PRO AGRO LECTORIUM

Miocrobiomes contribute to the health of wheat based on controlling wheat disease

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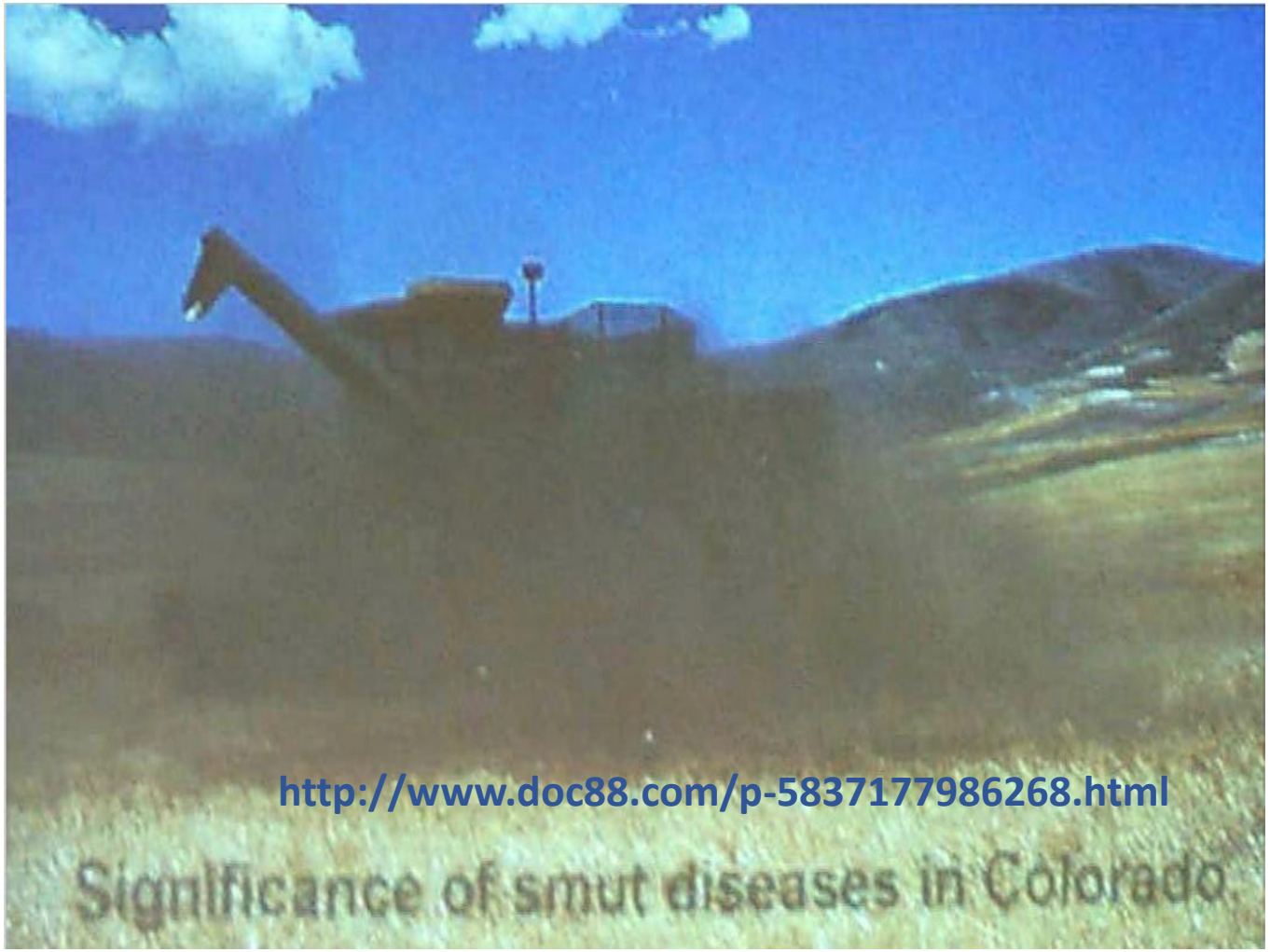
Contents

- Detection of wheat dwarf bunt
- Miocrobiomes contribute to control plant disease
- Miocrobiomes contribute to plant health

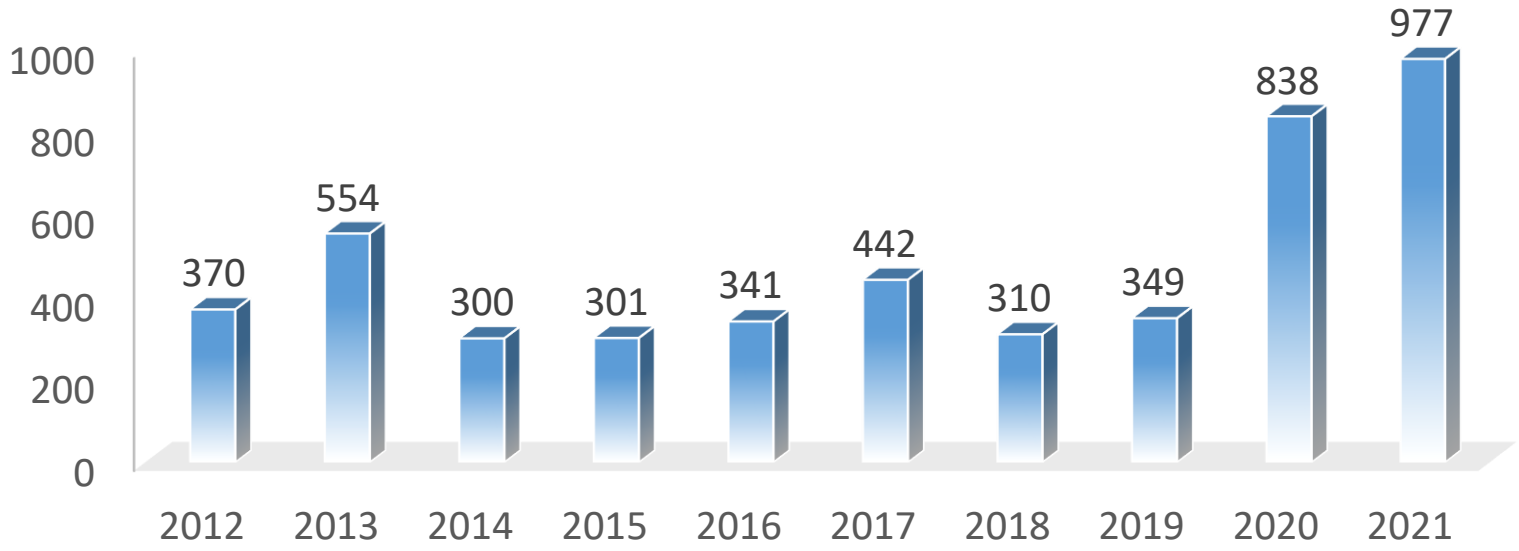
Background

Fig 1. Healthy and bunted (*T. controversa*).
（B.J. Goates）

Fig 2. Close-up of wheat ear smutted by *T. controversa*, showing smutted grains removed and broken open (Priekule, 2007)


http://www.doc88.com/p-5837177986268.html
Significance of smut diseases in Colorado

2012–2021 Import Wheat (10 kilotons)



- Wheat dwarf bunt, caused by **Tilletia controversa** Kühn, is an international quarantine disease that occurs worldwide and can lead to 80% or even total losses of wheat crops.
- Dwarf bunt of wheat affects the milled flour quality with a stinky smell.
- The teliospores of **T. controversa** are able to survive in soil for up to 10 years under favorable conditions (Harwood, 1987).
- The increasing demand for the quality of foods worldwide emphasizes the need to develop better friendly strategies for the efficient management of dwarf bunt disease.

Identification methods

- **T. laevis** and **T. caries** can cause wheat common bunt.
- **T. controversa, T. laevis and T. tritici** are morphologically so similar that they are difficult to distinguish.
- Many researches focused on the differentiation of these pathogens.

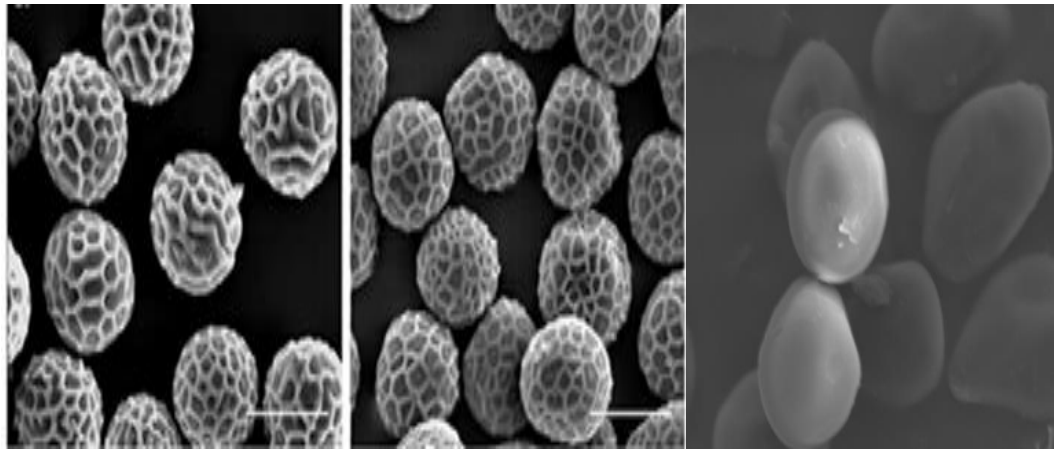
➢Morphological and biological characteristics

➢Immunological characteristics

➢Molecular detection

Morphological and biological characteristics

- **Network ridge 1.43 μm**



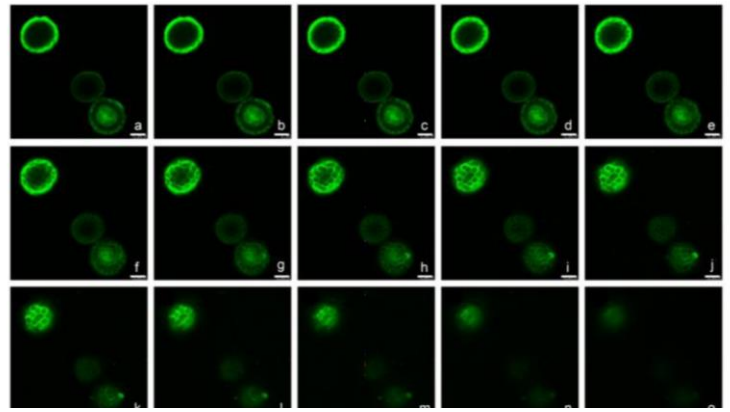
- **Germination of teliospores**

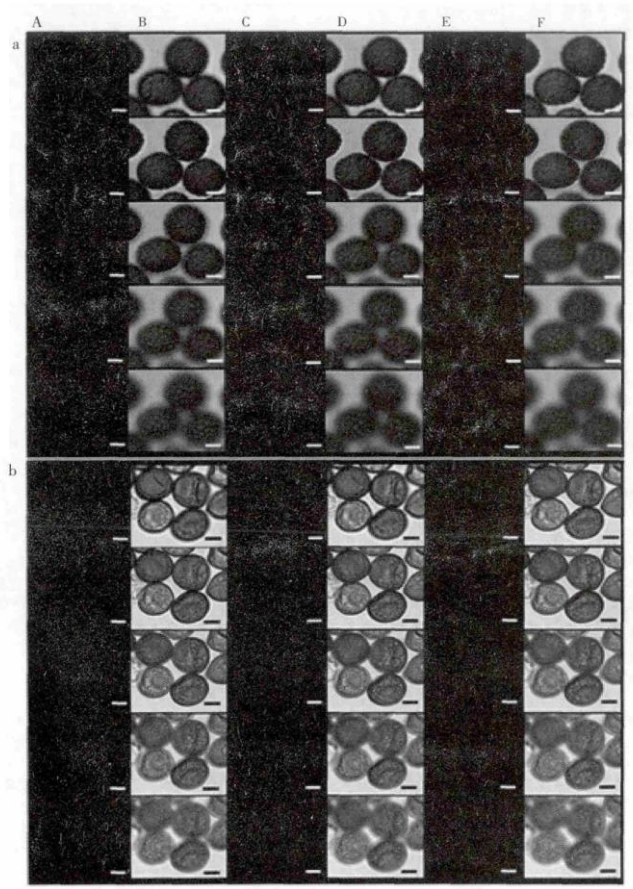
TCK：5℃ (one month) 17℃ not germinated

TCT：5℃,17℃ both germianated (one week)

TFL：17℃ germinated (one week) 5℃ not germinated

Fluorescence microscopic characteristics of teliospores

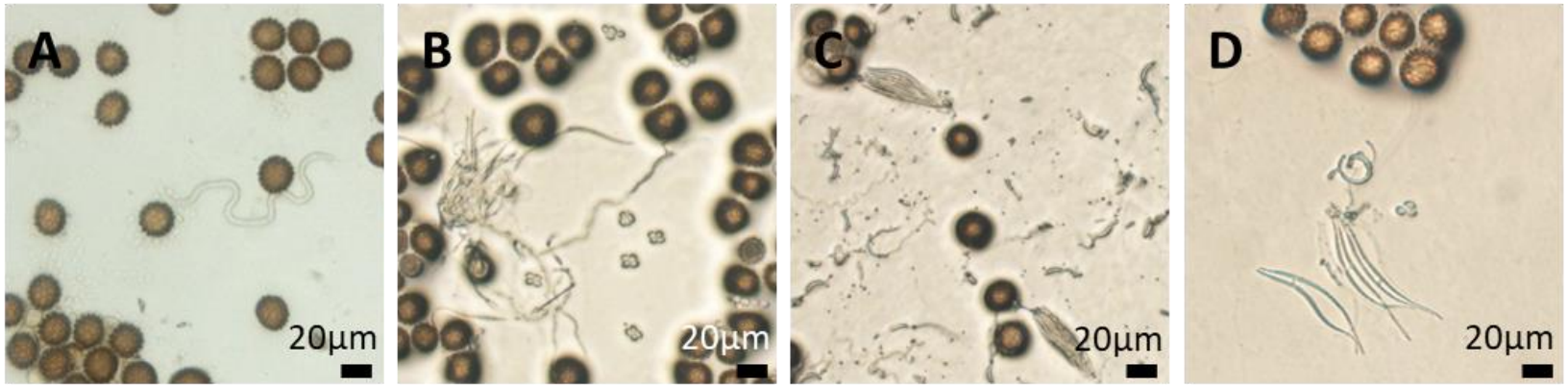




Discrimination of teliospores with laser scanning confocal microscope, the teliospores of *T. controversa* showed a reticulated green color, while The cell wall of the teliospores of *T. foetida* presents a smooth and uniform green color.

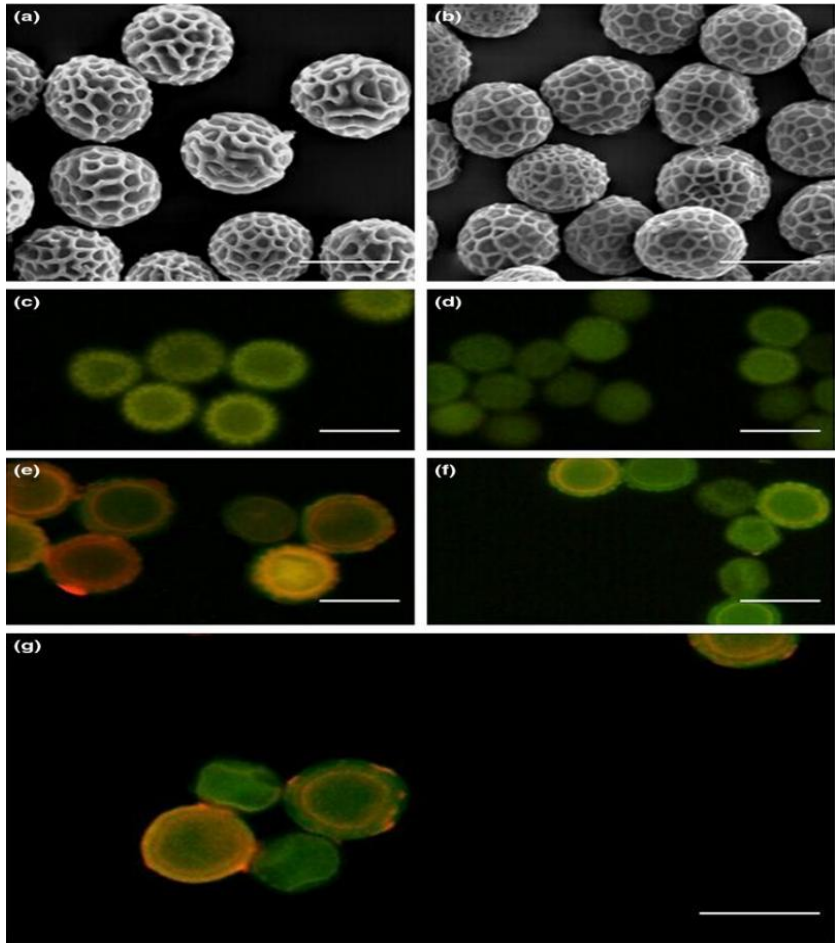
Mainly distributed on outer spore wall and net ridge, but less in protoplasm

Germination testing is an accurate method for detecting **T. controversa** telispores. However, the germination and cultivation of pathogens take a long time, **T. controversa** needs to be cultured at 5 ° C for 3–6 weeks to germinate.



Germination of *T. controversa* teliospores

Immunological characteristics



Journal of Applied Microbiology, 2015

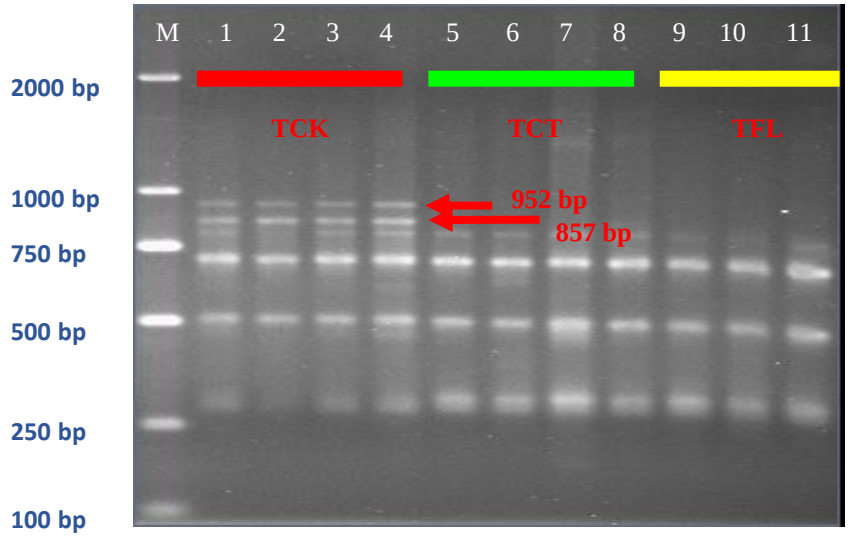
- The orange cycle fluorescent signal was stronger against **T. controversa** teliospores in the outer spore wall and net ridge, whereas only the green signal was observed for the protoplasm of **T. caries** teliospores.
- The detection limit of this method was 2·0 μg ml^{-1} of the D-1 monoclonal antibody.
- It can be used for on-site rapid identification of **T. controversa**, and with further development of technology, it will help to develop fungicides for disease control.

(Corresponding author)

Molecular detection methods

AFLP(1)\ISSR(3)

特异性引物序列	扩增片断	灵敏度
TCKSF1: 5'-CTCCGACGACGAAGTATAGCG-3' TCKSR1: 5'-GGTATACGCGGCACCATATGC-3'	367 bp	10.0 ng
TCKSF2: 5'-TGG TGG TCG GGA AAG ATT AGA-3' TCKSR2: 5'-GGG ACG AAG GCA TCA AGA AG -3'	372 bp	5.0 ng
TCKSF3: 5'-TTG CTG GCT CTT CGC CCT GA-3' TCKSR3: 5'-TTG CCC GTC TTG CGG TTG AT -3'	419 bp	5.0 ng
TCKSF4: 5'-CACACACACACAGGAAGCA-3' TCKSR4: 5'-CGAGGAAGCAGACAAGGCAT-3'	496 bp	1.0 ng



China Patents (ZL201110051411.X、ZL201110051404.X、

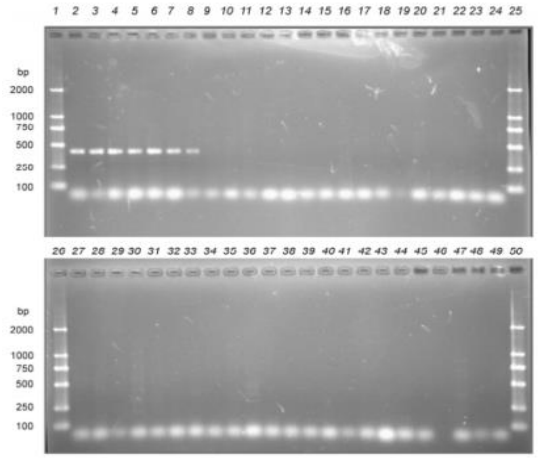
ZL200910085151.0、201518516.3 and 201517724.1)

Letters in Applied Microbiology, 2009

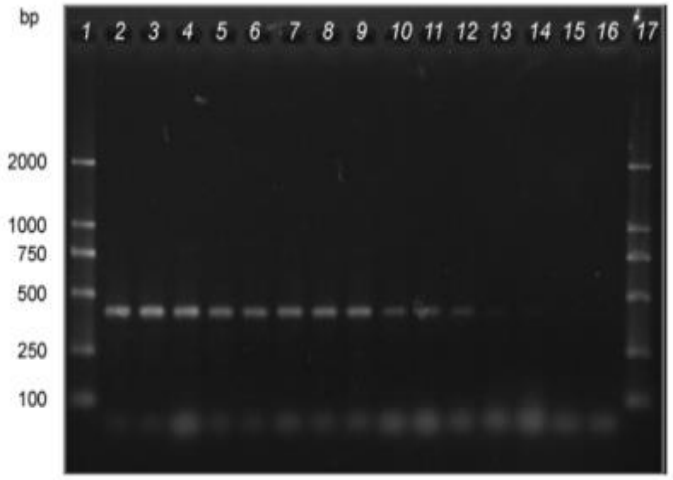
Journal of Phytopathology, 2010

Molecular marker technology detection — ISSR

Gao et al.(2010) designed a SCAR primers (TCKSF3/TCKSR3), based on ISSR-818 primer. The specific primers (TCKSF3/TCKSR3) was designed for use in PCR detection assays; they amplified a unique 419bp DNA fragment in all isolates of **T. controversa** but not in the related pathogens. The detection limit with the primer set (TCKSF3/TCKSR3) was 5 ng of DNA。



The universality and specificity of the SCAR marker.

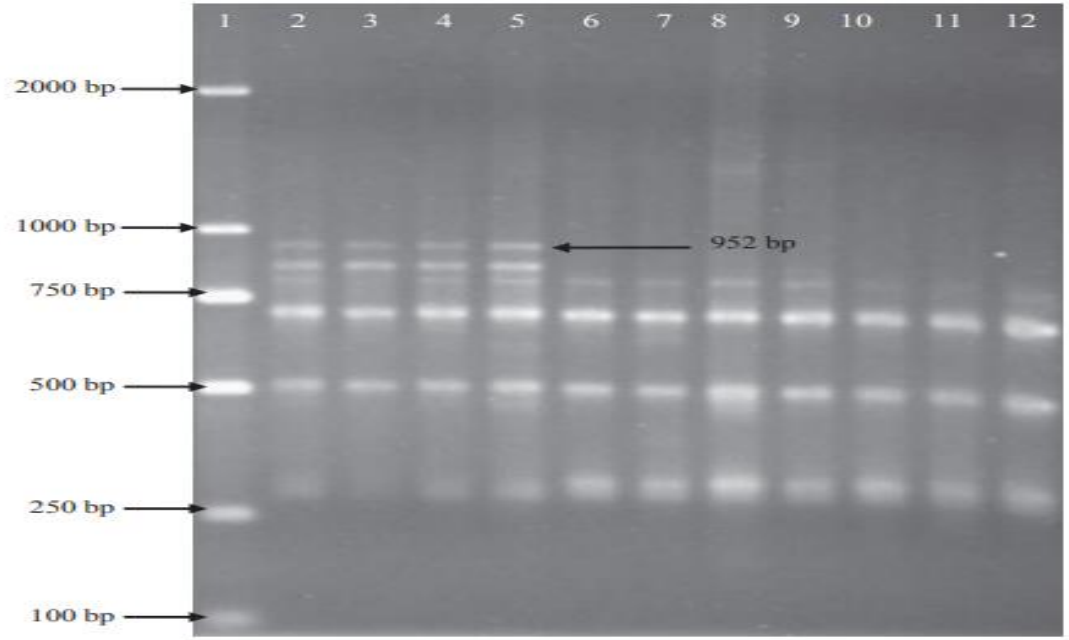


The sensitivity test of the SCAR marker with different amounts of DNA template .

Gao L et al. Development of a SCAR marker by inter-simple sequence repeat for diagnosis of dwarf bunt of wheat and detection of **Tilletia controversa** Kühn. Folia Microbiologica, 2010, 55(3), 258-264

Molecular marker technology detection – ISSR

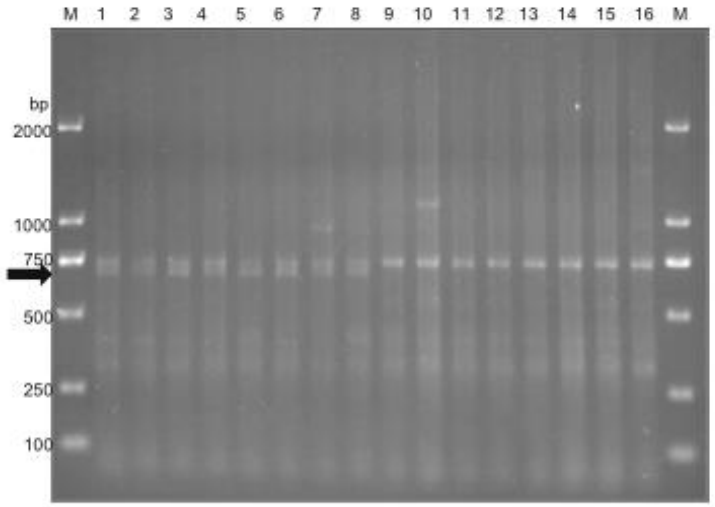
Gao et al. (2011) found primer ISSR-818 generated a polymorphic pattern displaying a 952bp DNA fragment specific for **T. controversa,** which could distinguish all isolates of **T. controversa** from **T. caries** and **T. foetida.**



Gao L et al., An ISSR-based approach for the molecular detection and diagnosis of dwarf bunt of wheat, caused by **Tilletia controversa** Kühn. Journal of Phytopathology, 2011, 159 (3), 155-158

Molecular marker technology detection — ISSR

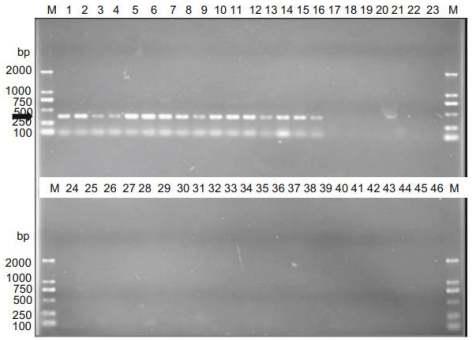
Gao et al. (2014) found primer of an inter-simple sequence repeat (ISSR) ISSR-859 was selected from 40 ISSR primers that could amplify specific DNA fragments in all **T. controversa** strains, while the DNA fragment was not amplified in the other strains tested.



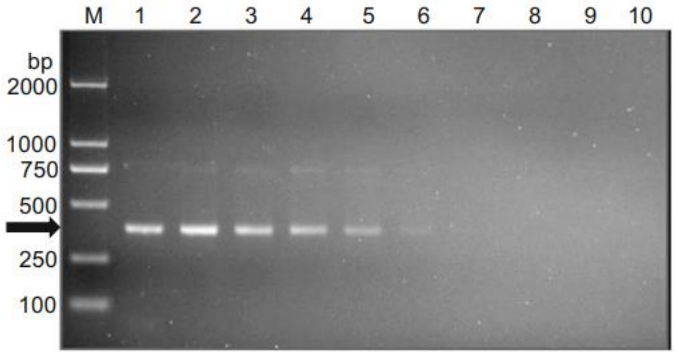
Gao L et al., Development of a SCAR marker for molecular detection and diagnosis of **Tilletia controversa** Kühn, the causal fungus of wheat dwarf bunt. World Journal of Microbiology and Biotechnology. 2014,30(12):3185-95.

Molecular marker technology detection — ISSR

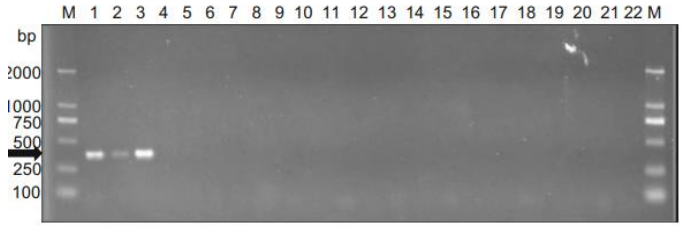
Gao et al. (2014) designed a SCAR primers (SSR140A/SSR511A) based on ISSR-859 primer, which produced a 372 bp DNA fragment specifically in **T. controversa** but not from any isolates of the common bunt fungi or other pathogenic fungi tested, and its detection limit is 1 ng template DNA, which can be used for rapid molecular detection of **T. controversa.**



PCR amplification with sequence characterized amplified region (SCAR)



Sensitivity test with sequence characterized amplified region (SCAR)

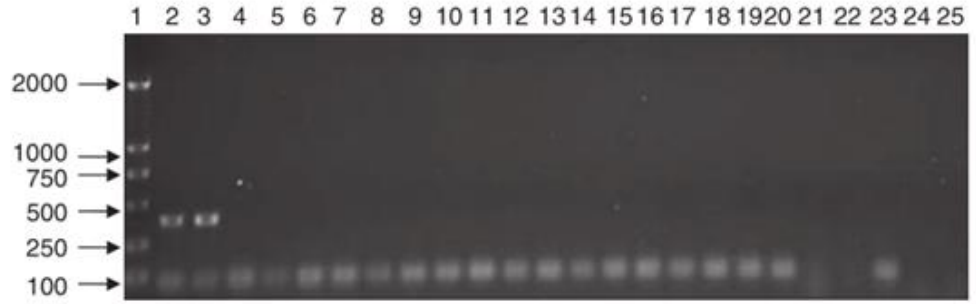


PCR amplification with sequence characterized amplified region (SCAR)

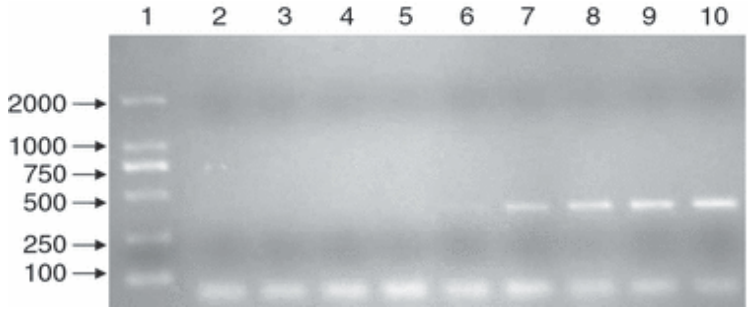
Gao L et al., Development of a SCAR marker for molecular detection and diagnosis of **Tilletia controversa** Kühn, the causal fungus of wheat dwarf bunt. World Journal of Microbiology and Biotechnology. 2014,30(12):3185-95.

Molecular marker technology detection — AFLP

We developed a sequence-characterized amplified region (SCAR) marker based on a specific primer E08/M02, and specific primers ($SC\text{-}01_{49}$/$SC\text{-}02_{415}$), designed for use in PCR detection assays. It amplified a unique 367bp DNA fragment in all isolates of **T. controversa,** but not in the related pathogens. The detection limit with the primer set $SC\text{-}01_{49}$/$SC\text{-}02_{415}$ was 10 ng. The **T. controversa** PCR detection kit based on this primer can be used for early diagnosis of diseases.



The universality and specificity of the SCAR marker

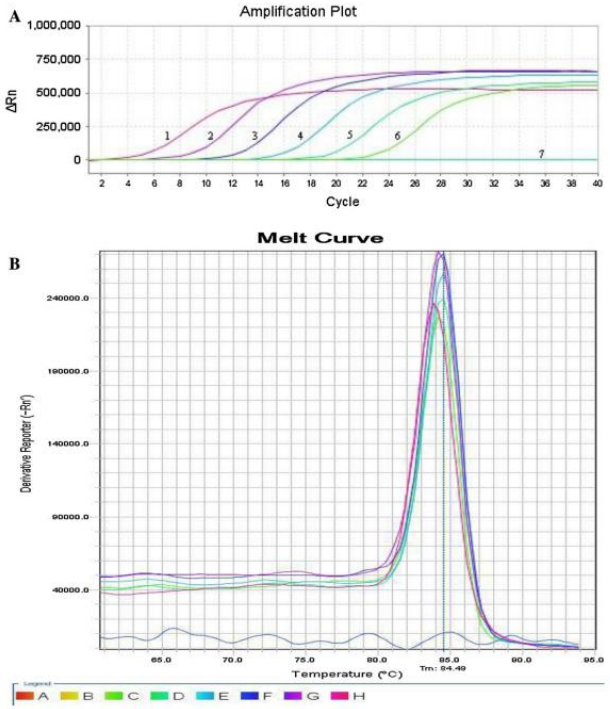


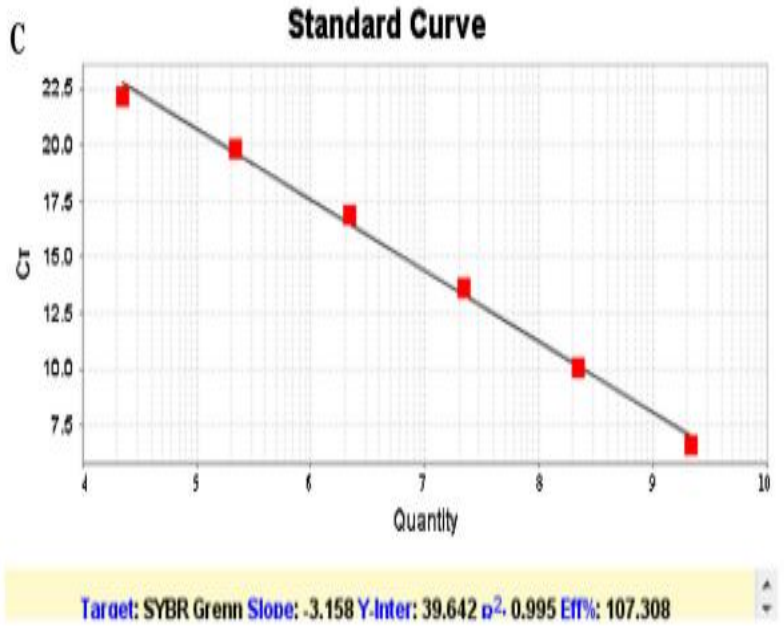
The sensitivity test of the SCAR marker

Development of a sequence-characterized amplified region marker for diagnosis of dwarf bunt of wheat and detection of Tilletia controversa Kühn. Letters in Applied Microbiology, 2009, 49(2), 235–240 (Corresponding author)

Fluorescence-based real-time quantitative PCR — SYBR Green

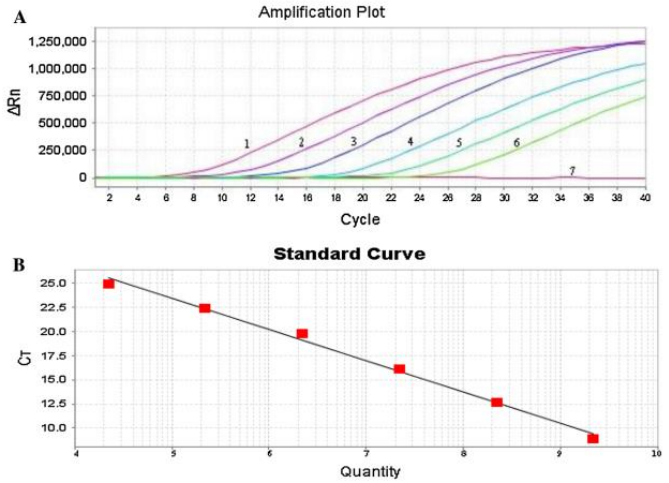
Gao et al.(2014) established a SYBR Green I real-time PCR method based on specific SCAR primers (SSR140A/SSR511A). The detection limit is 0.1fg/μ L.



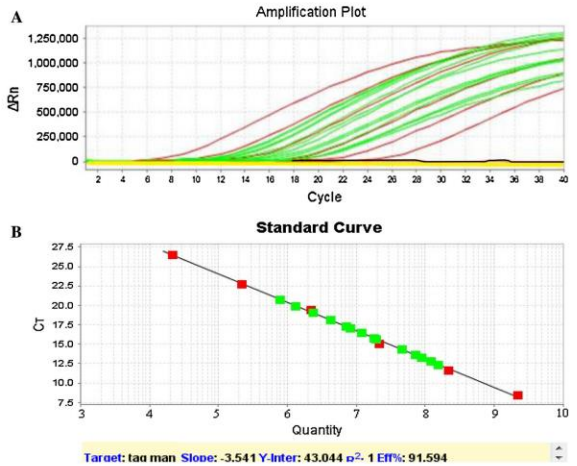


Fluorescence-based real-time quantitative PCR — TaqMan

Gao et al. (2014) also established a TaqMan probe real-time PCR method based on specific SCAR primers (SSR140A/SSR511A). The detection limit is 0.1fg/ μ L. From wheat plants grown from seeds artificially contaminated by teliospores at various growth stages.



Construction of standard curve by TaqMan probe real-time PCR.

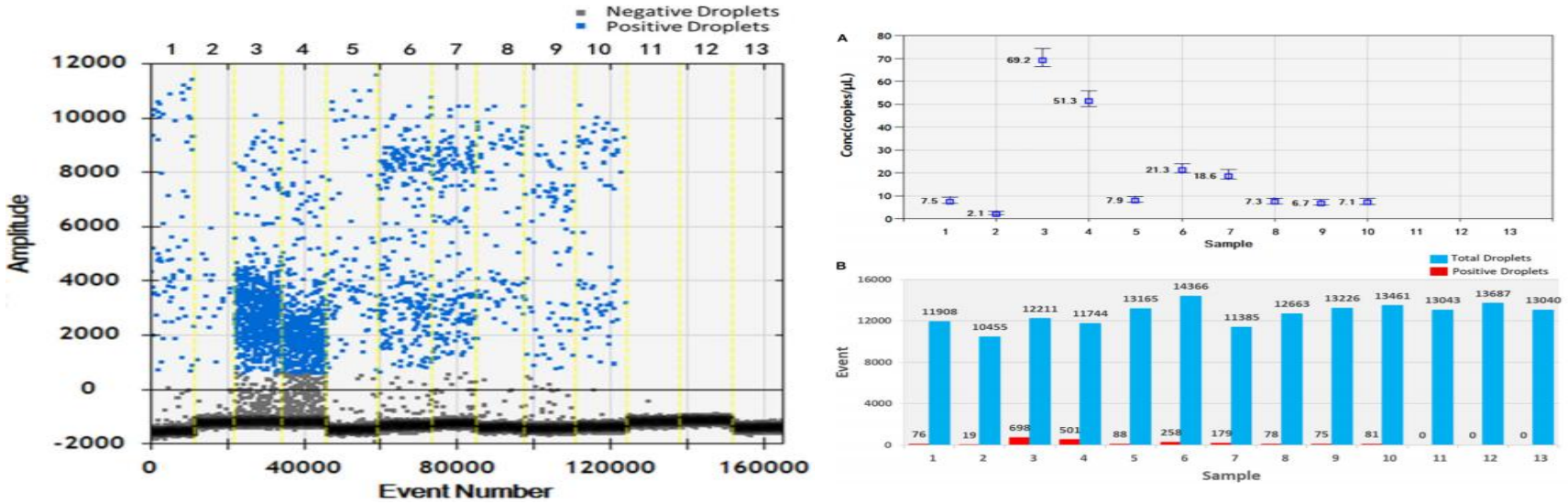


Detection of *T. controversa* and *T. caries* strains by TaqMan probe real-time PCR

Development of a SCAR marker for molecular detection and diagnosis of Tilletia controversa Kühn, the causal fungus of wheat dwarf bunt. World Journal of Microbiology and Biotechnology. 2014,30(12):3185-95. (Corresponding author)

Droplet digital PCR — ddPCR

We established ddPCR detection system to successfully detect **T. controversa** telispores in soil, sensitive detection at 2.1 copies/μL, which was 100 times more sensitive than that of simple PCR.



Development of the droplet digital PCR to detect the teliospores of **Tilletia controversa** Kühn in the soil with greatly enhanced sensitivity. Front. Microbiol 7.2020, 11, 1–9. （Corresponding author)

- Quick detection, acute detection and early detection
- Small sample, even a single teliospore

Summary of detection methods of T. controversa in our lab

Morphological characteristics	Immunological characteristics	Molecular detection technology
Advantages: Simple and quick. **Disadvantages:** • Need much more experience • Easily error for distinguish • Germination requires a long period of time.	**Advantages:** Fast and sensitive. **Disadvantages:** • Need to handle fluorescence microscope • Need to prepare monoclonal antidody previously	**Advantages:** Fast, high sensitivity, and strong specificity. **Disadvantages:** • Common PCR need some **special skills**, such as extract DNA, Run PCR and gels; • Real time quantitative PCR and ddPCR methods require **special equipment**.

Optimized concentration of difenoconazole fungicide for controlling of wheat dwarf bunt based on microbial communities for disease incidence

- Microbial diversity and composition play an important role in improving soil fitness and fertility.
- Rhizospheric microbes can enhance disease resistance in plants, thus protecting the plant from the development of disease.
- Plamt pathogen changes the composition of microbiomes in host and many plant-associated microbes, such as **Trichoderma spp.,** act as potential biocontrol agents against many pathogens such as Fusarium spp.
- Fungicide reduces the population density of bacterial (Bacillus spp.), fungal (Penicillium and Rhizopus spp.) species and soil population in cropping system.

Plant Materials and Treatments

Wheat varieties (16)and one highly susceptible variety (Morocco) to **T. controversa** were collected from the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, China.

List of the varieties used in this study

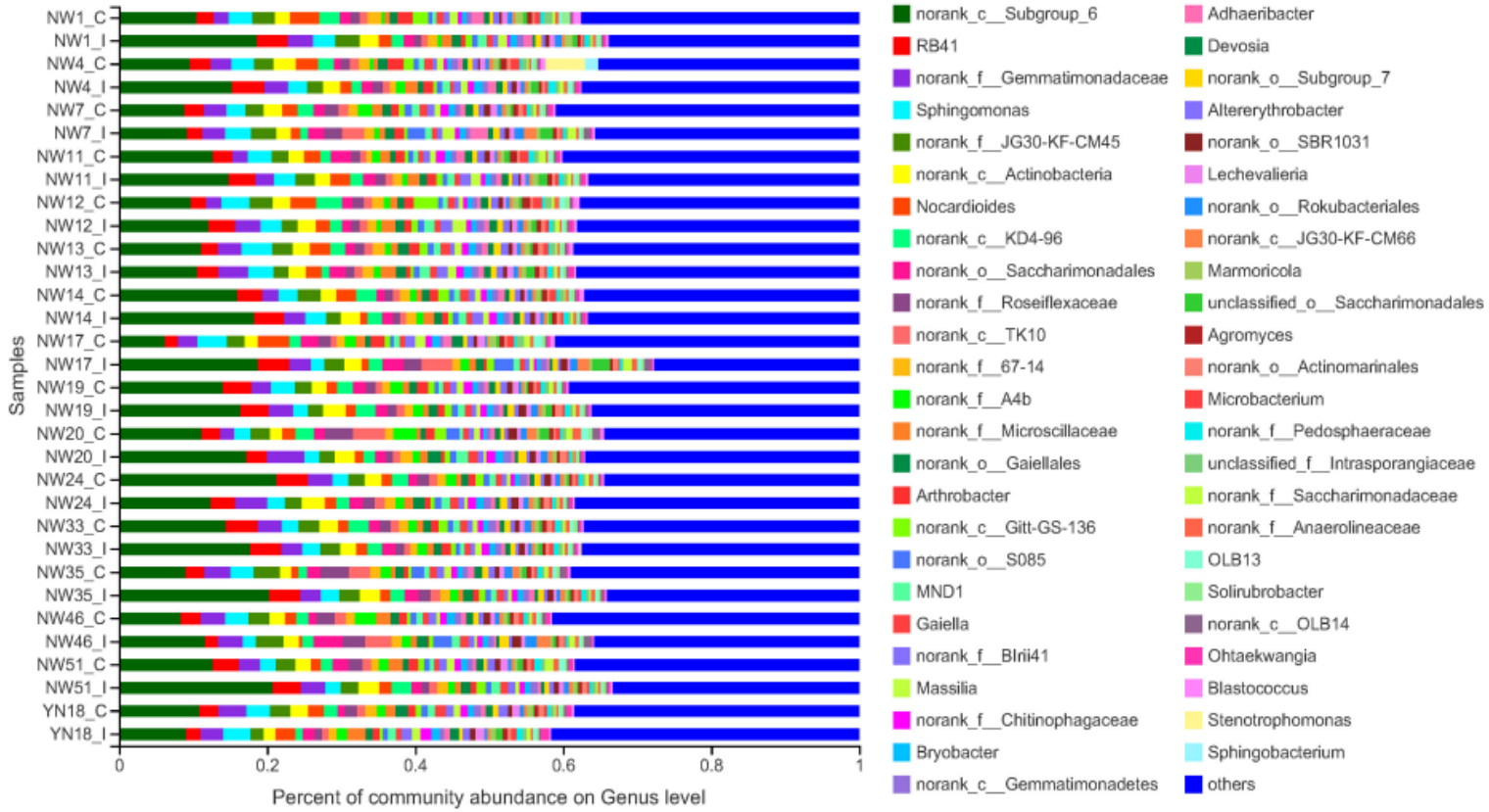
Variety name	Control	Infected*	Variety name	Control	Infected*
New Winter 1	NW1_C	NW1_I	New Winter 19	NW19_C	NW19_I
New Winter 4	NW4_C	NW4_I	New Winter 20	NW20_C	NW20_I
New Winter 7	NW7_C	NW7_I	New Winter 24	NW24_C	NW24_I
New Winter 11	NW11_C	NW11_I	New Winter 33	NW33_C	NW33_I
New Winter 12	NW12_C	NW12_I	New Winter 35	NW35_C	NW35_I
New Winter 13	NW13_C	NW13_I	New Winter 46	NW46_C	NW46_I
New Winter 14	NW14_C	NW14_I	New Winter 51	NW51_C	NW51_I
New Winter 17	NW17_C	NW17_I	Yinong 18	YN18_C	YN18_I

The seeds of the Morocco cultivar were coated with six different concentrations of difenoconazole fungicide. Seed dressing with difenoconazole application against **T. controversa** infected and non-infected wheat rhizosphere.

Fungicide (ratio)	Dose	Infected	Control
1.5% dose	2.25 mL/100 kg	IA	NG
3% dose	4.5 mL/100 kg	IB	NH
5% dose	7.5 mL/100 kg	IC	NI
Recommended dose	150 mL/100 kg	ID	NJ
1.5 times dose	225 mL/100 kg	IE	NK
No fungicide	No seed treatment	IF	NL

Relative abundance of the dominant rhizosphere soil bacterial

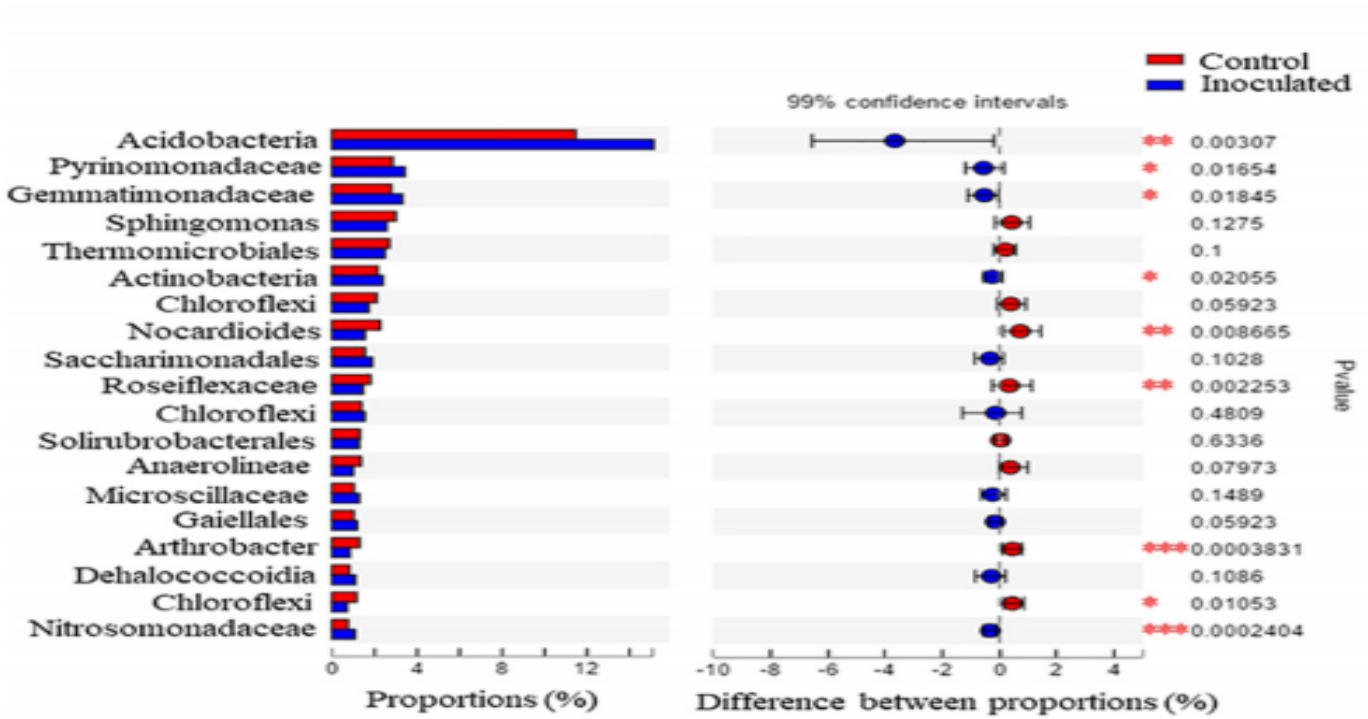
Dominant phyla: norank_c_Subgroup_6, RB41, norank_f_Gemmatimonadaceae, Sphingomonas, norunk f_JG30-KF-CM45, norunk_c_Actinobacteria, and Nocardiodes



Wilcoxon rank-sum test analysis for the rhizosphere soil microorganisms

Whether **T. controversa** inoculation and control variables influenced the structure of the microbial community.

Populations of *Arthrobacter* sp. and Nitrosomonadaceae were highly significant ($P \leq 0.001$); Acidobacteria, Nocardioides, and Roseiflexaceae were significantly different ($P \leq 0.01$)



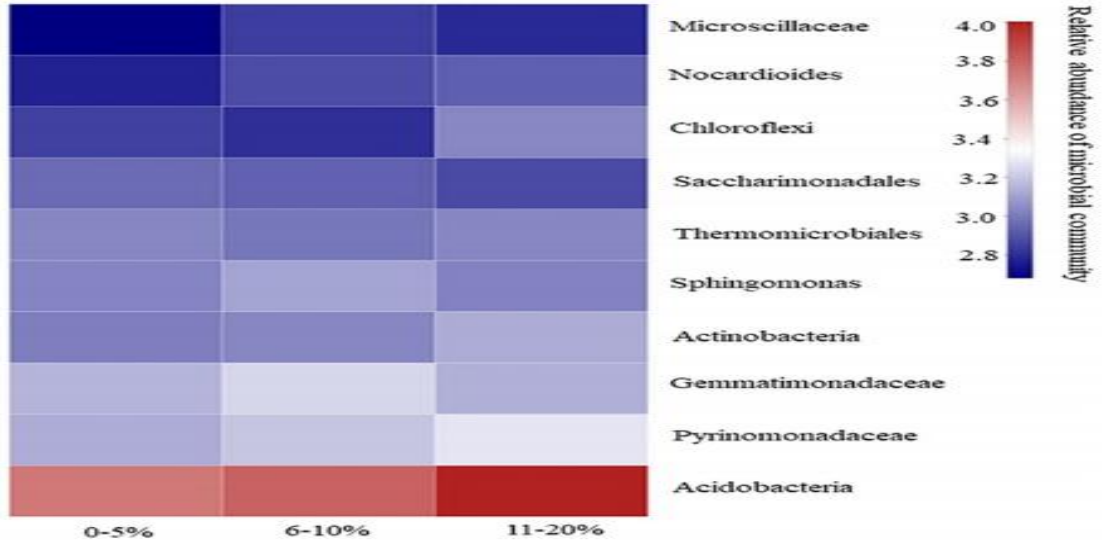
Community heatmap analysis of rhizosphere soil microorganisms

The disease incidence was correlated with the abundance of some microbial communities based on the heatmap analysis of the community.

Acidobacteria showed a direct proportional relationship with disease incidence, The same pattern was observed for the members of Pyrinomonadaceae, Actinobacteria, and Nocardioides.

For Sphingomonas, the disease incidence increased as the abundance level decreased.

The disease incidence was inversely proportional to the abundance of Saccharimonadales.



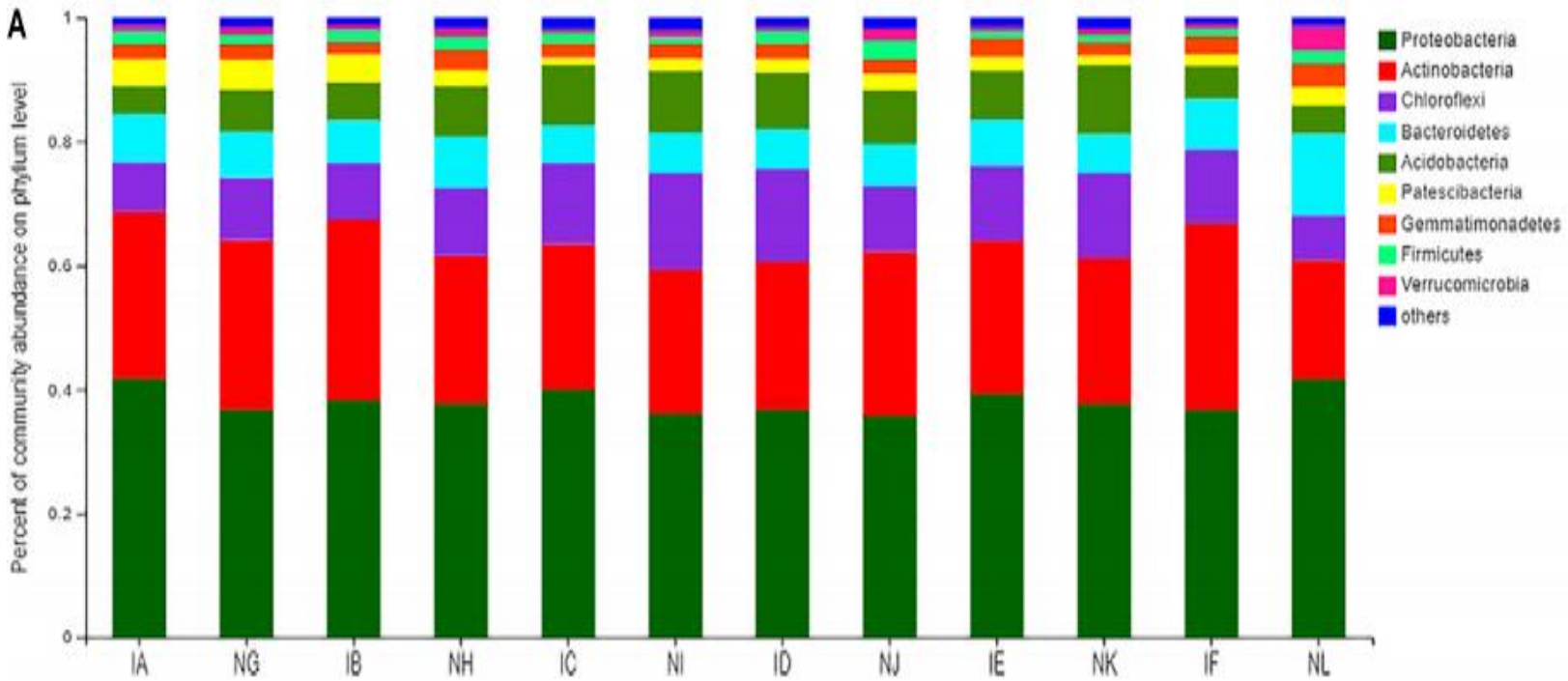
Disease incidence in different varieties treated with T. controversa

The study results indicated that the disease incidence varied between the cultivars. The maximum disease incidence was recorded in New Winter 51, and the minimum was recorded in New Winter 20 and New Winter 35, with values of 14 and 4%.

Variety name	Disease incidence	Variety name	Disease incidence
New Winter 1	–	New Winter 19	–
New Winter 4	10%	New Winter 20	4%
New Winter 7	–	New Winter 24	–
New Winter 11	–	New Winter 33	8%
New Winter 12	–	New Winter 35	4%
New Winter 13	–	New Winter 46	–
New Winter 14	–	New Winter 51	14%
New Winter 17	–	Yinong 18	6%

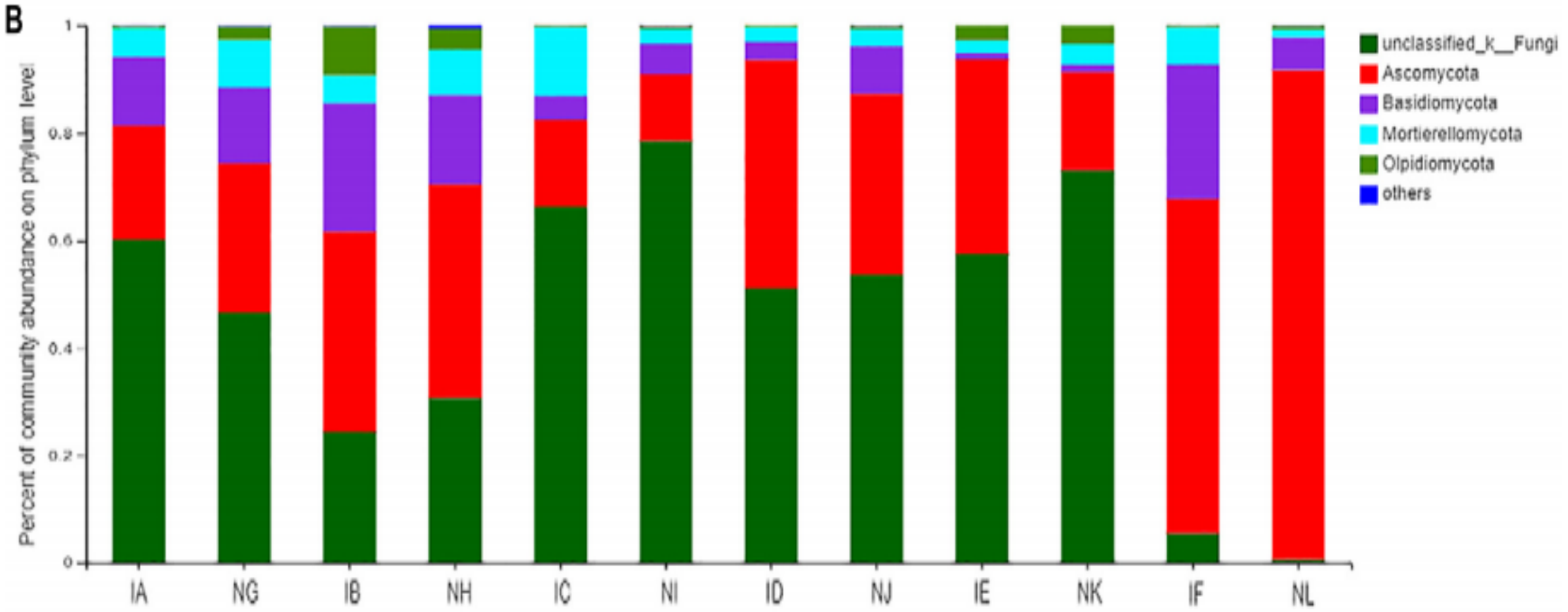
Comparison of taxonomic distributions of bacterial phyla between different concentrations of difenoconazole fungicide in T. controversa-infected and non-infected samples

These findings indicated that with the increase in the concentration of fungicide, the population of Acidobacteria and Actinobacteria also increased, while that of Chloroflexi and Patescibacteria decreased. Following infection with **T. controversa,** the number of Proteobacteria decreased, while that of Acidobacteria and Chloroflexi increased.



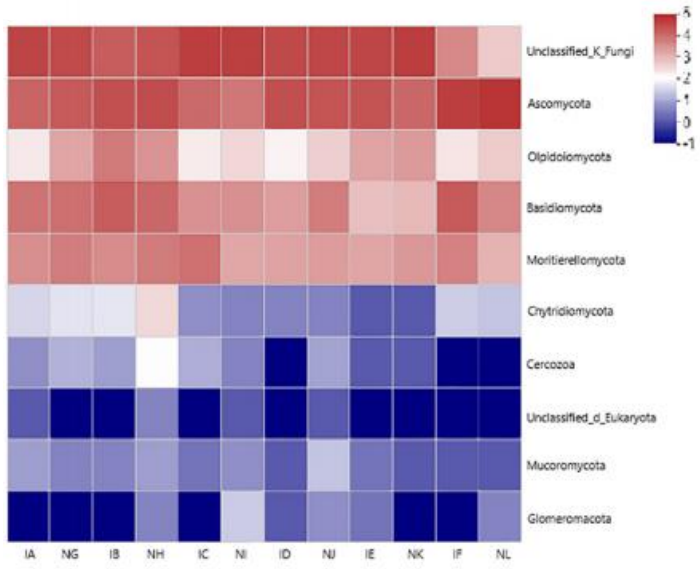
Comparison of taxonomic distributions of fungal phyla between different concentrations of difenoconazole fungicide in T. controversa-infected and non-infected samples.

The fungal communities were predominated by four dominant phyla (Ascomycota, Basidiomycota, Mortierellomycota, and Oipidiomycota) with some fungi belonging to the unclassified kingdom. We found that IC (*T. controversa* + 5% dose of difenoconazole) treatment highly decreased the Basidiomycota population, which may contain the pathogen of **T. controversa**.



Fungal distribution of the top eight abundant phyla among the 12 samples

The results showed The members of Ascomycota, Oipidiomycota, Basidiomycota, and Mortierellomycota showed significant differences, but the abundance of Basidiomycota in the IC (*T. controversa* + 5% dose of difenoconazole) sample was lower than observed in control, which means that this concentration of fungicide can decrease the population of **T. controversa.**



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3% dose	4.5 mL/100 kg	IB	NH
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Recommended dose	150 mL/100 kg	ID	NJ
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No fungicide	No seed treatment	IF	NL

Microbiome signature of endophytes in wheat seed response to wheat dwarf bunt

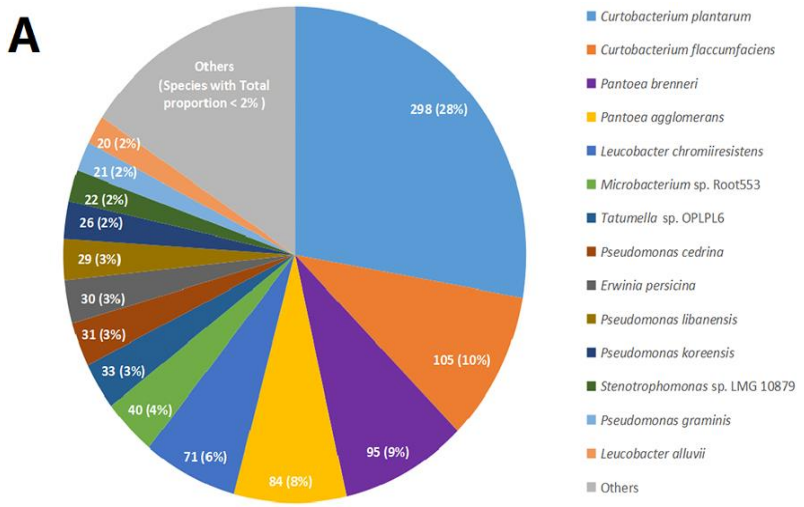
Variety name	Treatment	Groups	Sample ID
Resistant cultivar 1	Infected	RI	S1
Resistant cultivar 2	Noninfected	RH	S2
Resistant cultivar 3	Infected	RI	S3
Resistant cultivar 4	Noninfected	RH	S4
Resistant cultivar 5	Infected	RI	S5
Resistant cultivar 6	Noninfected	RH	S6
Resistant cultivar 7	Infected	RI	S7
Resistant cultivar 8	Noninfected	RH	S8
Resistant cultivar 9	Infected	RI	S9
Resistant cultivar 10	Noninfected	RH	S10
Resistant cultivar 11	Infected	RI	S11
Resistant cultivar 12	Noninfected	RH	S12
Susceptible cultivar1	Infected	SI	S13
Susceptible cultivar2	Noninfected	SH	S14
Susceptible cultivar3	Infected	SI	S15
Susceptible cultivar4	Noninfected	SH	S16
Susceptible cultivar5	Infected	SI	S17
Susceptible cultivar6	Noninfected	SH	S18
Susceptible cultivar7	Infected	SI	S19
Susceptible cultivar8	Noninfected	SH	S20
Susceptible cultivar9	Infected	SI	S21
Susceptible cultivar10	Noninfected	SH	S22
Susceptible cultivar11	Infected	SI	S23
Susceptible cultivar12	Noninfected	SH	S24

Medium for cultural isolates in this study

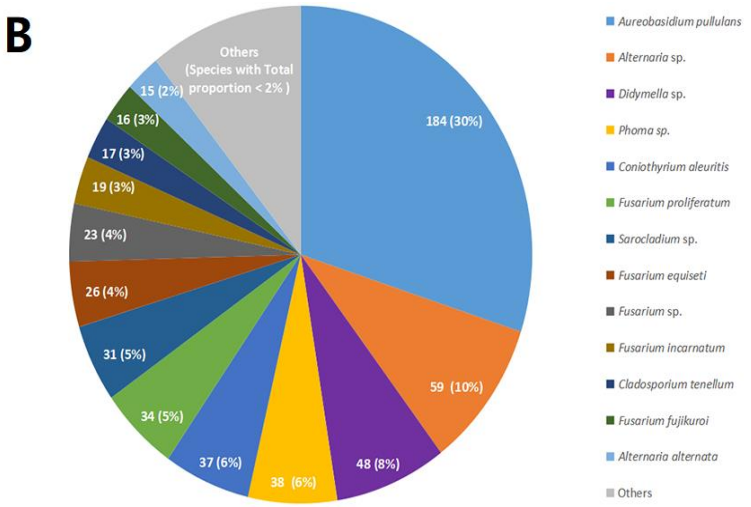
Medium	Formation
LB	Tryptone 10 g/L; Yeast extract 5 g/L; NaCl 10 g/L; Agar 15 g/L.
Per 25 NA	Peptone 1.25 g/L; Beef extract 0.75 g/L; NaCl 1.25 g/L; Agar 15 g/L.
PDA	Potato extract 200 g/L; glucose 20 g/L; Agar 15 g/L.
Per 25 PDA	Potato extract 200 g/L; glucose 20 g/L; Agar 15 g/L.
TSA	Tryptone 15 g/L; Soy papain hydrolyzate 5 g/L; NaCl5 g/L; Agar 15 g/L.
R2A	Yeast extract powder 0.5 g/L; Peptone 0.5 g/L; Casein Hydrolyzate 0.5 g/L; Glucose 0.5 g/L; Soluble Starch 0.5 g/L; $KH_2PO_4$0.3 g/L; $MgSO_4$0.024 g/L; $C_3H_3NaO_3$ 0.3 g/L; Agar 15 g/L.
Rice	Rice 300 g/L, Agar 20g/L.
TWYE	Yeast extract0. 25 g/L;$KH_2PO_4$0. 5 g/L;Agar 15 g/L.
CMA	Maize flour 5 g/L; Peptone 0.1 g/L; Glucose 1 g/L
DG 18	Casein peptone 5g/L; Anhydrous dextrose 10 g/L; $KH_2PO_4$1 g/L; $MgSO_4$0.5 g/L; Chlornitramine 0.002 g/L; Chloramphenicol 0.1 g/L; Agar 15 g/L.
MEA	Malt extract 30 g/L; Soybean peptone 3 g/L, Agar 15 g/L.
RBM	Peptone 5 g/L; Glucose 10 g/L; $KH_2PO_4$1 g/L; MgSO4 0.5 g/L; Bengal Red 0.03 g/L; Chloramphenicol 0.1 g/L; Agar 15 g/L.
V8	V-8 Juice 200 g/L $CaCO_3$g/L; Agar 15 g/L.

Endophytic microorganisms obtained based on isolation

- Endophytic bacteria (A): 1,392 isolates
- Endophytic fungi (B): 636 isolates



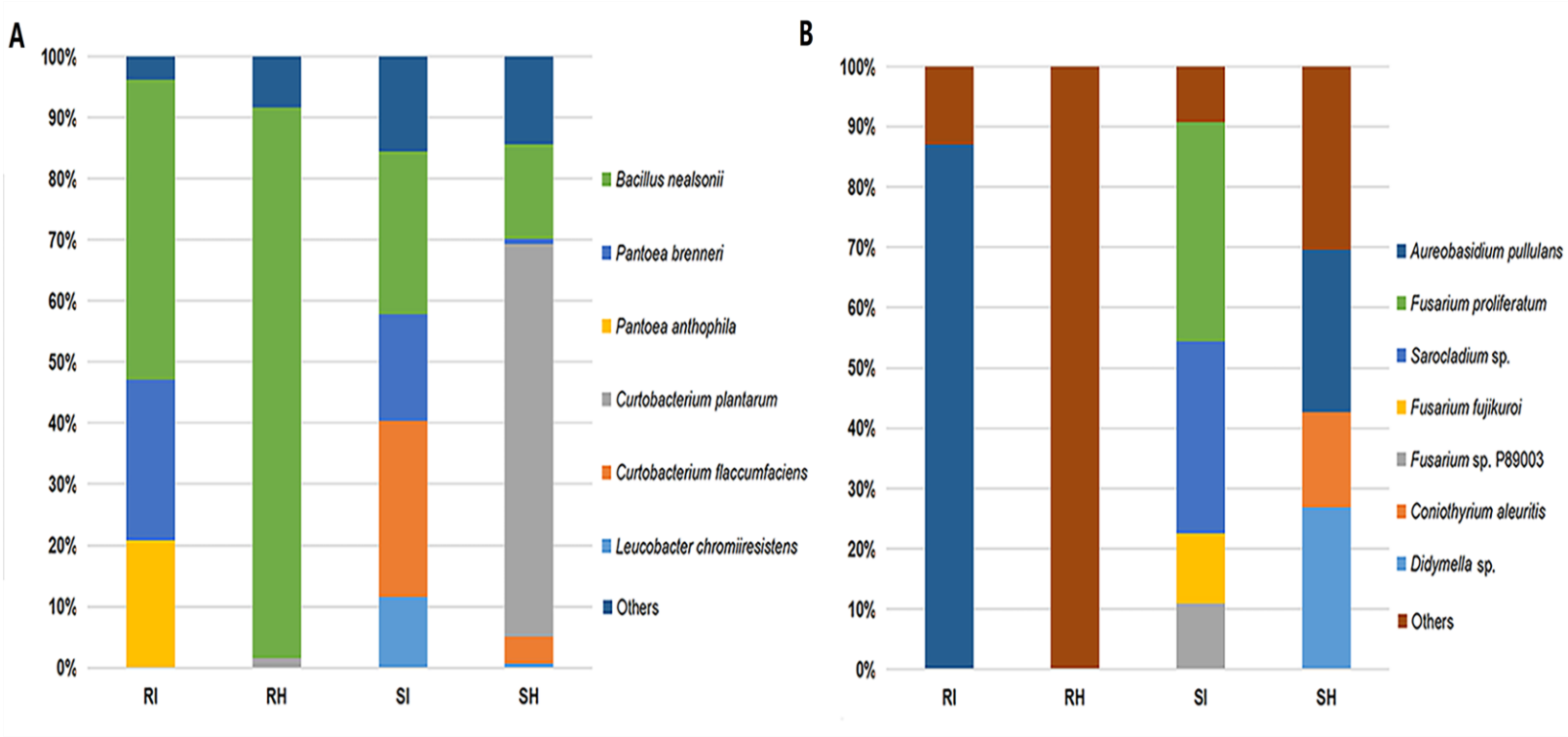
Bacterial isolates



Fungal isolates

Relative abundances of endophytic microorganisms

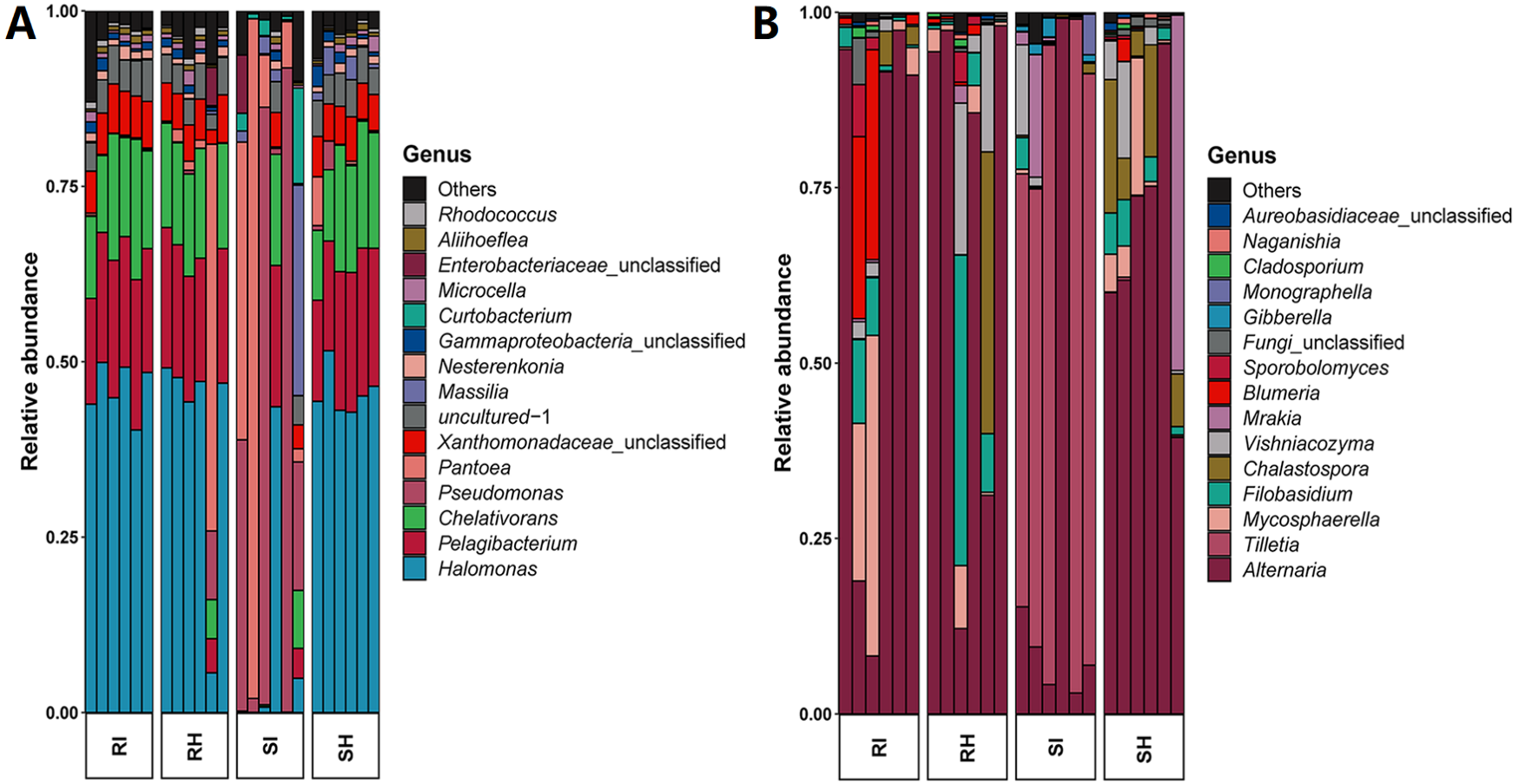
The total relative abundance of the main endophytic microorganisms were visualized, revealing that the relative abundances of endophytic microorganisms varied among cultivars and changed after **T. controversa** infection.



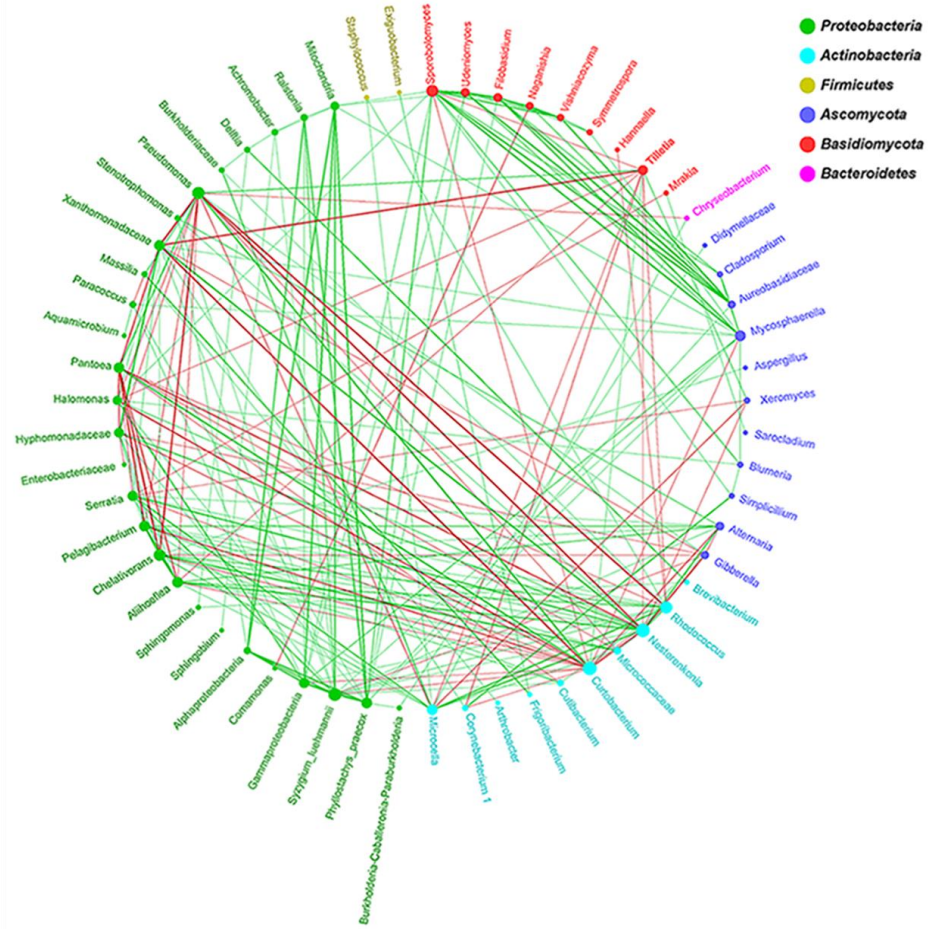
Bacterial communities

Fungal communities

Cultivars and infection-associated seed endophytes



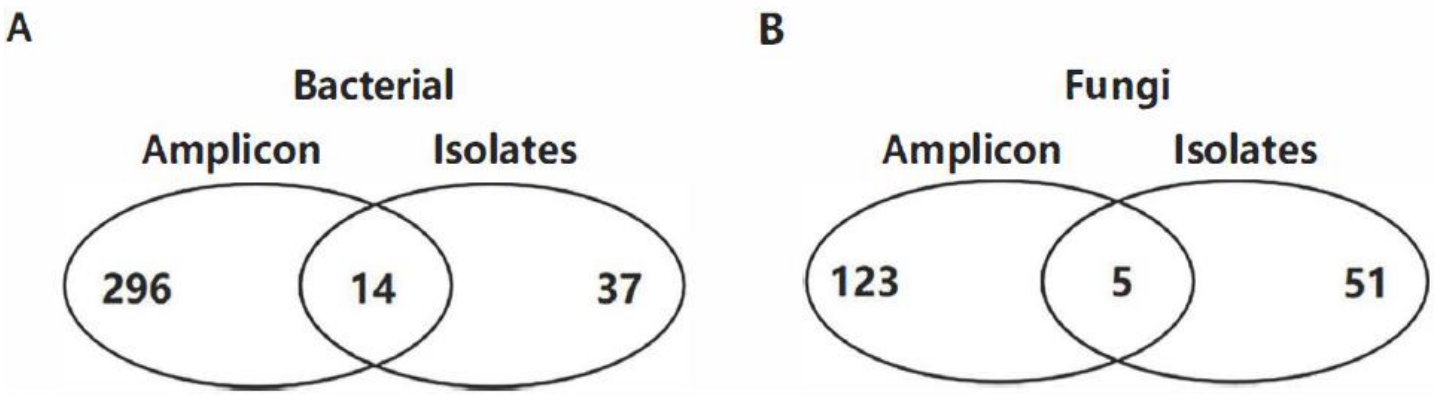
Cross-kingdom connectivity of endophytic microbiota



- Separate co-occurrence networks were constructed for all groups. The green lines indicated positive correlated and red lines indicated negative correlated.
- Results showed that **Xanthomonadaceae**, **Halomonas**, **Aliihoeflea**, **Microcella**, **Corynebacterium**, **Nesterenkonia** and **Rhodococcus** were negatively correlated with **Tilletia**.

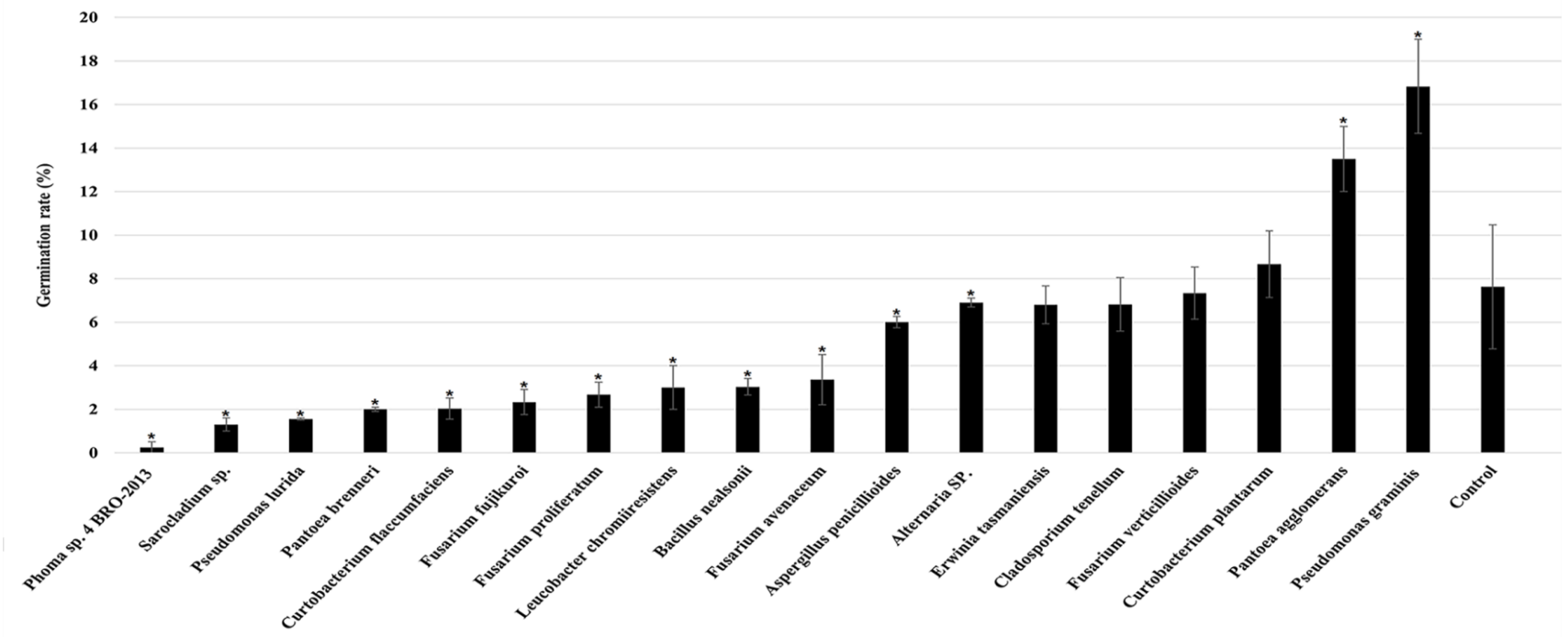
Overlapping endophytic microorganisms from isolation and amplification sequencing

- **For bacterial species:** 51 bacterial were isolated, 310 species were obtained by amplicon sequencing, 14 species were overlapping.
- **For fungi species:** 56 bacterial were isolated, 128 species were obtained by amplicon sequencing, 5 species were overlapping.



Antagonists against the germination of teliospores of T. controversa

The germination rates indicated that **Phoma** sp. strain 4 BRO-2013, **Sarocladium** sp., **P. lurida**, **P. brenneri**, **C. flaccumfaciens**, **F. fujikuroi**, **F. proliferatum**, **L. chromiiresistens**, **B. nealsonii**, **F. avenaceum**, **A. penicillioides**, and **Alternaria** sp. inhibited the germination of teliospores significantly.



Related publications

1. Corresponding author. Development of real-time PCR and droplet digital PCR based marker for the detection of Tilletia caries inciting common bunt of wheat, Frontiers in Plant Science 2022, 13, 1031611
2. Corresponding author. Development of the droplet digital PCR to detect the teliospores of Tilletia controversa Kühn in the soil with greatly enhanced sensitivity. Front. Microbiol 7, 2020, 11, 1–9.
3. Corresponding author. Development of droplet digital PCR for the detection of Tilletia laevis, which causes common bunt of wheat, based on the SCAR marker derived from ISSR and real-time PCR. Scientific Reports. 2020,10(1):16106.
4. Corresponding author. Development of ISSR-derived SCAR marker and SYBR Green I Real-time PCR method fordetection of teliospores of Tilletia laevis Kühn. Scientific Reports. 2019, 9(1):17651.
5. Corresponding author. Detection of Tilletia controversa using immunofluorescent monoclonal antibodies. Journal of Applied Microbiology, 2015, 118(2), 497-505
6. Corresponding author. Development of a SCAR marker for molecular detection and diagnosis of Tilletia controversa Kühn, the causal fungus of wheat dwarf bunt. World Journal of Microbiology and Biotechnology. 2014, 30(12):3185-95
7. First author. Development of a sequence-characterized amplified region marker for diagnosis of dwarf bunt of wheat and detection of Tilletia controversa Kühn. Letters in Applied Microbiology, 2009, 49(2), 235–240
8. First author. An ISSR-based approach for the molecular detection and diagnosis of dwarf bunt of wheat, caused by Tilletia controversa Kühn. Journal of Phytopathology, 2011, 159 (3), 155-158
9. First author. Development of a SCAR marker by inter-simple sequence repeat for diagnosis of dwarf bunt of wheat and detection of Tilletia controversa Kühn. Folia Microbiologica, 2010, 55(3), 258-264

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RESEARCH ARTICLE

Microbiome Signature of Endophytes in Wheat Seed Response to Wheat Dwarf Bunt Caused by *Tilletia controversa* Kühn

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Characterization of Rhizosphere Microbial Communities for Disease Incidence and Optimized Concentration of Difenoconazole Fungicide for Controlling of Wheat Dwarf Bunt

OPEN ACCESS

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Thank you!