

Supplementary Table 1. Strains used in this study

No.	Species	Host	Provenance
1	<i>Phytophthora nicotianae</i>	Tobacco	Henan, China
2	<i>Thielaviopsis basicola</i>	Tobacco	Henan, China
3	<i>Fusarium oxysporum</i>	Tobacco	Henan, China
4	<i>Alternaria alternata</i>	Tobacco	Henan, China
5	<i>Botrytis cinerea</i>	Tobacco	Henan, China
6	<i>Pseudomonas syringae</i> pv. <i>angula</i>	Tobacco	Henan, China
7	<i>Cercospora nicotianae</i>	Tobacco	Henan, China
8	<i>Peronophythora litchii</i>	Litchi	Hainan, China
9	<i>Rhizoctonia solani</i>	Corn	Henan, China

Supplementary Table 2. Gene sequences for the primer design of *P. nicotianae*

Gene	Sequence	Length (bp)	Accession Number in NCBI
<i>Hgd</i>	CGAGTGCTGCTTACGAAGACCGCGACTGCGAATACTTGGTCGTCAA- CAAAGTATGCTCTTTTCCATTATTTTCATCGTGGAAAAATGACTGAGTCTCA- CAATCGTTTTTTTTTTTTTTCAGTTTGGTGGCCAACTGTTCTCCGCTCGTATGG- CCTTCTCTCCGTTCAACGTCGTGGCTTGGCATGGAACTACGTGCCGTATA- AATACAATCTGGACAAATTCTGCACGATAAACTCAGTGAGCTTCGAC- CACCCGGTAAGAAGAATTGCAAACTATTA TCCCATCCCAAAGTATTTTGGCT- AACAAACTTTAAATATTTTCAGGATCCTTCGATCTACTGTGTCTCACGTGC- CAAACGGAGGAAGCAGGCAACGC	375	**
<i>rDNA-ITS</i>	TCCGTAGGTGAACCTGCGGAAGGA TCATTACCACACCTAAAAAACT TTC- CACGTGAACCGTTTCAACCCAATAGTTGGGGGCTTATTTGGCGGCGGCT- GCTGGCTTAATTGTTGGCGGCTGCTGCTGAGTGAGCCCTATCAAAAAAAG- GCGAACGTTTGGGCTTCGGCCTGATTTAGTAGTCTTTTTTTCTTTTA- AACCCATTCTTAATACTG AATATACTGTGGGGACGAAAGT CTCTGCTTT- TAAGTAGATAGCAACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGAT- GAAGAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGATTCAGT- GAGTCATCGAAATTTTGAACGCATATTGCACTTCCGGGTTAGTCCTGGAAG- TATGCCTGTATCAGTGTCCGTACATTAAACTTGACTTTCTTCTTCCGTG- TAGTCGGTGGAGGAGATGTGAGATGTGAAGTGTCTTGGCATTGGTCTTCG- GATCGGCTGCGAGTCCTTTTAAATGTAATAAACTGAACTTCTCTTTGCTC- GAAAAGTGGTGGCGTTGCTGGTTGTGAAGGCTGCTATTGTGGCAAATTG- GCGACTGGTTTGTCTGCTGCGGCGTTAATGGAAGAGTGTTCGATTTCGTG- GTATGGTTGGCTTCGGCTGAACAATGCACTTATTGGACGTTTTTCTGCT- GTGGCGTGATGGACTGGTGAACCATAGCTCGGTGGCTTGGCTTTTGAATTG- GCTTTGCTGTTGCGAAGTAGGGTGGCAGCTTCGGTTGTGCGAGGGTCGATC- CATTTGGAACTTAATGTGTA CTTCGGTATGCATCTCAATTGGACCTGATAT- CAGGCAAGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA	892	KJ494903.1

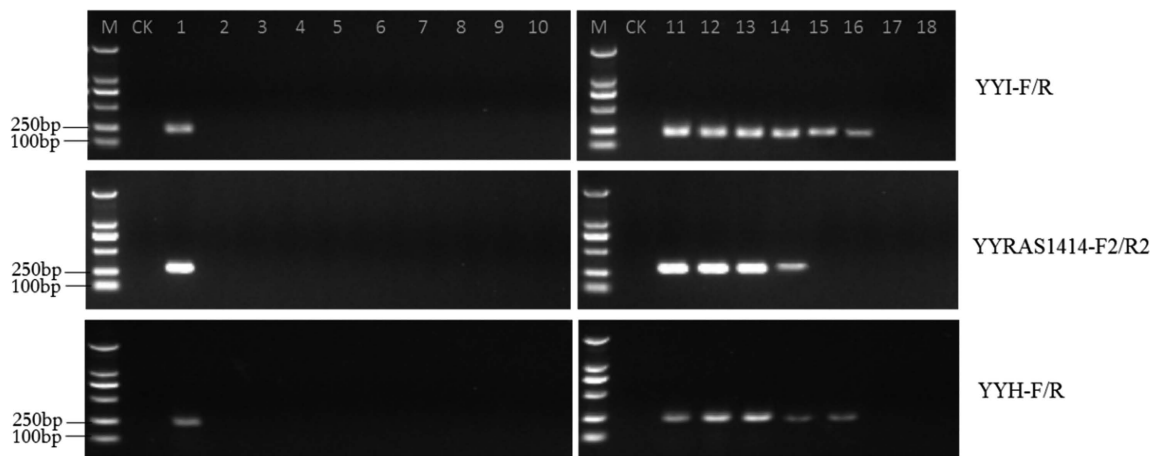
Ras	GATTGAAGGCTGGATCGACTGACGCTCGTATCTTGTGTGCAGTGAC-TACCTCTTTAAGCTGCTGCTGATCGGTGACTCTGGCGTCGGCAAGTCGT-GTCTGCTGCTTCGTTTCGCCGACGACACTTACACGGAGAGCTACATCTC-GACCATTGGTGTGGACTTTGTAAGTGCCACCATACTTAGATCCAG-GTTTTTCATCAGGATTGCTGACATGATATCAACTGTTCTGCAGAAAATC-CGTACGATCGAGCTGGACGGCAAGACCATCAAGCTCCAGATTGTAC-GTCTTTCAATAGACTTGTATCACTGCAGTTTTTTTTTCTATTCAACTAAC-CGATGTACCAAATCACGTGTGTGTCTGTAGTGGGACACGGCCGGACAG-GAGCGTTTCCGCACGATCACTAGCAGTTACTACCGCGGTGCACACG-GCATTATTGTGGTGTACGACGTGACGGACCAGGAGTCATTCAACAATGT-GAAACAGTGGCTGCATGAGATCGATAGGTACGTCCGACTTATCGAGA-GAAGATCCAAGGAAAAGAGCTGAGTGATATTCCACTGATTTACAGATAC-GCCTGTGAGAATGTGAACAAGCTGCTGGTCGGTAACAAGAGCGATCT-GACTGCCAAGCGCGTCTGAGCACTGACGCCGCCAAGGAGTTCGCC-GAAAGCCTGGGCATTGAATTCCTGGAGACCAGTGCAAAGAACGCTGC-CAATGTGCGAGAAGGCCCTTCATGATGATGGCCGCCAGATCAAGAAGCG-CATGGCCAACGCTCCCGTGGCCCCAAGGCCGCGTTAAGCTGACGCCTG-GTCAGCAGGTTCCGTCCAATGGCGGGTCAAGTGC	844	KJ567642.1
-----	--	-----	------------

**This sequence has been submitted to NCBI by our lab (Zheng et al. 2018). Red color indicates forward primers. Blue color indicates reverse primers.

Supplementary Table 3. Gene sequences for the primer design of *T. basicola*

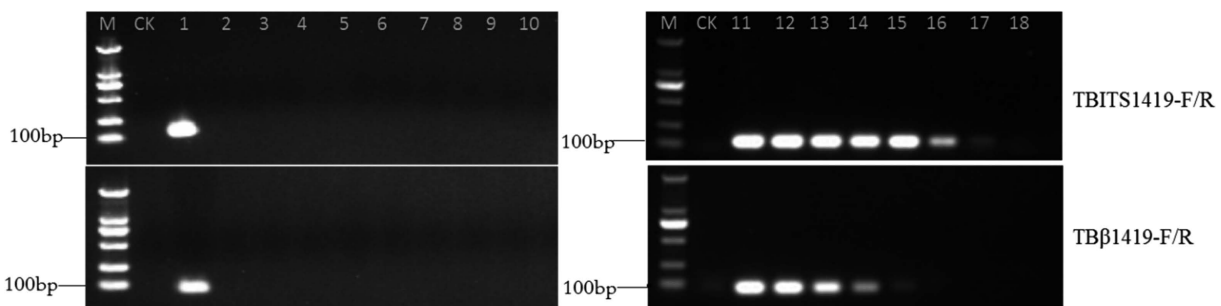
Gene	Sequence	Length (bp)	Accession Number in NCBI
rDNA-ITS	TCCGTAGGTGAACCTGCGGAGGGATCATTATCGAGTTTTTAACCTTTA-AACCATATGTGAACGTACCTTTTCTAGCTGCTTTGGCAGGTGCCTCTC-GGGGCTTCTGCCGGTAGCATTATAAACTCTTTATATTTCTATAGAAT-TATTCATTGCTGAGTGGCATTAACTAAATAAGTTAAAACCTTTCAACAACG-GATCTCTTGGCTTAGCATCGATGAAGAACGCAGCGAAATGCGATAAG-TAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATT-GCGCCTGGCAGTATTCTGCCAGGCATGCCTGTCCGAGCGTCATTTCAC-CACTCAAGCTCTGCTTGGTGTGGAGGACCCGCGTTAGTCGCGGGC-CGCCGAAATGCATCGGCTGTTGTATATACAGCTTCCCTGTGTAGTAAAT-GCTTAGCTTTACACTTTGAACTTTTATATAACATGCCGAAAAACCCT-CAACTTTTGAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACT-TAAGCATATCAATAAGCGGAGGA	556	KT696597.1
<i>β-tubulin</i>	TGAACTCCATCTCGTCCATACCCTCACCAGTGACCAATG-CAAGAAAGCCTTGCGACGGAACATGGCAGTGAAGTCTCGCCAATGC-GCTTGAACAGCTCCTGGGTGGCGGTCGAGTTACCGACAAAGGTCGACGA-CATCTTGAGGCCACGGGGAGGAATTGAGCACAGGGCGGTCTGGACGTT-GTTGGGGATCCACTCGACGAAGTACGACGAGTTCTTGTCTGAACGTT-GCGCATCTGGTCTCAACCTCCTTCATGGCAACCTTGCCACGGCTGTTGT-GTAGAAAAGAGACGGGTTAGCAATGTGTGTTTCGGGATAAGCGGATTGG-TAGGGCAGTACGTACAAGATGGCAGAGCAAGTCAGGTAGCGGCCGTTGC-GGAAATCAGAGGCAGCCATCATGTTCTTGGGGTCAACATCTGCTGGGT-GAGCTCAGGAACACTGACAGCGCGGAAGGAGTGAGCACCGC	469	**

**This sequence has been submitted to NCBI by our lab (Zheng et al. 2018). Red color indicates forward primers. Blue color indicates reverse primers.



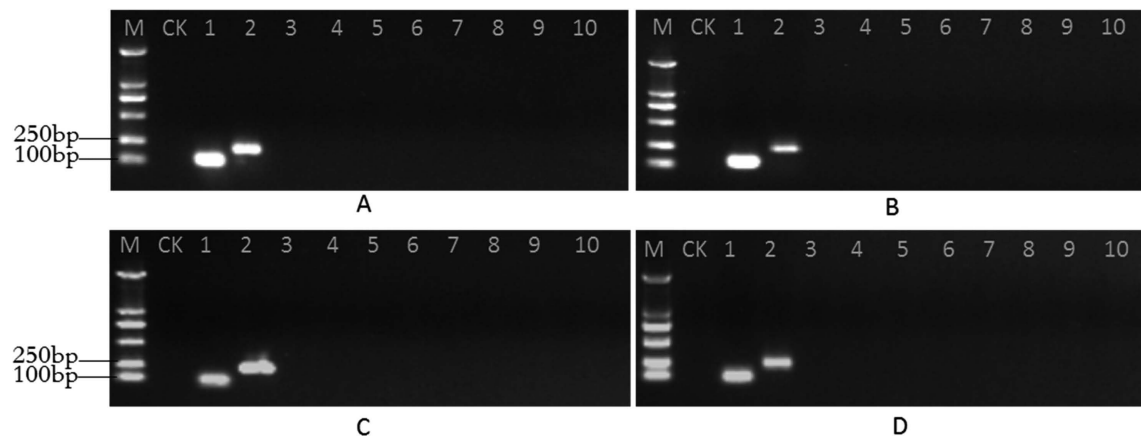
Supplementary Fig. 1. Identification of primer specificity and sensitivity for *P. nicotianae*.

1: *P. nicotianae*; 2: *T. basicola*; 3: *F. oxysporum*; 4: *A. alternate*; 5: *B. cinerea*; 6: *N. tabacum L* (Healthy plant DNA); 7: *P. syringae* pv. *angula*; 8: *C. nicotianae*; 9: *P. litchi*; 10: *R. solani*; 11-18: a serial dilution of total DNA (100 ng/ μ l) for *P. nicotianae* as 1, 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7} fold change, respectively; M: DM2000 DNA Marker; CK: control (distilled water).



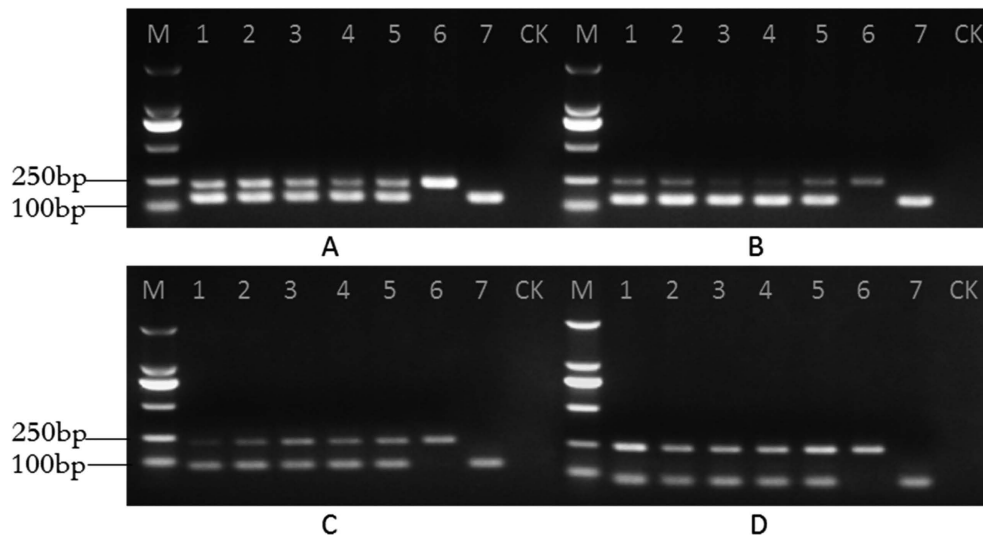
Supplementary Fig. 2. Identification of primer specificity and sensitivity for *T. basicola*.

1: *T. basicola*; 2: *P. nicotianae*; 3: *F. oxysporum*; 4: *A. alternate*; 5: *B. cinerea*; 6: *N. tabacum L* (Healthy plant DNA); 7: *P. syringae* pv. *angula*; 8: *C. nicotianae*; 9: *P. litchi*; 10: *R. solani*; 11-18: a serial dilution of total DNA (104 ng/ μ l) for *T. basicola* as 1, 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7} fold change, respectively; M: DM2000 DNA Marker; CK: control (distilled water).



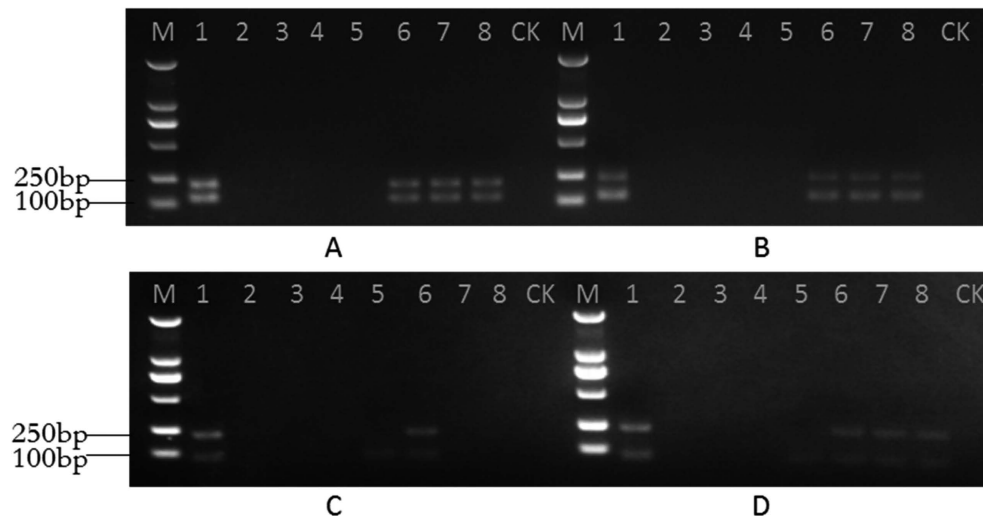
Supplementary Fig. 3. Specificity detection of duplex PCR for *P. nicotianae* and *T. basicola*.

(A, B): Duplex PCR for primer pair TBITS1419-F/TBITS1419-R of *T. basicola* combined with YYI-F/YYI-R and YYH-F/YYH-R of *P. nicotianae*. (C, D) Duplex PCR for primer TBβ1419-F/TBβ1419-R of *T. basicola* combined with YYI-F/YYI-R and YYH-F/YYH-R of *P. nicotianae*. 1: *T. basicola*; 2: *P. nicotianae*; 3: *F. oxysporum*; 4: *A. alternate*; 5: *B. cinerea*; 6: *N. tabacum L* (Healthy plant DNA); 7: *P. angula*; 8: *C. nicotianae*; 9: *P. litchi*; 10: *R. solani*; M: DM2000 DNA Marker; CK: control (distilled water).



Supplementary Fig. 4. Resolution of duplex PCR with different template ratios of *T. basicola* and *P. nicotiana* at the given PCR protocol.

A, B) Duplex PCR for primer TBITS1419-F/TBITS1419-R of *T. basicola* combined with YYI-F/YYI-R and YYH-F/YYH-R of *P. nicotiana*. (C, D) Duplex PCR for primer TB β 1419-F/TB β 1419-R of *T. basicola* combined with YYI-F/YYI-R and YYH-F/YYH-R of *P. nicotiana*. 1-7: DNA template ratios of *P. nicotiana* and *T. basicola* were 0.2 μ l:0.8 μ l, 0.4 μ l:0.6 μ l, 0.5 μ l:0.5 μ l, 0.6 μ l:0.4 μ l, 0.8 μ l:0.2 μ l, 1.0 μ l:0 μ l and 0 μ l:1.0 μ l; M: DM2000 DNA Marker; CK: control (distilled water). Original DNA concentrations of *P. nicotiana* and *T. basicola* were 100 ng/ μ l and 104 ng/ μ l, respectively.



Supplementary Fig. 5. Duplex PCR Detection of infected tobacco tissue.

(A, B) Duplex PCR for primer TBITS1419-F/TBITS1419-R of *T. basicola* combined with YYI-F/YYI-R and YYH-F/YYH-R of *P. nicotiana*. (C, D) Duplex PCR for primer TB β 1419-F/TB β 1419-R of *T. basicola* combined with YYI-F/YYI-R and YYH-F/YYH-R of *P. nicotiana*. 1-8: DNA mixture of *P. nicotiana* and *T. basicola*, soil DNA 1 (50 ng/ μ l), soil DNA 2 (50 ng/ μ l), soil DNA 3 (50 ng/ μ l), NC297 (no diseased symptom observed, 100 ng/ μ l), VL297 (diseased symptom observed, 100 ng/ μ l), 005 (diseased symptom observed, 100 ng/ μ l), XL003 (no diseased symptom observed, 100 ng/ μ l); M: DM2000 DNA Marker; CK: control (distilled water).