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Fusarium Species Complex Causing Pokkah Boeng in China

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<http://dx.doi.org/10.5772/intechopen.73133>

Abstract

Sugarcane is one of the most important crops for sugar production in sugarcane-growing areas. Many biotic and abiotic stresses affected the sugarcane production which leads to severe losses. Pokkah boeng is now playing a very important role due to its economic threats. Currently, the occurrence and rigorousness of pokkah boeng disease have been spread like wildfire from major sugarcane-growing countries. Pokkah boeng is a fungal disease that can cause serious yield losses in susceptible varieties. Infection of the disease is caused either by spores or ascospores. It may cause serious yield losses in commercial plantings. However, there have been many reported outbreaks of the disease which have looked spectacular but have caused trade and industry loss. *Fusarium* species complex is the major causal agent of this disease around the world, but some researchers have documented the increased importance of *Fusarium*. Three *Fusarium* species have been identified to cause the sugarcane pokkah boeng disease in China. Moreover, *Fusarium* may be accompanied of its mycotoxin production, genomic sequencing, and association with nitrogen application in China. Many studies on disease investigations, breeding of disease-resistant varieties, and strategy of disease control have also been carried out in China.

Keywords: sugarcane, pokkah boeng, *Fusarium* species complex, nitrogen, secondary metabolism

1. Introduction

Sugarcane is a major crop in Southern China, and it is the third biggest sugarcane producer in the world. Sugarcane is one of the most important crops grown commercially in the tropical and subtropical region. Sugarcane belongs to the genus *Saccharum* L. composed of hybrids [1, 2]

derived from *Saccharum officinarum* (noble clones), *S. sinense* (Chinese clones), *S. barberi* (North Indian clones), and *S. spontaneum*. This species has C4 photosynthesis, resulting in a vigorous biomass accumulation under tropical conditions, but it also implies a less growth in temperate regions. It grows well in deep, well-drained soils of medium fertility of sandy loam soil textures with a pH range from 6.0 to 7.7. It plays a major role in the economy of sugarcane-growing areas. Sugarcane can be affected from different organisms with various factors such as environmental and physiological disorders and nutritional deficiencies.

Many biotic and abiotic stresses affected the sugarcane production and are known to be one of the oldest cultivated plants in the world. Improving sugarcane production will greatly help in economic prosperity of the farmers and others associated with sugarcane cultivation. Large numbers of sugarcane pathogens have been recorded all over the world. One of the current major diseases affecting sugarcane and sugar production is pokkah boeng. It is caused by *Fusarium* species complex, a destructive fungal disease in sugarcane-growing regions.

Fusarium is a devastating phytopathogenic fungi belonging to Division: Ascomycota, Class: Sordariomycetes, Order: Hypocreales and Family: Nectriaceae. The fungal genus *Fusarium* is composed of a large number of species that can be pathogenic on plants. Within the genus the following 16 sections have been recognized: Eupionnotes, Macroconia, Spirarioides, Submicrocera, Pseudomicrocera, Arachnites, Sporotrichiella, Roseum, Arthrosporiella, Gibbosum, Discolor, Lateritium, Liseola, Elegans, Martiella, and Ventricosum. However, many *Fusarium* species are abundant in fertile cultivated and rangeland soils rather than in forest soils [3]. *Fusarium* species are causal agents of various diseases affecting many economically important cereals, crops, etc. Airborne *Fusarium* species are rarely found in the cultures obtained from soil or the roots of plants.

Fusarium species can grow on a variety of substrates and have efficient dispersal mechanisms owing to their worldwide distribution. Plant debris in soils plays a very important role as nutrient reservoir for *Fusarium* species to continue living in soils as saprotrophs [4]. *Fusarium* spp. also produce gibberellic acid [5], fusaproliferin, and beauvericin [6]. Fusaproliferin and beauvericin have been found to be toxic to insects [7, 8]. The pathogens are difficult to control by conventional strategies such as the use of resistant host cultivars and synthetic fungicides.

Pokkah boeng disease on sugarcane has been recorded in almost all countries where sugarcane is grown commercially. It normally appears during periods of hot humid conditions when the cane is growing rapidly. This disease was originally described in Java in 1896, denoting a malformed or distorted top. The temperature, light, and fertilizer regimes are optimized for maximal plant growth, but these conditions may also be favorable for pathogens. Walker and Went (1896) were the first ones who describe the pokkah boeng disease on sugarcane. Generally, it appears that slowly growing fungi, which are less efficient than quickly growing fungi at escaping competition by entering specific niches, have a higher prevalence of enmity against competing fungi. Geh [9] first reported the presence of the disease in Malaysia. It may cause substantial damage to the crop and not severe except in very susceptible varieties.

Pokkah boeng is a reemerging disease of sugarcane—which has been found recently to cause major yield losses—in most sugarcane-producing regions, including South Africa, Malaysia,

India, and China [10–14]. Pokkah boeng disease of sugarcane has associated with several diseases of sugarcane such as sett rot, root rot, and wilt [15]. The pathogen is transmitted by air currents, and airborne spores will colonize the leaves, flowers, and stems of the plant [16]. Pokkah boeng causes serious yield losses in commercial plantings. Reported outbreaks of the disease, while looking spectacular, have caused economic losses. The fungus was reported to occur systemically in all plant parts of sugarcane.

Pokkah boeng diseases are dependent upon the environmental conditions, quality of setts, and handling of the plants, e.g., exposing sugarcane plants to stress either from water stress, temperature, pH, or soil nutrition. Hail damage can cause cane plants to be easily susceptible to diseases due to the bruised stalks and broken leaves, giving the diseases access to the damaged setts. Some of the favorable conditions for disease development included drenched conditions of the soil, lack of cultural practices that result in the growth of weeds, constant cultivation of same variety in the field, and existence of susceptible varieties in the surroundings. It is very important for a farmer to prevent and control such pests and diseases to avoid losses. *Fusarium* species complex can produce many kinds of toxic secondary metabolites known as mycotoxins, which can easily enter humans and animals through food and feed because of their resistance to milling, processing, and heating [17].

The taxonomy of *Fusarium* species complex (FSC) is based on phylogenetic, biological, and morphological species concepts [18, 19]. Species in the FSC produce a wide range of mycotoxins that contaminate food and are harmful to human and animal health. *Fusarium* species are common and can survive for long periods in soil. The nature of *Fusarium* disease is that they often become a problem after plant stress occurs. It is now well known that *Fusarium* causes two different diseases, one in stalk and the other in leaves/spindle, and two different species, namely, *F. sacchari* and *F. verticillioides*, respectively, were associated with these diseases. Conventional field-based screening for resistance to pests and diseases is a key component of the breeding program prior to release of a commercial cultivar [20].

Several control measures may be implemented to minimize potential sugarcane yield loss caused by pests and diseases, but an integrated approach is often recommended. Good farming practices are essential but do not guarantee eradication of infections. The planting of resistant cultivars is recommended as the best and most economical approach for controlling pests and diseases, having the least impact on the environment and increasing productivity without the need for other inputs, such as costly chemical applications or labor. Breeding sugarcane that is resistant to multiple pests and diseases is difficult due to the complex genome of sugarcane [21]. Additional genome-scale comparative and functional studies are needed to elucidate the evolution and diversity of pathogenicity mechanisms, which may help inform novel disease management strategies against *Fusarium* pathogens.

2. Manifestation of pokkah boeng

The initial symptoms were easy to recognize the disease since they attack the top parts and are chlorotic areas at the base of young leaves. Heavily infected plants showed a malformed or

damaged top, and stalk may occur in highly susceptible varieties. The base of affected leaves is often narrower than that of normal leaves. Ladder-like lesion on the spindle leaves pronounced yellowing, wrinkling of the spindle, twisting or tangling appearance of the spindle, marketing red stripes, and shortening of the leaves accompanied the malformation or distortion of the young leaves. The most advanced and serious stage of pokkah boeng is a top rot phase. Leaf infection sometimes continued to downward and penetrates in the stalk by way of a growing point. The young spindles are killed and the entire top dies. Leaf sheaths may also become chlorotic and develop asymmetrical necrotic areas of reddish color.

The reddish tissue form ladder-like lesions, often with dark edges. These lesions sometimes break through the surface of the rind. Occasionally, the pathogen also attacks the spindle, and from there it moves down the terminal portion of the stalk causing top rot. The pathogen makes its entry into the host tissues through any sort of injury made by insects or borers or natural growth cracks, etc. The severity of symptoms varies with the susceptibility of a variety and with the congenial environmental conditions and governs the development of the causal organism. During fungal penetration and growth inside the plant, *Fusarium* proteases and mycotoxins act in a kind of strategic cooperation during spike and core colonization by featuring complementary roles during the host defense suppression and the intracellular colonization of spikelet.

3. Mode of transferal

The pathogens of pokkah boeng disease are transmitted by the movement of spores through airflow. For spores to take off, it depends on the environmental situation that requires different strategies to disperse. Fungal species that dispersed by rain splash are based on the “puff” and “tap” mechanisms that will cause the dry spores to become airborne, and usually the spores are curved like *Fusarium* species.

The growth of sugarcane is the most important factor in the biological control and prevention and land and natural environmental factor. The processes for controlling are limited, and there is an increasing need for novel and environmental strategies to control diseases of sugarcane. There will be four sections in this chapter, including *Fusarium* species complex (FSC) and their distribution, comparative genomics of *Fusarium* species complex (FSC), FSC and nitrogen, and sugarcane resistance to FSC.

3.1. *Fusarium* species complex (FSC) and their distribution

Fusarium is a genus of filamentous fungi that includes many toxin-producing plant pathogens of agricultural significance and opportunistic human pathogens. The *Fusarium* collectively represents the most important group of fungal plant pathogens, causing various diseases on nearly every economically important plant species. Besides their economic importance, species of *Fusarium* also serve as key model organisms for biological and evolutionary research. It is the most common and significant pathogen which spread pokkah boeng disease all over the world. Pokkah boeng disease of sugarcane can drastically reduce crop yield and quality. *Fusarium* species produce a number of secondary metabolites that are dependent on different

physiological responses in plants and animals. It also produces a variety of other compounds such as other mycotoxins, pigments, antibiotics, and phytotoxins.

Fusarium species are commonly identified based on their micro- and macroscopic features. But these features are mostly unstable and render the taxonomy of the group problematic. The presence of different taxonomic systems for the genus also contributes to this problem. A number of molecular tools have been used to circumvent these limitations and also to characterize *Fusarium* isolates in terms of their genetic diversity, population biology, and phylogeny. In the studies presented here, *Fusarium* strains isolated from agricultural soils and plant tissues were characterized using different DNA-based tools.

Fusarium fujikuroi (formerly *Gibberella fujikuroi*) species complex (FFSC) members cause important diseases in gramineous crops. The FFSC becomes compatible with the species concept of *F. moniliforme* as described by Snyder and Hansen or section *Liseola* as defined by Wollenweber and Reinking. *Fusarium fujikuroi* is known to produce a broad spectrum of secondary metabolites.

To recognize and define species in the FFSC, various operational species concepts have been applied. However, a variety of genetic, ecological, and biological traits and properties may be used for this purpose. Only morphological species recognition (MSR), biological species recognition (BSR), and phylogenetic species recognition (PSR) have contributed significantly to the classification of *Fusarium* species in the FFSC. Of these, the MSR was the most widely used and has dominated *Fusarium* taxonomy since its establishment in 1809. The MSR also takes into account physiological characters such as growth rates at different temperatures, host associations, and secondary metabolite production. The majority of the current GFC species definitions and descriptions are based on such polyphasic or integrative taxonomic approaches that incorporate various types of data. Till now, two species of FFSC have been identified to cause sugarcane pokkah boeng disease in China.

3.1.1. *Fusarium verticillioides*

Fusarium verticillioides is the most commonly reported fungal species infecting sugarcane. *F. verticillioides* is the accepted species, which was also known as *Fusarium moniliforme*. It can able to produce the chemical agent fusaric. Among the *Fusarium* species, *F. verticillioides* is the most prominent *Fusarium* species in China. It is regulated by the fumonisin biosynthetic gene cluster (*FUM*), responsible for transport proteins. In our previous study, a total of 101 isolates were recovered from the sugarcane plants affected by pokkah boeng, which were collected from the major sugarcane-producing areas (Guangxi, Yunnan, Guangdong, Fujian, Hainan) in China throughout 2012 and 2013. More than 90% of the isolates (94 isolates) belonged to *F. verticillioides*, which was closely related to *F. sacchari*, using the morphological observation and the phylogenetic tree of rDNA-ITS region sequence amplified using fungus-conserved ITS1 and ITS4 primers.

Fusarium verticillioides causes seedling decay, stalk rot, and mycotoxin contamination in sugarcane. This destructive disease occurs virtually everywhere that sugarcane is grown worldwide. Airborne spores (conidia) arising from fungal growth on plant debris or current growth on silks or leaves may cause infection. *F. verticillioides* (teleomorph *Gibberella moniliformis*) is a filamentous fungus that produces two types of conidia—macroconidia and microconidia. The fungal colony of the *F. verticillioides* isolate (CNO-1) appeared to be pale in color but became

orange at the top as it aged, while it was initially white at the bottom which later changed into a yellow color. The fungus is distributed throughout the world but predominant in humid tropical and subtropical regions and also present in the temperate regions.

3.1.2. *Fusarium proliferatum*

Fusarium proliferatum is grouped in FFSC and can be found on a wide host range as well as pathogenic on various agricultural crops. *F. proliferatum* is a common pathogen infecting numerous crop plants and occurring in various climatic zones. It occurs worldwide as a moderately aggressive pathogen of multiple plant species. *F. proliferatum* is well documented as a fumonisin-producing species, and some strains can produce large quantities of fumonisins, a group of polyketide-derived mycotoxins. *F. proliferatum* causes diseases on a remarkably wide range of plant species, including asparagus, banana, date palm, fig, mango, pine, and sorghum. *F. proliferatum* causing sugarcane pokkah boeng disease was firstly detected in 2012 in China. *F. proliferatum* in sugarcane is important for resistance, for estimating the evolutionary risk of the pathogen, and for planning the agricultural management practices.

During winter or in dry periods, *F. proliferatum* survives in the soil and on plant debris. It also produces other mycotoxins, including beauvericin, enniatins, fusaric acid, fusarin, fusaproliferin, and moniliformin. *F. proliferatum* can be distinguished from other species of the FFSC by analysis of molecular markers. Most recent assessments of fungal pathogens have used multilocus markers to detect populations. The ability of strains and species from geographically separated locations to recombine poses the danger of introducing virulence or toxigenic genes into local pathogen populations. The most commonly observed in human infections are *F. proliferatum*. However, members of FFSC are increasingly identified in especially invasive and disseminated infections in hemato-oncological patients. Many environmental *Fusarium* species and the human infections they cause have a worldwide distribution. The knowledge of the genetic structure of the *F. proliferatum* populations might be useful in order to establish effective strategies for controlling the disease.

3.1.3. The other members of *Fusarium fujikuroi* species complex (FFSC)

Other FFSC, viz., *F. sacchari*, *F. verticillioides*, *F. proliferatum*, and *F. subglutinans*, have been isolated from sugarcane. The fungus *F. sacchari* grows on decaying plant material and produces a large number of conidia that are spread by wind and rain. The stem borer *D. saccharalis* was shown to carry the fungus from plant to plant in different locations and provide access of conidia in the wind and rain to the inner stem, through their damage made to the stalk. Sugarcane infestation by the stem borer *E. saccharina* is a major problem in the sugar industry. The lepidopteran's infestation of sugarcane by boring the stalk rind permits *Fusarium* species access to the stem tissue. As a result, *E. saccharina* infestation is usually associated with *Fusarium* infection, which can cause stem rot in sugarcane.

3.1.4. *Fusarium oxysporum* species complex (FOSC)

Fusarium oxysporum is one of the most economically important pathogens in the genus, but members of this species complex are generally considered to be non-toxicogenic. *F. oxysporum* comprises over 120 known strains or "special forms," each of which is specific to a unique

plant host in which it causes disease. From a traditional taxonomic point of view, *F. oxysporum* isolates are differentiated from each other based on the pathogenicity as *formae speciales*, but this has been shown to be an unreliable approach. Vegetative compatibility groups (VCG) have been useful in the FOSC to characterize strains with similar pathogenic properties, and their genetic basis is an active area of research on the toxigenic species and mycotoxins in FOSC.

Fusarium oxysporum can spread short distances by irrigation water and contaminated agricultural machinery and via air or long distances by infected seeds and planting material. This prevents transport of water and nutrients to the rest of the host, causing wilting, discoloration, and ultimately death of the plant. Some strains of *Fusarium oxysporum* are pathogenic to different plant species; they penetrate into the roots and provoke the vascular system, causing severe damage on many plant species of economic importance.

Fungal growth initiated with white mycelium which subsequently turned pale violet. Ten isolates were recovered from the single-spore cultivation. The mycelia were floccose, sparse, or abundant. The microconidia were oval, elliptical, or kidney shaped and with 0 septate, while the macroconidia usually had three septa. The apical cell was tapered and basal cell was foot shaped. The morphological features and sporulation pattern were consistent with the description of *Fusarium oxysporum* (Leslie et al., 2006). The pairwise alignment and phylogenetic tree based on three genes (rDNA-ITS, GenBank Accession No. KU863663; pgx4, KU863663; tef, KU933831) and other reference sequences from GenBank also showed that our isolate gx3 belonged to *Fusarium oxysporum*, close related to FFSC.

3.2. Comparative genomics of *Fusarium* species complex (FSC)

Comparative genomics allows investigating many questions of evolutionary and functional significance of sequence features. By associating the species-specific genes with the unique characteristic of that species, researchers can find the potential relationship between genotype and phenotype. Various forward and reverse genetic methods have been developed to explore the repertoire of *Fusarium* genes contributing to disease formation, mycotoxin production, and sporulation. Phylogenetic tree is another application of comparative genomics to infer evolutionary relationship and to estimate diverge time based on the sequence similarity.

The whole genome of three fungal isolates (CNO1, YN41, and BS2–BS6) from the *Fusarium* species complex (FSC) that caused pokkah boeng disease of sugarcane was sequenced by Illumina and PacBio platforms. The genome coverages ranged from 100× to 200×. The newly sequenced genomes, along with five previously sequenced isolates (*F. fujikuroi* IMI58289, *F. verticillioides* 7600, *F. mangiferae*, *F. circinatum* FSP34, and *F. oxysporum* 4287), were selected based on their incidence in geographical locations, taxonomy/species, host isolation, toxin production, and pathology. Overall, the eight sequenced genomes were comparable in size and structure. The sizes of the eight sequenced genomes ranged from 41.9 to 61.4 Mb with approximately 48.0 of GC content (from 47.3 to 48.3). The CDS (protein-coding genes) ranged from 10,522 to 17,753. The gene density ranged from 284 to 356 per Mb.

The development of genomics is allowing the incorporation of new tools and resources to address the important new challenges for agriculture. The commercial sugarcane cultivars used today resulted from crosses of *S. officinarum* and *S. spontaneum*. However, the reproductive

biology and complex genome of sugarcane complicate breeding of genetically improved varieties by conventional means. The global relationship between the linear sequence of nucleic acid bases in the DNA of the gene and the sequence of amino acids in the protein encoded by the DNA of *S. officinarum* and *S. spontaneum* associated these chromosome exchanges occurred through recombinations between the chromosomes of the two species.

A comparative genomics approach was effective in resolving the genetic relationship among fungal species and isolates. The *Fusarium fujikuroi* species complex (FFC) causes a wide spectrum of devastating diseases on diverse agricultural crops, like sugarcane. This is in part due to the complexity of the sugarcane genome which is probably the most complex of all plant crops. Sugarcane complex genome structure is due to a number of interesting developments that resulted in the sugarcane varieties grown.

FFC species can produce structurally diverse secondary metabolites (SMs), including the mycotoxins fumonisins, fusarins, fusaric acid, and beauvericin and the phytohormones gibberellins, auxins, and cytokinins. *Fusarium*-induced crop diseases as well as mycotoxin contamination problems result in significant economic losses to world agriculture every year. The major discoveries contributed by genomic analyses of *Fusarium* focused on plant pathogenicity, production of mycotoxins, and other secondary metabolites. A key theme is the finding that a *Fusarium* genome is compartmentalized into core and adaptive regions that encode functions associated mostly with primary growth versus adaptation to specific niches (e.g., virulence on specific hosts, growth in specific environments). This genome compartmentalization should enable functional studies focused on the development of improved means for controlling *Fusarium* diseases and toxin contamination.

Secondary metabolites are very important in mediating interactions between fungus and host plant. The genes encoded the secondary metabolite often involve particular types of key enzymes, including polyketide synthase (PKS), non-ribosomal peptide synthetase (NPS), and terpenoid synthase. These key enzymes are clustered along with various combinations of additional enzymes for further metabolite catalyzing and with transporters and transcription factors that are essential for the regulation of most of the clustered genes. Based on the fungal SM analyses by antiSMASH software, some SM biosynthetic gene clusters were shared in all *Fusarium* species complex causing sugarcane pokkah boeng, including aflatrem, fusaric acid, fusarubin, asperfuranone, bikaverin, acetylaranotin, fusaridione A, equisetin, nivalenol/deoxynivalenol/3-acetyldeoxynivalenol, and fujikurins. Ustilagic acid was only produced in *F. oxysporum* 4287. The gene clusters for azaphilone, fumonisin, and apicidin biosynthesis were only available in *F. proliferatum*. Gibberellin acid was only produced in *F. verticillioides* CNO1, but not in *F. proliferatum* YN41 because the genome of *F. proliferatum* YN41 lacked one of the key genes (p450) in GA biosynthetic gene cluster, which regulated GA production.

The genotype *F. verticillioides* isolates exposed the event of non-toxicogenic strains and confirmed that their phenotype was likely the deletion of genes which are requisite for fumonisin biosynthesis. Some *Fusarium* secondary metabolite gene clusters exhibit a discontinuous distribution that does not correlate with phylogenetic relationships of species. For example, the intently distributed fumonisin and gibberellin gene clusters are present in some but not all species of the *F. fujikuroi* and *F. oxysporum* species complexes. Along with genes responsible for the

biosynthesis of the secondary metabolite, genes with regulatory and transport functions are usually also present in these clusters.

The *Fusarium* comparative genomics highlighted the existence of lineage-specific chromosomes that are enriched for transposable elements and encoded genes that are pathogenicity related. These lineage-specific chromosomes play significant roles in adaptation to changing environments among this species complex. The well-defined genetic model system, will help to redefine the control strategies for pathogens with such genetic pathogenicity; qualitative genes are more often inherited dominantly and are also normally found clustered together in certain chromosome arms. These genes have major effects and are expressed throughout the life of a plant, tending to produce a plant completely resistant to one or more strains of a particular pathogen.

Modern sugarcane cultivars were derived from the interspecific crosses among a few clones of *S. officinarum*, *S. barberi*, and *S. sinense* and the wild relatives of *S. spontaneum* and *S. robustum*. The wild species has played an important role in the development of adaptation or tolerance to varied abiotic and biotic stresses. After the initial interspecific crosses, sugarcane breeders concentrated their attention on intercrossing of the hybrid derivatives. The direct contribution of the chromosomes to pathogenicity is indicated by the fact that they encode known virulence factors such as effector proteins, necrosis-inducing peptides, and a large array of enzymes targeting plant substrates but lack genes involved in primary metabolism. Effectors are “secreted proteins and other molecules which allow plant-associated organisms to modulate plant defense circuitry and enable colonization of plant tissue” [22]. *F. verticillioides*, *F. proliferatum*, and several other species can produce a variety of mycotoxins (e.g., trichothecenes or fumonisins) that are associated with plant disease.

3.3. *Fusarium* species complex and nitrogen source

Nitrogen is one of the most important nutrients for crop growth and production. It is a major component in chlorophyll, which is the most important pigment needed for photosynthesis, as well as amino acids, the key building blocks of proteins. Nitrogen accelerates growth, gives vitality to plants, and promotes dark green color in leaves due to better chlorophyll synthesis. Sugarcane is the world’s largest sugar crop and an economically important crop in China. The symptoms of sugarcane pokkah boeng tend to develop during periods in which high concentrations of nitrogen are applied.

Fungi are able to respond to quantitative and qualitative changes in nitrogen availability through complex regulatory mechanisms. The source of nitrogen has been isolated from sugarcane (*Saccharum* spp.) as beneficial interaction that promotes plant growth. Nitrogen is the most essential factor having direct effect on cane growth, sugarcane yield, and juice quality. However, nitrogen application at high rates exceeding sugarcane plant utilization has adverse effect on cane quality. The beneficial plant microorganism association has unique features that remain to be characterized. This living microorganism, which has potential for the development of plant growth by civilizing the nutrient condition of the plant and inadequate supply of nitrogen, decreases the plant metabolism and growth.

Nitrogen availability has significant effects not only on physiological and morphological characteristics of the fungus but also on the biosynthesis of secondary metabolites, such as mycotoxins in *Fusarium* species complex. It is difficult to determine exact N requirements of sugarcane crop. The use of organic nitrogen base may reduce the disease outbreaks and improve antagonistic to pathogens on certain fungi and microorganisms.

On the other hand, it has been observed that in some plants as the N content is increased beyond sufficient levels, the amount of antifungal compounds decreases. Nitrogen fixation is a biological process that reduces molecular N_2 into ammonia (NH_3), which can be easily absorbed by plants. During this adaptation, nitrogenase plays a very important role in catalysis. Strains with nitrogenase activity were identified on the basis of their phenotypic and 16S rDNA sequence analysis and concluded that isolates had potential for regulation of plant growth. To synthesize the secondary metabolites of nitrogen molecules, ammonia plays the vital role in plant growth and development.

Nitrogen supply can bang plant-pathogen interactions through consequence on pathogen virulence. The well-established virulence factor of *Fusarium oxysporum* was found to repress the capacity of the fungal species to penetrate cellophane membrane through the nitrogen source. Nitrogen Utilization may be either regular or impartial with phosphorus and potassium so that nitrification can take place properly. Due to swift mobility of nitrogen, its effect is quite visible in the form of rapid growths due to its presence and rapid retardation in growth and of the crop due to its deficiency. The profuse of nitrogen can enhance the production of young, luscious growth, an expanded vegetative period, and tardy ripeness. The N requirements vary with climate, crop growth, cane yield pattern, irrigation frequency and distribution, land preparation, soil types, and soil behavior. The sustainability of prospective crops is strongly reliant on minimization of fertilizer inputs that can be achieved by enhancement of plant-associated nitrogen fixation. The various applications of nitrogen have been allied with increase in yield. Similarly, the heavy use of nitrogen can promote lodging which can reduce potential yield. In exacting, the nitrogen accessibility directly modulates the regulation of nitrogen source.

Biofertilizers are based on effective strains of microorganisms in sufficient numbers, which are useful for nitrogen fixation in plants and synthesis of growth-promoting substances like hormones, vitamins, and auxins. Besides being essential as a source of cheap protein for human nutrition and animal feed, symbiosis with rhizobia is essential in crop rotation to maintain soil fertility. Poultry manure and other animal waste products were used as a source of supplemental nitrogen long before inorganic nitrogen fertilizer came into popular use. The utilization of BNF for agricultural purposes has long been the dynamic force behind N-fixation research. The environmental benefits from using biological N-fixation are seen to be associated with the proxy of chemical-based technologies with a biological system. Some of the main benefits provided through crop rotation include the prevention of soil erosion, increased soil microorganism diversity, decreased pest prevalence, and increased field fertility. The importance of field fertility in the process of growing crop is immense. The process of BNF can be defined as the reduction of dinitrogen to ammonia by means of a prokaryote. BNF is accomplished by a wide variety of prokaryotes; some can accomplish this as free living organisms, while others require a symbiotic association with plants.

The secondary metabolism, also called specialized metabolism, is part of the metabolism of fungi which is not essential for direct survival; such gene will rely on regulatory mechanisms for biosynthesis and their perpetual relations with the nitrogen regulation of other pathways in *Fusarium*, a paradigmatic model fungus for secondary metabolism. The screening of plant genotypes for their enhanced ability to acquire nitrogen by BNF can reduce the use of expensive nitrogen fertilizers in several important cash crops like sugarcane. In fact, the condition of biologically fixed nitrogen plays a key role in crop production in world cultivation. To understand the role of biological nitrogen fixation, more research work is needed for improving efficiency of nitrogen in order to reduce the use of synthetic fertilizer for production.

Fusarium species having plant growth-promoting activities are exploited for growing agricultural needs. The *Fusarium* spp. range in their pathogenicity, but they can produce mycotoxins in sugarcane. Such *Fusarium* includes the *Gibberella fujikuroi* species complex especially *F. verticillioides* and *F. proliferatum*, which can cause stalk rot. Under alternating drought/wet conditions, *F. verticillioides* produces toxic fumonisins and under warm in certain crop variety. The effects of nitrogen sources on *F. verticillioides* will lead to pigmentation variation derived from bikaverin, fusarubins, and carotenoids. The gene expression in *F. verticillioides* was established to analyze processes modulated by different sources of nitrogen and to identify new regulatory mechanisms. Historically, a small amount of N fertilizers was recommended at planting to aid in early season fall growth as well as a mid-season N fertilization application in early spring. Excess N can lead to prolonged vegetative growth and reduced sucrose concentration mainly due to increased moisture in stalks. Crop response to immunization with symbiotic nitrogen has established their important role in supplementing nitrogen to the plant, allowing a sustainable use of nitrogen fertilizers. Today, nitrogen source of applications on sugarcane tends to be a complicated issue due to previous research showing contradicting results. However, economic factors and soil reaction must be considered while selecting the forms of fertilizers.

3.4. Preventive and control measures

Sugarcane is a highly industrious crop which suffers from numerous diseases caused by different organisms and factors such as environmental and physiological disorders and nutritional deficiencies. Historically, planting susceptible varieties in a large area encouraged the outbreak of a certain diseases in a particular period of time. Several control measures may be implemented to reduce potential sugarcane yield loss caused by pests and diseases, but an incorporated approach is often recommended.

Disease control in sugarcane is based on an integration of legislative control, resistant cultivars, and other management procedures. Short-term spraying options are available, but their economic viability may not be sustained. Machine harvest can also transmit disease. Many sugarcane diseases are also managed through the use of disease-free planting material supplied through Cane Protection and Productivity Boards. The genetical resistant cultivars is the most cost-effective method to control the disease, and the presence of genetic variations against pokkah boeng and its associates is well documented. Because of the more serious disease

problem, a progressive effort to socialize and conduct integrated management for controlling the disease.

Several control measures may be implemented to reduce potential sugarcane yield loss caused by pests and diseases, but an incorporated approach is often recommended. To remove and destruct of infected plants on the first appearance of the disease in case of pokkah boeng established that frequent breakdown of varietal resistance against pokkah boeng is due to the appearance of new pathotypes matching the resistance of cane genotypes.

Successive ratoons are characterized by reductions in cane yield due to systemic diseases or physical damage to stools, and the number of ratoons obtained from a single harvest also depends on genotypic and environmental factors. Ratoon productivity has been proved to increase with proper management involving timely agricultural operations, proper nutrition management and integrated pest management, and maintenance of adequate plant population. A number of ratoon management practices currently in use, such as inter-row ripping, burning of crop residues at harvest, harvesting under wet conditions, and using heavy infield transport, were found to be contrary with the substantial, chemical, and biological properties of the soil. The incidence was figured out as five grades (**Figure 1**).

Based on the disease severity index (DSI) of pokkah boeng disease of sugarcane, the resistance of sugarcane against pokkah boeng was classified into five levels from 0 to 5. Level 0 was defined as highly resistant (HR) with $DSI \leq 1.0$, Level 1 as resistant (R) with DSI ranged from 1.1 to 5.0, Level 2 as moderately resistant (MR) with DSI from 5.1 to 10.0, Level 3 as moderately susceptible (MS) with DSI from 10.1 to 15.0, Level 4 as susceptible (S) with DSI from 15.1 to 20.0, and Level 5 as highly susceptible (HS) with $DSI > 20.0$.

The disease severity index (DSI) was calculated as follows:

$$DSI = \left[\frac{\text{Sum of all numerical grades}}{(\text{Total number of plants counted} \times \text{maximum grade})} \right] \times 100 \quad (1)$$

Conidial suspensions of the isolates (CNO-1 and YN41, 10^6 conidia mL^{-1} , 100 μL) were dripped into the young spindle of 89 sugarcane germplasm, and the symptoms were observed on the inoculated plants in 6–8 days post-inoculation, respectively. Our results showed that 34 of 89 tested clones (38.2%) were susceptible to both CNO1 and YN41, 32 clones (36.0%) susceptible to CNO-1 but resistant to YN-41, 14 clones (15.7%) susceptible to YN41 but resistant to CNO-1, and only 8 clones (9.0%) resistant to both CNO-1 and resistant YN41. Both these resistant clones included CP84-1198, GT94-40, GT05-3846, ROC1, ROC27, YC58-14, YC64-173, and YT94-128. Moreover, our results also showed that CNO-1 had higher infection than YN-41 by this inoculation with a success of up to 89.8%.

Chemical control is often expensive and has downstream unconstructive effects on the environment. Nine compounds were tested at three concentrations (100, 50, and 10 ppm) for their ability to inhibit mycelial growth of *Fusarium* species complex. Two antibacterial compounds including copper 8-hydroxyquinoline and validamycins had no effect on the mycelial growth of *Fusarium* species complex at 10 ppm and partially inhibited mycelial growth at the concentration of 50 and 100 ppm. In addition, two compounds, including thifluzamide and chloroisobromine



Grade 1: Chlorotic or generate slight etiolation symptom at the base of young leaves; slight shrinkage; very few irregular reddish specks or stripes.



Grade 2: Narrower or shorter; distinct distortion (wrinkling and twisting); the reddish areas develop into lens-shaped holes or form ladder-like lesions often with dark edges.



Grade 3: The infection in the spindle continues downward into the stalk and dark reddish streaks may be found extending through several internodes; or the infection may form long lesions with cross depressions that give them a ladder-like appearance.



Grade 4: In the stem, the fungus causes a dark-brown discoloration of the infected tissues. The ladder-like lesions are due to rupturing of the diseased cells which cannot keep up with the growth of the healthy tissue.



Grade 5: The entire top (growing point) of the plant dies (referred to as "top rot")

Figure 1. Symptoms of sugarcane pokkah boeng disease in China.

cyanuric acid, had great effect on inhibition of fungal growth at 10 ppm rather than at 50 and 100 ppm.

In the field test, spraying of different fungicides like Bavistin or Blitox or copper oxychloride or carbendazim is efficient for reducing the pokkah boeng disease. Planting of healthy seed, the use of resistant varieties, and following the integrated disease management practices are the best ways to prevent disease incidence. The use of resistant cultivars is particularly useful, as it reduces the use of harmful chemicals which can disturb the balance of nature and result in other pests becoming a problem. Furthermore, *Fusarium* spp. prevalence in the soil can be affected considerably by crop rotation practices. Although the use of resistant varieties is the best means of control, some strains have been found to overcome resistance, and the once-resistant varieties were reported to be susceptible.

Host plant resistance shows major advantages compared to chemical, biological, and cultural control components for management programmes. However, it needs to be supported with additional management practices to ensure durability in the field. Biological control of plant pathogens is an attractive alternative to the strong dependence of modern agriculture on chemical fungicides, which cause environmental pollution and development of resistant strains. The endophytic bacterial community associated with sugarcane harbors multiple genera with potential for plant growth promotion and disease control.

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