Investigation on types of corn rust in eastern Yunnan ecology and analysis of population genetic structure of its rusty

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ABSTRACT
Corn rust disease can be classified into four types: common corn rust, southern corn rust, tropical corn rust and stem corn rust. In this paper, 270 samples of corn rust gathered from Yunnan province were identified by observation of symptom and pathogen morphology, detection of specific molecular markers between Puccinia sorghi Schw and Puccinia polysora Unedrw. The results showed that 180 samples of corn rust collected form Qujing, Zhaotong, Kunming, Honghe (Mile, Kaiyuan and Jianshui) were common corn rust caused by Puccinia sorghi Schw; and 90 samples of corn rust collected form Wenshan and Honghe (Pinbian) were southern corn rust. Morphology and aspect ratio compared Puccinia sorghi Schw with Puccinia polysora Unedrw. The uredospores of Puccinia polysora Unedrw were oval and the aspect ratio of 81.6% of all uredospores from Puccinia polysora Unedrw was greater than 1.2. The uredospores of Puccinia sorghi Schw were nearly round, and their aspect ratio was 1.0-1.3. In addition, the population genetic structure of all corn rust samples was analyzed by ISSR molecular marker. Concerning Puccinia polysora Unedrw, the genetic diversity was larger in Wenshan than in Honghe. In Puccinia sorghi Schw, the population genetic diversity was larger in Zhaotong and the lowest in Kunming.

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Introduction
Corn is an annual herbaceous plant of Zea mays L. The planting area and yield of corn are second only to rice and wheat, among the three major grain crops in the world. Corn is also an important grain, economic and forage crop in China, playing a significant role in industrial raw materials and animal husbandry (Li et al. 2017). In many countries where corn is grown, its production can reach millions of tons per year (Dániel and Györi 2000). In Yunnan Province, China, the planting area of corn accounts for 23–28% of the total cultivated land area, and the yield accounts for 28% of the total grain production of Yunnan Province (Zhou et al. 2011; Ramirez-Cabral et al. 2017). Uredospores, in essence, perform the same function as summer spores in terms of fast population spread. Their coverings do not prevent the pathogen from severe dehydration or environmental fluctuations.

Corn rust is a common fungal disease in many grain crops, cash crops, etc. Rust fungi causing plant rust belongs to Pucciniomycotina fungi, which has 14 families, 166 genera and about 7800 species (Yamaoka 2014). The fungus Puccinia sorghi causes widespread rust. Infections that arise later in the season have a minor effect on productivity. During the growth season, the fungus overwinters on vegetation in southern states, and dispersed spores are wind-blown to northern states. All across the season, corn faces a variety of disease concerns, including grey leaf spot, northern corn bacterial leaf, oil spot, and common and southern rust. Early disease management is critical for maintaining your corn crop strong and maintaining yields. The infection can move from the foliage to the stalks, or it might infect the main stem through the base of the plant or root(Balamurugan et al. 2020; Hsu et al. 2020). There are four kinds of rust fungi causing corn rust, including Puccinia sorghi Schw, which causes common corn rust; Puccinia polysora Unedrw, which causes southern corn rust; Physopilla zeae (mains) Cummins and Pamaxhar, which causes tropical corn...
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In 2007, 10 counties (cities and districts) in Zhoukou vince covered an area of 785,200 hm², accounting for 20.17%. In 2004, the outbreak of corn rust in Henan Province covered an area of 785,200 hm², accounting for 19.29% of the planting area, and 66,600 hm² in 2009, accounting for 16.31% of the planting area (Zhou et al. 2011). Corn rust can be transmitted by airflow. It occurs in a wide range in China, covering the main corn production areas in the north and south, causing increasingly serious damage to corn (Fu et al. 2011). At present, there are few studies on genetic diversity of corn rust. In 2011, Xing Guozheng et al. analyzed the molecular genetic diversity of southern corn rust population in Hainan and Henan by molecular biology. The results showed that there was no obvious regional differentiation between the isolates of Hainan and Henan, the overall genetic diversity was low, and there was no obvious sub-population genetic differentiation between regions (Xing 2011). It is becoming harder to regulate due to its widespread dispersion, long-distance migration, various physiological races, and rapid evolution, all of which have linked to an increased likelihood of linked outbreaks. The similarity coefficient was computed utilising five inter-simple sequence repeat (ISSR) indicators and the findings of genetic variety investigations.

The climate of Yunnan Province has seven temperate zone types, such as the North Tropic, the South Tropic, the Middle Subtropics and the North Subtropics. It has abundant precipitation, distinct wetness and dryness areas, and a wide variety of plant species, which is very prone to the occurrence and epidemic of rust. People often distinguish the two kinds of rust by the symptoms of common corn rust and southern corn rust, but it is difficult to distinguish them in the areas where the two kinds of rust occur together. Therefore, in this study, the symptoms and spore morphology of corn rust in Yunnan Province were observed, and the fungal ribosomal gene transcription spacer (ITS) marker cloning and sequencing were used to identify the pathogenic fungi of corn rust with different symptoms. Inter simple sequence repeat interval amplified polymorphism (ISSR) markers were used to analyze the population genetic structure of corn rust pathogen in different areas of Yunnan Province, which provided a reliable method for accurate identification of two kinds of corn rust and theoretical basis for corn rust resistance breeding and scientific control. ISSR markers were used to analyses the population genetic structure of the corn rust pathogen providing a reliable method for accurate determination of two types of corn rust as well as a theoretical background for corn rust resistance breeding and scientific control.
Materials and methodologies

Materials

Samples

The 270 samples of corn rust used in this study were collected from different areas of Yunnan Province from July to August 2018. The geographic information of the samples collected is shown in Table 1. Detailed information of the samples collected is shown in Table 1.

Primers

The primers used in this experiment were specific molecular marker primers: for common corn rust, forward primer: TTTAGTAGTCTCTACTTCAACAACA, reverse primer: AAGACTCTTTTGATGGTTT; for southern corn rust, forward primer: CTCCAAGAACCTCTACTTCAACAACA, reverse primer: TGACATGAAGATTCT (Xing et al. 2017); for fungal universal primer, ITS1-TCCGTAGGTGAACCTGCGG and ITS4-TCCTCCGCTTATTGATATGC (Chen and Zheng 2007); and six ISSR primers for analysis of population genetic structure by software Primer 5.0 (Table 2). The primers used in this experiment were synthesised by Kunming Qingke Biotechnology Co., Ltd. A genetic marker is a gene or a gene product that has a known position on a chromosome and may be utilised to classify individuals or organisms. It can be either a brief Genome, like that around a single base-pair alteration (nucleotide sequences, SNP), else a lengthy structure, including minisatellites.

Instruments used

The main instruments used in this experiment are: ultraclean workbench, autoclave, drying box, electronic balance, HH-4 digital constant temperature water bath pot, Microfuge18 centrifuge, Blue Shield 522 visible light gel electrophoresis transmission meter, ABI-9700 PCR, 78HW-1 constant temperature heating magnetic stirrer, GeneQuant Pro protein nucleic acid analyzer, Imagequant300 gel imaging system, ABI-3730XL genetic analytical system, Lycra fluorescence microscope and stereomicroscope.

Methodologies

Collection of samples

In this experiment, 270 samples of corn rust were collected from different sites of Yunnan Province from July to August 2018, and each site has multiple different corn fields. For sampling, samples with a clean leaf surface and complete spores of the rust fungus were chosen. After sampling, each sample was packed separately in a self-made bag to prevent mildew and cross-contamination of rust fungus between samples. Sample collection information such as location, time, number, type of rust and elevation should be indicated and samples be brought back to the laboratory for storage at −20°C.

Observation of morphology

Fresh rust samples were observed the symptoms (size, colour and morphological characteristics) of different types of corn rust under stereomicroscope. The spore

Table 1. Collection information of corn rust samples.

| Collection location   | Number of sample | Time of collection | Longitude and latitude | Altitude (metre) | Sr. no. of sample |
|-----------------------|------------------|--------------------|------------------------|------------------|------------------|
| Qujing – Huize        | 15               | 2018.8.1           | 103.513E, 26.812N       | 1991             | QJ-HZ-1-15       |
| Zhaotong – Ludian      | 15               | 2018.8.1           | 103.568E, 27.215N       | 1923             | ZT-LD-1-15       |
| Qujing – Xuanwei       | 15               | 2018.8.1           | 103.385E, 26.247N       | 1862             | QJ-XW-1-15       |
| Kunming – Panlong District | 30    | 2018.8.23          | 102.727E, 25.104N       | 1934             | KM-PL-1-30       |
| Kunming – Shillin      | 15               | 2017.7.24          | 103.289E, 24.774N       | 1685             | KM-SL-1-50       |
| Yuxi – Chengjiang      | 15               | 2017.7.21          | 102.392E, 23.998N       | 1741             | YX-CJ-1-15       |
| Yuxi – Jiangchuan      | 15               | 2017.7.21          | 103.362E, 24.967N       | 1695             | YX-JC-1-15       |
| Yuxi – Tonghai         | 15               | 2017.7.21          | 102.709E, 24.176N       | 1783             | YX-TH-1-15       |
| Honghe – Jianshui      | 15               | 2017.7.22          | 102.837E, 23.891N       | 1423             | HH-JS-1-15       |
| Honghe – Kaiyuan       | 15               | 2017.7.22          | 103.255E, 23.608N       | 1368             | HH-KY-1-15       |
| Honghe – Mile          | 15               | 2017.7.22          | 103.491E, 24.243N       | 1469             | HH-ML-1-15       |
| Honghe – Pibingang      | 25               | 2017.7.23          | 103.686E, 22.988N       | 1305             | HH-PB-1-25       |
| Honghe – Baizhai       | 15               | 2017.7.23          | 103.763E, 23.014N       | 385              | HH-BZ-1-15       |
| Wenshan – Maguan 1     | 25               | 2017.7.23          | 103.941E, 22.901N       | 503              | WS-MG-1-25       |
| Wenshan – Maguan 2     | 25               | 2017.7.23          | 104.014E, 22.913N       | 1606             | WS-MG-26-50      |

Total 270

Table 2. Primers used in genetic analysis.

| Primer name | Primer sequence | Tm |
|-------------|-----------------|----|
| ISSR1       | AGAGAGAGAGAGAGTC | 50 |
| ISSR2       | AGAGAGAGAGAGAGYC | 50 |
| ISSR3       | AGAGAGAGAGAGAGKG | 45 |
| ISSR4       | ACACACACACACACTG | 55 |
| ISSR5       | ACACACACACACACWC | 45 |
| ISSR6       | ACACACACACACACYA | 50 |
morbidity of different forms of corn rust was observed by Lycra fluorescence microscope. The spore size was measured by Lycra fluorescence microscope’s own measuring tool. Ten spores were measured for each sample, and a total of 20 samples from different regions were measured. At the same time, the rust samples measured spore size were recorded and used for molecular detection. Molecular approaches allow for the rapid determination of resistance mechanisms, eliminating the need for the lengthy delays associated with successive civilisation classification as well as vulnerability screening.

**Extraction of DNA**

The DNA of corn rust was extracted by CTAB. The methods were as follows: (1) A fresh infected leaf was selected and rust spores were scraped with small blades and placed in a 1.5 mL centrifugal tube. (2) The liquid nitrogen was added into the centrifugal tube and grinded with a grinding rod (2–3 times until the spores were powdered). (3) 500 μL CTAB was absorbed into the centrifugal tube by a 1000 μL pipette. (4) Put the centrifugal tube in a 65°C water bath pot for 1 h (shake every 15 min). (5) 500 μL Isoamyl chloroform (24:1) was absorbed from the fume hood by a 1000 μL pipette and added into the centrifugal tube (chloroform reagent is toxic solvent, operated with a mask). (6) Oscillating on the oscillator for 20 s and centrifuging for 10 min at 13,000 g. (7) The supernatant was sucked into a new 1.5 mL centrifugal tube with a 200 μL pipette. (8) Add ice isopropanol of equal volume and shake it upside down for 10 min. (9) Place for 1 h at −20°C (or for 5 min at −80°C). (10) centrifugation for 10 min at 13,000 g. (11) Abandon the supernatant, wash twice with 75% alcohol and dry it. (12) Dissolve DNA with 50 μL ddH₂O (in a 65°C water bath pot beforehand) by a 100 μL pipette. The resulting liquid is the sample genomic DNA. (13) Store in a refrigerator at −20°C for reserve.

**Specific molecular marker sequence analysis of corn rust**

The total volume of PCR reaction for specific molecular marker analysis of corn rust was 50 μL, including: 10 × Phanta max × Buffer 25 μL, dNTP Mix 1.0 μL, Phanta max 1.0 μL, forward primer and reverse primer 2 μL each, ddH₂O 18 μL, template DNA (25 ng/μL) 1 μL, ddH₂O as negative control group was used for each reaction. The amplification was done on ABI-PCR. This Applied Biosystems polymerase chain reaction device is a second-generation sequence detection tool that uses fluorescent-based PCR chemicals to analyze qualitative descriptive data. This equipment is able of both quantities’ detection using real-time analysis and end-point and dissociation-curve analyses. The reaction procedures were as follows: pre-denaturation for 3 min at 95°C, denaturation for 15 s at 95°C, annealing for 15 s at 57°C, extension for 30 s at 72°C, running for 35 cycles; extension for 5 min at 72°C, preservation at 16°C; after the end of PCR, 5 μL PCR product was mixed with 2 μL Loading-buffer, conducted electrophoresis with 1.5% agarose gel for 30 min under 120 V, and then photographed with the bio macromolecule analyzer to analyze PCR product.

**Population genetic structure analysis of corn rust**

The total volume of ISSR-PCR reaction for population genetic structure analysis of corn rust was 30 μL, including: 10 × Easy Buffer (containing MgCl₂) 3.0 μL, 2.5 mM dNTPs 2.4 μL, ISSR primer 1.2 μL, TaqE 0.3 μL, template DNA (25 ng/μL) 1 μL, ddH₂O 22.1 μL. Wheat stripe Coleosporium was used as positive control group and ddH₂O as negative control group each time. The reaction procedures were as follows: pre-denaturation for 5 min at 95°C, denaturation for 45 s at 94°C, annealing for 1 min at 50°C (the specific annealing temperature of each primer was shown in Table 2), extension for 1 min at 72°C, running for 35 cycles; extension for 10 min at 72°C, preservation at 4°C; after the end of PCR, 5 μL PCR product was mixed with 2 μL Loading-buffer, conducted electrophoresis with 1.5% agarose gel for 30 min under 120 V, and then analyzed with the bio macromolecule analyzer. Samples with PCR products were further analyzed with ABI-3730XL genetic analysis system.

**Data analysis**

ISSR-PCR amplified fragments were analyzed by genetic analysis system. The size of ISSR-PCR amplified fragments ranged from 100 bp to 700 bp (internal standard ABI-LIZ1200). The fragments of PCR products obtained by genetic analysis system were transformed into 01 matrix. Utilising PCR, a technique for preparing randomised DNA fragments has been created. The continuous primer is inserted throughout this third cycle, as well as double segments containing the consistent primer sequences at both extremities are generated throughout DNA template. The PCR products were packed in a box with a width of 10 bp. Popgene and NTsis software were used for population structure analysis.
Results and analysis

Morphology of corn rust

Scale and proportion are both size-related design aspects. The dimension of one thing in connection towards the other elements in a composition or image is referred to as scale. The size of one section of an item in comparison to other sections with similar thing is referred to as proportionality. The author carefully observed the collected samples of corn rust, and found that the symptoms of the collected samples could be divided into two types: common corn rust (caused by the infection of \textit{Puccinia sorghi} Schw) and southern corn rust (caused by \textit{Puccinia polysora} Unedrw). The spores of two types of corn rust were selected and observed under the microscope. The morphology of summer spores of \textit{Puccinia sorghi} Schw and \textit{Puccinia polysora} Unedrw were different. The symptoms and spore morphology were shown in Figure 1. As can be seen from Figure 1, the symptoms of common corn rust and southern corn rust are different as follows: blister spots are produced on both sides of leaves of common corn rust, while southern corn rust usually produces blister spots on the front side. Summer spore heap of common corn rust is larger than that of southern corn rust, summer spore heap of southern corn rust is small and dense; summer spores of \textit{Puccinia sorghi} Schw causing common corn rust are mostly round, while those of \textit{Puccinia polysora} Unedrw causing southern corn rust are mostly long ellipse. In addition, the distribution of protoplasts in summer spores of common corn rust was slightly different at different stages. In

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{Symptoms of corn rust. Note: A–D are samples of common corn rust (ferruginous color); E–H are samples of common corn rust (deeply ferruginous color); I–M are samples of southern corn rust; B, F, G, J and K were picture of Stereo-Microscope, the proportional scale is 0.1 mm.}
\end{figure}
the early stage, the protoplasts in summer spores were evenly distributed. In the middle and late stages, the protoplasts in summer spores were clustered (Figure 2).

**Morphological characteristics of pathogens of corn rust**

In order to observe the morphological characteristics of different spores of corn rust pathogens, the spores of corn rust were made into temporary slides and observed under Lycra fluorescence microscope. The size of summer spores of corn rust was measured. The results are shown in Table 3. From Table 3, it can be seen that the size of summer spores of *Puccinia sorghi* Schw is ranged between $26.78 \times 35.57$ and $18.38 \times 23.68$, and that of summer spores of *Puccinia polysora* Unedrw is ranged between $18.61 \times 36.24$ and $16.06 \times 28.25$.

By measuring and comparing the length and width of summer spores of southern corn rust and common corn rust, we can see that the ratio of length/width of summer spores of *Puccinia sorghi* Schw is smaller than that of *Puccinia polysora* Unedrw, the average ratio of length/width of summer spores of *Puccinia sorghi* Schw is 1.360, and the average ratio of length/width of summer spores of common corn rust is between 1.106 and 1.147 in different stages. Summer spores whose ratio of length/width is larger than 1.2 accounts for 81.6% of the total spores of *Puccinia polysora* Unedrw, while the ratio of length/width of summer spores of *Puccinia*...
Table 4. Aspect ratio of uredospore about different symptoms of corn rust.

| Range of length/width ratio | Corn common rust (Tended to yellow) | Corn common rust (Early stage) | Corn common rust (Middle and final stage) |
|-----------------------------|-------------------------------------|--------------------------------|------------------------------------------|
| <1.1                        | 4.6                                 | 40.0                           | 24.2                                    |
| 1.1–1.2                     | 13.8                                | 50.0                           | 54.5                                    |
| 1.2–1.3                     | 16.1                                | 10.0                           | 20.2                                    |
| 1.3–1.4                     | 21.8                                | 1.0                            |                                         |
| 1.4–1.5                     | 31.0                                |                                |                                         |
| 1.5–1.6                     | 6.9                                 |                                |                                         |
| 1.6–1.7                     | 3.4                                 |                                |                                         |
| >1.7                        | 2.3                                 |                                |                                         |
| Average Width Ratio         | 1.360                               | 1.118                          | 1.147                                    |

Puccinia polysora Unedrw. The results showed that: when conducting PCR amplification on samples of common corn rust with Puccinia sorghi Schw specific molecular markers, PCR products whose fragment size is around 540 bp can be obtained, no PCR products can be obtained from samples of southern corn rust; when conducting PCR amplification on samples of southern corn rust with Puccinia polysora Unedrw specific molecular markers, PCR products whose fragment size is around 480 bp can be obtained, no PCR products can be obtained from samples of common corn rust (Figure 3).

Genetic diversity of corn rust populations

The population genetic diversity of Puccinia polysora Unedrw in samples collected from Honghe and Wenshan was analyzed by ISSR primers. Inter-simple sequence repeats (ISSRs) are microsatellite-flanked sections in the chromosome. ISSR markers are simple to employ, low-cost, and analytically less difficult than other attribute is a characteristic, making them a specific genetic marker for novices and species with less genetic data. The results showed that the Nei, s genetic diversity H (0.1906 and 0.2068), Shannon information index I (0.3103 and 0.3312) and polypeptide loci percentage P (86.11%) of Puccinia polysora Unedrw collected from Wenshan - Maguan were higher than those of samples collected from Honghe - Pingbian, which were, Nei, s genetic diversity H (0.1400 and 0.1620), Shannon information index I (0.2305 and 0.2627) and polypeptide loci percentage P (69.75% and 70.99%); that is to say, the genetic diversity of Puccinia polysora Unedrw collected from Wenshan - Maguan is richer than that collected from Honghe – Pingbian (Table 6). The genetic diversity of Puccinia sorghi Schw in different areas was analyzed by ISSR primers. The results showed that the population genetic diversity of Puccinia sorghi Schw in different areas was different. Nei, s genetic diversity H (0.2153), Shannon information index (0.3410) and polypeptide loci percentage P (81.17%) of Puccinia sorghi Schw collected from

Table 5. Comparison and analysis of ITS sequence of different symptoms of corn rust.

| Symptoms          | No | Homologous species | PCR Product (bp) | Query cover (%) | E-value | Ident% | Accession          |
|-------------------|----|--------------------|------------------|-----------------|---------|--------|--------------------|
| Spotted           | Pb-2| Puccinia polysora  | 727              | 100             | 0.0     | 99.86  | HM467909.1         |
|                   | Pb-9-2| Puccinia polysora | 727              | 100             | 0.0     | 99.86  | HM467909.1         |
|                   | Pb-10-1| Puccinia polysora | 727              | 100             | 0.0     | 99.86  | HM467909.1         |
|                   | Lj-h-1| Puccinia sorghi   | 712              | 100             | 0.0     | 99.72  | AY114291.1         |
| Partial yellow    | Lj-h-2 | Puccinia sorghi   | 712              | 100             | 0.0     | 99.72  | AY114291.1         |
|                   | Lj-h-3 | Puccinia sorghi   | 712              | 100             | 0.0     | 99.72  | AY114291.1         |
|                   | Km-5-1| Puccinia sorghi   | 712              | 100             | 0.0     | 99.44  | AY114291.1         |
|                   | Km-6-2| Puccinia sorghi   | 712              | 100             | 0.0     | 99.44  | AY114291.1         |
|                   | Km-8-1| Puccinia sorghi   | 712              | 100             | 0.0     | 99.44  | AY114291.1         |
| Partial dark      | Lj-h-2 | Puccinia sorghi   | 712              | 100             | 0.0     | 99.72  | AY114291.1         |
|                   | Lj-h-3 | Puccinia sorghi   | 712              | 100             | 0.0     | 99.72  | AY114291.1         |
|                   | Km-5-1| Puccinia sorghi   | 712              | 100             | 0.0     | 99.44  | AY114291.1         |
|                   | Km-6-2| Puccinia sorghi   | 712              | 100             | 0.0     | 99.44  | AY114291.1         |
|                   | Km-8-1| Puccinia sorghi   | 712              | 100             | 0.0     | 99.44  | AY114291.1         |
Zhaotong-Ludian were the largest, and the genetic diversity of *Puccinia sorghi* Schw was the richest. Nei's genetic diversity \( H \) (0.1071 and 0.1115), Shannon information index (0.1686 and 0.1802) and polypeptide loci percentage \( P \) (41.36% and 50.31%) were the smallest in Kunming-Panlong District, and their population genetic diversity was the lowest (Table 6).

### Discussion

Corn rust is one of the most important diseases, which can be divided into four types according to its symptoms and pathogens: common rust, southern rust, tropical rust and stem rust (Luo 2011). However, the specific distribution of these four corn rust diseases in Yunnan has not yet been reported. In this paper, the spore morphology, ITS sequence analysis, *Puccinia sorghi* Schw and *Puccinia polysora* Unedrw specific molecular marker analysis were studied on corn rust samples collected from Qujing, Zhaotong, Kunming, Yuxi, Honghe and Wenshan in Yunnan Province in 2018. It was found that the 180 samples collected from Qujing, Zhaotong, Kunming, Yuxi, Honghe-Mile, Kaiyuan and Jianshui all belong to common corn rust caused by...
Puccinia sorghi Schw, while 90 samples collected from Honghe- Pingbian, Baizhai and Wenshan- Maguan belong to southern corn rust caused by Puccinia polysora Unedrw. Only Puccinia polysora Unedrw was detected in Wenshan, but no Puccinia sorghi Schw was detected. Puccinia sorghi Schw was detected from four samples collected from Qujing, Zhaotong, Kunming and Yuxi, but no Puccinia polysora Unedrw was detected. Both were detected from samples of Honghezhou area. Yet only one type of pathogen was detected in each county of Honghezhou area. It can be seen that the distribution of common corn rust and southern corn rust in Yunnan is different in different regions. This is different from that reported by Zhou Huiping in 2011 that the main cause of maize rust in Yunnan province is mainly common rust (Zhou et al. 2011). This is mainly due to the different areas and methods of disease investigation. In our study, we not only distinguished the symptoms of rust on maize, but also used the specific molecular markers of Puccinia sorghi Schw and Puccinia polysora Unedrw to further identify, and the results were more accurate. However, further sampling and investigation are needed to understand the detailed distribution of different corn rust diseases in Yunnan, as well as the cross infection of common corn rust and southern corn rust in Yunnan.

At the same time, through the observation of the morphology of summer spores of Puccinia sorghi Schw and Puccinia polysora Unedrw, it was found that there were great differences. The summer spores of Puccinia sorghi Schw were round, bright yellow to light brown. The length/width ratio of summer spores was less than 1.3, and the length/width ratio of summer spores less than 1.2 in different stages accounted for 78% of the total spores measured. On the contrary, most of the summer spores of Puccinia polysora Unedrw are elliptic, and the colour is light yellow to bright yellow. The length-width ratio of summer spores is large, and the length/width ratio of more than 80% of summer spores is greater than 1.2. This provides a basis for identification of Puccinia sorghi Schw and Puccinia polysora Unedrw by morphology of summer spores in the future.

Population genetic structure of Puccinia sorghi Schw and Puccinia polysora Unedrw collected from different areas was studied. It was found that the population genetic polymorphism of Puccinia sorghi Schw and Puccinia polysora Unedrw was different in different areas. This is mainly due to the different varieties of maize planted in different regions. In addition, in the process of sampling, it was found that the incidence of different maize varieties in the same area was different, some varieties were more resistant, some varieties were more susceptible. Therefore, we will further analyze whether maize varieties, altitude and climate will affect the population genetic structure of Puccinia sorghi Schw and Puccinia polysora Unedrw, as well as the influence of different population genetic structures on maize incidence, so as to lay a foundation for rational distribution of maize varieties and control of maize rust.

Conclusions

1. The morphology of summer spores of Puccinia sorghi Schw and Puccinia polysora Unedrw was observed. It was found that summer spores of Puccinia sorghi Schw were nearly round with the average length/width ratio ranging between 1.106 and 1.147. The measured length/width ratio of summer spores was less than 1.3, and the length/width ratio of more than 90% of summer spores was between 1.0 and 1.2. The majority of summer spores of Puccinia polysora Unedrw were elliptical, and the average length/width ratio of summer spores was 1.360 with 80% of summer spores being larger than 1.2. Therefore, the length/width ratio of summer spores of corn rust can be used as a basis for preliminary identification of Puccinia polysora Unedrw and Puccinia sorghi Schw.

2. The distribution of common corn rust caused by Puccinia sorghi Schw and southern corn rust caused by Puccinia polysora Unedrw is regional. Southern corn rust is mostly distributed in Kaiyuan Pingbian area and Wenshan area, south of Yunnan Province. Therefore, further sampling and investigation are needed to understand the detailed distribution of different corn rust diseases in Yunnan, as well as the cross infection of common corn rust and southern corn rust in Yunnan.

3. By analyzing the population genetic structure of rust fungi in different areas, the results showed that the genetic diversity of Puccinia polysora Unedrw collected from Wenshan was richer than that of Honghe; the genetic diversity of Puccinia sorghi Schw collected from Zhaotong was richest while that collected from Kunming was the lowest.

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Disclosure statement
No potential conflict of interest was reported by the author(s).

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