Development and validation of PCR tests to diagnose the new Trichoderma ear rot

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Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

in cooperation with

GEORG-AUGUST-UNIVERSITÄT GÖTTINGEN

Mais Tricho

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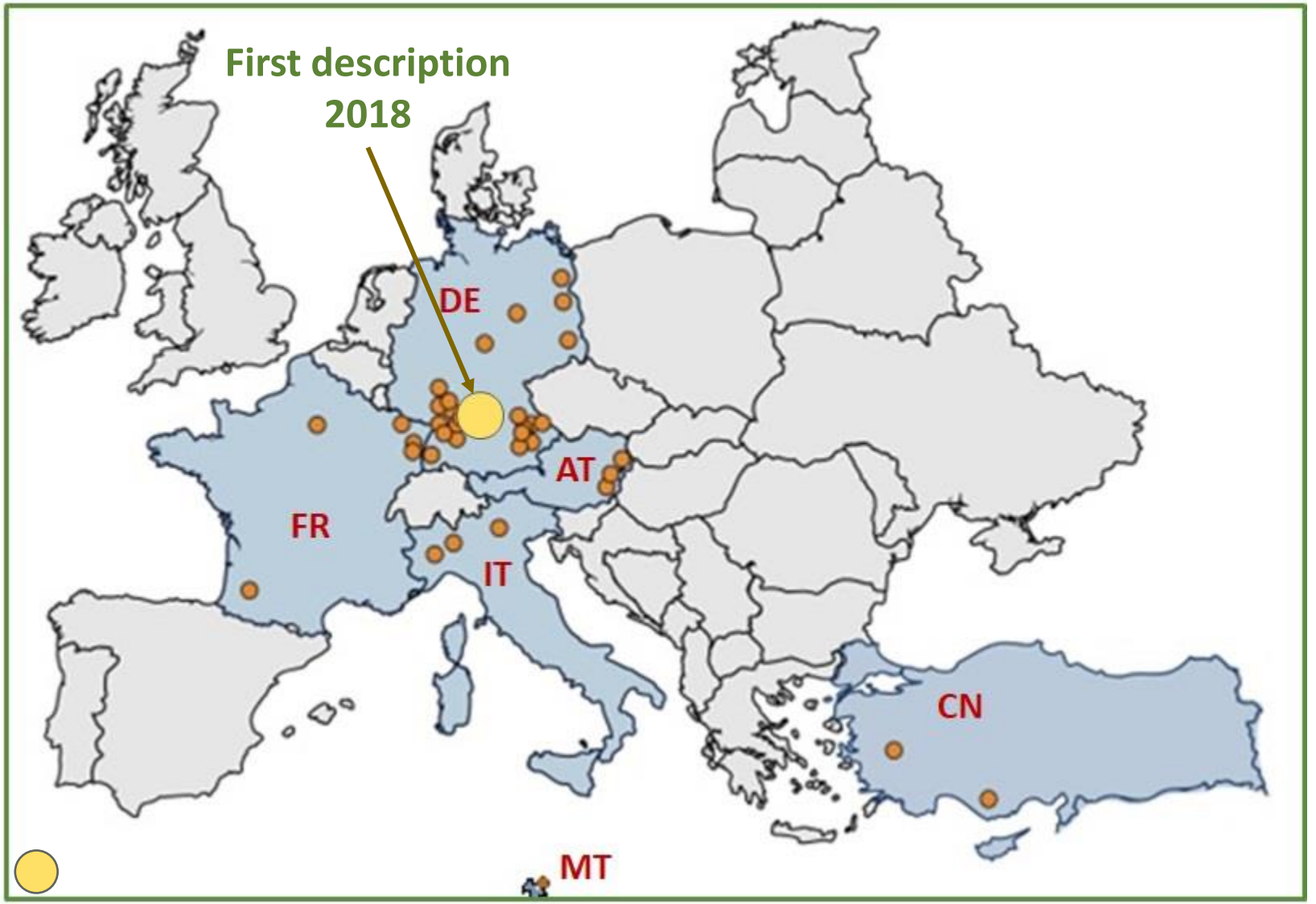
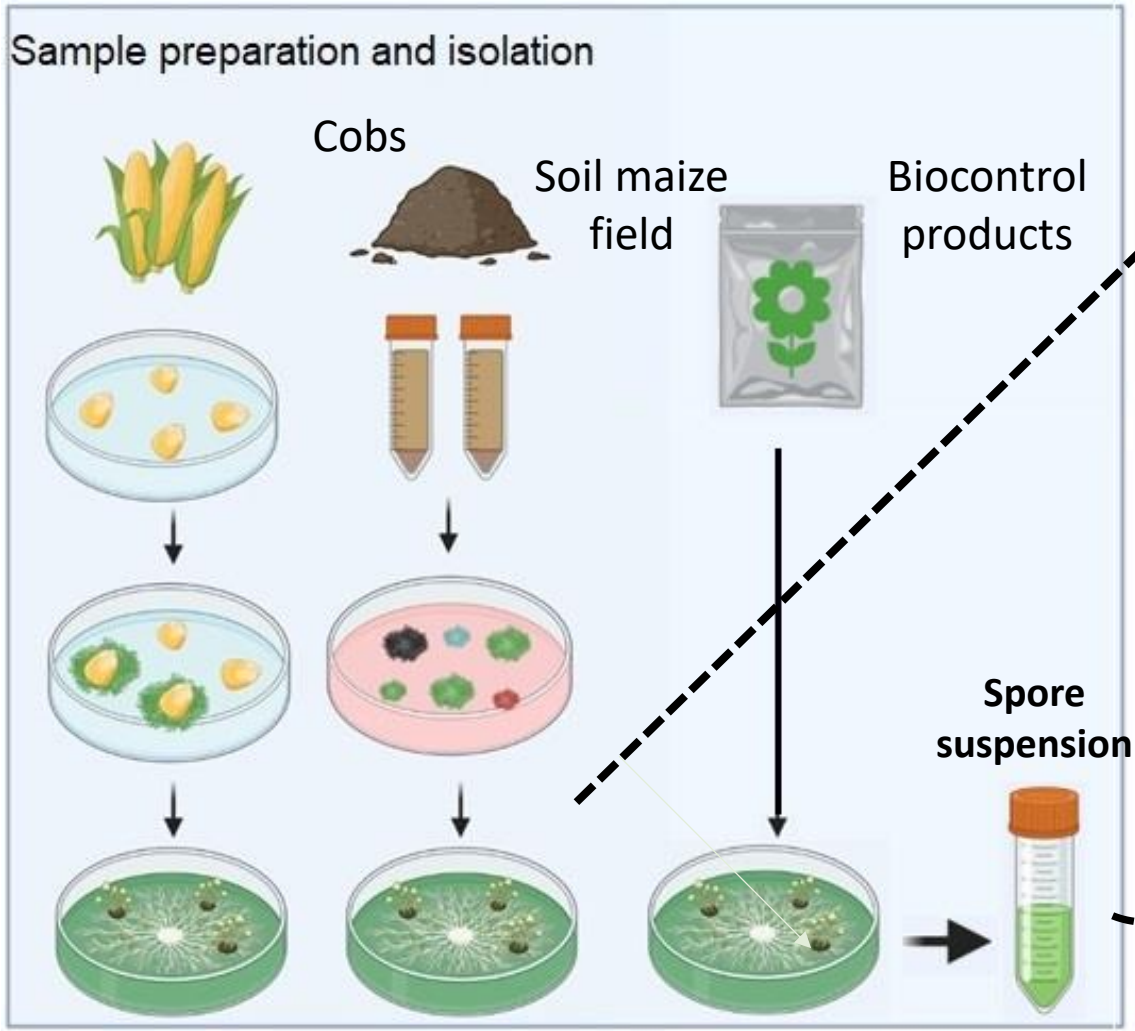
Symptome

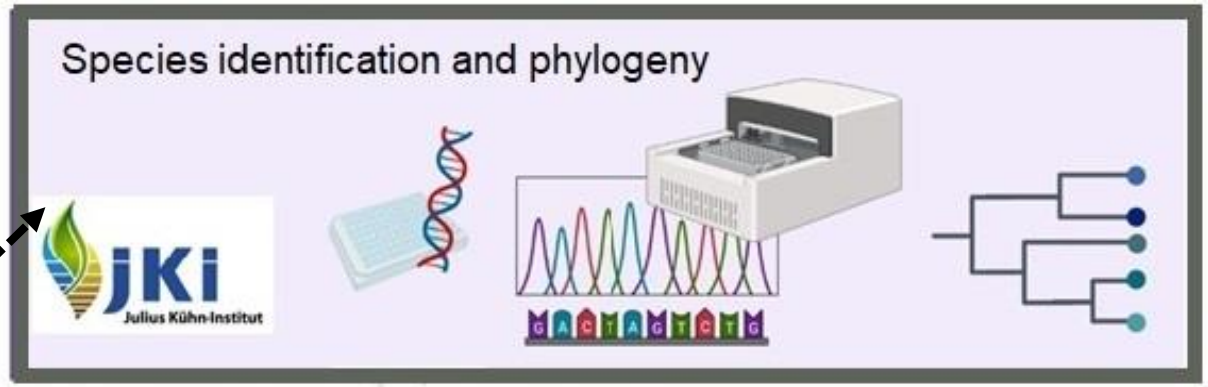
Known distribution

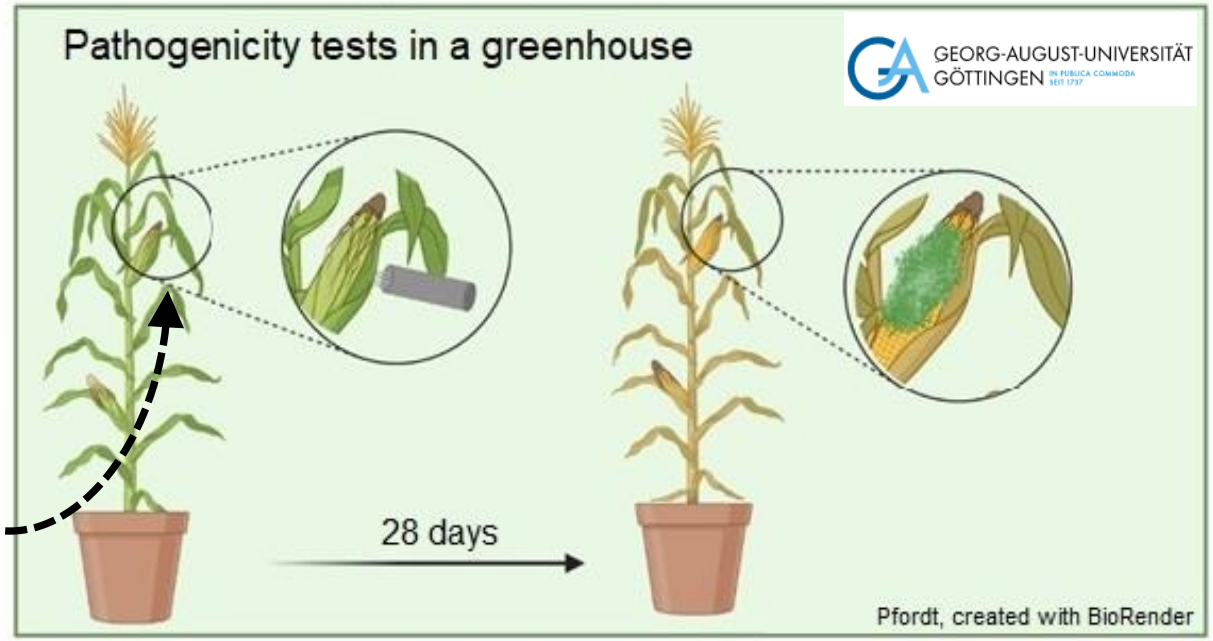


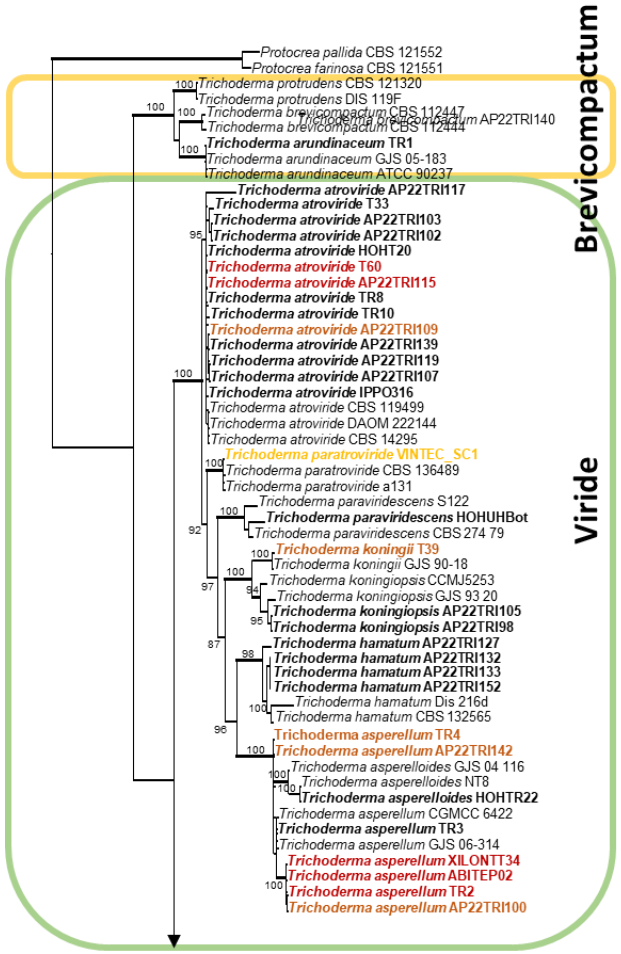
Objectives

. Distribution
. Epidemiology/ Mycotoxins
. Detection



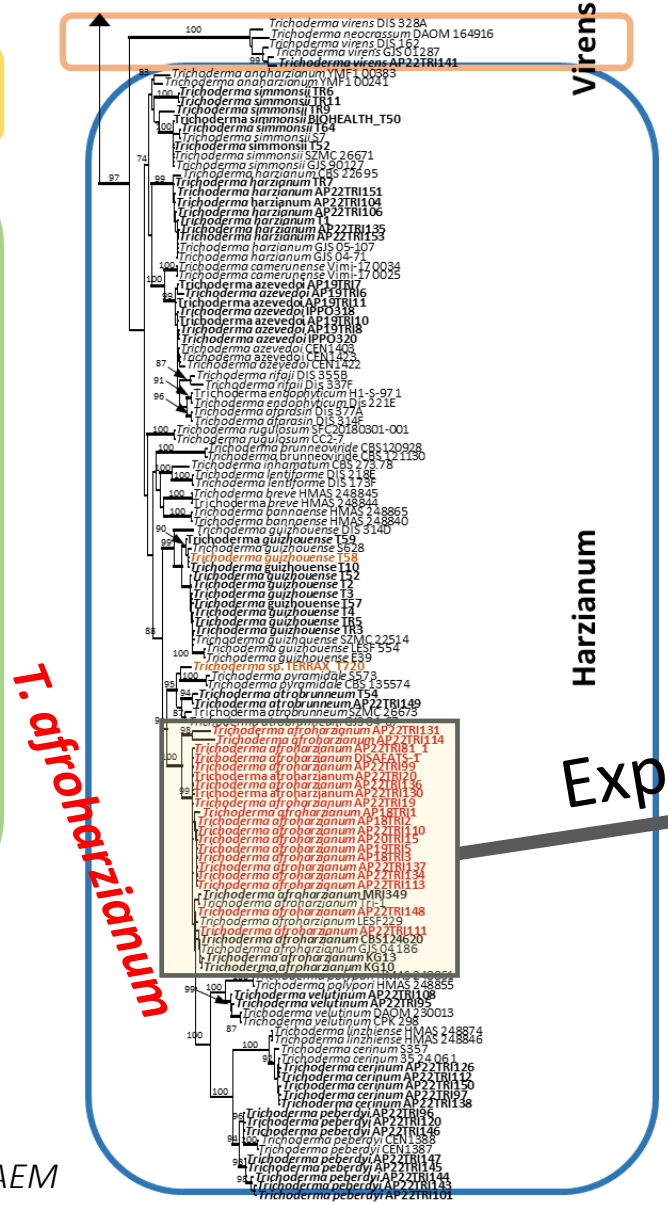






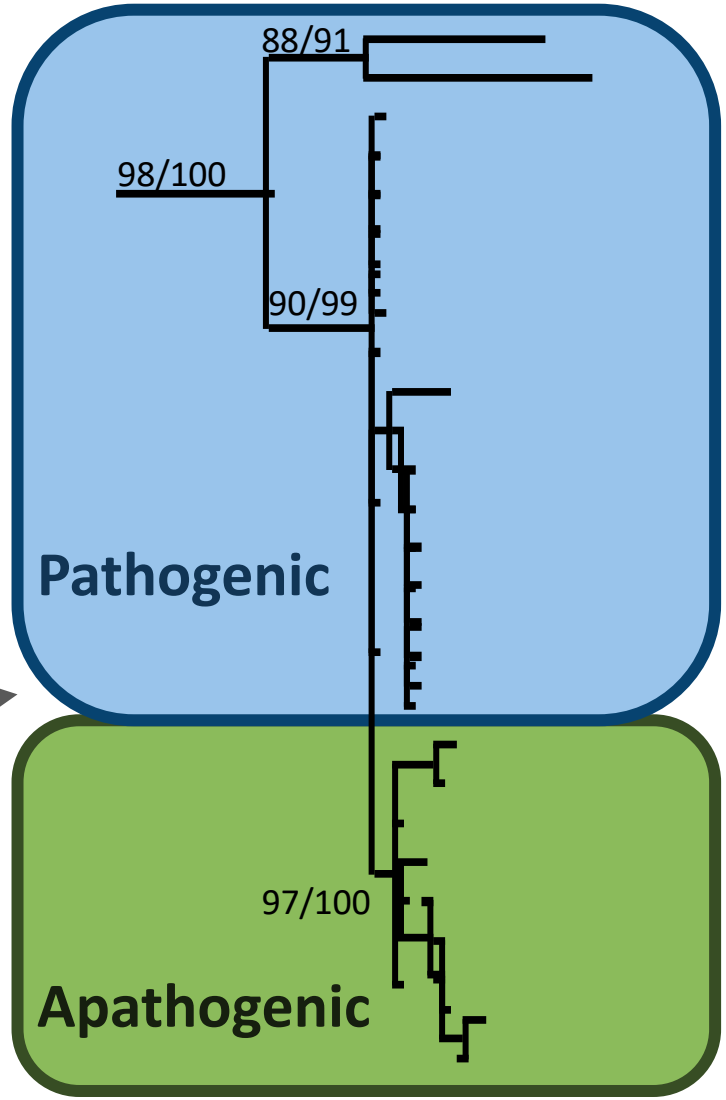
ITS+TEF1+RPB2 Phylogeny

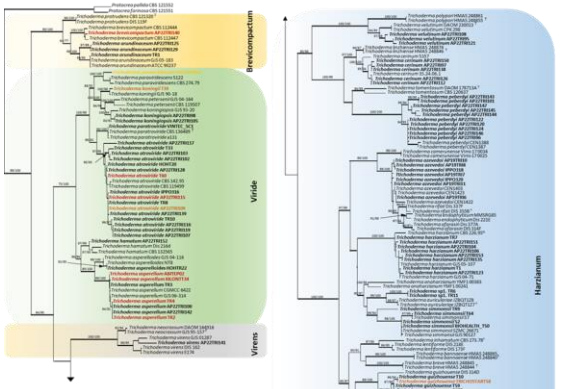
Pfordt et al., 2025; AEM



Expected

Not all *T. afroharzianum* are pathogenic





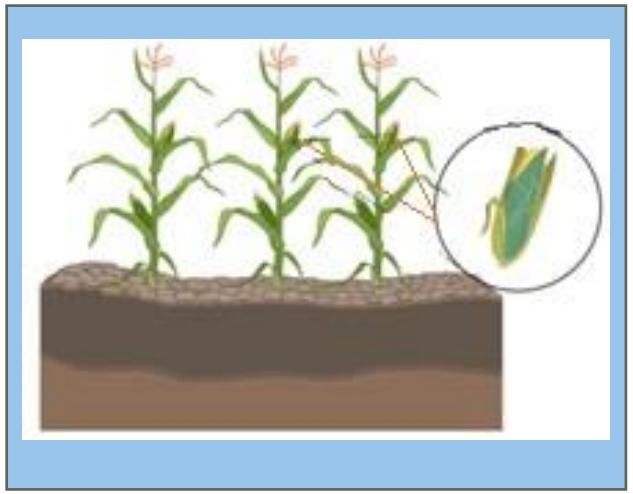
ITS+TEF1+RPB2
Phylogeny

All *T. afroharzianum* isolates from maize and maize fields are pathogenic

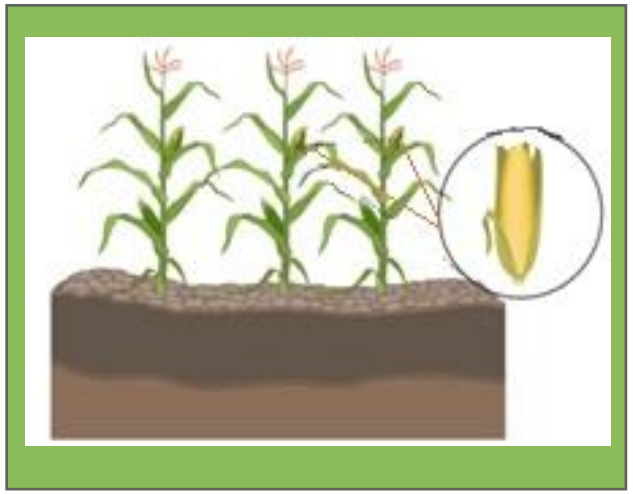
***T. afroharzianum* clade observed**

	Source/Host
88/91 — *Trichoderma afroharzianum* AP22TRI131	Soil maize field
Trichoderma afroharzianum AP22TRI114	Soil maize field
Trichoderma afroharzianum AP22TRI81_1	Symptomatic cobs
98/100 — *Trichoderma afroharzianum* DISAFATS-1	Symptomatic cobs
Trichoderma afroharzianum AP22TRI99	Soil maize field
Trichoderma afroharzianum AP22TRI20	Symptomatic cobs
Trichoderma afroharzianum AP22TRI136	Soil maize field
90/99 — Trichoderma afroharzianum AP22TRI130	Soil maize field
Trichoderma afroharzianum AP22TRI19	Symptomatic cobs
Trichoderma afroharzianum AP18TRI1	Symptomatic cobs
Trichoderma afroharzianum AP18TRI2	Symptomatic cobs
Trichoderma afroharzianum AP22TRI110	Soil maize field
Trichoderma afroharzianum AP20TRI15	Symptomatic cobs
Trichoderma afroharzianum AP19TRI5	Symptomatic cobs
Trichoderma afroharzianum AP18TRI3	Symptomatic cobs
Trichoderma afroharzianum AP22TRI137	Soil maize field
Trichoderma afroharzianum AP22TRI134	Soil maize field
Trichoderma afroharzianum AP22TRI113	Soil maize field
Trichoderma afroharzianum MRI349	Biostimulant
Trichoderma afroharzianum Tri-1	-
Trichoderma afroharzianum AP22TRI148	Symptomatic cobs
Trichoderma afroharzianum LESF229	Soil *Atta bisphaerica*
97/100 — *Trichoderma afroharzianum* AP22TRI111	Soil maize field
Trichoderma afroharzianum CBS124620	*Theobroma cacao*
Trichoderma afroharzianum GJS 04 186	*Moniliophthora roreri*
Trichoderma afroharzianum KG13	*Pleurotus ostreatus* substrate
Trichoderma afroharzianum KG10	*P. ostreatus* substrate

Pathogenic



Apathogenic



PCR tests

Primer name	Sequence (5‘ to 3‘)		Locus	Product size (bp)
TrafTef1c-F	TTCAGCGACGCTAACCACTT	**cPCR**	TEF1α	**217**
TrafTef1c-R	TGTTAGCACTGGTCCGCAAT		TEF1α	
TrafRpb2q-F	GAGGAGACGGCCATGATCTG	**qPCR**	RPB2	**138**
TrafRpb2q-R	GTGAGTTGTCGGGTTCGTCT		RPB2	
TrafRpb2q-P	Fam -CGTCTTCAGAAGGCCGGTAT- BHQ1		RPB2	

Validation (EPPO, PM 7/98)

- Analytical specificity
- Analytical sensitivity
- Repeatability
- Reproducibility
- Robustness
- Transferability

StepOnePlus qPCR (Applied Biosystems)

Biometra thermal cycler (Analytic, Jena)

PCR reaction conditions

- Initial denaturation at 94°C for 3 min,
- 35 cycles of 45 s at 94°C, 45 s annealing with a gradient run from 56°C to 64°C and 1 min at 72°C
- final extension of 7 minutes at 72°C

- Initial denaturation at 95°C for 2 min
- 40 cycles of at 95°C for 15 and annealing with a gradient run from 60°C to 66°C for 1 min

Matrices:

- **Culture**
- **Maize kernels**
- Soil

37 isolates *T. afroharzianum*

100% | 100%

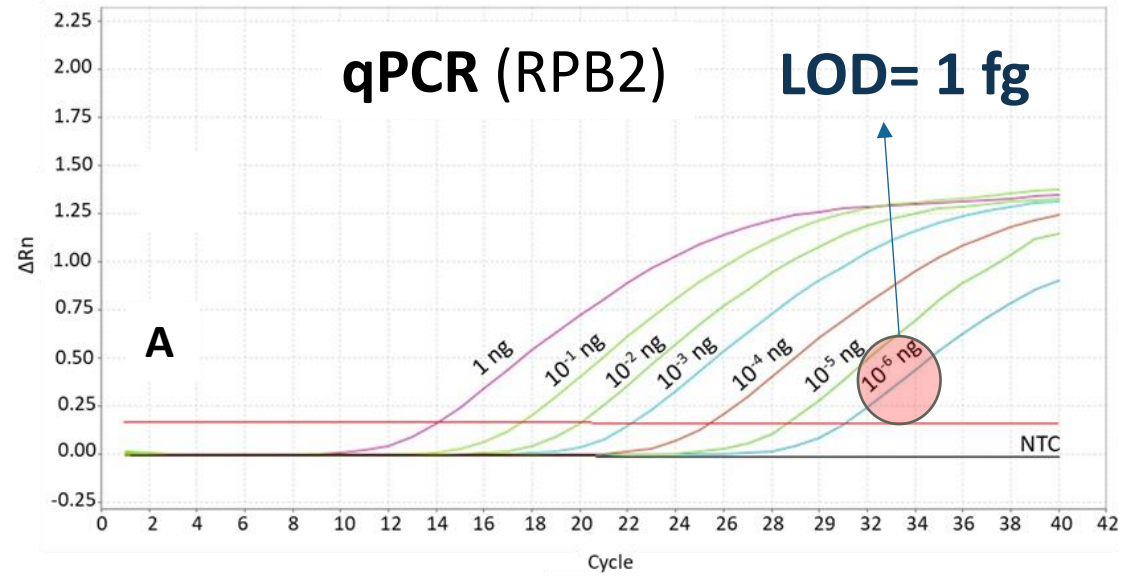
Species	Source/Host	Location	qPCR	cPCR
			+	+
13 Isolates[P]	Symptomatic corncobs	Germany	+	+
1 Isolate[P]	Symptomatic corncobs	France	+	+
1 Isolate[P]	Symptomatic corncobs	Italy	+	+
5 Isolate[P]	Corncobs	Germany	+	+
13 Isolate[P]	Soil maize field	Germany	+	+
1 Isolate[NP]	Biostimulant	-	+	+
1 Isolate[NP]	*Marchantia polymorpha*	-	+	+
1 Isolate[NP]	*M. polymorpha*	-	+	+
1 Isolate[NP]	*Theobroma cacao*	Peru	+	+
1 Isolate[NP]	*P. ostreatus* substrate	-	+	+

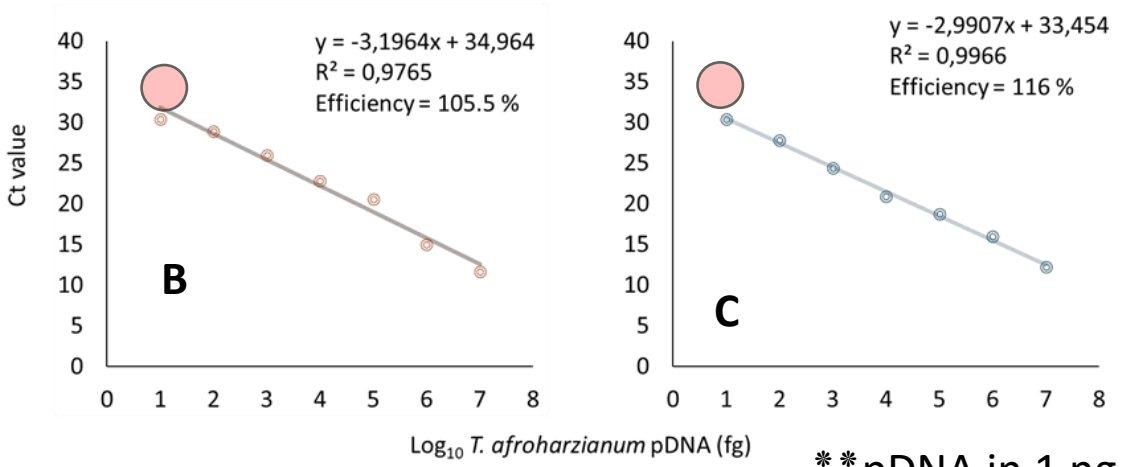
Inclusivity

51 isolates of non-target species

N-1/N% | 100%

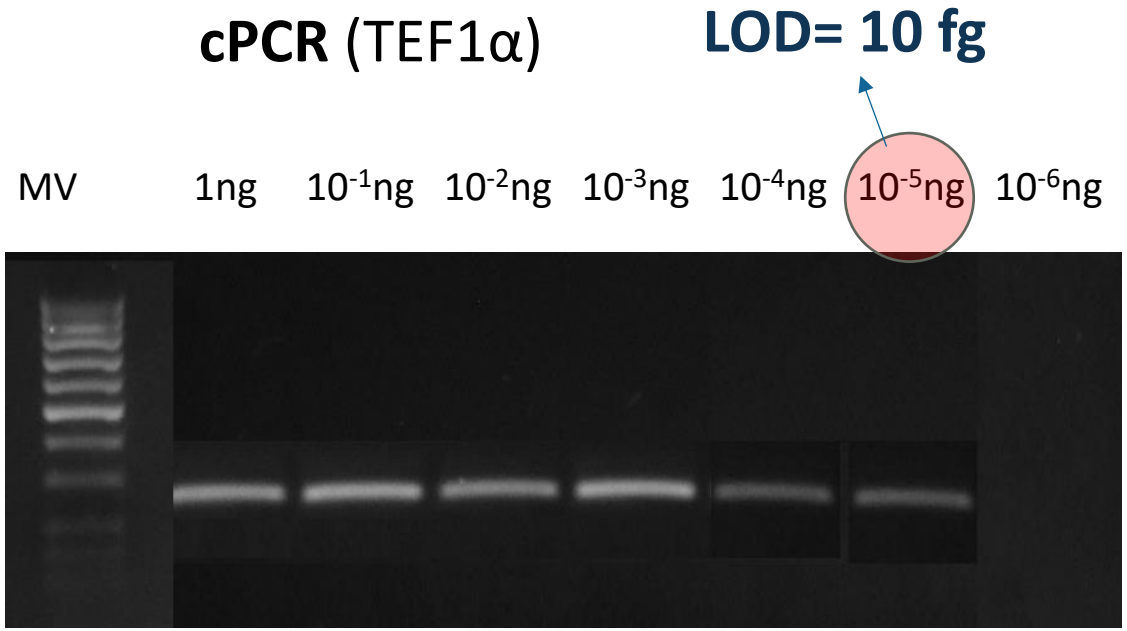
Species	Source/Host	Location	qPCR	cPCR
T. asperelloides[NP]	-	Germany	-	-
T. asperellum [P*]	Apricot, fruit	France	-	-
T. asperellum[P]	Biostimulant	Italy	-	-
T. asperellum[P]	ABiTEP GmbH Berlin	Germany	-	-
T. asperellum [P*]	Soil maize field	Germany	-	-
T. atrobrunneum[NP]	Soil maize field	-	-	-
T. atrobrunneum[NP]	*A. bisporus* fruitbodies	-	-	-
3x ***T. atroviride***[NP]	*A. bisporus* fruitbodies	Serbia	-	-
T. atroviride [P*]	Vintec	Biostimulant	-	-
2x ***T. atroviride***[NP]	Soil maize field	Germany	-	-
T. azevedoi[NP]	Soil	Thailand	-	-
2x ***T. azevedoi***[NP]	Corncobs	KWS	-	-
T. brevicompactum[NP]	Soil maize field	Germany	-	-
T. cerinum[NP]	Soil maize field	Germany	-	-
3x ***T. gamsii*** [NP]	Corncobs	Germany	-	-
1x ***T. gamsii***[NP]	Soil maize field	Germany	+	-
T. hamatum[NP]	Soil maize field	Germany	-	-
2x ***T. harzianum***[NP]	Corncobs	Germany	-	-
2x ***T. koningii***[NP]	Spore suspension	Trichodex	-	-
T. koningiopsis[NP]	Soil	Germany	-	-
T. paratroviride [P*]	Biostimulant	Vintec	-	-
T. paraviridescens[NP]	Uni. Hohenheim	-	-	-
2x ***T. peberdyi***[NP]	Soil maize field	Germany	-	-
T. simmonsii[NP]	Biostimulant	Biohealth	-	-
2x ***T. velutinum***[NP]	Soil maize field	Germany	-	-
T. virens[NP]	Soil maize field	Germany	-	-
2x***Fusarium proliferatum***	Symptomatic corncobs	Germany	-	-
3x ***F. graminearum***	Symptomatic corncobs	Germany	-	-
3x ***F. temperatum***	corncobs	Germany	-	-
F. subglutinans	Symptomatic corncobs	Germany	-	-
Penicillium paneum	Symptomatic corncobs	Germany	-	-
P. brevicompactum	corncobs	Germany	-	-
2x ***Pseudogymnoascus* sp.**	corncobs	Germany	-	-





*pDNA in TE buffer

Using plasmid DNA of the type strain CBS 124620



Using *;**

**pDNA in 1 ng µL^{-1} of DNA from healthy maize kernels.

Maize material has no detectable effect on analytical sensitivity

Douanla-Meli et al., Sc. Rep.

No effect on assay performances

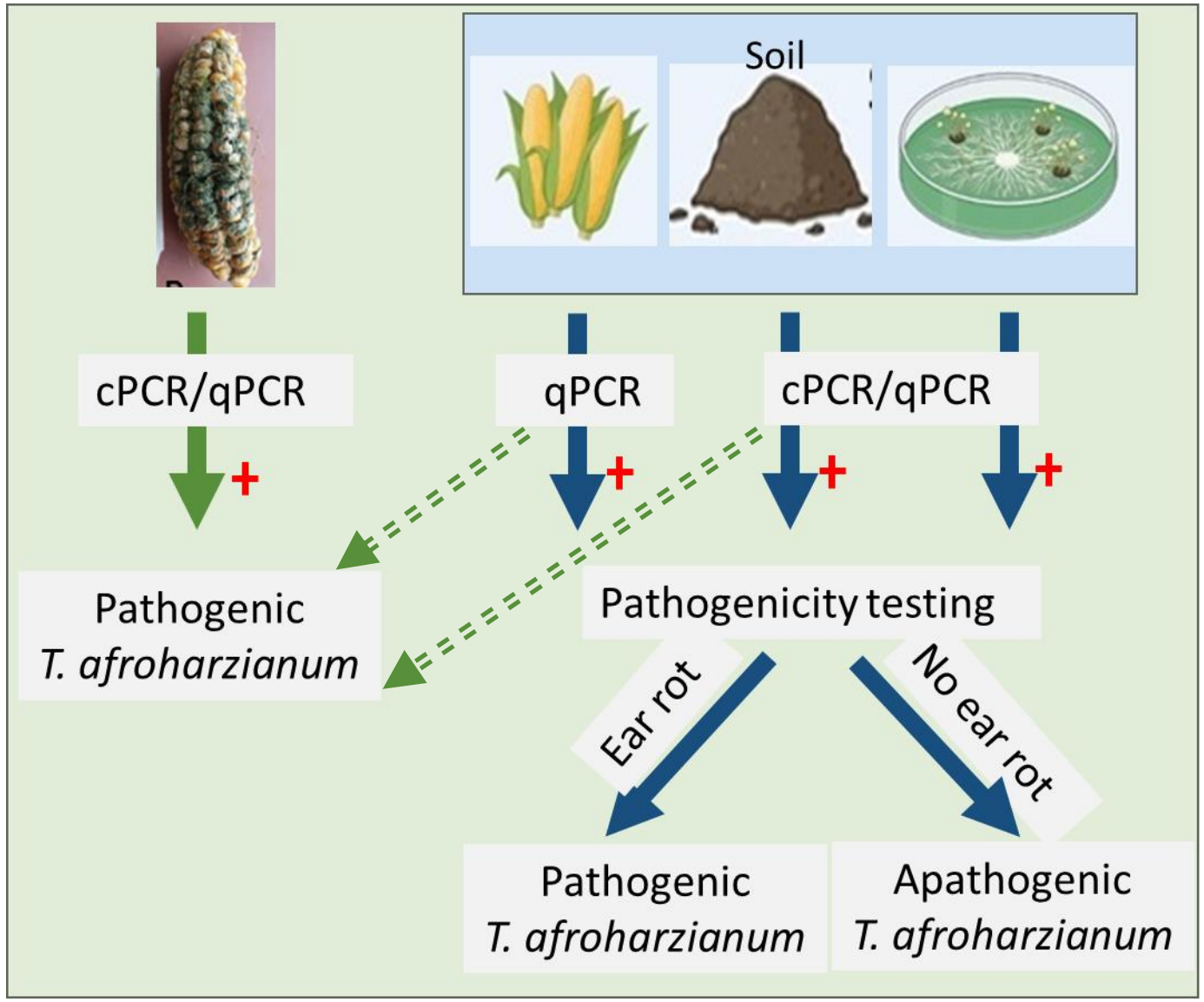
	Repeatability		Reproducibility		Robustness				
DNA template	*qPCR*	*cPCR*	*qPCR*	*cPCR*	**T°C**	60°C	64°C	62°C	62°C
	CV (%)		CV (%)		**Volume**	12.5 µL	12.5 µL	11.25 µL	13.75 µL
target Plasmid DNA 100xLOD (1:10.000)	0.9	High	0.72	High	CV (%)	0.19	0.15	0.27	0.18
target Plasmid DNA 10xLOD (1:100.000)	1.05		0.49			0.25	0.86	0.22	0.23
***T. afroharzianum* CBS124620 (0.1 ng/µl)**	1.74		0.60			0.21	0.08	0.14	0.09
***T. afroharzianum* 159.1 (0.1 ng/µl)**	1.30		0.84			0.26	0.25	0.11	0.24
***T. asperellum* 1 ng/µl**	-		-			>40	>40	>40	>40
***T. brevicompactum* 1 ng/µl**	-		-			>40	>40	>40	>40

Transferability	**StpOnePlus**		**qTower³ thermocycler**	
	Maxima Probe/ROX qPCR Master Mix/ TaqMan Universal Master Mix II/ TaKaRa Premix Ex Taq Probe qPCR +		iTaq Universal Probes Supermix/ TaqMan Universal Master Mix II	
DNA template	Mean Ct ± SD	CV	Mean Ct ± SD	CV
target Plasmid DNA 100xLOD (1:10.000)	24.59 ± 0.43	1.75	29.24 ± 0.83	2.83
target Plasmid DNA 10xLOD (1:100.000)	28.49 ± 0.62	2.18	33.77 ± 0.98	2.90
***T. afroharzianum* CBS124620 (0.1 ng/µl)**	24.46 ± 0.29	1.18	30.46 ± 0.68	3.32
***T. afroharzianum* 159.1 (0.1 ng/µl)**	26.15 ± 0.11	0.42	29.92 ± 0.55	2.76
***T. asperellum* 1 ng/µl**	>40	-	>40	-
***T. brevicompactum* 1 ng/µl**	>40	-	>40	-

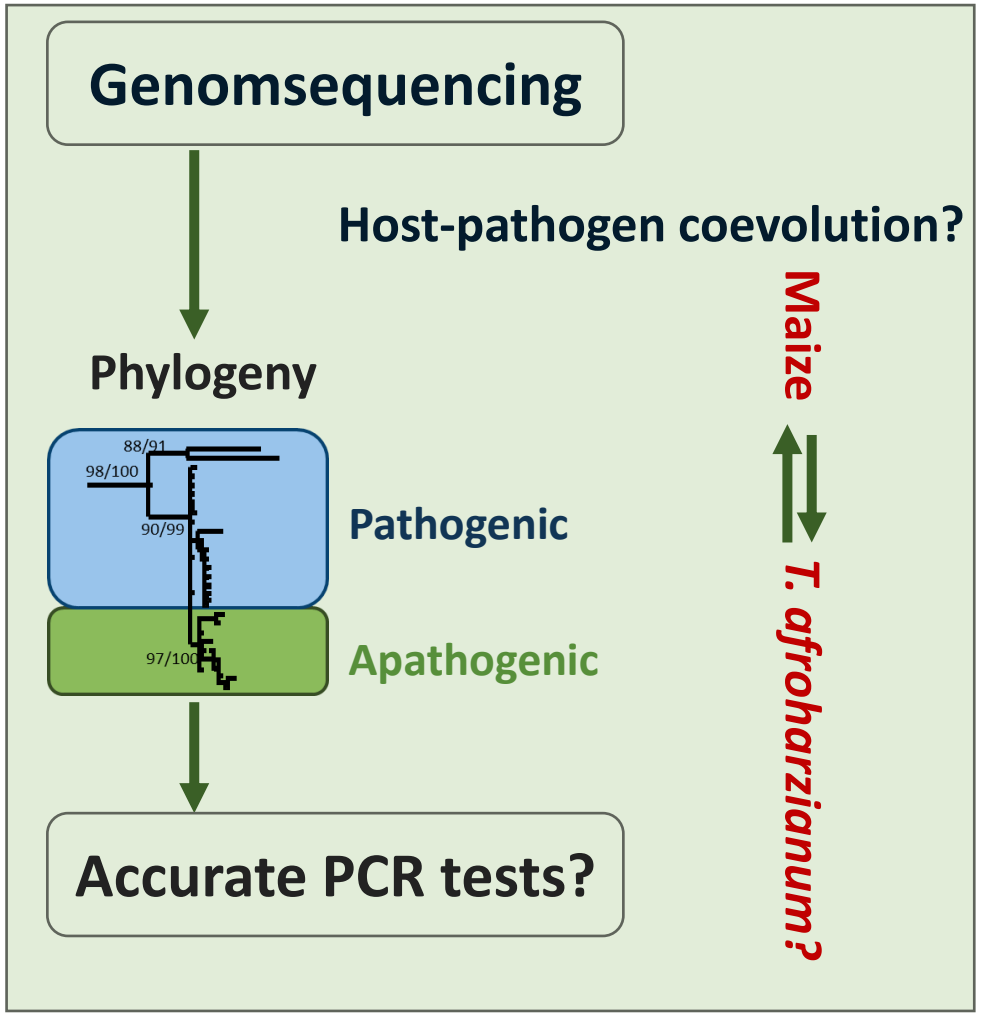
- **Late Ct**
- **Reduced robustness**

Verification of qPCR assay prior to implementation, when using platforms other than the StepOnePlus

Suggested testing procedure (≠ different lifestyles)



Outlook/Underway



- We have developed and validated a molecular tool based on TaqMan qPCR (RPB2) and endpoint PCR (TEF1α) for efficiently diagnosing the newly emerging Trichoderma ear rot
- This tool enables the detection of *T. afroharzianum* directly from infected maize tissue
- The high sensitivity of the qPCR assay allows the detection of latent infections, essential for early diagnosis

However, not all *T. afroharzianum* strains are cause disease, and detection of this species in asymptomatic cobs does not constitute evidence of ear rot. *T. afroharzianum* can only be confirmed as the causal agent of ear rot when disease symptoms are present or following pathogenicity testing

Mais Tricho

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