Comparison of three DNA extraction methods suitable for PCR-based detection of *Acidovorax citrulli* in watermelon and melon seeds

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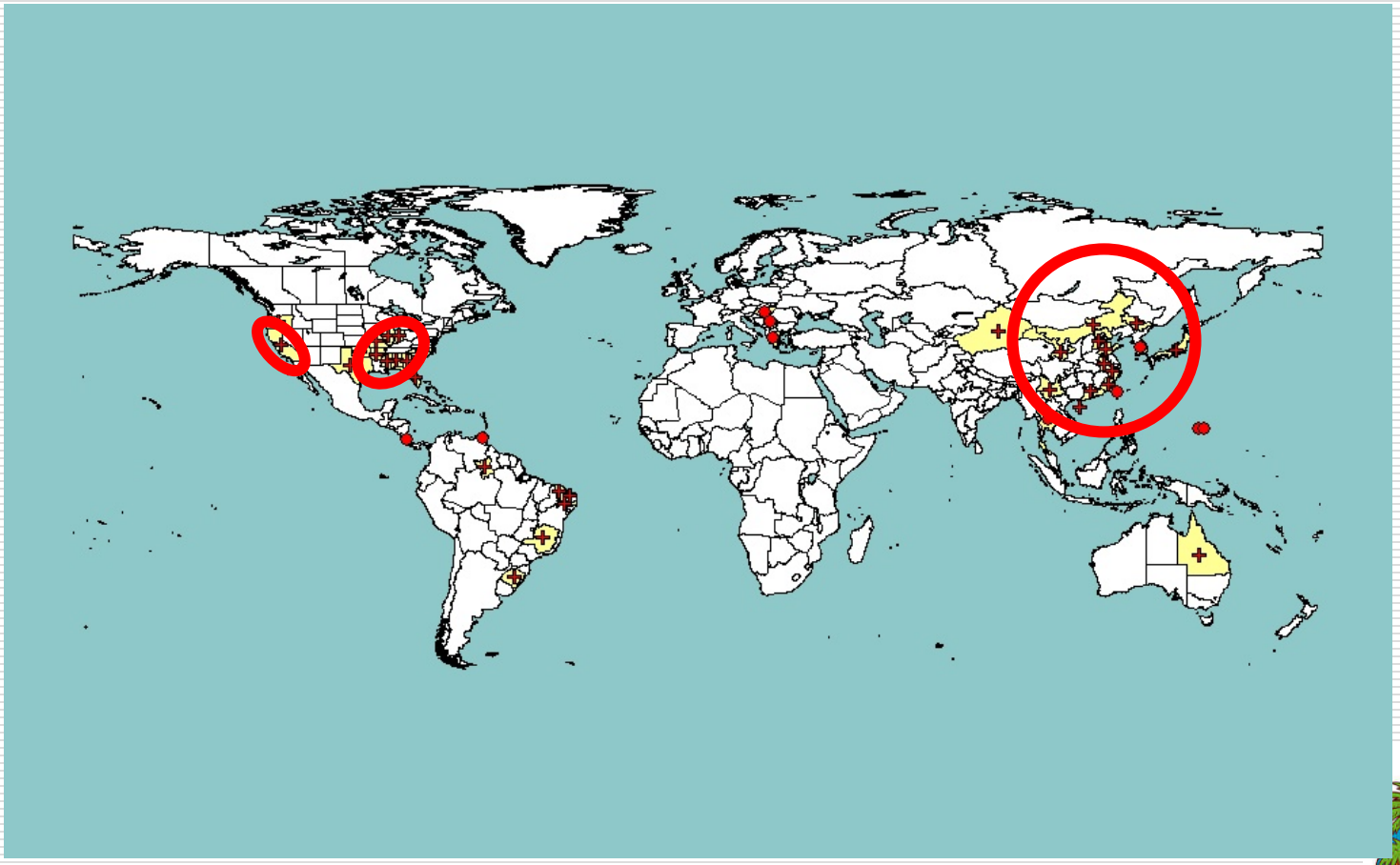
The disease

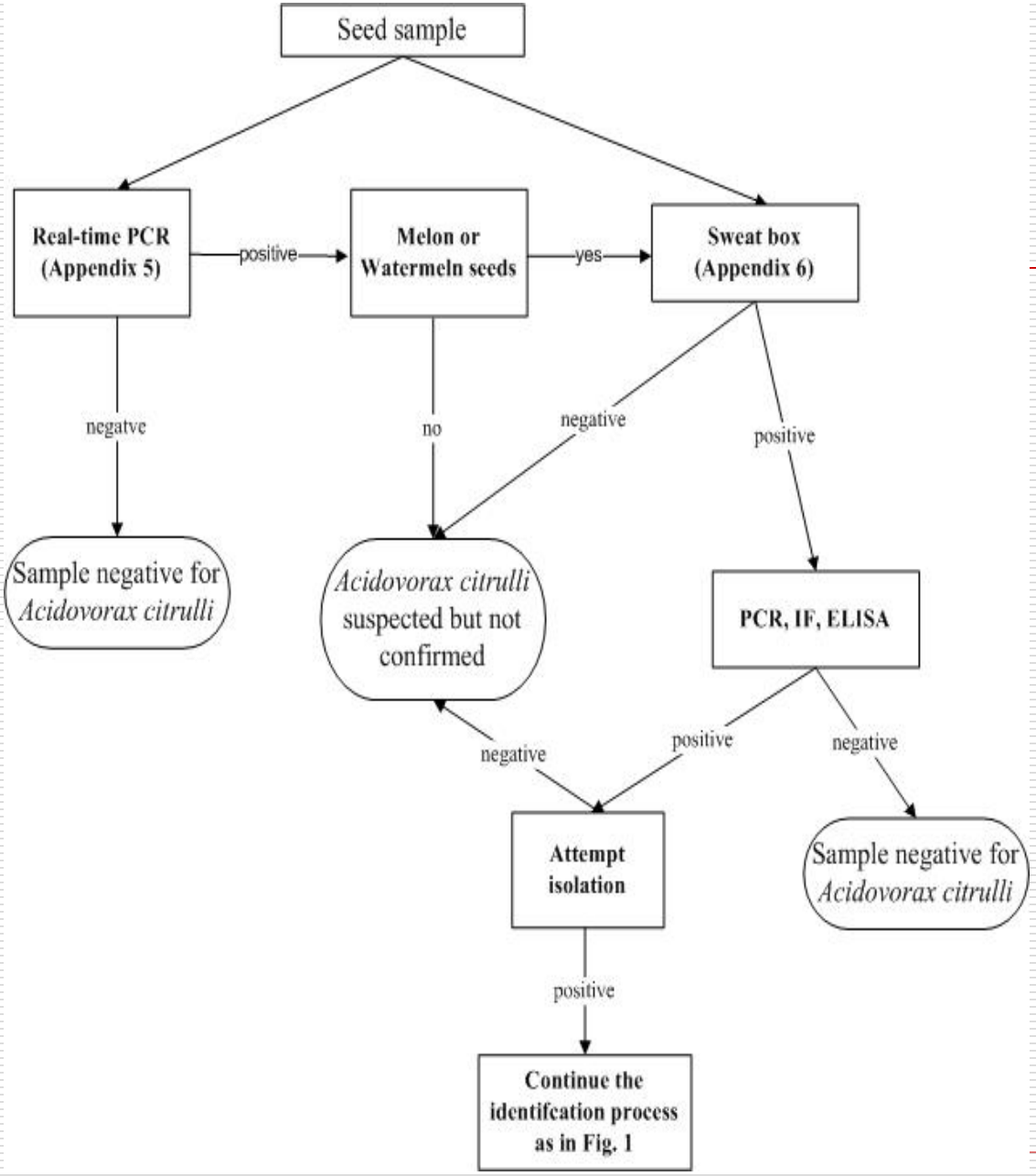
- The bacterial fruit blotch (BFB) is the most severe bacterial disease of cucurbts.
- It mainly affects watermelon, but epidemics have been reported on melon and cantaloupe as well.
- It's an emerging disease in the Mediterranean.

The causal agent

- The BFB is caused by the Gram-negative rod *Acidovorax citrulli*
- It is in the EPPO A1 list
- Regulated in Turkey (A2)
- Quarantine pest in Israel
- *Acidovorax citrulli* is <u>seed transmitted</u>
  - Pathogen location: perisperm/endosperm and embryo

EPPO distribution map





The EPPO diagnostic protocol (draft)

Aim of our studies

- Implement extraction of the pathogen from seeds
- Compare three DNA extraction kits in order to ensure/improve the performance of PCR protocols

Material and Methods

- Watermelon and Cantaloupe seeds:
  - (5.000, different contamination rates)
- Two seed treatments:
  - Seed overnight soaking in PBST buffer
  - Seed crushing by hammering
- Three DNA extraction and purification methods:
  - DNeasy Plant Mini Kit (Qiagen)
  - DNeasy Blood and Tissue (Qiagen)
  - Wizard Magnetic 96 DNA Plant System (Promega)

Material and Methods

- Three seed inoculation rate:
  - 1:10, 1:100, 1:1000
- Two simplex-PCR protocols compared:
  - Primer pair Seq3/Seq4 (Schaad *et al*., 2000)
  - Primer pair WFB1/WFB2 (Walcott *et al*., 2000)
  - NIC, NAC, PIC, PAC

- *All experiments were repeated three times, in different days and by different operators*

Results: cantaloupe

		Dneasy® Plant mini Kit (QIAGEN)		Dneasy® Blood & Tissue (QIAGEN)		Wizard® Magnetic 96 Plant System (Promega)	
Host	**Primer Pairs**	***soaking***	***hammering***	***soaking***	***hammering***	***soaking***	***hammering***
Melon	***Seq3/Seq4***	**1:1000**	**1:1000**	1:1000	1:100	1:1000	1:100
	WFB1/WFB2	**1:1000**	**1:1000**	1:1000	1:1000	1:1000	1:100

Results: watermelon

		Dneasy® Plant mini Kit (QIAGEN)		Dneasy® Blood & Tissue (QIAGEN)		Wizard® Magnetic 96 Plant System (Promega)	
Host	**Primer Pairs**	***soaking***	***hammering***	***soaking***	***hammering***	***soaking***	***hammering***
Watermelon	***Seq3/Seq4***	**1:1000**	**1:1000**	Negative	1:10	Negative	Negative
	WFB1/WFB2	**1:1000**	**1:1000**	Negative	1:1000	1:1000	1:100

Results: summary

		Dneasy® Plant mini Kit (QIAGEN)		Dneasy® Blood & Tissue (QIAGEN)		Wizard® Magnetic 96 Plant System (Promega)	
Host	**Primer Pairs**	***soaking***	***hammering***	***soaking***	***hammering***	***soaking***	***hammering***
Melon	***Seq3/Seq4***	**1:1000**	**1:1000**	1:1000	1:100	1:1000	1:100
	WFB1/WFB2	**1:1000**	**1:1000**	1:1000	1:1000	1:1000	1:100
Watermelon	***Seq3/Seq4***	**1:1000**	**1:1000**	Negative	1:10	Negative	Negative
	WFB1/WFB2	**1:1000**	**1:1000**	Negative	1:1000	1:1000	1:100

Discussion and conclusion

- Seed treatment prior to analysis:
  - Overnight soaking performed better than crushing any single seed (approx. 1 hour time required for 5000 seeds)
- DNA extraction and purification:
  - Better results with DNeasy Plant Mini Kit (Qiagen)
- Simplex-PCR:
  - WFB1/WFB2 primers more sensitive

Discussion and conclusion

- Melon seeds easier to analyse than watermelon seeds
  - Inhibitors
  - Seed size
- Analytical procedure showed same sensitivity as Real-Time PCR
  - Developed by NAKT, validated in TESTA)
- Validation in progress


Thank you for your attention!