Establishment and Application of Prevention and Control Techniques of New Sugarcane White Leaf Disease

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Abstract Aiming at the basic and key technical problems in prevention and control of sugarcane white leaf disease (SCWL), this study systematically overcame key technical bottleneck of prevention and control of new SCWL after 10 years of collaborative research, and achieved several innovative achievements. SCWL phytoplasmas newly recorded in China and the new subgroup of SCWL phytoplasmas (16SrXI-D) were discovered for the first time in Yunnan, and the whole genome analysis of the epidemic subgroup was completed. The main transmission source of SCWL pathogens has been identified as infected seed canes, and *Tettigoniella viridis* and *Clovia conifer* were newly discovered as vectors for virus transmission. The disease resistance of 25 main varieties was identified, and 10 control varieties were selected. The prevention and control strategy of "emphasizing early warning, strictly carrying out quarantine, blocking the vectors and controlling residual plants" was put forward, and a comprehensive prevention technique was established through integration of various techniques, and standardized technical regulations were formulated for demonstration application. The promotion and application of these achievements have realized scientific prevention and control of SCWL, effectively curbed the spread of SCWL, and ensured the safety of sugarcane producing areas in China, achieving great economic, social and ecological benefits and providing technical support for high-quality development, loss reduction and efficiency improvement of China's sugar industry.

Key words Sugarcane white leaf disease; Comprehensive prevention and control; Application

DOI:10.19759/j.cnki.2164-4993.2024.04.005

Project Background

Grain, cotton, oil and sugar are important agricultural products in China, and the development of sugar is related to national food safety, farmers' income increase and rural revitalization. Yunnan is the second largest sugar base in China, with 333 333 hm^2 of sugarcane planted over the years[1]. Sugar industry is one of the most effective industries in precision poverty alleviation in border areas of Yunnan Province. There are more than 6 million sugarcane farmers in 49 counties and cities in 10 prefectures and cities in the province. Most sugarcane areas are border ethnic minority areas (34 national-level poverty-stricken counties and 21 border counties), with a regional poverty population of 607 000 and 101 000 registered households. During the 2019/2020 crushing season, 196 000 people were lifted out of poverty with sugarcane planting. Sugar industry has become a pillar industry for poverty alleviation and rural revitalization in Yunnan border areas and frontier ethnic areas, and plays an important role in promoting economic development and increasing farmers' income and local financial growth in frontier ethnic areas[1].

Yunnan is located in the low-latitude plateau, and the sugarcane areas have the characteristics of diverse ecosystems and complex types of disease pathogens. Sugarcane white leaf (SCWL) is the first dangerous and important new disease caused by phytoplasmas, which can cause devastating disasters to sugarcane and pose a serious threat to safety production of sugarcane[2-4]. The incidence rate of susceptible varieties is over 50%, and as high as 100% in severe cases. After infection with SCWL phytoplasmas, plant height, stem diameter and stem formation rate significantly decrease, resulting in a significant reduction in cane yield and sugar production. The yield of newly planted crop in serious fields is 90 – 105 t/hm^2, and total crop failure will occur in the second year[4-5]. The spread of SCWL has become an unfavorable factor restricting the sustainable development of sugarcane industry in Yunnan. If it is not effectively prevented and controlled, it will also bring potential threats to sugarcane areas such as Guangxi and Guangdong. Overcoming the technical problem of SCWL prevention and control and realizing scientific prevention and control of SCWL is the fundamental guarantee for high-quality development of sugar industry. In view of basic and key technical problems in SCWL prevention and control, sugar enterprises and pharmaceutical companies led by Sugarcane Research Institute, Yunnan Academy of Agricultural Sciences have conducted in-depth systematic research from the aspects of diagnosis and detection, pathogen groups, disaster characteristics, disaster-causing factors, monitoring and early warning, and comprehensive prevention and control

Received: April 28, 2024 Accepted: June 30, 2024

Supported by National Natural Science Foundation of China (31760504); China Agriculture Research System of MOF and MARA (CARS-17); Special Fund for the Construction of Modern Agricultural Industrial Technology System in Yunnan Province.

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techniques, aiming to clarify the prevention and control foundation, overcome the bottleneck of prevention and control, and integrating prevention and control techniques. We also hope to realize scientific prevention and control of the disease, reduce pesticide application and sugarcane loss, and improve efficiency, so as to support high-quality development of sugar industry.

Technological Innovation Points

SCWL phytoplasmas newly recorded in China were discovered for the first time

In 2012 – 2013, the quarantine disease of SCWL phytoplasmas was detected by nested PCR for the first time on sugarcane in Baoshan and Lincang sugarcane areas of Yunnan Province, which confirmed that SCWL phytoplasmas had been introduced into Yunnan. Twenty-seven positive Nest-PCR products were selected for sequencing analysis, and the results showed that the sizes of 17 fragments in Baoshan were all 210 bp, and the sequences were completely consistent. The 10 fragments in Lincang were all 202 bp in size, and the sequences were identical. BLAST search results showed that the sequences were the spacer sequence of 16S rRNA gene in the genome of phytoplasmas causing SCWL, and they were highly homologous to sequences of corresponding fragments of SCWL phytoplasma genome published in GenBank, with homology between 99.05% and 100%. Through DNAman multiple sequence alignment, it could be known that the Baoshan isolate showed eight base deletions compared with the Lincang isolate, and the homology was 99.51%. It showed no base difference from Thailand isolate (accession number HQ917068), and the homology was 100%. There were two base deletions and one base substitution compared with Hawaiian isolate (accession number JN223448), and the homology was 99.05%. There was a base deletion compared with Sri Lanka isolate (accession number JF754447), and the homology was 99.52%. Lincang isolate had eight base deletions compared with the Thailand isolate, and the homology was 99.51%. There were eight base deletions and one base substitution compared with the Hawaiian isolate, and the homology was 98.53%. Although there were seven base deletions compared with the Sri Lanka isolate, and the homology was 100%. Representative 16S – 23S rRNA ISR sequences of phytoplasma in various groups of 16Sr of phytoplasmas in GenBank were downloadeded, and the homology with the 16S – 23S rRNA ISR sequences of SCWL phytoplasmas in Baoshan and Lincang was analyzed, and a phylogenetic tree was constructed using Mega4.1 software. It could be seen from the phylogenetic tree that the SCWL phytoplasmas from Baoshan and Lincang were in the same branch as those of Thailand and Sri Lanka in GenBank, and both belonged to the XI group of 16Sr, and their genetic relationship was closer than that with the SCWL phytoplasma from Hawaii. Based on the results of field symptom observation, molecular detection and sequence analysis, it was confirmed that the symptoms of SCWL in Yunnan sugarcane area were caused by SCWL phytoplasmas in the XI group of 16Sr, which was the first report of newly recorded pathogens of SCWL in China.

A new subgroup of SCWL, 16Sr XI-D subgroup, was reported of for the first time

"A PCR-RFLP identification method for different subgroups of phytoplasmas of sugarcane white leaf disease" was invented, and granted a national invention patent in 2019. The 16S rDNA of SCWL phytoplasmas from Baoshan, Lincang, and Myanmar, Philippines, Thailand, France and other countries were combined with reported 16S rDNA sequence data of SCWL phytoplasmas from other countries to carry out phylogenetic analysis and virtual RFLP analysis. Lincang isolate and Baoshan isolate belonged to different branches, respectively. It was found that the phytoplasma of Baoshan isolate was a new subgroup of 16Sr XI group, namely 16Sr XI-D subgroup, which revealed for the first time that SCWL could be caused by two subgroups of the 16Sr XI group of SCWL phytoplasmas, namely 16Sr XI-B subgroup and new 16Sr XI-D subgroup.

Two different populations, ST1 and ST2, were revealed in SCWL phytoplasmas in China

The established multi-locus sequence typing method based on seven housekeeping genes was applied for analysis, which revealed that there were two different populations, ST1 and ST2, belonging to different clonal complexes, respectively, in 87 samples from areas where SCWL occurred in China. ST1-type SCWL phytoplasma is the most widely distributed in China, and most isolates belong to ST1 type, while ST2 type is only found in Baoshan, China. The analysis on 16Sr RNA gene sequences of different ST-type SCWL phytoplasmas showed that ST1-type SCWL phytoplasmas all belong to the 16Sr XI-B subgroup, while ST2-type phytoplasmas belong to the new 16Sr XI-D subgroup. At present, the new 16Sr XI-D subgroup is only reported in China, but all the SCWL phytoplasmas identified in other countries in the world belong to subgroup 16Sr XI-B. ST1 is not only the main type of SCWL phytoplasmas in China, but also the main type of SCWL phytoplasmas in the world.

The first whole genome sequence of SCWL phytoplasma, SCWL1 strain was obtained

"A purification method for genomic DNA from phytoplasmas of sugarcane white leaf disease" was invented, and granted a national invention patent in 2022. The genome sequencing of SCWL phytoplasmas was carried out by using the invention combined with Illumina and Nanopore techniques, and the whole genome sequence of the first SCWL phytoplasma SCWL1 strain was obtained. The genome of SCWL1 strain consists of a 538 951 bp circular chromosome with a G + C content of 20.54% and a 2 976 bp plasmid with a G + C content of 21.00%. The genome of SCWL1 strain is also the complete genome of phytoplasma with fewest coding genes. According to annotation, the chromosome of SCWL1 strain contains 459 coding sequences (CDSs), two complete 5S-23S-16S rRNA gene operators and 27 tRNA genes. The CDSs have a total length of 413 403 bp, and an average length of 901 bp, accounting for 76.71% of the total chromosome length.

Infected seed canes were ascertained as the main source of transmission for SCWL pathogens

In order to find out new transmission vectors of SCWL, "a field device for determining the transmission vectors of sugarcane white leaf disease phytoplasmas" and "a method for determining the transmission vectors of sugarcane white leaf disease phytoplasmas" were invented, and granted two patents at home and two patents abroad. By using this patented technique, systematic synchronous comparison and analysis was conducted on new planted/ratoon cane of five sugarcane varieties inside and outside insect-proof nets in the same field, and it was determined that the transmission vectors of SCWL phytoplasmas in diseased sugarcane areas in Yunnan were mainly virus-carrying seed canes, providing a basis for formulating prevention and control measures.

***Tettigoniella viridis* and *Clovia conifer* were newly discovered as vectors for virus transmission**

Field investigations were carried out in main occurrence areas where SCWL occurs in Yunnan. Insect vectors were collected by light trapping and collection method and identified in laboratory combined with nested PCR detection technique. It was found that two insect vectors, *T. viridis* and *C. conifer*, were the vectors of SCWL phytoplasmas in low-latitude plateaus. Combined with the investigation and determination of transmission vectors, the incidence law of SCWL in different sugarcane varieties at different planting stages was further clarified, and the effects of sugarcane variety resistance and virus-carrying rate of seed canes on the occurrence and epidemic of disease were found out, providing a scientific basis for the selection of production seeds and disease prevention and control.

The accurate identification method of SCWL resistance was pioneered, and 10 control varieties were selected

The 1 – 5 level evaluation and grading standards for SCWL resistance were successfully developed, and two sets of accurate identification methods for SCWL resistance were optimized and created. The methods are simple, efficient, stable in pathogenicity, standardized and practical, and they filled the blank of SCWL resistance breeding research in China and provide key technical support for SCWL resistance breeding. The resistance of 25 main sugarcane varieties to SCWL was identified by the invented disease resistance identification method combined with natural resistance, and 10 standard varieties resistant to SCWL were selected, laying a foundation for variety layout and reliability of resistance identification. Based on these achievements, rapid and accurate evaluation of excellent sugarcane germplasm resources, common hybrid parents and new varieties resistant to SCWL can be realized, and the accuracy and the efficiency of breeding for resistance to SCWL can be effectively improved.

The geographical distribution (epidemic area) of SCWL was found out, providing a basis for formulating prevention and control strategies

SCWL is a devastating disease of sugarcane caused by phytoplasmas. It was first discovered in Thailand in 1954, and now it has widely occurred in sugarcane-growing countries around China, such as Vietnam, Myanmar, Philippines, Pakistan, India and Sri Lanka. In 2011 and 2012, Sugarcane Research Institute, Yunnan Academy of Agricultural Sciences detected SWCL phytoplasmas from sugarcane varieties introduced from abroad such as Myanmar, Philippines, Thailand and France. In 2012, SCWL was found in suspected SCWL sugarcane plants collected in Shidian County and Longyang District of Baoshan City, Yunnan Province for the first time, and the occurrence area was over 1 333 hm^2. In 2013, suspected SCWL sugarcane plants were collected from Gengma, Zhenkang, Shuangjiang, Linxiang and other sugarcane areas in Lincang, Yunnan Province, and SCWL was found, with an area of over 10 000 hm^2. Meanwhile, suspected SCWL sugarcane plants were also found in the investigation of Lancang sugarcane area in Pu'er, Yunnan. During the investigation of sugarcane diseases and pests in Ximeng sugarcane area of Yunnan Province in 2018, suspected SCWL sugarcane plants were found, and diagnosed as SCWL caused by phytoplasmas of the 16Sr XI-B subgroup by nested PCR. To sum up, the results of extensive field investigation and sampling tests in 49 counties and cities in eight major producing prefectures and cities in Yunnan show that by 2022, the geographical distribution (epidemic areas) of SCWL has involved nine counties and districts in Yunnan, namely Gengma, Zhenkang, Shuangjiang, Linxiang, Cangyuan, Shidian, Longyang, Ximeng and Lancang, among which Mangweng and Hepai of Gengma are the most serious areas.

The prevention and control strategy of "emphasizing early warning, strictly carrying out quarantine, blocking the vectors and controlling residual plants" and integrated comprehensive prevention technique were put forward, and standardized technical regulations was formulated for demonstration application

According to the transmission routes and disaster characteristics of SCWL, the prevention and control strategy of "emphasizing early warning, strictly carrying out quarantine, blocking the vectors and controlling residual plants" was put forward. Through experiments and demonstrations, a comprehensive prevention and control technical system centered on "warning about disasters, implementing quarantine, planting disease-resistant varieties, selecting disease-free seedlings, mainly promoting warm water detoxification technique, preventing insect vectors, removing residual diseased plants and residues, and killing infectious sources with chemicals" was integrated in sugarcane areas in low-latitude plateaus. Standardized technical regulations were formulated, which

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Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU

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successfully solves the key technical problems of comprehensive prevention and control of new SCWL in large area in sugarcane production. A cooperative extension model of "sugar factory + scientific research + agricultural department + farmers" was constructed and has been successfully applied in a large area, realizing the scientific prevention and control of SCWL, preventing new SCWL phytoplasmas from invading from abroad with introduction from the source. These measures have effectively curbed the spread of SCWL phytoplasmas in domestic inter-provincial sugarcane areas, and provide key technical support and safety guarantee for high-quality development, loss reduction and efficiency improvement of sugar industry in major producing areas such as Yunnan, Guangxi and Guangdong.

Popularization and Application of Prevention and Control Techniques has Achieved Remarkable Social and Economic Benefits

Over the years, based on "enterprise as the main body and industry as the guidance", the collaborative promotion model of "sugar factory + scientific research + agricultural department + farmers" has been built for demonstration and promotion. Prevention and control techniques of new SCWL have been widely promoted and applied in nine counties and cities of three main producing prefectures and cities in Yunnan Province, where SCWL occurs. The harm has been controlled and the prevention and control effect is significant. From 2021 to 2023, the techniques were promoted and applied in 151 khm^2 of producing areas totally, saving 1.81 Mt of sugarcane harvest from pest control and reducing 233 kt of "sugar loss". The sales revenue was increased by 2.156 billion yuan, and the profit was increased by 805 million yuan, and the tax was increased by 93 million yuan. The promotion and application of these achievements have successfully solved the bottleneck of comprehensive prevention and control of new SCWL in sugarcane production, achieved scientific prevention and control of SCWL, and effectively curbed the spread of SCWL, making significant contributions to the development of border ethnic groups, frontline economy, farmers' income increase, enterprise efficiency improvement, and rural revitalization.

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Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU