °C when presence of continues light and 28 °C in total dark for race O is necessary for conidia sporulation, 20°C and 24 °C for race T, sequentially. *B. maydis* conidia are detached only in presence of wind speed at 18 km/h.

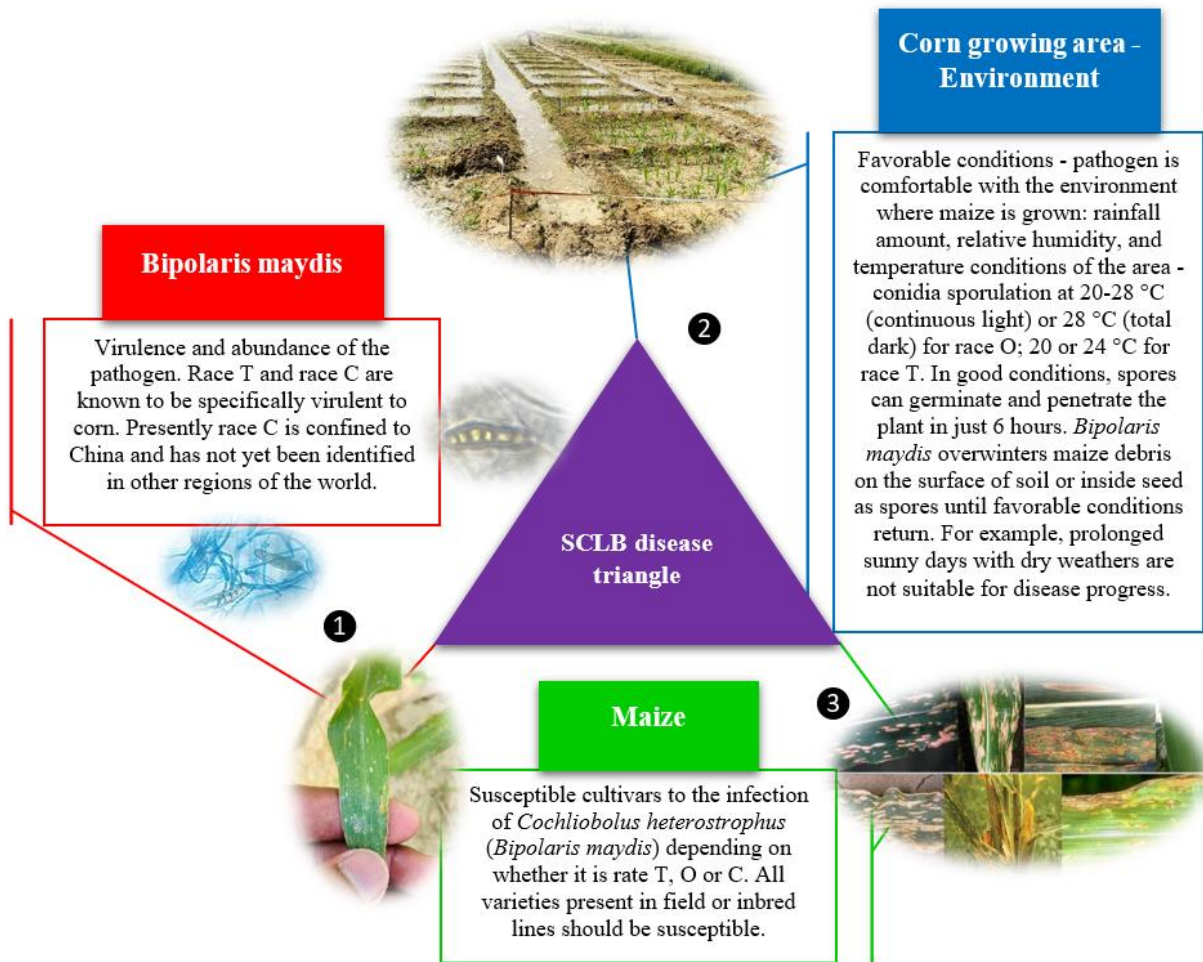


Fig. 2. SCLB disease triangle adaptation. When these three elements coincide, SCLB disease will occur. But, eliminating one of them will keep corn cultivars healthy.

Cytoplasmic male sterility, variability, and races diversity

C. heterostrophus has three races and can use ascospores or conidia to cause infection in maize, such as race T, O and C. Pathogen type like race T was found in India (Sharma et al., 1978). Around the world, race O is mostly occurred and wider in distribution (Pavan and Shete, 2021). Male sterility in corn was first discovered in Peru from seedings of an ear of an open-pollinated cultivar grown by Emerson and Richey (Duvick, 1965) and in Argentina by Gini (1940), but like the one from Peru it too has been lost. A cross was made to a plant from Chile that was unrelated, producing 45 F1 kernels that were all male sterile but, when fertilized with viable pollen, produced well-filled ears with no indication of female sterility (Bruns, 2017).

The origin of cms-T in corn began with a discovery of male sterility in a field of white corn Mexican June (Rogers and Edwardson, 1952). According to what is described by Bruns (2017), SCLB resulted from an over reliance on cytoplasmic Texas male sterile (cms-T) lines in hybrid seed production and a natural mutation of a race of the pathogen, that for years was seldom of economic importance. This mutation discovered in the Philippines in 1961 first appeared in the Corn Belt in 1969, damaging not only leaves, but stalks, ears, and developing kernels of hybrids containing cms-T genetics. A favorable environment, combined with >85% of the hybrids grown being of cms-T genetics set the stage for an epidemic. The cms-T was discontinued in 1971 and hybrid seed production returned to using detasseling for the female parent. According to Dewey et al. (1987), for cms-T plants, two restorer genes *Rf1* and *Rf2*, are known to be involved in restoring phenotypic male fertility. A plant that is heterozygous *Rf1 rf1 Rf2 rf2* at both loci will produce viable pollen. A second cytoplasmic male sterile group is cms-O. Race O of SCLB, as stated previously, will attack the leaf blade tissue of corn causing the development of small tan lesions usually 0.6 by 2.5 cm (Agrios, 1997). A third cytoplasmic male sterile group cms-C, discovered in a Brazilian cultivar Charrua, was reported after the SCLB-race T epidemic by Beckett (1971). This cytoplasm has been used in more modern hybrids but has succumbed to SCLB race C, which was first described by Wei et al. (1988). Presently this race is confined to China and has not yet been identified in other regions of the world. Detailed description of SCLB race-C is currently limited in part to this reason previously mentioned.

Race T of SCLB is more destructive to host plants than race O because of its tendency to form lesions on the leaf sheaves, ear husk, developing grain on the ear, as well as leaf blades (Carson, 2016). For Smith et al. (1970), those two most commonly races O and T are responsible to cause SCLB. Recall that, lesions produced by T strain are oval and larger than those produced by O strain. T strain affects husks and leaf sheaths, while O strain does not. Race O still significant problem in parts of the world, including Africa, India, USA, and Europe.

Sharma et al. (2001) reported that races O and T significantly differ in expression of symptoms produced, cytoplasmic specificity, production of toxins, optimum growth temperature regimes, reproductive rates, and the site of infection in the plants. Though race T has been detected in India, its distribution and incidence are not widespread whereas race O has been the most prevalent one.

4. Mycotoxin's production

Mycotoxins are toxic secondary metabolites of fungal origin which have a major effect on agriculture and ecology, as well as human and animal health. *C. heterostrophus* race T produces T-toxin. The genetics of T toxin production is complex, and the evolutionary origin of associated genes is uncertain. It is known that ability to produce T toxin requires three genes encoded at two unlinked loci, Tox1A and Tox1B (Kodama et al., 1999; Rose et al., 2002; Baker et al., 2006; Inderbitzin et al., 2010), which map to the breakpoints of a reciprocal translocation.

However, generally two physiologic races, T and O of the pathogen cause the disease. The two races are morphologically similar, but race T is specifically pathogenic to corn containing cms-T (Texas male-sterile) cytoplasm while race O is not. Race T produces a pathotoxin in culture that is highly toxic only to susceptible cms-T corn (Hooker et al., 1970), whereas race O produces only a small amount of a toxin non-specific to cytoplasms. A study of intraspecific crosses among three wild-type isolates of *C. heterostrophus* from various geographical areas showed that toxin production amount is under genetic control (Smedegard-Peterson and Nelson, 1969).

According to Lim and Hooker (1971), both the selective pathogenicity and the specific pathotoxin production of race T to plants with cms-T cytoplasm for male sterility are monogenic in inheritance. The severity of pathogenicity expressed and the amount of pathotoxin produced may be polygenic in inheritance. The two characters are highly associated. Miller and Koeppe (1971) support this statement.

5. Host-pathogen interaction

The overall understanding of host-pathogen-environment relationships (**Fig. 3.**) is fundamental in the study of SCLB disease. It wants to demonstrate the passage of the pathogenic fungus from plant parts liable or not known to carry the pathogen in transport to corn and vice versa. The nature of the various interactions that occur in the pathological host-pathogen system, likely to influence the epidemiology of the SCLB disease.

Plant parts liable to carry the pathogen in transport are through (1) seeds (grains) colonized by hyphae and spores of fungi where the pathogen (or its parts) or symptoms usually invisible, (2) leaves by hyphae and spores where signs or symptoms usually visible to the naked eye, (3) flowers, inflorescences, cones, or calyx colonized by hyphae and spores with signs and symptoms usually

visible to the naked eye. There exist also some plant parts not known to carry the plant pathogenic fungus in transport such as (4) bark, (5) bulbs, tubers, corms, or rhizomes, (6) fruits (pods), (7) growing medium accompanying plants, (8) roots, (9) seedlings or micropropagated plants, (10) stems (above ground), shoots, trunks, or branches, and (11) wood.

All these plant parts could play a role of potential reservoirs of the fungal pathogen *B. maydis* during the period the maize is absent in the area, by symbiosis and on which the pathogen can still acquire virulence for transmission to maize plants once present in the growing area.

6. Diagnosis of the SCLB based on disease symptoms

There is a lot of overlap between fungal, bacterial, and viral disease symptoms. Also, abiotic diseases, pesticide injury and nematode problems must be considered possibilities when an unknown plant problem appears. Generally, two concepts that always claim confusion in identification of plant fungal infection based on disease symptoms, are diagnosis and detection.

Diagnosis can be defined as a careful examination to determine underlying cause of the disease; whereas **detection** consist of finding out the presence or absence of a pathogen, which can be a fungus, bacteria, or virus.

For instance, following first steps are useful for diagnosing a plant fungal infection: observe disease in the field, determine affected plant species and cultivars, disease incidence and distribution within field (random-, clustering-, peripheral-, uniform-distribution of infected plants); record the symptoms and compare in literature for any similar descriptions on the same host in-country or elsewhere. This step is called visual examination.

7. Leaf sampling, isolation, characterization, and inoculation

The steps described above can lead to the collection of infected plant samples of corn showing typical symptoms, with purpose of identification of the fungal pathogen. In this case, examine maize leaf sample under microscope to detect any spore (conidia or ascospore) (**Fig. 3.**), this only after isolation of the pathogen; otherwise, use of lactophenol to stain the leaf is required. Apart from that, the standard tissue isolation method which can be used for isolation and characterization of *B. maydis* are described by different scientists (Nelson, 1957; Slesman et al., 1974; Fry et al., 1983; Zaitlin et al. 1993; Chang and Peterson, 1995; Tskiboshi et al., 1996; Anjos et al., 2004;

Shekhar and Kumar, 2012; Gogoi et al., 2014; Pal et al., 2015; Azra and Hussain, 2019; Aregbesola et al., 2020; Ferreira et al., 2022).

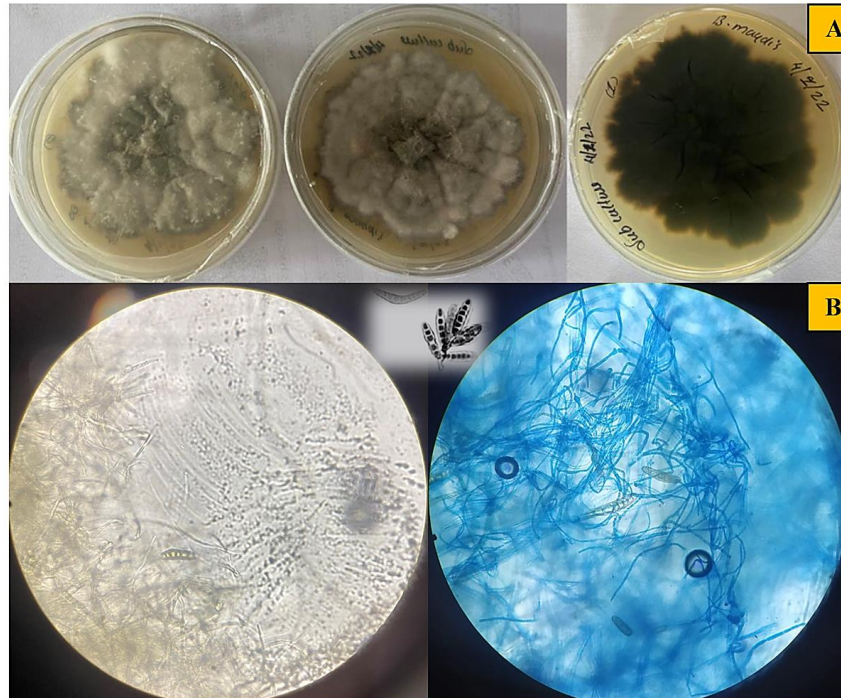


Fig. 3. Mycelial (A) and conidia (B) formation of *B. maydis*

The pure culture of the pathogen can be confirmed based on the morphological and molecular methods. The pathogenicity of the causal organism can be proved by using Koch's postulates including association of the fungal pathogen with the typical symptoms, isolation, inoculation, and re-isolation of the pathogen. In other words, inoculate (by spraying) the *B. maydis* to a range of test corn cultivars (health plants) and back inoculate to a parallel range of test cultivars to check possible multiple infections and to determine host range and symptoms; compare symptoms observed on experimental host range in literature for clues to identify or confirm the fungal pathogen; select systemically infected host for plant fungal propagation for purification purpose, local lesion host for fungus assays, and diagnostic species, which react uniquely to that causal fungus *B. maydis*. Thus, for purification and maintenance of the culture, the pathogen can be purified separately by transferring the tip of the mycelia into fresh PDA plates and maintained on PDA slants which must be stored at 4 °C.

Here are two detailed inoculation methods by Shekhar and Kumar with contributions of Gowda, Gogoi, Rai, Shetty, Sharma, Shekhar, Kumar, Shekhar, and Hooda (Shekhar and Kumar, 2012) for SCLB and other foliar diseases:

First Method. The pathogens are isolated by collecting diseased leaf lesions and placing in a moist chamber. After two-three days newly formed spores on the surface of the lesions are picked up with the help of fine flattened needle under a dissecting microscope, placed in a droplet of sterile water and streaked across the surface hardened, acidified water agar in Petri plates. After few hours the spores start to germinate, and they are cut out of the agar and transferred to hard, acidified PDA. After two weeks of incubation at 20-25 °C, this culture may be transferred to fresh plates of acidified PDA for multiplication. When the fungus growth covers the surface of Petri plate fully, the cultures are ready for use. About 20 Petri dishes of full-grown cultures are macerated in a warring blender for 15-30 seconds, strained through a layer of cheese or muslin cloth and made up to four five liters of suspension. This stock suspension is taken to the field and diluted in a compressed air sprayer (which is not ever used for pesticidal spray) 1 liter/12 liters of water. Spray should be done into the whorls of the plants where it will be retained for longer period/enough to permit the spore germination. If inoculation is sprayed over the leaves it evaporates before germination. Inoculation should be made twice a week for three weeks, when plants are 30-45 cm high, 120 Petri dishes of pure culture will be enough for 1000 plants.

Second Method. This is the easiest method to prepare inoculum by collecting heavily infected leaves collected in the previous year. This should be done before leaves become fully mature. Infected leaves should be stored in large gunny bags in dry conditions protected from moisture and rodents. To prepare inoculum, the dry leaves are ground into a meal about the coarseness of wheat bran. Inoculation is done by placing a pinch of leaf meal into whorl of each plant, when plant attains the height of 30-45 cm. A second inoculation may be made five to ten days later. This method of inoculation will be ineffective if dry weather prevails following application of the leaf meal. To overcome this situation, 10-12 ml of water can be applied in the whorls by means of sprayer. High humid weather is congenial for inoculation and disease spread. In case of rain soon after inoculation, water spray is not at all required.

These two techniques are effective for inoculum preparation and field inoculation to create artificial epiphytotic condition to screen maize germplasm for SCLB. However, for genetic

variability in isolates of *B. maydis* these steps can be followed, based on what Gogoi et al. (2014) reported: (1) collection of disease specimen and pathogen isolation; (2) isolation of genomic DNA from *B. maydis*; (3) nucleic acid extraction; (4) qualitative and quantitative analysis of *B. maydis* genomic DNA, (5) internal transcribed spacer-polymerase chain reaction (ITS-PCR), (6) random amplified polymorphic DNA analysis, (7) internal transcribed spacer-restriction fragment length polymorphism (ITS-RFLP), as well as data analysis with cluster.

8. SCLB disease assessment

Standard cultural practices throughout the growing season are highly recommended in controlling the behavior of maize cultivars under field conditions: land preparation, sowing, plant protection, weeding and irrigation, fertilizer application, hoeing and thinning. For artificial inoculation, the inoculum should be always prepared before any observation.

To assess the SCLB disease, observations and data collection must include disease severity based on disease severity scale (reaction and scoring of disease), marking of monitored plants, leaf disease incidence, percentage disease index, disease intensity, percentage of leaf infection, percentage plant infection, area under disease progress curve, number of harvested ears, thousand Kernel Weight (TKW) or test Weight, harvesting, threshing and yield, shelling percentage, grain moisture content, grain yield, phenological study SPAD (Soil Plant Analysis Development), and other important agronomical characters like height, diameter and leaf area.

Leaf area, $LA = L \times W \times A$ where L=leaf length, W=leaf maximum width and A, constant (A=0,75, a K-coefficient for determination of leaf area for maize).

Leaf disease incidence, $LDI = \frac{iL}{tL} \times 100$ where iL is number of infected leaves, tL is total number of leaves assessed.

Percentage disease index, $DI(\%) = \frac{n_1 \times 1 + n_2 \times 2 + n_3 \times 3}{\text{Total number of leaves observed} \times \text{Maximum rating value}} \times 100$

where, n, number of days after lesion observes (interval), 5, 0, 5 etc., x, number of lesions after interval.

Disease intensity, $DI_n(\%) = \left\{ \frac{n.v}{N.G} \right\} \times 100$ where, (n.v) - Sum of score, N - Total number of leaves counted, G - Highest score.

Percentage of leaf infection, $PLI = \frac{\text{number of leaves infected}}{\text{total number of leaves}} \times 100$

Percentage plant infection, PPI = $\frac{\text{number of plants infected}}{\text{total number of plants}} \times 100$

Area under disease progress curve, AUDPC = $\sum_{i=1}^{n-1} \left[\left\{ \frac{Y_i + Y_{i+1}}{2} \right\} x (t_{i+1} - t_i) \right]$ where, Y_i = disease

severity on the i^{th} date, t_i = time on which Y_i will be recorded and n = number of times observations will be taken. The average data of each score at five days' interval has always to be converted to percent leaf area for computation of AUDPC. This parameter gives a quantitative measure of disease development and intensity of disease (Reynolds and Neher, 1997). Thus, the variety having the lowest AUDPC value must be categorized as the most resistant, while susceptible one has higher values (Bhandari et al., 2017). AUDPC values is also used to identify disease resistance in maize (Leath et al., 1990). Based on the mean AUDPC values, the genotypes must be categorized into 4 groups of resistance level as below (Magar et al., 2015). The resistance category code (mean AUDPC value) is as follows: resistant (1-30), moderately resistant (31-60), susceptible (61-90) and highly susceptible (> 90). This parameter was highly used by many scientists like Campbell and Madden (1990) and Ceballos et al. (1991) in the past years.

Confirmation of SCLB disease. Infected leaf should be stained with lactophenol and observed under microscope. Several branched conidiophores and elliptical conidia and few septa and pseudosepta will be observed to confirm the presence or not the disease.

Number of harvested ears. Total number of ears harvested from net harvestable area must be recorded as harvested ears per plot and being converted to hectare basis.

Thousand Kernel Weight (TKW) or Test Weight. One thousand shelled maize grains from each plot can be randomly taken, weighed, and recorded as test weight and expressed in gram (g). The kernels used for test weight should be adjusted to around 15% moisture content.

Reaction and scoring of disease. O- Highly resistant - no visible infection, R- Resistant - necrotic areas with or without minute uredia, MR- Moderately resistant - small uredia present surrounded by necrotic areas, MS - Moderately susceptible - medium uredia with no necrosis or distinct chlorosis, and S – Susceptible - large uredia and little or no chlorosis present severity (Razzaq et al., 2019). All the leaves on infected plants should be scored using 0-5 scale adopted by maize pathology unit (CIMMYT, 2004) as 0 = no visible lesion, 1= one to few scattered lesions on leaves covering up to 10% of leaf area, 2= lesions on leaf covering 11- 25% leaf area, 3= lesions on leaf covering 26-50% leaf area, 4 = lesions abundant on leaf covering 51-75% leaf area, 5 = lesions abundant on almost all leaf, plant prematurely, dried with 76-100% leaf area covered.

Harvesting, Threshing and Yield. Crop will can be harvested manually from net plot area, i.e., after plants turning yellow, ear husk turned brown, and a black layer appeared at the base of each kernel when scratched. Grain yield/plot can be taken by weighing all dehusked cobs (*shelling percentage* considering to be 80%). Randomly selected cobs can be shelled, and the grains being used for moisture recording by a moisture meter, and grain yield (Kg/ha) being adjusted generally to 15% MC. $Yield = \frac{FW(Kg) \times (100 - MC\%) \times 0.8 \times 10}{NHA \times 85}$ where, FW= fresh weight, NHA= net harvested area (m²) and MC= moisture content. The shelled grains should be always cleaned, and sun dried to maintain recommended moisture level and TKW can be taken.

Shelling percentage. It is the ratio of grain to ear (grain: ear) and expressed in percentage. Five to ten randomly selected ears can be weighed with grains. All grains should be shelled out and the weights of grain being taken. $Shelling\ percentage = \frac{Grain\ yield\ (kg)}{Cob\ yield(kg)} \times 100$

Grain moisture content (%). Depending on the field superficies, ten to twenty ears can be selected randomly and central two kernel rows being shelled out. The kernels can be bulked from all ears and moisture can be measured, for example, by multigrain moisture meter.

Grain yield, Grain yield (Kg/ha) = $\frac{FEW \times SP \times (100 - GMC)}{NHA \times 85 \times 10}$ where FEW= filled ears weight (Kg), SP= shelling percentage (%), GMC = grain moisture content at harvest (%) and NHA = net harvested area (m²).

Phenological Study-SPAD (Soil Plant Analysis Development). SPAD is used to measure the chlorophyll content of leaf. 15 SPAD readings of 5 to 10 randomly plants to be selected from each plot can be recorded and average SPAD above/ below cob will be calculated.

In addition, the general formula is that Disease Severity, DSI (%)= [sum (class frequency × score of rating class)] / [(total number of plants) × (maximal disease index)] × 100, and Disease Incidence, DI= number of infected plants or leaves / total number of observed plants or leaves.

All these observations can be modified according to objectives pursued by a specific study. Here we shed light important information to be considered in SCLB disease assessment and gain more knowledge regarding the same. In case, a researcher wants to go for screening of genotypes against SCLB and estimation of yield loss in maize cultivars, all these observations should be evaluated. Otherwise, there will be lack of information.

9. Real impact of SCLB disease on maize crop

9.1. Reduction in crop yield, costliest fungal disease

According to Mubeen et al. (2015), SCLB is considered the most devastating disease of maize crop, which causes noticeable reduction in crop yield. Based to what said Bruns (2017), it was one of the costliest disease outbreaks to affect North American agriculture, destroying 15% of the crop at a cost of US\$1.0 billion (Ullstrup, 1972), similar to \geq \$6.0 billion by 2015 standards (Bruns, 2017). If the seeds are eventually exposed to Race T (Bruns, 2017), then the estimated losses can extend up to 100%, a total loss as reported many years ago by Southeast Farm Weekly (Ullstrup, 1972). For *C. heterostrophus* race O, grain yield loss was evaluated at \geq 40%, by Gregory et al. (1979) and Byrnes et al. (1989). According to Rehman et al. (2021), it was not unusual for few farmers to bear 80 to 100% losses and average losses of about 35 and 50% have been reported in corn belts. Tatum (1971) reported that losses to SCLB approached 710 million bushels. This amount is equivalent to 25,019,739.82 tones, i.e., economic loss around US\$ 6,004,737,556.8 (with 1 tone being about \$240 by 2022).

9.2. Increasing in corn price and demand, lack of grow seeds, affection of human healthy

Since its apparition (Ullstrup, 1972), Chicago Tribune and Wall Street Journal reported that SCLB epidemic increased the price of corn "futures" on the Chicago grain market in USA, soared from about \$1.35 per bushel in late July to \$ 1.68 per bushel (1bushel is about 8 gallons, equivalent to 36.4 liters or >64 US pints) by September 20, 1970, as well as from about \$1.30 per bushel in late May to about \$1.63 per bushel in late June in 1971. Demand in maize and its price increased also in United Kingdom at that time. This was the first time a disease had seriously affected the price of corn. Seed supplies in the United States were estimated to consist of about 25% normal-cytoplasm hybrids, 25% Terns hybrids and 40% blends. The demand for normal-cytoplasm seed was far beyond the supply. Seed was also imported regarding insufficient quantity and cost a lot to the country. Many farmers became concerned over the possible hazard of feeding infected grain or corn forage to livestock. Storage and milling problems with infected corn were anticipated but did not prove to be serious. When properly dried, infected corn stored as well as healthy corn. Milling difficulties were overcome by blending infected with noninfected corn. Respiratory difficulties, including symptoms of asthma and hay fever as well as some skin irritations were attributed to the fungus. Inhalation of spores can be prevented by wearing a respirator and thorough washing usually will allay skin irritation.

10. Prophylactic measures in fields and Integrated SCLB management

SCLB is most prevalent though in the warm temperate and humid subtropical regions. Controlling the disease and its pathogen involve the use of botanical extracts, biocontrol agents, fungicides, resistant hybrids, tillage practices, and crop rotation with a non-host (**Fig. 4.**).

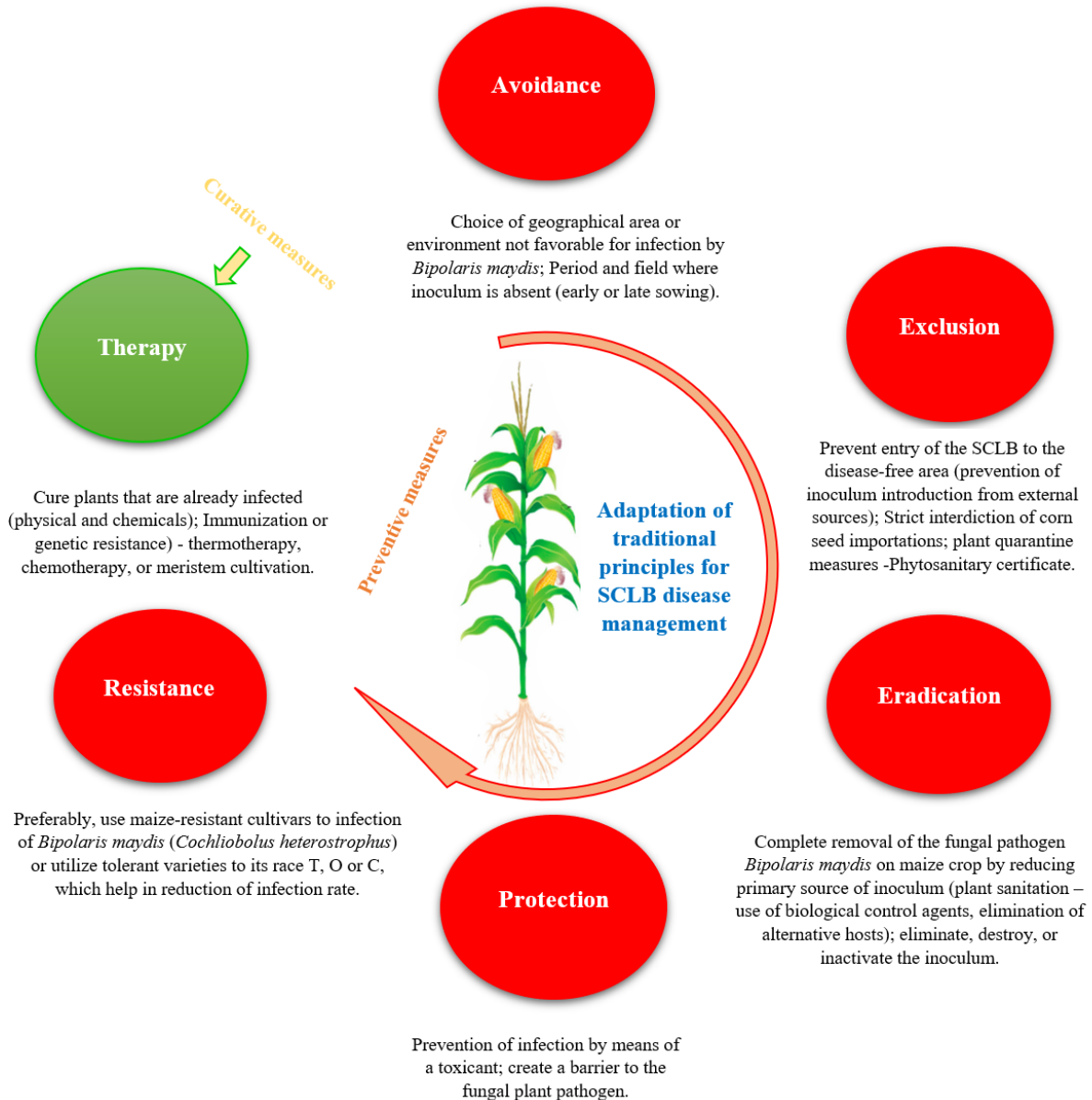


Fig. 4. Principles applicable in SCLB disease management

For different botanical extracts like bael (*Agelemarmelos*) very toxic to many fungi, including SCLB pathogen (Karande et al., 2007), garlic (*Allium sativum*) in which was found 72.65% mycelial development suppression (Khamari et al., 2015), neem (*Azadirachta indica*), the best one in management of the fungi (Gurjar et al., 2012; Kumar et al., 2021), onion (*Allium cepa*), ginger, turmeric (Kumar et al. 2021) and eucalyptus, their efficiency was tested individually against *B. maydis*. Above all, individual extracts are evaluated against the spore growth of *B. maydis*, and all extracts were found good in inhibition of spore germination at the range of 50% to 100% when compared to control (Jha et al., 2004). Thus, the garlic clove extracts are very effective in suppressing the growth of pathogen causing leaf blight up to around 85% (Kumar et al., 2009) or fully suppressed at 10% both in field and laboratory conditions (Meena et al., 2003).

Many species of the genus *Trichoderma* are mainly used as biocontrol agents against the pathogen. The *Trichoderma* populations are suppressing the growth of *B. maydis*. Natural enemies of the fungus are *Trichoderma harzianum* (Wang et al., 2019; Ashlesha et al., 2019; Harman, 2000), *Burkholderia cepacia* which both control the pathogen by mechanism of antagonism while *Chaetomium globosum*, is a pathogen and *Hypocrea rufa*, a mycoparasite of *B. maydis*. Kumar et al. (2021) conducted an *in vitro* study and reported that all the isolates of *Trichoderma* reduced the mycelial growth of *B. maydis*.

Scientists have proved that fungicides such as carbendazim, mancozeb, chlorothalonil (Harlapur, 2005; Harlapur et al., 2007; Kumar et al., 2021), propiconazole (Harlapur et al., 2007; Dai et al., 2018; Kumar et al. 2021), pyraclostrobin, fluxapyroxadare (Chapara et al., 2012), diniconazole and prochloraz (Dai et al., 2018), carboxin, thiram (Kumar et al., 2021) are effective against *B. maydis*. In the past years of disease apparition, controlled and replicated field experiments with fungicidal sprays showed significant reductions in disease severity and a corresponding increase in yield with applications of Dithane M-45, Manzate 200, and Citcop 4E (Ullstrup, 1972).

For instance, between growing seasons, it is necessary to check for the crop debris. As in leaf or leaf sheath debris, *B. maydis* overwinters. The crop rotation with some other non-host crops is another method that is used to reduce this disease. Further, foliar fungicides can be used. The management of this disease is crucial from 15 days before to 21 days after the tasseling, which is the most important and susceptible period for damage (Rehman et al., 2021).

For integrated management proposed by Rehman et al. (2021), breeding of resistant host is the best method for handling SCLB. Both origins of polygene and single gene resistance have been studied. The distribution of all races can be avoided by other methods of regulation.

11. Effective approaches and research trends for disease control

11.1. Genes as sources of resistance

The only well-known means of controlling the SCLB disease is the use of resistant hybrids.

Resistance is the ability to prevent infection or limit parasite replication (Råberg, 2014). For example, fungal cell wall is source of resistance (Díaz-Jiménez et al., 2012). Based on what said Bilichak et al. (2020), the ability to make specific modifications to a genetic material of a plant creates lots of opportunities for the rapid development of high-quality cultivars with desired characteristics, including resistance and yield increasing. The Department of Agriculture and the agricultural experiment stations in Indiana, North Carolina, and Georgia had discovered by 1950 several sources of resistance that scientists have used to improve inbred lines (Robert, 1953). According to Sharma et al. (2001) CM 104 and CM 105 have been considered as the two sources of resistance to SCLB during the last two decades, their resistant reaction being controlled by additive genes with negative effects appeared to be highly efficient in transferring high level of resistance in a direct crossing programme for hybrid development under this genetic situation. Thus, selection of cultivars for disease resistance is with great interest (Shukuru et al., 2022; Shukuru et al., 2023).

Among the twenty maize genotypes experimented in Rampur, Chitwan, Nepal by Magar et al. (2015), RML-32/RML-17, RML-4/NML-2 and RML-4/RML-17 appeared resistant to SCLB. Accordingly, Santos et al. (2020) reported that the hybrids L61xL76, L61xL77 and L76 x P1 were the most promising for resistance to SCLB and other the fungal diseases. In the same way, lines like SE-013, SAM, PR-023, PARA-172, PA-170-R, PA-091, CHZM-13-134, BOZM-260, ARZM-07-04 are resistant to the pathogen (Saluci et al., 2020). Shekhar and Kumar (2012) said that, for minimizing the losses due to diseases and simultaneously increasing the production to meet the burgeoning demand, it is necessary to introgress an adequate level of genetic resistance against SCLB and other maize diseases of economic importance. Kraja et al. (2000) identified several tropical and temperate maize germplasm accessions as sources of alleles to improve disease resistance *Bipolaris* species among them tropical×Mo17, a germplasm highly resistant to SCLB.

The works conducted by Martinez and Bruni (1972), Gengenbach and Green (1975), Sharma et al. (1982), Burnette and White (1985), Dey et al. (1988), Liang et al. (1988), Holley and Goodman (1989), Tsukiboshi et al. (1992), Kump et al. (2011), Chandrashekara et al. 2014), Sharma et al. (2021) reported different new resistance sources, with distinct resistant genotypes. Cai et al. (2003) found that the co-dominant AFLP marker, p7m36, combined with agrP144, may be useful for map-based cloning of the *rhm* gene and marker-assisted selection for *rhm*. Accordingly, Zhao et al. (2012) suggested that LHT1 is the best candidate gene for *rhm1* against SCLB.

11.2. Challenges and upcoming outlooks

No doubt that there are several methods to control fungal diseases including SCLB. Fungicides are by far effective, giving rapid response by suppressing the fungal pathogen populations, compare to other management techniques, but most of fungicides are continuously being banned and withdrawn for use in disease management. Also, few fungicides are effective against the pathogen, *B. maydis*, after it has infected the maize crop. Biofungicides should therefore be privileged instead of chemical fungicides; that for many reasons such as phytotoxicity, environmental hazards and human health. For example, *Bacillus subtilis* DZSY21 isolated from the leaves of *Eucommia ulmoides* Oliv. was found to effectively colonize the leaves of maize plant. DZSY21 and its lipopeptides had antagonistic activity against *B. maydis*, as well as high biocontrol effects (Ding et al., 2017). Several biological agents described above play an important role in SCLB management.

Presently, as well as in future, disease resistant sources of male-sterility may be given priority and attention, and rapidly recommend its employment by the Scientific Committee. The truth is that such sources are at hand, but their usefulness and potential hazards still posing a barrier to their use, because an effective examination should be done carefully. Ullstrup (1972) reported that, carefully tested, chemical gametocides may become useful in hybridization. He also gave a statement that so far, no chemical has been found that will destroy or sterilize pollen with the thoroughness needed for large scale seed production. For Bruns (2017), Ullstrup's warning is as true today as it was in 1972: "never again should a major cultivated species be molded into such uniformity that it is so universally vulnerable to attack by a pathogen, an insect, or environmental stress. Diversity must be maintained in both the genetic and cytoplasmic constitution of all important crop species". The duplex PCR based method was found to be effective to prevent

infections in maize by detecting infected seeds or maize and discarding them. Besides saving time and effort, early diagnosis can help to prevent infections, establish comprehensive management systems, and secure healthy seeds (Kang et al., 2018).

12. Concluding remarks

This chapter explored different ways that can be used to control SCLB disease. Understanding recent approaches and research trends are helpful; considering the economic yield loss registered in maize crop is key in SCLB management. SCLB is caused by the fungus *B. maydis*. There are three races of the pathogen. Race O normally attacks only leaves. Lesions are tan, somewhat rectangular in shape, and have reddish-brown margins. Race T attacks leaves, husks, stalks, leaf sheaths, shanks, ears, and cobs. The yield losses in maize reported in the world due to SCLB disease and its pathogen since its discovery are still worrying scientists. Resistant hybrids and inbred, or pure lines are available. Some foliar fungicides labeled for SCLB are available. Regarding restrictions required in hybrid seed production, and in use of chemical fungicides, integrated disease management is most encouraged nowadays, especially using crop rotation, tillering system, natural enemies including biofungicides and resistant cultivars.

Declaration of Competing Interest

The one and only author declares that he has no known competing personal relationships that could have appeared to influence this golden endeavor.

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