

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

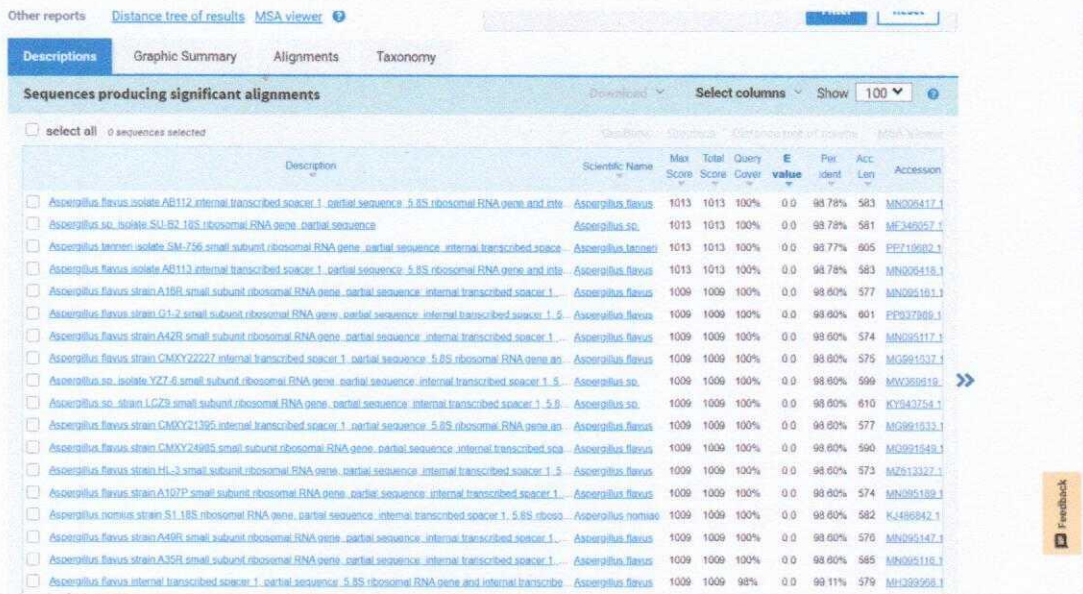


Figure 2 ITS sequence alignment results of Weichu 902-3 sample

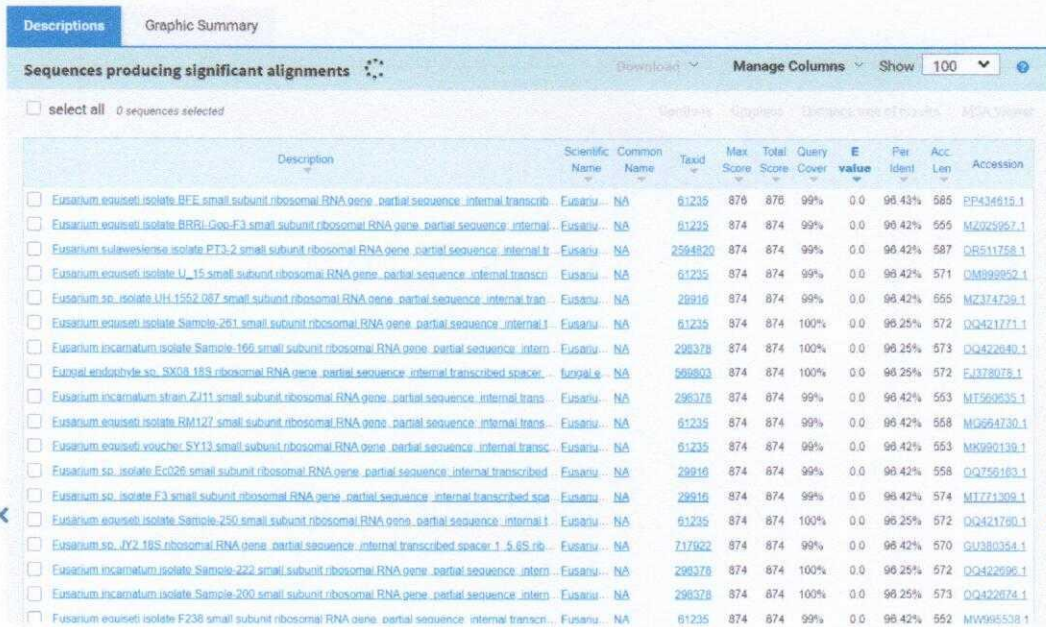


Figure 3 ITS sequence alignment results of Yuan's 8 sample

Sequences producing significant alignments Download Select columns Show 100

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	Acc. Len	Accession
<input checked="" type="checkbox"/> Fusarium equiseti isolate Sample-245 small subunit ribosomal RNA gene, partial sequence, internal transcribed sp...	<i>Fusarium equiseti</i>	1053	1053	100%	0.0	99.83%	573	QQ422854.1
<input checked="" type="checkbox"/> Fusarium equiseti isolate Sample-233 small subunit ribosomal RNA gene, partial sequence, internal transcribed sp...	<i>Fusarium equiseti</i>	1053	1053	100%	0.0	99.83%	573	QQ421745.1
<input checked="" type="checkbox"/> Fusarium sacchari culture collection NCCPF 580033 18S ribosomal RNA gene, partial sequence, internal transcrib...	<i>Fusarium sacchari</i>	1046	1046	100%	0.0	99.05%	572	KM921664.1
<input checked="" type="checkbox"/> Fusarium incarnatum isolate Sample-177 small subunit ribosomal RNA gene, partial sequence, internal transcribed...	<i>Fusarium incarna...</i>	1042	1042	100%	0.0	99.48%	574	QQ422681.1
<input checked="" type="checkbox"/> Fusarium incarnatum isolate Sample-168 small subunit ribosomal RNA gene, partial sequence, internal transcribed...	<i>Fusarium incarna...</i>	1042	1042	100%	0.0	99.48%	573	QQ422842.1
<input checked="" type="checkbox"/> Fusarium equiseti isolate Sample-240 small subunit ribosomal RNA gene, partial sequence, internal transcribed sp...	<i>Fusarium equiseti</i>	1042	1042	100%	0.0	99.48%	574	QQ421752.1
<input checked="" type="checkbox"/> Fusarium incarnatum isolate Sample-225 small subunit ribosomal RNA gene, partial sequence, internal transcribed...	<i>Fusarium incarna...</i>	1042	1042	100%	0.0	99.48%	574	QQ422699.1
<input checked="" type="checkbox"/> Fusarium equiseti isolate Sample-263 small subunit ribosomal RNA gene, partial sequence, internal transcribed sp...	<i>Fusarium equiseti</i>	1042	1042	100%	0.0	99.48%	574	QQ421773.1
<input checked="" type="checkbox"/> Fusarium sp. strain KUMCC 21-0450 small subunit ribosomal RNA gene, partial sequence, internal transcribed spa...	<i>Fusarium sp.</i>	1040	1040	100%	0.0	99.48%	571	QN426849.1
<input checked="" type="checkbox"/> Fusarium incarnatum isolate Sample-185 small subunit ribosomal RNA gene, partial sequence, internal transcribed...	<i>Fusarium incarna...</i>	1040	1040	100%	0.0	99.48%	572	QQ422659.1
<input checked="" type="checkbox"/> Fusarium sulawesiense isolate PT3-1 small subunit ribosomal RNA gene, partial sequence, internal transcribed sp...	<i>Fusarium sulawe...</i>	1040	1040	100%	0.0	99.48%	589	QR511757.1
<input checked="" type="checkbox"/> Fusarium sp. 1 TMS-2011 voucher SC28d100p16-14 18S ribosomal RNA gene, partial sequence, internal transcribe...	<i>Fusarium sp. 1 T...</i>	1040	1040	100%	0.0	99.48%	587	HQ631005.1
<input checked="" type="checkbox"/> Fusarium incarnatum isolate Sample-217 small subunit ribosomal RNA gene, partial sequence, internal transcribed...	<i>Fusarium incarna...</i>	1040	1040	100%	0.0	99.48%	573	QQ422691.1
<input checked="" type="checkbox"/> Fusarium equiseti isolate FUS2940 small subunit ribosomal RNA gene, partial sequence, internal transcribed spac...	<i>Fusarium equiseti</i>	1038	1038	99%	0.0	99.47%	586	MH578583.1
<input checked="" type="checkbox"/> Fusarium sp. isolate ASPSP44 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1...	<i>Fusarium sp.</i>	1038	1038	100%	0.0	99.30%	572	PQ248744.1
<input checked="" type="checkbox"/> Fusarium incarnatum isolate Sample-178 small subunit ribosomal RNA gene, partial sequence, internal transcribed...	<i>Fusarium incarna...</i>	1037	1037	100%	0.0	99.30%	573	QQ422652.1
<input checked="" type="checkbox"/> Fusarium incarnatum isolate Sample-203 small subunit ribosomal RNA gene, partial sequence, internal transcribed...	<i>Fusarium incarna...</i>	1037	1037	100%	0.0	99.30%	573	QQ422677.1
<input checked="" type="checkbox"/> Fusarium incarnatum isolate Sample-205 small subunit ribosomal RNA gene, partial sequence, internal transcribed...	<i>Fusarium incarna...</i>	1037	1037	100%	0.0	99.30%	574	QQ422679.1
<input checked="" type="checkbox"/> Fusarium equiseti isolate Sample-237 small subunit ribosomal RNA gene, partial sequence, internal transcribed sp...	<i>Fusarium equiseti</i>	1037	1037	100%	0.0	99.30%	575	QQ421749.1
<input checked="" type="checkbox"/> Fusarium incarnatum isolate Sample-180 small subunit ribosomal RNA gene, partial sequence, internal transcribed...	<i>Fusarium incarna...</i>	1037	1037	100%	0.0	99.30%	574	QQ422654.1
<input checked="" type="checkbox"/> Fusarium sp. strain AQ16 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S...	<i>Fusarium sp.</i>	1037	1037	100%	0.0	99.30%	573	MH084157.1