Characterization of Cassava Witches Broom Disease in Lao PDR

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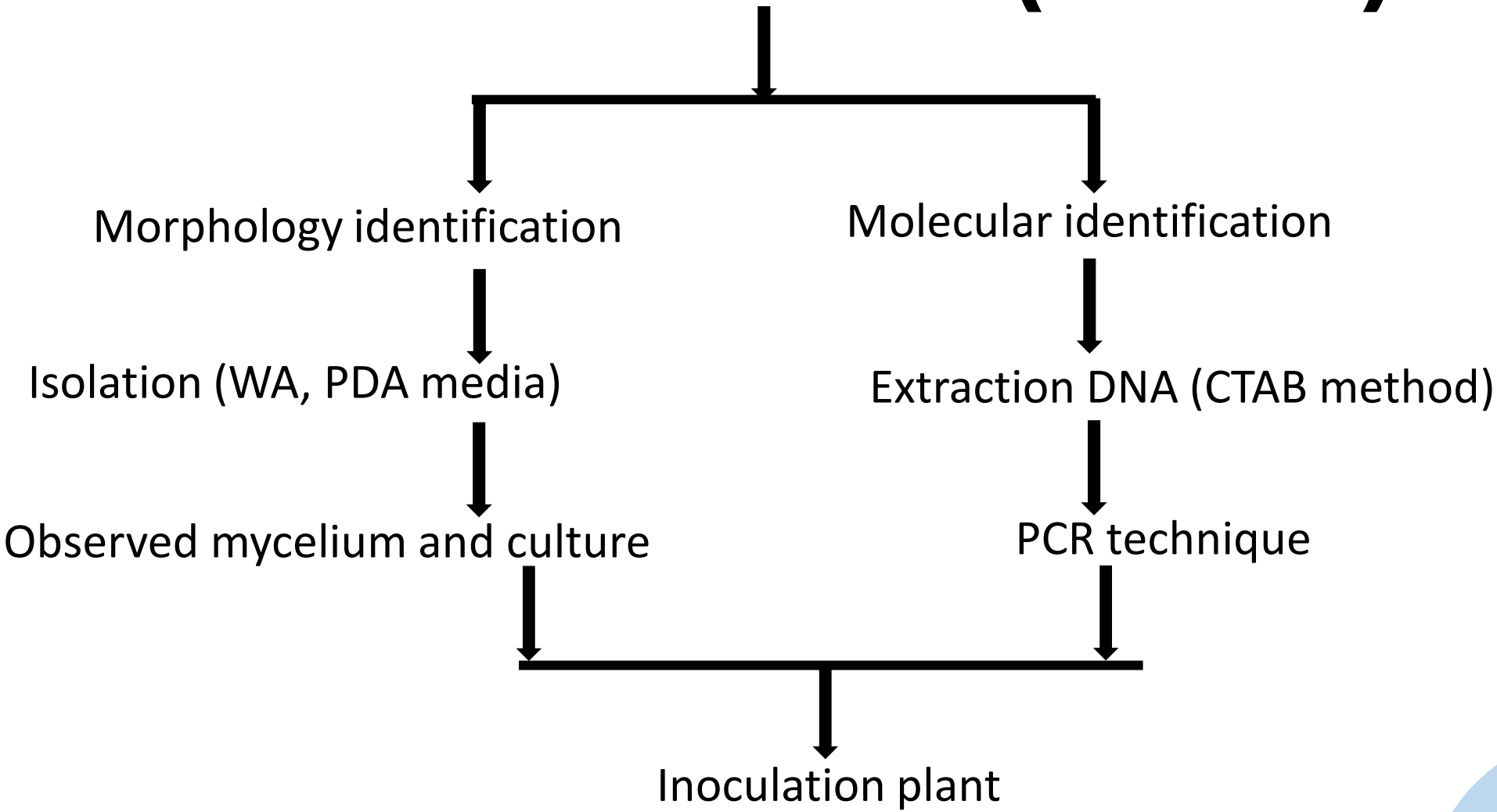
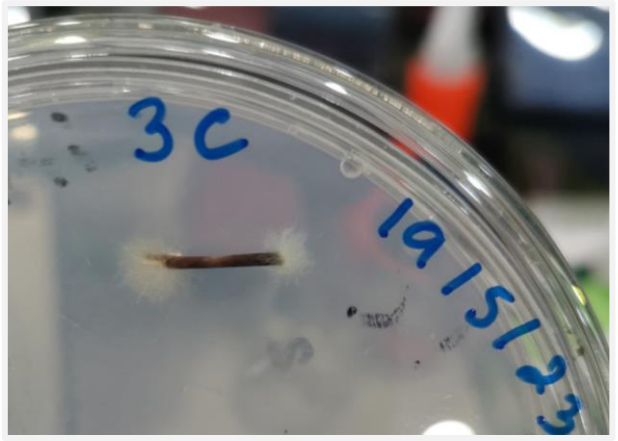
Characterization (CWBD)



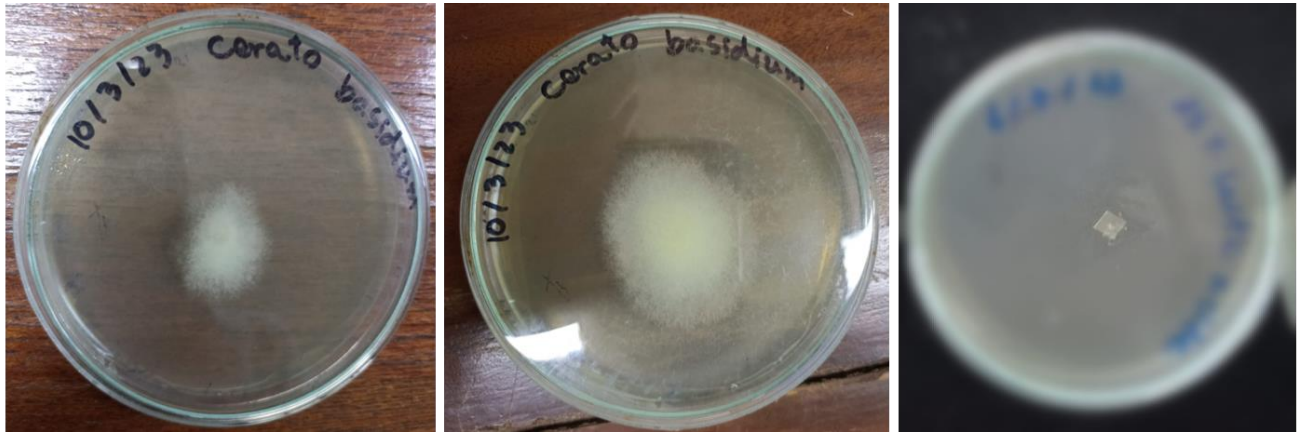
Morphology identification (**CWBD Isolation)**

Cerato inoculum production



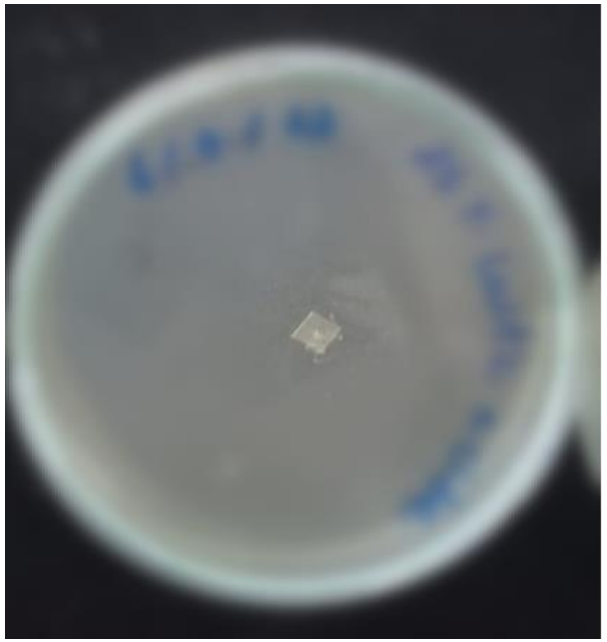
Isolation on WA 3-5 days
Using petiole sterilize on 3% hypochlorite

31/3/2023 10/4/2023 19/5/2023

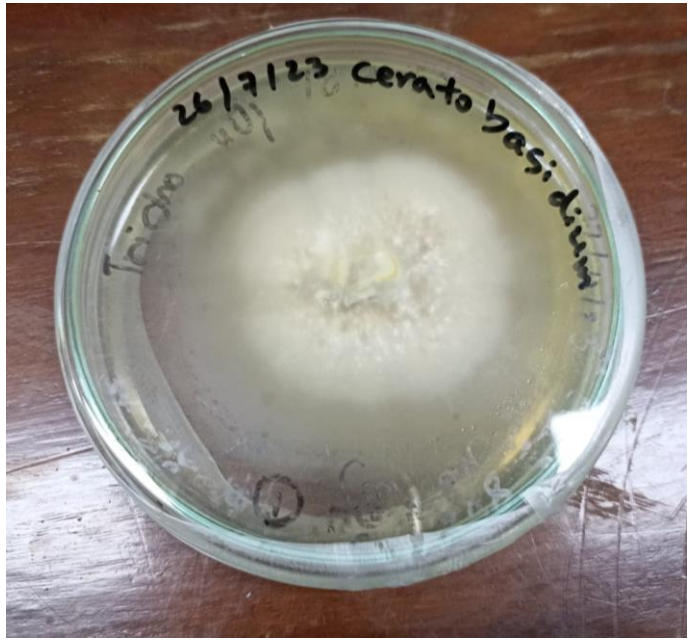


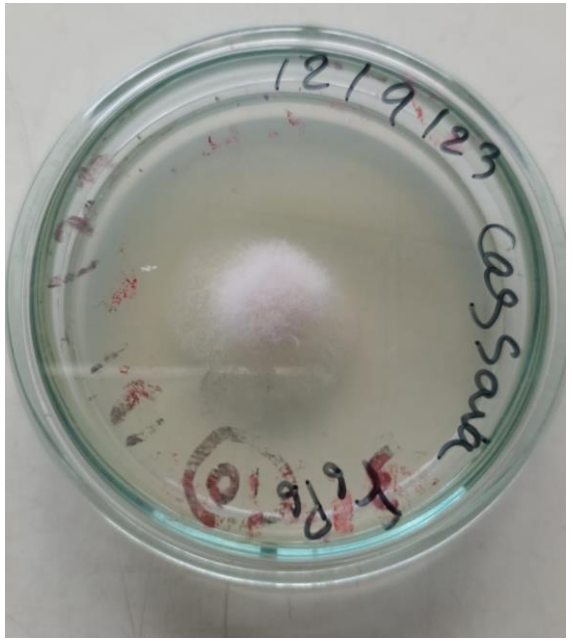
Wooly yellow isolate from KU 50

Major drawbacks: Contamination, slow growth, and lack of sporation

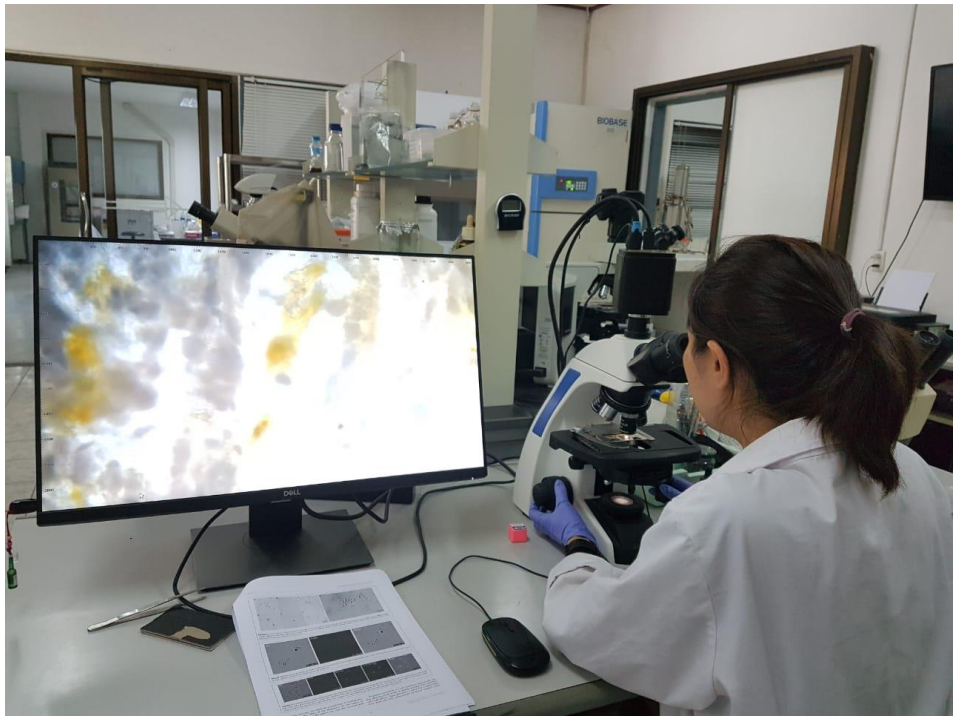


Stops growing after several subculturing





Contaminated culture



Observation mycelium on Microscope

Any fungal structures on infected plants?

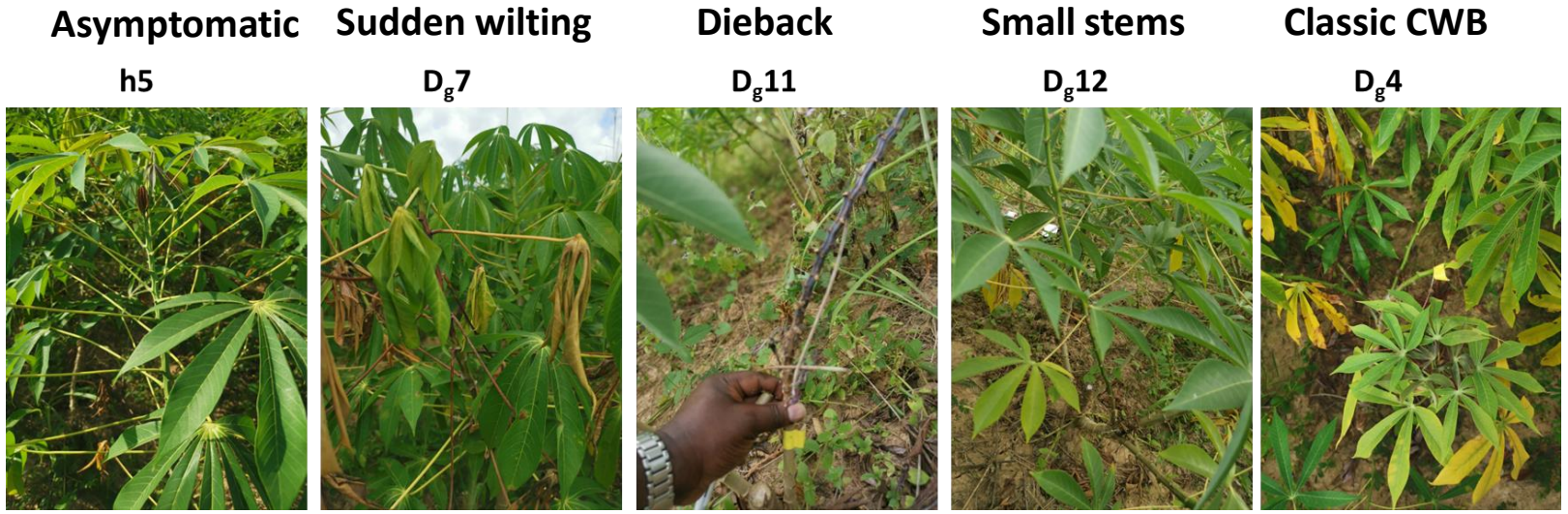
Protocol for stem vascular tissues and discrimination of symptoms

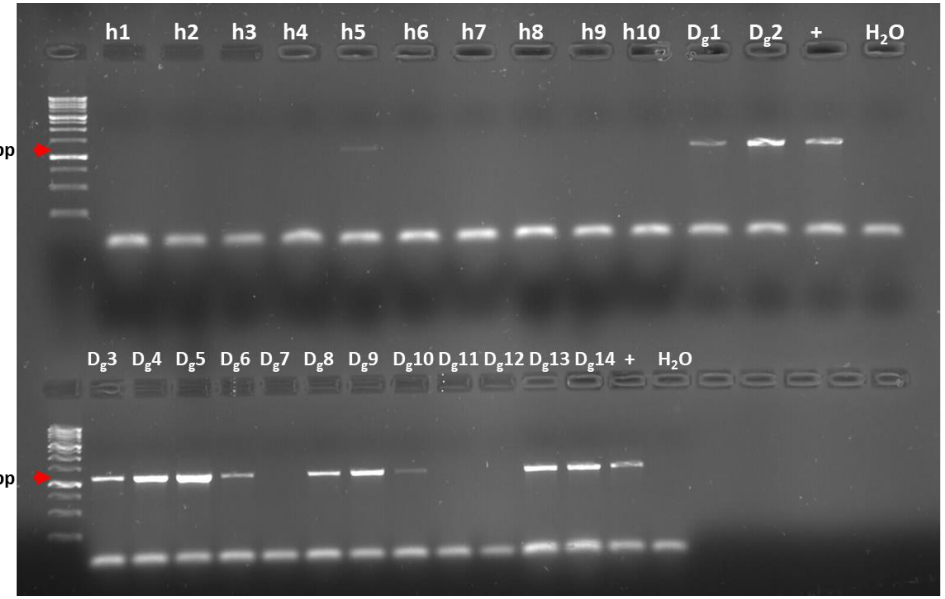
1. Sampled cassava of different ages with/without CWB and other symptoms

2. Collect 0.3g of vascular tissue
3. Extracted DNA using CTAB with modification

4. Quantify DNA and standardize to 60ng/ul

5. PCR with 1ul





Is Ceratobasidium associated with CWBD?

Laos

Phenotype	Number of samples	PCR (-)	PCR (+)
Asymptomatic	123	122	1
Classic witches broom	41	1	40
Other symptoms	3	0	0
Unclear symptoms	4	0	0
Total	**171**	**123**	**41**

Note: These are fresh samples!

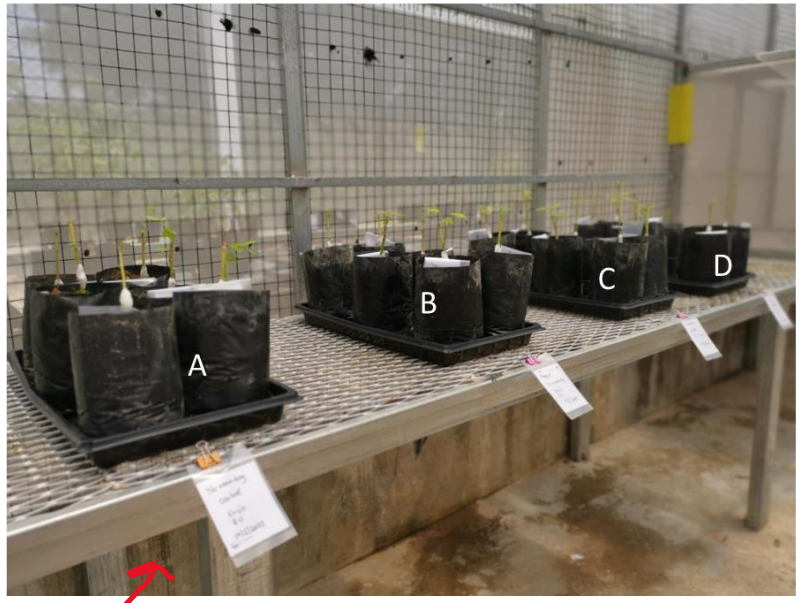
Ongoing work= transmission

Different methods

- mycelium culture

This was three-month culture

Treatments
A. No Wounding + PDA-CCM agar; **B**. No Wounding +Fungal mycelium
C. Wounding + PDA-CCM agar; **D**. Wounding +Fungal mycelium
2 Varieties: KU 50 and Rayong 11



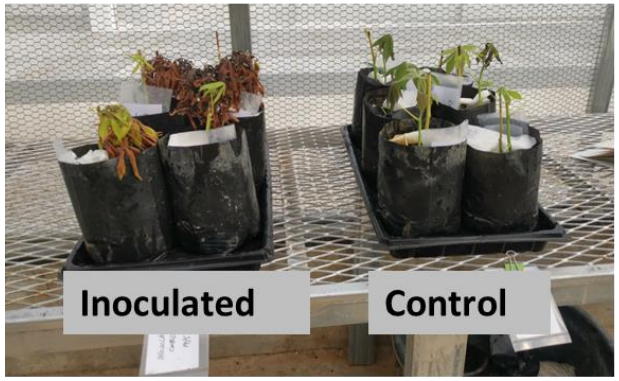
Point of inoculation. The site was kept moistened with water-soaked sterile cotton wool. Inoculum fastened with parafilm

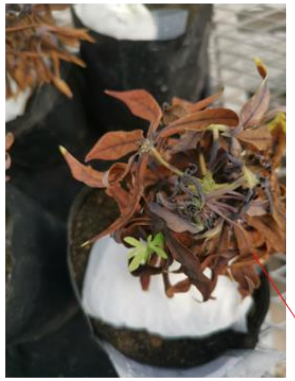
Not successful with no wounding

With wounding 1/6 wilted after 9 days

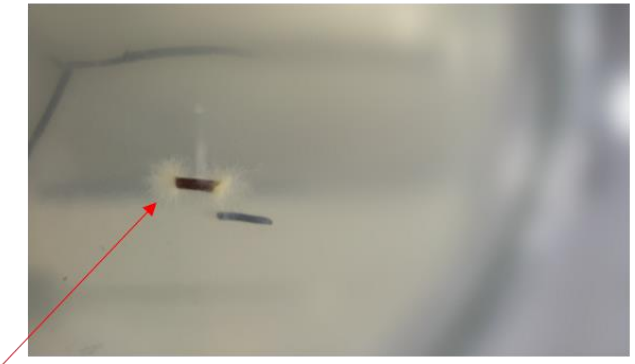
Enclose health plants together with infected plants

Inoculate → Humidify → Normalize and observe





Inocula isolated for confirmation



2/6 infection rate, but wilted suddenly!

Identify

An improved method of the above is ongoing the greenhouse.

Summary

- ✓ We have a good marker for detecting CWBD using PCR
- ✓ Pureculture of a fungus isolated, although it grows slowly and does not sporulate.
- ✓ Inoculation studies using different methods ongoing
- ✓ We now have a clear under of different CWBD symptoms

Next steps

- **Continue with the optimization of CWBD transmission**
- **Diversity of fungus across regions, different crops including weeds**
- **Chemical and biocontrol**
- **Check for resistant varieties.**
- **Farmer sensitization**


Thank you