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Analysis of Agronomic Traits and Quarantine Diseases of Sugarcane Germplasms Newly Introduced from USA and Vietnem

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Abstract The purpose of this study was to detect the status of Fiji disease virus (FDV) and leaf scald (*Xanthomonas albilineans*) in the newly introduced sugarcane germplasms from USA and Vietnam, and observe their agronomic performances in Guangxi sugarcane growing area, thus to provide references for screening healthy sugarcane germplasm. Following the quarantine procedure, 41 newly introduced sugarcane germplasm accessions were planted in isolated quarantine area for FDV and leaf scald detection. The PCR (RT-PCR) results showed the reaction to FDV and leaf scald were both negative and no quarantine disease occurred. Further, field experiments were conducted to observe their agronomic performance and resistance to commom diseases, like red rot, yellow spot, ring spot, pokkah boeng, mosaic, smut, rust and brown stripe diseases, and the results showed that 18 germplasm accessions were resistant to these diseases. Based on the experimental results, 8 accessions from USA and 4 accessions from Vietnam were selected for the next variety test. This study would provide excellent resistant germplasm materials and scientific references for further sugarcane disease resistance breeding.

Keywords Sugarcane germplasm, Disease, Quarantine, Molecular detection, Agronomic trait

Sugarcane (*Saccharum officinarum*) is the main sugar crop and cash crop in tropical and subtropical areas (Chen et al., 2015; Li et al., 2015). The conventional breeding of sugarcane is a long and hardworking research. It usually takes 10 years to develop a new variety. Therefore, breeders often introduce new sugarcane varieties (lines) from abroad and screen out healthy germplasm under local climate conditions. It is one of important methods of germplasm innovation and application in sugarcane breeding. Sugarcane introduction is an effective way to make rapid and easy use of sugarcane varieties from all over the world, to optimize the structure of sugarcane varieties in China and increase genetic resources. However, all sugarcane materials from abroad must be quarantined for diseases that recorded in the List of Import Phytosanitary Pests in the People's Republic of China, like sugarcane Fiji disease virus (FDV), sugarcane leaf scald, phytoplasma and sugarcane downy mildew that are not seriously infected in the main production area of Guangxi, thus to prevent serious losses from the introduction of foreign diseases into China.

Sugarcane FDV and leaf scald diseases are the main objects of worldwide quarantine. Sugarcane FDV disease is infected by Fiji leaf gall (FLG). It was first found in Fiji, 1886 and also in Australia, 1890. It is now found in the Philippines, Papua New Guinea, Thailand, Western Samoa and other countries, since it is regarded as the most important disease in Australia, Asia and Pacific region (Smith and Candy, 2004). After sugarcane infected with FDV, the cane stalk was significantly dwarfed and the upper leaf was short as a sword, finally the whole leaves showed fan-shaped. Sugarcane infected with FDV grew a cluster of plants, usually short leaves were found without cane stalks, so that it caused serious reduction of yield (Egan and Ryan, 1986). Leaf scald disease is

caused by *Xanthomonas albilineans* (Ashby Dowson). It has been reported in many countries and regions, such as USA, Cambodia, Vietnam, Thailand and so on, where outbreaks have caused huge economic losses (Hoy and Grisham, 1994; Rott et al., 1995; Saumtally et al., 2004). It also has been reported in Guangxi, Hainan and Fujian of China, especially in recent years, sugarcane varieties easily infected with leaf scald have been popularized and planted, resulting in more severe leaf scald disease (Li et al., 2018; Wei et al., 2018).

In order to understand the disease resistance of 41 sugarcane varieties (materials) introduced by Suguarcane Research Institute of Guangxi Academy of Agricultural Sciences from USA and Vietnam as well as their characteristics under local climate conditions in Guangxi, sugercane FDV and leaf scald diseases were detected and important agronomic traits were observed in this study, which would help explore the utilization potential of these introduced sugarcane germplasms in sugarcane breeding in Guangxi and provide important information and reference for sugarcane hybrid parents.

1 Results and Analysis

1.1 Status of diseases in quarantine cultivation

During the period of quarantine monitoring, the status of diseases was recorded once a month. After two growth cycle of monitoring, 41 introduced sugarcane varieties grew well and no quarantine diseases occurred.

1.2 Detection of sugarcane leaf scald disease by PCR

The detection of leaf scald disease by PCR was carried out in 41 sugarcane varieties. The results showed that no bands were amplified in the negative control, while the target band of 600 bp was amplified in the positive control. At the same time, the target band of 600 bp was also not amplified in 41 sugarcane materials, and the identification result was negative (Figure 1; Table 1). The results indicated that 41 sugarcane germplasms introduced from USA and Vietnam were not infected with leaf scald disease.

1.3 Detection of sugarcane Fiji disease by RT-PCR

The detection of sugarcane FDV by RT-PCR was carried out in 41 introduced sugarcane varieties. The target band of 450 bp was not amplified and the results were negative (Figure 2; Table 1).

1.4 Observation of other diseases

Besides leaf scald and FDV diseases, other common diseases such as red rot, yellow spot, ring spot, pokkah boeng, mosaic, smut, rust and brown stripe disease were also observed in the field. The results showed that 18 of 41 imported materials were not infected with these diseases, other 29 materials were infected with one or more diseases (Table 1).

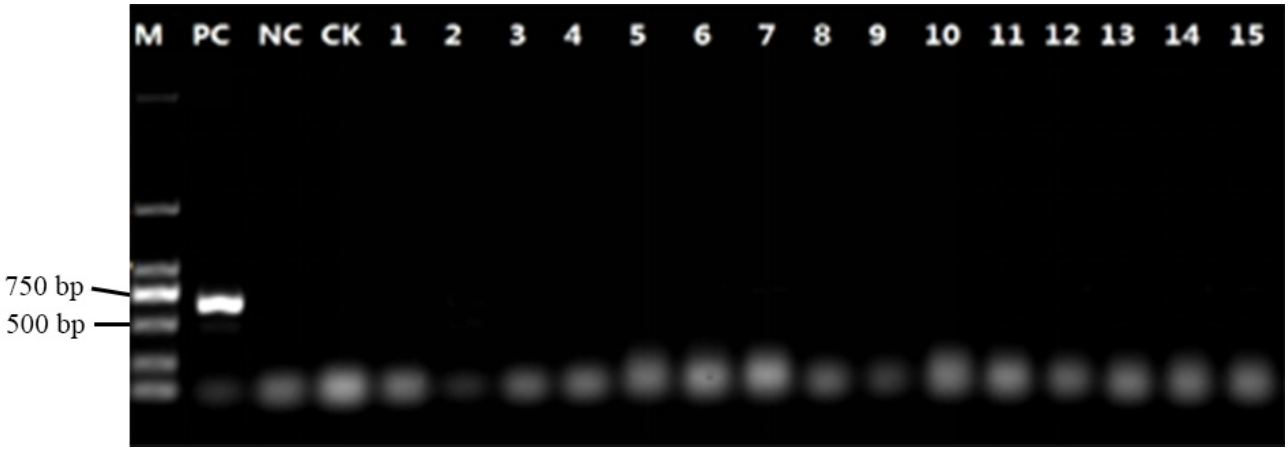

Figure 1 PCR amplification of sugarcane leaf scald

Note: M: DNA ladder marker (D2000); PC: Positive control; NC: Negative control; CK: Blank control; 1~15: No.1~15 materials as listed in Table 1

Table 1 Disease detection of 41 introduced sugarcane materials

Code	Materials	PCR detection results		Field survey results	Importing country
		Fiji disease	Leaf scald	Other diseases	
1	CPCL97-2730	-	-	Not found	USA
2	CPCL02-6848	-	-	Red rot+, yellow spot+, ring spot+, pokkah boeng+	USA
3	CPCL00-4111	-	-	Not found	USA
4	CPCL02-1295	-	-	Red rot++	USA
5	CP05-1526	-		Not found	USA
6	CPCL05-1791	-	-	Not found	USA
7	CPCL99-1401	-	-	Not found	USA
8	CPCL95-2287	-	-	Mosaic+	USA
9	CPCL02-0926	-	-	Not found	USA
10	CPCL05-1201	-	-	Ring spot++	USA
11	CP00-1446	-	-	Ring spot+	USA
12	CP06-2400	-	-	Not found	USA
13	CP06-2402	-	-	Yellow leaf virus+++	USA
14	CP09-4229	-	-	Smut++	USA
15	CP04-1566	-	-	Not found	USA
16	CP04-1844	-	-	Yellow spot++, ring spot++	USA
17	CP04-1935	-	-	Yellow leaf virus++, yellow spot++, smut++	USA
18	L03-371	-	-	Mosaic+	USA
19	L01-299	-	-	Not found	USA
20	Ho05-961	-	-	Mosaic+++	USA
21	LCP85-384	-	-	Ring spot+	USA
22	Ho07-613	-	-	Not found	USA
23	HoCP00-950	-	-	Mosaic+, red rot++	USA
24	HoCP85-845	-	-	Ring spot++	USA
25	HoCP96-540	-	-	Not found	USA
26	LCP04-838	-	-	Smut+	USA
27	L01-283	-	-	Not found	USA
28	VN08-207	-	-	Pokkah boeng++	Vietnam
29	VN66-03	-	-	Not found	Vietnam
30	VN84-4137	-	-	Rust+++	Vietnam
31	VN09-213	-	-	Not found	Vietnam
32	VN66-06	-	-	Not found	Vietnam
33	VN09-391	-	-	Not found	Vietnam
34	VN99-838	-	-	Smut+	Vietnam
35	VN09-450	-	-	Rust++, ring spot++	Vietnam
36	VN08-260	-	-	Smut+++	Vietnam
37	VN84-1745	-	-	Ring spot++	Vietnam
38	VN08-05	-	-	Yellow leaf virus++	Vietnam
39	VN09-284	-	-	Not found	Vietnam
40	VN08-235	-	-	Ring spot++	Vietnam
41	VN99-317	-	-	Smut+, brown stripe+	Vietnam

Note: -: PCR result was negative; +: Mild disease; ++: Moderate disease; +++: Severe disease

The fields agronomic characters of 41 introduced sugarcane germplasm were observed and tested (Table 2). The results showed that 12 germplasm (CPCL97-2730, CPCL02-6848, CPCL02-1295, CP05-1526, CPCL02-0926, CP04-1935, LCP85-384, LCP04-838, VN66-03, VN84-4137, VN09-213 and VN66-06) had better comprehensive traits and higher yield potential, which will be entered the next round of variety comparison trials continue to screen. The other 29 germplasm showed poor performance, and we suggested entering the sugarcane resource nursery for further study and utilization.

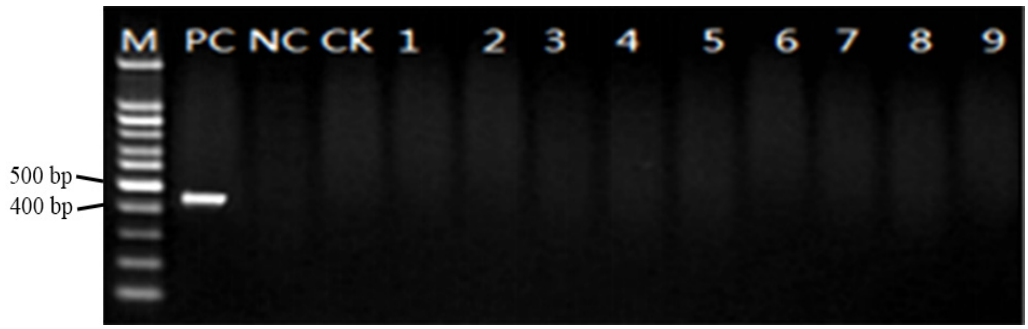

Figure 2 RT-PCR amplification of sugarcane Fiji disease

Note: M: DNA ladder marker (D1500); PC: Positive control; NC: Negative control; CK: Blank control; 1~9: No.1~9 materials as listed in Table 1

Germplasm resources of sugarcane are important basic materials for sugarcane genetics and breeding. The introduction of different sugarcane germplasm with higher heterogeneity from abroad could greatly enrich the gene pool of sugarcane germplasm in China, which might be of great significance for sugarcane breeding. The introduced varieties often perform well at abroad, but in China, with the different domestic climatic conditions, they are difficult to accept. Therefore, it is necessary to carry on field experiment observation to introduce germplasm, and to screen excellent germplasm with good agronomic characters, high yield, high sugar content, and strong ratooning ability for production or for sugarcane genetic breeding.

The introduction of germplasm resources from abroad must be quarantined. The import and export of sugarcane must go through strict quarantine to exchange germplasm resources. Otherwise, it would be easy to cause new diseases to break out in sugarcane area. Leaf scald is a worldwide disease, the potential threat is greater, and there is a tendency to aggravate the disease in recent years. Some new sugarcane varieties (lines) have been found to be infected with this disease in Guangxi sugarcane area (Li et al., 2018; Wei et al., 2018). PCR detection and sequencing were effective methods for identification of leaf scald. Wei et al. (2018) and Li et al. (2018) successfully confirmed that sugarcane leaf scald in sugarcane region of Guangxi was caused by Xanthomonas albicans in Xanthomonas using PCR technique. Sugarcane Fiji disease virus is a kind of virus disease which is one of the important diseases of sugarcane like sugarcane leaf scald and has great influence on sugarcane yield. Sugarcane Fiji disease virus has been reported in Fujian experimental nursery and germplasm resource nursery in China (Zhou et al., 1987). It has also been reported in sugarcane varieties imported from abroad in Guangxi (Deng, 1997). At present, the disease has not been found in domestic production. In Yunnan province, RT-PCR technique was used to detect 11 sugarcane varieties from Australia, and no Fiji disease virus was found (Lu et al., 2009). The most fundamental way to control sugarcane Fiji disease virus and leaf scald would be to strengthen quarantine and prohibit the transfer of sugarcane species from endemic areas and plant resistant varieties. In this study, 41 sugarcane materials were introduced from the United States and Vietnam. After two growth cycles of isolated planting, there were no specific bands for sugarcane leaf scald (600 bp) and Fiji disease virus (450 bp) be detected by laboratory test using mature PCR technique. Therefore, agronomic characters were observed. Field experiments results showed that 17 introduced germplasm had strong resistance to disease, except for sugarcane leaf scald and Fiji disease virus, and there was no common disease was found, like red rot, yellow spot, ring spot, pokkah boeng, mosaic, smut, rust, brown stripe diseases and so on. The disease resistance and agronomic characters of eight American sugarcane germplasms (CPCL97-2730, CPCL02-6848, CPCL02-1295, CPCL05-1526, CPCL02-0926, CP04-1935, LCP85-384, LCP04-838), and four Vietnamese sugarcane germplasms (VN66-03, VN09-213, VN66-06, VN09-284) were all good. These materials could be used as hybrid parents in the next round of comparative trials. Other unselected materials would be evaluated for agronomic characters, such as without superior traits (high yield or high sugar content) would be suggested to enter the resource nursery for planting and preservation, and for those with excellent agronomic traits would be considered put in the sugarcane parent nursery for future sugarcane cross breeding.

Table 2 The agronomic traits in 41 introduced sugarcane germplasm materials

Code	Material	Agronomic trait	Note
1	CPCL97-2730	Medium stalk, tight leaf sheath, lodging, dwarf, rich millable stalks, vigorous growth	Enter next field trial
2	CPCL02-6848	Medium stalk, easily defoliated leaf sheaths, erect, tall, good tillering, vigorous growth	Enter next field trial
3	CPCL00-4111	Medium small stalk, rich millable stalks, leaf sheath, average growth	Enter germplasm nursery
4	CPCL02-1295	Medium –medium big stalk, more millable stalks, defoliate, tall, vigorous growth	Enter next field trial
5	CP05-1526	Medium stalk, rich millable stalks, easily defoliate leaf sheaths, vigorous growth	Enter next field trial
6	CPCL05-1791	Medium stalk, average millable stalks, leaf sheath, dwarf, average growth	Enter germplasm nursery
7	CPCL99-1401	Small stalk, leaf sheath, dwarf, average growth	Enter germplasm nursery
8	CPCL95-2287	Small stalk, rich millable stalks, leaf sheath, booting stage	Enter germplasm nursery
9	CPCL02-0926	Medium small stalk, rich millable stalks, tall, leaf sheath, vigorous growth	Enter next field trial
10	CPCL05-1201	Small stalk, average millable stalks, dwarf, leaf sheath	Enter germplasm nursery
11	CP00-1446	medium to small stalk, average millable stalks, leaf sheath	Enter germplasm nursery
12	CP06-2400	Medium stalk, average millable stalks, dwarf, leaf sheath	Enter germplasm nursery
13	CP06-2402	Medium small stalk, rich millable stalks, dwarf	Enter germplasm nursery
14	CP09-4229	Medium stalk, rich millable stalks, bad growth	Enter germplasm nursery
15	CP04-1566	Medium stalk, rich millable stalks, bad growth	Enter germplasm nursery
16	CP04-1844	Medium stalk, poor millable stalks, bad growth	Enter germplasm nursery
17	CP04-1935	Medium small stalk, rich millable stalks, plant erect, easily defoliate, tiller good, vigorous growth	Enter next field trial
18	L03-371	Medium stalk, rich millable stalks, leaf sheath, average growth	Enter germplasm nursery
19	L01-299	small stalk, rich millable stalks, leaf sheath, average growth	Enter germplasm nursery
20	Ho05-961	small stalk, rich millable stalks, average growth	Enter germplasm nursery
21	LCP85-384	Medium stalk, rich millable stalks, vigorous growth	Neter next field trial
22	Ho07-613	small stalk, rich millable stalks, vigorous growth	Enter germplasm nursery
23	HoCP00-950	small stalk, poor millable stalks, average growth	Enter germplasm nurser
24	HoCP85-845	Medium small stalk, poor millable stalks, bad growth	Enter germplasm nursery
25	HoCP96-540	Medium small stalk, poor millable stalks, average growth	Enter germplasm nursery
26	LCP04-838	Medium stalk, rich millable stalks, average growth	Enter next field trial
27	L o01-283	small stalk, bad growth	Enter germplasm nursery
28	VN08-207	Medium stalk, poor millable stalks, bad growth	Enter germplasm nursery
29	VN66-03	Medium stalk, rich millable stalks, average growth	Enter next field trial
30	VN84-4137	Medium stalk, rich millable stalks, vigorous growth	Enter next field trial
31	VN09-213	Medium stalk, rich millable stalks, average growth	Enter next field trial
32	VN66-06	Medium samll stalk, rich millable stalks, average growth	Enter next field trial
33	VN09-391	Medium stalk, poor millable stalks, bad growth	Enter germplasm nursery
34	VN99-838	Medium stalk, rich millable stalks, average growth	Enter germplasm nursery
35	VN09-450	Medium stalk, rich millable stalks, average growth	Enter germplasm nursery
36	VN08-260	small stalk, rich millable stalks, dwarf, bad growth	Enter germplasm nursery
37	VN84-1745	Medium stalk, average millable stalks, leaf sheath, bad growth	Enter germplasm nursery
38	VN08-05	Medium to small stalk, average millable stalks, bad growth	Enter germplasm nursery
39	VN09-284	Medium to small stalk, rich millable stalks, average growth	Enter germplasm nursery
40	VN08-235	Medium stalk, rich millable stalks, leaf sheath, average growth	Enter germplasm nursery
41	VN99-317	Small stalk, poor millable stalks, medium growth	Enter germplasm nursery

The PCR technology has been widely used in the disease detection of various crops, and gradually became a necessary means for the inspection of import and export plant quarantine (Lu et al., 2009; Li et al., 2016; Peng et

al., 2018). It is a guarantee for the healthy development of sugarcane industry and the cooperation and exchange at home and abroad to apply quarantine detection techniques and standardized quarantine procedures, and carry on the quarantine of Disease and insect pests for import and export sugarcane germplasm resources. In this study, the disease surveillance was carry out in strict accordance with the quarantine procedure of the import and quarantine of the sugar cane, disease symptom observation combined with molecular monitoring and the detection of sugarcane pests & diseases to eliminate new pests and diseases caused by introduction, which was of great significance for the healthy production of sugarcane industry and breeding of sugarcane disease resistance in China.

3 Materials and Methods

3.1 The tested material

41 sugarcane germplasm materials were introduced from the United States and Vietnam, including 27 sugarcane germplasms from the United States and 14 sugarcane germplasms from Vietnam (Table 3). The samples were collected in the quarantine greenhouse of Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences.

3.2 Epidemic surveillance

Strict quarantine monitoring of introduced sugarcane germplasm was carried out. In the pretreatment room of quarantine room, the imported sugarcane germplasm was first unsealed and appearance inspected, then treated with hot water, soaked in hot water at 50 ℃ for 2 hours, then soaked with carbendazim 50% visibility powder 800 times solution for 10 min. Then, the sterilizing soil at 120 ℃ was used to plant and cultivated. We put them in different separate greenhouses according to the batches and sources. Observe and monitor diseases and pests on a monthly basis. After 6 ~ 10 months of planting, 50% of the sugarcane plants were cut and replanted in barrels. Plant cane and ratoon planted for 6-10 months. Samples were taken for molecular bioassay of Fiji disease virus and leaf scald during cultivation.

3.3 Sampling and sample preparation

The sugarcane growing in quarantine greenhouse which was at fourth month fresh +1 leaf of plant cane and ratoon was taken for the detection of Fiji disease virus. The sugarcane cane near the growing point at tenth month was taken for the detection of leaf scald. Sample about 1 g. The sampling tool was washed with water and then disinfected with 75% alcohol at every time of sampling. The sample was cut into small pieces with knife or scissors, put in a mortar, added liquid nitrogen to grind quickly 4~5 times to powder, and rapidly transferred to 1.5 mL centrifuge tube, saved at -80 ℃.

3.4 DNA extraction and RNA extraction of samples

Wolact® Plant Genomic DNA Purification Kit was used to extract total DNA from leaves for PCR molecular detection of sugarcane leaf scald. Wolact® Plant RNA Isolation Kit was used to extract the sample RNA for RT-PCR detection of sugarcane FDV. The specific steps were carried out according to the operation of the specification.

Table 3 The sugarcane germplasm materials from USA and Vietnam

Source	Time	Number	Name of material
USA	2014-11	17	CPCL97-2730, CPCL02-6848, CPCL00-4111, CPCL02-1295, CP05-1526, CPCL05-1791, CPCL99-1401, CPCL95-2287, CPCL02-0926, CPCL15-1201, CP00-1446, CP06-2400, CP06-2402, CP09-4229, CP04-1566, CP04-1844, CP04-1935
USA	2015-4	10	HoCP96-540, HoCP85-845, HoCP00-950, Ho07-613, LCP85-384, Ho05-961, L01-283, L01-299, L03-371
Vietnam	2015-5	14	VN 08-05, VN 08-207, VN 08-235, VN 08-260, VN 84-4137, VN 66-06, VN 09-213, VN 09-284, VN 09-391, VN 09-450, VN 66-03, VN 84-1745, VN 99-317, VN 99-838

3.5 Primer design and synthesis

X. allbilineans specific primers for PCR detection of sugarcane leaf scald were designed with reference to Birch (2001). Forward Primer XAF1 ： 5'-CCTGGTGATGACGCTGGGTT-3'; downstream primer XAR1: 5'-CGATCAGCGATGCACGCAGT-3'. The size of pre-amplified product was about 600 bp.

The primers used for RT-PCR detection of sugarcane Fiji disease virus were Fontana et al. (2013) conserved sequence specific primers of sugarcane Fiji virus. Forward primer FDV7F: 5'-CCGAGTTACGGTCAGACTGTTCTT-3'; downstream primer FDV7R: 5'-CAGTGGTGACGAAATGATGGCGA-3'. The size of the pre-amplified product was about 450 bp.

3.6 PCR amplification and electrophoretic detection of sugarcane leaf scald

The growth point of sugarcane DNA was used as a template, and the PCR system referred to the method of Wei et al. (2018). System (20.0 μL): DNA sample was 3.0 μL, 2×PCR *Taq* mix 8.0 μL , each of the forward primer and downstream primer was 0.2 μL (20 μg/μL), ddH_2O 8.6 μL. Amplification program: Pre-denaturation at 95℃ for 5 min; 94℃ for 45s, 65℃ for 1 min, 72℃ for 1 min, 10 circles. 94℃ for 45 s, 65℃ for 1 min, 72℃ for 2 min, 10 circles; 94℃ for 45 s，65℃ for 1 min，72℃ for 3 min，10 circles; extended at 72℃ for 10 min. The experiment was repeated 3 times.

The amplified products of 8.0 μ L were detected by 1.5% agarose gel electrophoresis. The amplified bands of about 600 bp were positive and the results of no amplified bands were negative. If there were specific bands, they would be taken for recovery and purification, sequencing, BLAST search and analysis.

3.7 RT-PCR amplification and electrophoretic detection of sugarcane Fiji virus

+1 leaf RNA samples extracted were detected by one-step RT-PCR with C. therm RT-PCR kit (Li et al., 2016). The actions were as follows: 1.0 μ L RNA template was added to the 0.5 mL PCR tube, the upstream and downstream primer (20 μ g / μ L) were 0.25 μ L and ddH2O 11.0 μ L, respectively. Mix well and place on ice immediately after denaturation at 99 ℃ for 2 min, on PCR. In the above mixture, 5×buffer 5 μL, 10% PVP 2.5 μL, 100 mmol/L DDT 1.25 μL, 100% DMSO 1.25 μL, 5% BSA 1.0 μL, 20 mmol/L dNTPs 0.5 μL and C. therm enzyme 1.0 μL were added in proper order, the total volume of the reaction was 25 μL. The PCR amplification program: 57℃ for 30 min, 95℃ for 2 min, 95 ℃ for 1 min, 57℃ for 1 min, 72℃ for 1 min, 35 circles, extended at 72℃ for 10 min, with three times of repeated amplification.

The amplified products of 8.0 μ L were detected by 1.5% agarose gel electrophoresis. Taking photos at Image maker VDS Image, the amplified bands of about 450 bp were positive and the results of no amplified bands were negative. If there were specific fragments, recovery and purification, sequencing, BLAST search analysis would be carried on.

3.8 Evaluation of agronomic traits of sugarcane

After the introduced sugarcane germplasm was isolated and quarantined, the agronomic character evaluation test was carried out in the field. The introduced sugarcane varieties were harvested after two growing cycles, soaked in flowing cold water for 24 hours, then soaked in hot water of 52℃ for 30 min, to observe agronomic and other characters in the field. Each variety was planted in 3 rows, each of which had 7m, 80 buds per row. The varieties were arranged randomly, and the control was the main cultivar ROC22. The agronomic characters were evaluated at the mature stage of the new plant, and the better materials were selected for the subsequent variety comparison test.

Authors' Contributions

WJJ was the main executors of this research and the writer of this article. SXP, ZXQ, TZQ, ZRH, WCY, GYY, ZH, THW, HDL and LHB completed data collection and analysis. LXH and LYR were the architect and in charge of the project, who guided paper writing and revision. All the authors read and agree to the final text.

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