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Isolation and Identification of Disease Pathogens in Plant Root

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1 The fresh tissure was washed and sterilized by 75% ethanol. Its interior milky tissue was sliced into 0.5 cm2 pieces. Then square blockages were incubated on the PDA medium (potato 200 g, glucose 30 g, agar 5 g, MgSO4 1 g, KH2PO4 5 g) with 32 ℃ condition. When the hyphal grew to about 2-3 cm, they were purified 3 times.

2 For identifying the isolated pathogens from the root tissues, the ITS barcode PCR and sequencing test were done. The pathogen DNA was extracted by E5038-1KT plant fungal DNA Extraction Kit. The ITS sequence amplified primers were (ITSlF) 5’-CTT GGT CAT TTA GAG GAA GTA A-3’ and (ITS4B) 5’-CAG GAG ACT TGT ACA CGG TCC AG-3’. The PCR reaction systems contained PCR Mix (SsoFast TM Eva Green® Superemix, Bio-Rad, USA) 25 μl, ITS1F 2 μl, ITS4B 2 μl, gDNA 1 μl and ddH2O 20 μl. And the reaction procedure was 94 ℃ 3 min ，35 cycles (94 ℃ 40 sec, 55 ℃ 40 sec, 72 ℃ 90 sec) and 72 ℃ 5 min. Then sequencing was finished. After ITS sequence alignment in NCBI database, the pathogens could be identified with homology ＞ 90%.