

Report on Pathogen Detection of Rice Seeds

1.1 Materials

On February 11, 2025, the laboratory received 5 hybrid rice seed samples, each weighing approximately 0.3 kg, named as Weichu 901, Weichu 902-3 and Yuan's 8.

1.2 Sample Inspection and Microscopic Examination

Place rice seed samples in sterilized white porcelain plates according to their numbers, check and select seeds suspected to contain rice kernel smut. Rice kernel smut generally presents three symptoms: the first two types of grains do not change color, with the difference being that the former has a broken outer grain back line and a black powdery substance at the crack; The latter cracked at the inner part, revealing a conical black angular substance, and a black powdery substance adhered to the cracked area; The third symptom is that the granules are dark green or dark yellow in color, the husk does not crack, and there is a black powdery substance inside.

Select 50 g of seed samples with similar symptoms mentioned above, disinfect the surface with alcohol, pour them into a 250 ml sterilized triangular flask, add 100 ml of sterilized water, 1 drop of Tween-20, seal and shake on a shaker for 5 minutes. Then inject the washing suspension into a centrifuge tube, centrifuge at 1000 r/min for 3 minutes, discard the supernatant and leave a precipitate. After collecting enough, suspend in sterile water and take 20 ul of the sample for observation.

The microscopic examination results showed that no rice kernel smut spores were found in the three microscopic samples.

1.3 Molecular detection

Select the above seeds with similar symptoms, grind them with liquid nitrogen, extract DNA with CTAB method, use primer ITS4/ITS5 for PCR amplification and the target band is sequenced by Youkang Biotechnology Co., Ltd. after the product is electrophoresis on agarose gel. The sequences obtained from sequencing were concatenated and subjected to homologous sequence (BLAST) alignment analysis with relevant sequences in the NCBI website GenBank. After sequence alignment, no *Tilletia barclayana* species were found, as shown in the attached figure.

1.4 Result Analysis

Through sample inspection, microscopic examination, and molecular testing, the pathogen *Tilletia barclayana* was not detected in the sample.

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Figure 1 ITS sequence alignment results of Weichu 901 sample

Other reports Distance tree of results MSA viewer 

Descriptions Graphic Summary Alignments Taxonomy

Sequences producing significant alignments Download Select columns Show 100 

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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per Ident	Avg Len	Accession
<i>Aspergillus flavus</i> isolate AB112 internal transcribed spacer_1 partial sequence 5.8S ribosomal RNA gene and inte	<i>Aspergillus flavus</i>	1013	1013	100%	0.0	98.78%	583	MN006417.1
<i>Aspergillus</i> sp. isolate SU-B2 16S ribosomal RNA gene partial sequence	<i>Aspergillus</i> sp.	1013	1013	100%	0.0	98.78%	581	MF348057.1
<i>Aspergillus tenuipes</i> isolate SM-756 small subunit ribosomal RNA gene partial sequence internal transcribed space	<i>Aspergillus tenuipes</i>	1013	1013	100%	0.0	98.77%	605	EP710682.1
<i>Aspergillus flavus</i> isolate AB113 internal transcribed spacer_1 partial sequence 5.8S ribosomal RNA gene and inte	<i>Aspergillus flavus</i>	1013	1013	100%	0.0	98.78%	583	MN006418.1
<i>Aspergillus flavus</i> strain A161 small subunit ribosomal RNA gene partial sequence internal transcribed spacer_1	<i>Aspergillus flavus</i>	1009	1009	100%	0.0	98.60%	577	MN095161.1
<i>Aspergillus flavus</i> strain G1-2 small subunit ribosomal RNA gene partial sequence internal transcribed spacer_1_2	<i>Aspergillus flavus</i>	1009	1009	100%	0.0	98.60%	601	EP037809.1
<i>Aspergillus flavus</i> strain A429 small subunit ribosomal RNA gene partial sequence internal transcribed spacer_1	<i>Aspergillus flavus</i>	1009	1009	100%	0.0	98.60%	574	MN095117.1
<i>Aspergillus flavus</i> strain CMXY2227 internal transcribed spacer_1 partial sequence 5.8S ribosomal RNA gene no	<i>Aspergillus flavus</i>	1009	1009	100%	0.0	98.60%	575	MG591537.1
<i>Aspergillus</i> sp. isolate YZ7-6 small subunit ribosomal RNA gene partial sequence internal transcribed spacer_1_5	<i>Aspergillus</i> sp.	1009	1009	100%	0.0	98.60%	599	MW380819.1
<i>Aspergillus</i> sp. strain LC79 small subunit ribosomal RNA gene partial sequence internal transcribed spacer_1_5_8	<i>Aspergillus</i> sp.	1009	1009	100%	0.0	98.60%	610	KY343754.1
<i>Aspergillus flavus</i> strain CMXY21395 internal transcribed spacer_1 partial sequence 5.8S ribosomal RNA gene no	<i>Aspergillus flavus</i>	1009	1009	100%	0.0	98.60%	577	MG591533.1
<i>Aspergillus flavus</i> strain CMXY24995 small subunit ribosomal RNA gene partial sequence internal transcribed spa	<i>Aspergillus flavus</i>	1009	1009	100%	0.0	98.60%	590	MG591549.1
<i>Aspergillus flavus</i> strain H1-3 small subunit ribosomal RNA gene partial sequence internal transcribed spacer_1_5	<i>Aspergillus flavus</i>	1009	1009	100%	0.0	98.60%	573	MZ613327.1
<i>Aspergillus flavus</i> strain A107P small subunit ribosomal RNA gene partial sequence internal transcribed spacer_1	<i>Aspergillus flavus</i>	1009	1009	100%	0.0	98.60%	574	MN095169.1
<i>Aspergillus nomius</i> strain S1-18S ribosomal RNA gene partial sequence internal transcribed spacer_1 5.8S rRNA	<i>Aspergillus nomius</i>	1009	1009	100%	0.0	98.60%	582	KJ468642.1
<i>Aspergillus flavus</i> strain A46H small subunit ribosomal RNA gene partial sequence internal transcribed spacer_1	<i>Aspergillus flavus</i>	1009	1009	100%	0.0	98.60%	576	MN095147.1
<i>Aspergillus flavus</i> strain A35R small subunit ribosomal RNA gene partial sequence internal transcribed spacer_1	<i>Aspergillus flavus</i>	1009	1009	100%	0.0	98.60%	585	MN095116.1
<i>Aspergillus</i> sp. internal transcribed spacer_1 partial sequence 5.8S ribosomal RNA gene and internal transcri	<i>Aspergillus</i> sp.	1009	1009	98%	0.0	98.11%	579	MH393568.1

Figure 2 ITS sequence alignment results of Weichu 902-3 sample

Descriptions Graphic Summary

Sequences producing significant alignments Download Manage Columns Show 100 

select all 0 sequences selected

Description	Scientific Name	Common Name	Taxid	Max Score	Total Score	Query Cover	E value	Per Ident	Avg Len	Accession
<i>Eusaccharomyces</i> isolate BEE small subunit ribosomal RNA gene partial sequence internal transcri	<i>Eusaccharomyces</i>	NA	61235	876	876	99%	0.0	96.43%	585	PF434615.1
<i>Eusaccharomyces</i> isolate BRRI-Coc-F3 small subunit ribosomal RNA gene partial sequence internal	<i>Eusaccharomyces</i>	NA	61235	874	874	99%	0.0	96.42%	565	MZ025957.1
<i>Eusaccharomyces</i> isolate PT3-2 small subunit ribosomal RNA gene partial sequence internal tr	<i>Eusaccharomyces</i>	NA	2594820	874	874	99%	0.0	96.42%	587	Q5511758.1
<i>Eusaccharomyces</i> isolate U_15 small subunit ribosomal RNA gene partial sequence internal transcri	<i>Eusaccharomyces</i>	NA	61235	874	874	99%	0.0	96.42%	571	MG599552.1
<i>Eusaccharomyces</i> isolate UH-1552-087 small subunit ribosomal RNA gene partial sequence internal tra	<i>Eusaccharomyces</i>	NA	26916	874	874	99%	0.0	96.42%	565	MZ374739.1
<i>Eusaccharomyces</i> isolate Samcole-261 small subunit ribosomal RNA gene partial sequence internal	<i>Eusaccharomyces</i>	NA	61235	874	874	100%	0.0	96.25%	572	Q2421771.1
<i>Eusaccharomyces</i> incarnatum isolate Sample-160 small subunit ribosomal RNA gene partial sequence intern	<i>Eusaccharomyces</i>	NA	295378	874	874	100%	0.0	96.25%	573	Q2422640.1
Fungal endophyte sp. SX08-183 ribosomal RNA gene partial sequence internal transcribed spacer	fungal	NA	569803	874	874	100%	0.0	98.25%	572	EJ378078.1
<i>Eusaccharomyces</i> incarnatum strain Z111 small subunit ribosomal RNA gene partial sequence internal trans	<i>Eusaccharomyces</i>	NA	296378	874	874	99%	0.0	96.42%	553	MT560935.1
<i>Eusaccharomyces</i> isolate RM127 small subunit ribosomal RNA gene partial sequence internal trans	<i>Eusaccharomyces</i>	NA	61235	874	874	99%	0.0	96.42%	558	MZ5664730.1
<i>Eusaccharomyces</i> sp. voucher SY13 small subunit ribosomal RNA gene partial sequence internal trans	<i>Eusaccharomyces</i>	NA	61235	874	874	99%	0.0	96.42%	553	MK990139.1
<i>Eusaccharomyces</i> sp. isolate Ec026 small subunit ribosomal RNA gene partial sequence internal transcribed	<i>Eusaccharomyces</i>	NA	29916	874	874	99%	0.0	96.42%	554	Q2756163.1
<i>Eusaccharomyces</i> sp. isolate F3 small subunit ribosomal RNA gene partial sequence internal transcribed spa	<i>Eusaccharomyces</i>	NA	29916	874	874	99%	0.0	96.42%	574	MT771109.1
<i>Eusaccharomyces</i> sp. isolate Sample-250 small subunit ribosomal RNA gene partial sequence internal	<i>Eusaccharomyces</i>	NA	61235	874	874	100%	0.0	96.25%	572	Q2421780.1
<i>Eusaccharomyces</i> sp. JV2 16S ribosomal RNA gene partial sequence internal transcribed spacer_1 5.8S rRNA	<i>Eusaccharomyces</i>	NA	717922	874	874	99%	0.0	96.42%	570	GU380354.1
<i>Eusaccharomyces</i> incarnatum isolate Sample-222 small subunit ribosomal RNA gene partial sequence intern	<i>Eusaccharomyces</i>	NA	295378	874	874	100%	0.0	96.25%	572	Q2422696.1
<i>Eusaccharomyces</i> incarnatum isolate Sample-200 small subunit ribosomal RNA gene partial sequence intern	<i>Eusaccharomyces</i>	NA	295378	874	874	100%	0.0	96.25%	573	Q2422674.1
<i>Eusaccharomyces</i> incarnatum isolate F238 small subunit ribosomal RNA gene partial sequence internal transcri	<i>Eusaccharomyces</i>	NA	61235	874	874	99%	0.0	96.42%	562	MW9965530.1

Figure 3 ITS sequence alignment results of Yuan's 8 sample

Sequences producing significant alignments									
		Download		Select columns		Show		100	?
<input checked="" type="checkbox"/> select all 100 sequences selected		GenBank		Graphics		Distance tree of results		MSA Viewer	
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per Ident	Len	Acc	Accession
<input checked="" type="checkbox"/> <i>Fusarium equiseti</i> isolate Sample-245 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium equiseti</i>	1053	1053	100%	0.0	99.83%	573	QQ422854.1	
<input checked="" type="checkbox"/> <i>Fusarium equiseti</i> isolate Sample-233 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium equiseti</i>	1053	1053	100%	0.0	99.83%	573	QQ421745.1	
<input checked="" type="checkbox"/> <i>Fusarium sacchari</i> culture collection NCCPF 500033 16S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium sacchari</i>	1046	1046	100%	0.0	99.85%	572	KM921684.1	
<input checked="" type="checkbox"/> <i>Fusarium incarnatum</i> isolate Sample-177 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium incarnatum</i>	1042	1042	100%	0.0	99.48%	574	QQ422651.1	
<input checked="" type="checkbox"/> <i>Fusarium incarnatum</i> isolate Sample-168 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium incarnatum</i>	1042	1042	100%	0.0	99.48%	573	QQ422642.1	
<input checked="" type="checkbox"/> <i>Fusarium equiseti</i> isolate Sample-240 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium equiseti</i>	1042	1042	100%	0.0	99.48%	574	QQ421752.1	
<input checked="" type="checkbox"/> <i>Fusarium incarnatum</i> isolate Sample-225 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium incarnatum</i>	1042	1042	100%	0.0	99.48%	574	QQ422699.1	
<input checked="" type="checkbox"/> <i>Fusarium equiseti</i> isolate Sample-263 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium equiseti</i>	1042	1042	100%	0.0	99.48%	574	QQ421773.1	
<input checked="" type="checkbox"/> <i>Fusarium</i> sp. strain KUMCC 21-0450 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium</i> sp.	1040	1040	100%	0.0	99.48%	571	DN426849.1	
<input checked="" type="checkbox"/> <i>Fusarium incarnatum</i> isolate Sample-185 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium incarnatum</i>	1040	1040	100%	0.0	99.48%	572	QQ422659.1	
<input checked="" type="checkbox"/> <i>Fusarium sulawesiense</i> isolate PT3-1 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium sulawesiense</i>	1040	1040	100%	0.0	99.48%	569	DR311757.1	
<input checked="" type="checkbox"/> <i>Fusarium</i> sp. 1 TMS-2011 voucher SC#d100n16-14 18S rRNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium</i> sp. 1 T	1040	1040	100%	0.0	99.48%	587	HQ631095.1	
<input checked="" type="checkbox"/> <i>Fusarium incarnatum</i> isolate Sample-217 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium incarnatum</i>	1040	1040	100%	0.0	99.48%	573	QQ422691.1	
<input checked="" type="checkbox"/> <i>Fusarium equiseti</i> isolate FUS2940 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium equiseti</i>	1038	1038	99%	0.0	99.47%	586	MH578583.1	
<input checked="" type="checkbox"/> <i>Fusarium</i> sp. isolate ASPSP44 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium</i> sp.	1038	1038	100%	0.0	99.30%	572	EQ249744.1	
<input checked="" type="checkbox"/> <i>Fusarium incarnatum</i> isolate Sample-178 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium incarnatum</i>	1037	1037	100%	0.0	99.30%	573	QQ422652.1	
<input checked="" type="checkbox"/> <i>Fusarium incarnatum</i> isolate Sample-203 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium incarnatum</i>	1037	1037	100%	0.0	99.30%	573	QQ422677.1	
<input checked="" type="checkbox"/> <i>Fusarium incarnatum</i> isolate Sample-205 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium incarnatum</i>	1037	1037	100%	0.0	99.30%	574	QQ422679.1	
<input checked="" type="checkbox"/> <i>Fusarium equiseti</i> isolate Sample-237 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium equiseti</i>	1037	1037	100%	0.0	99.30%	575	QQ421749.1	
<input checked="" type="checkbox"/> <i>Fusarium incarnatum</i> isolate Sample-180 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium incarnatum</i>	1037	1037	100%	0.0	99.30%	574	QQ422654.1	
<input checked="" type="checkbox"/> <i>Fusarium</i> sp. strain AQ16 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S	<i>Fusarium</i> sp.	1037	1037	100%	0.0	99.30%	573	MH084157.1	