10.1071/CP24053

Crop & Pasture Science

Supplementary Material

A novel *Leifsonia xyli* subsp. *xyli* quantitative LAMP-based diagnostic correlated with sugarcane ratoon stunting disease rating

Moutoshi Chakraborty[A,B,*], *Shamsul A. Bhuiyan*[C,D], *Simon Strachan*[B,C], *Sithi Saha*[D], *Rabbee G. Mahmudunnabi*[B,C], *Nam-Trung Nguyen*[C], *Muhammad J. A. Shiddiky*[B,C,E], *and Rebecca Ford*[A,B]

[A]Centre for Planetary Health and Food Security (CPHFS), Nathan Campus, Griffith University (GU), Nathan, Qld 4111, Australia.

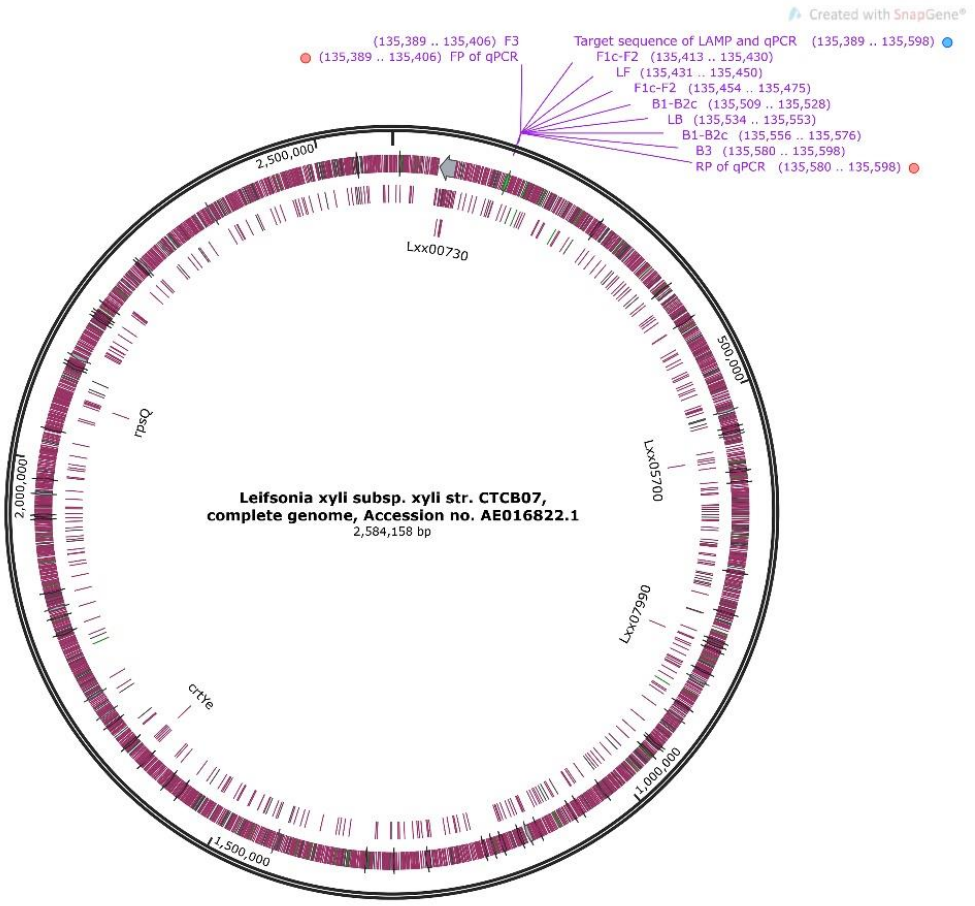
[B]School of Environment and Science (ESC), Nathan Campus, Griffith University, Nathan, Qld 4111, Australia.

[C]Queensland Micro and Nanotechnology Centre (QMNC), Nathan Campus, Griffith University, Nathan, Qld 4111, Australia.

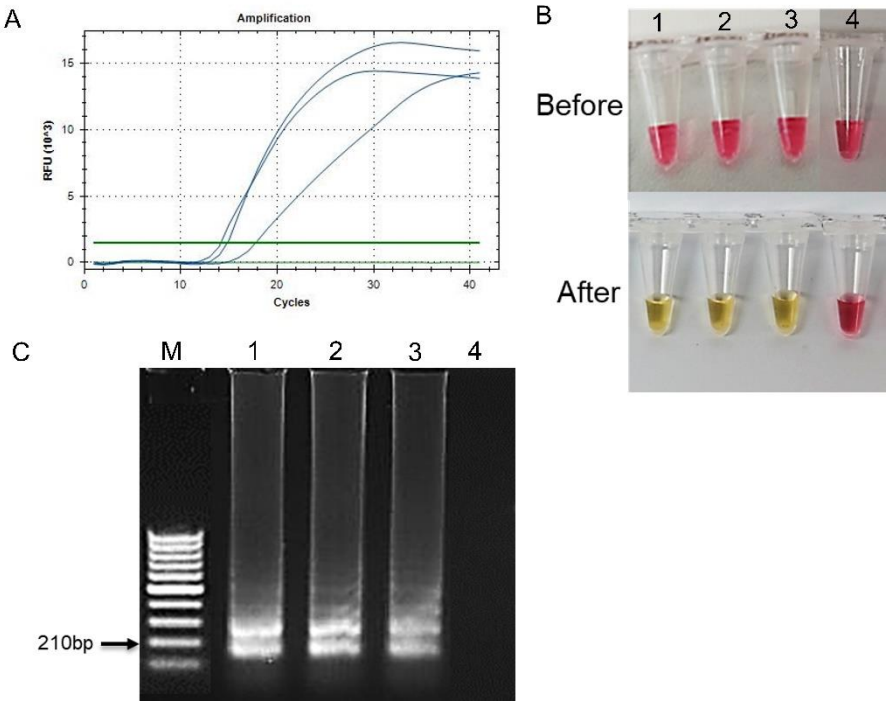
[D]Sugar Research Australia (SRA), 90 Old Cove Road, Woodford, Qld 4514, Australia.

[E]Rural Health Research Institute (RHRI), Charles Sturt University, Orange, NSW 2800, Australia.

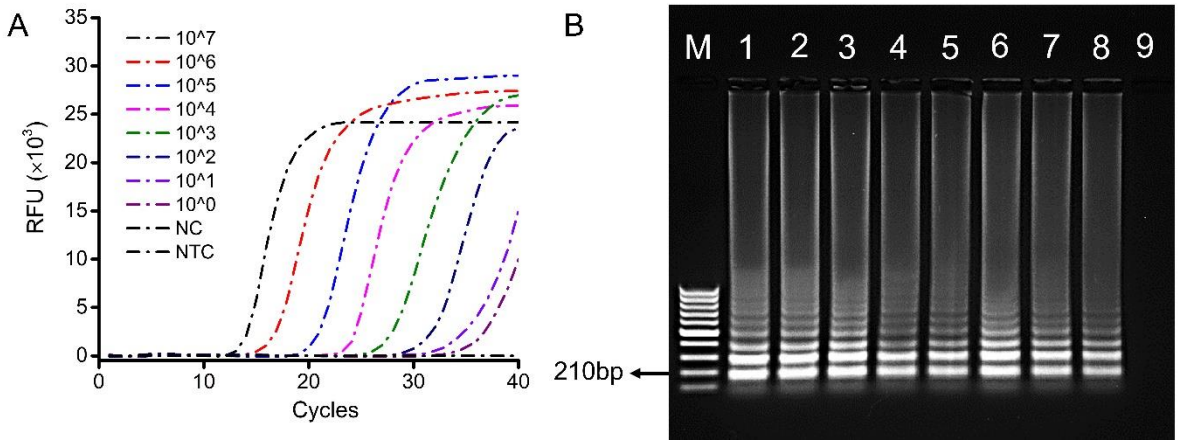
[*]Correspondence to: Moutoshi Chakraborty Centre for Planetary Health and Food Security (CPHFS), School of Environment and Science (ESC), Nathan Campus, Griffith University, Nathan, Qld 4111, Australia Email: moutoshi.chakraborty@griffithuni.edu.au



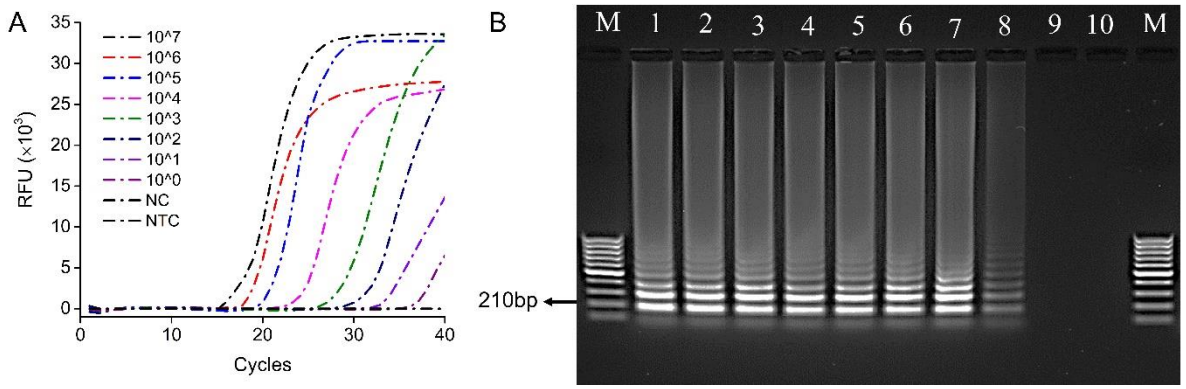
Supplementary Figure S1: SnapGene view of Selected 210bp conserved section of the intergenic spacer (IGS) region between 16S and 23S rRNA genes (GenBank accession no. AE016822.1) corresponding to positions 1,35,389–1,35,598 in *Leifsonia xyli* subsp. *xyli* strain CTCBO7 complete genome along with designated LAMP and qPCR primers.



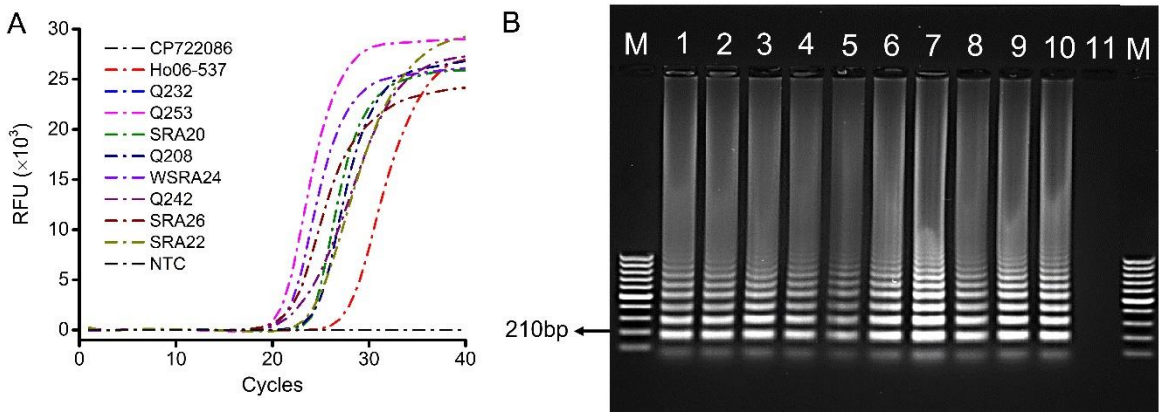
Supplementary Figure S2: LAMP primer pairs checking with synthetic target. (*a*) Fluorescence LAMP detection for known concentration of synthetic target (10^7 copies/μL or 10 pg/μL); (*b*) Colorimetric detection of the sample. (*c*) Agarose gel electrophoresis of the sample. Lane M: 100bp$^+$ marker; Lanes 1 to 3: 10^7 copies/μL (10pM) of synthetic target; Lane 4: No Target Control (NTC).



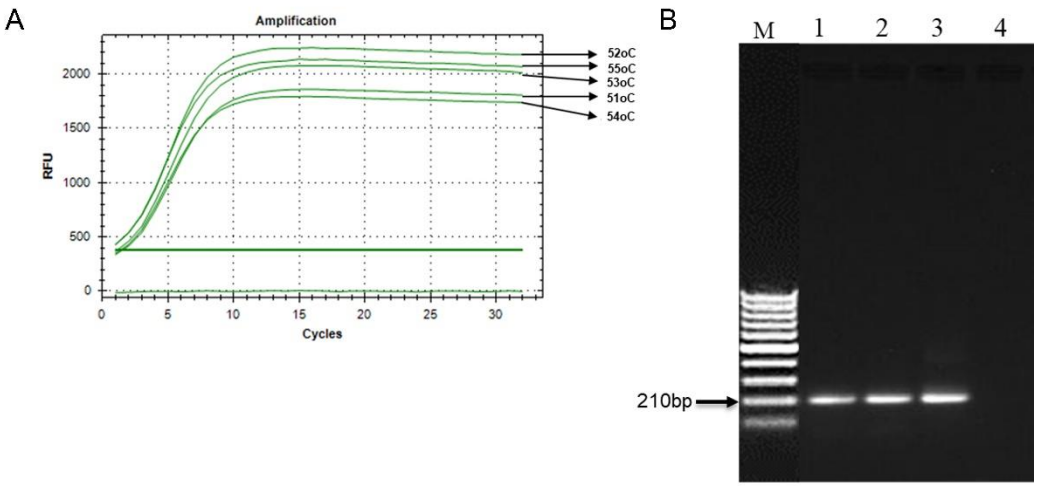
Supplementary Figure S3: (*a*) Florescent LAMP detection and (*b*) Agarose gel electrophoresis of LAMP amplified products for designated concentrations of synthetic target. Lane M: 100bp+ marker; Lanes 1 to 8: 1:10 dilutions of synthetic target (10^7-10^0 copies/μL, or 10 pg/μL-1ag/μL); Lane 9: No Target Control (NTC).



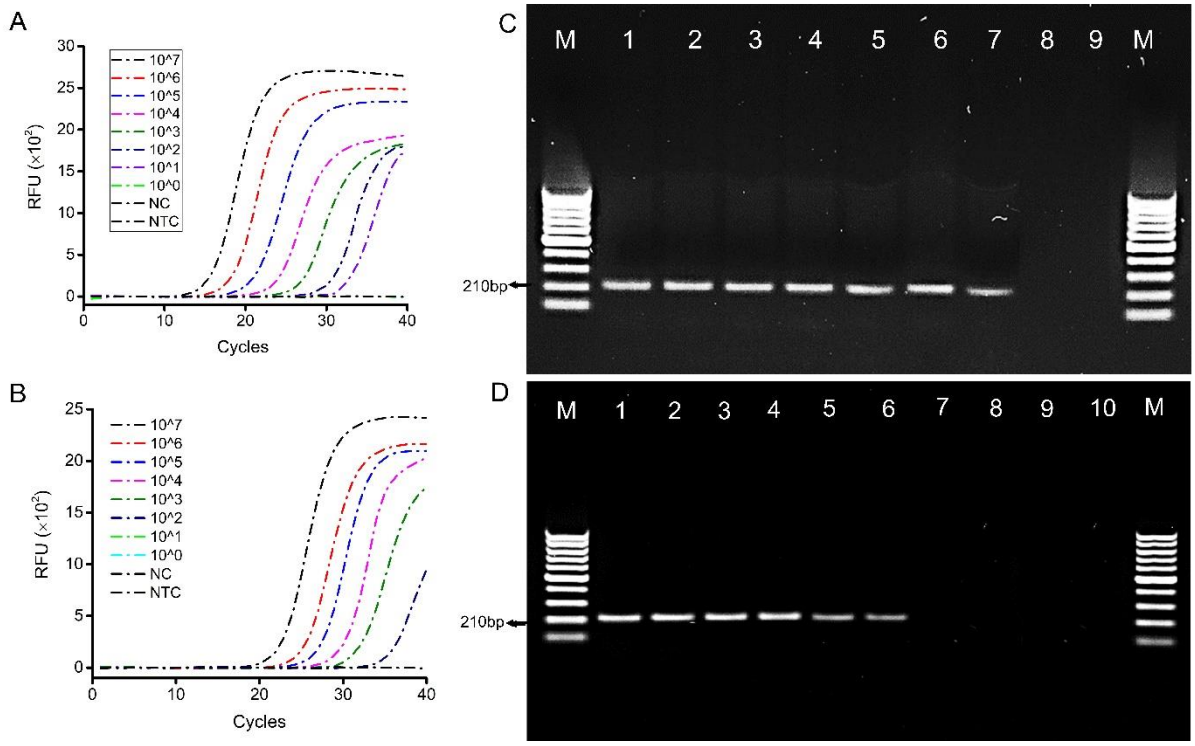
Supplementary Figure S4: (*a*) Florescent LAMP detection and (*b*) Agarose gel electrophoresis of LAMP amplified products for designated number of *Lxx* cells (10^7-10^0 cells/μL) spiked in fresh xylem sap. Lane M: 100bp+ marker; Lanes 1 to 8: 1:10 dilutions of spiking *Lxx* cells (10^7-10^0 cells/μL); Lane 9: No Target Control (NTC); Lane 10: Known number of *Xalb* cells (10^7 cells/μL).



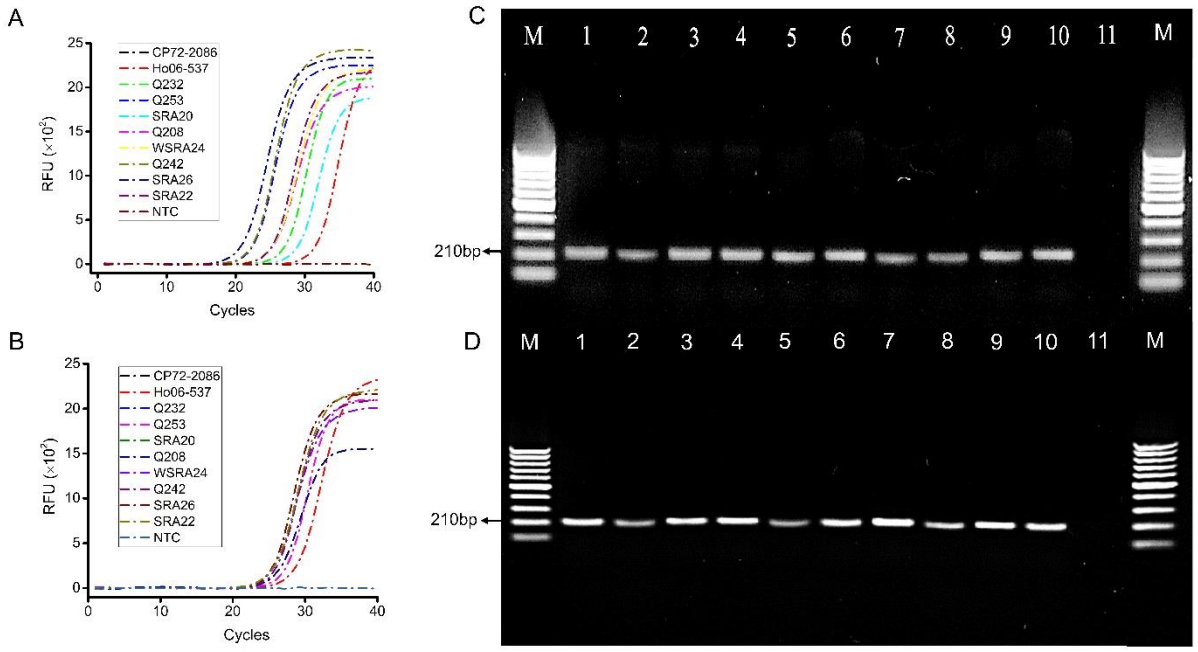
Supplementary Figure S5: Field application of assay. (*a*) Florescent LAMP detection and (*b*) Agarose gel electrophoresis of LAMP amplified products for all the xylem sap samples collected from SRA Woodford RSD screening trials. Lane M: 100bp+ marker; Lanes 1 to 10: RSD-infected xylem sap samples- CP72-2086, Ho06-537, Q232, Q253, SRA20, Q208, WSRA24, Q242, SRA26, and SRA22; Lane 11: No Target Control (NTC).



Supplementary Figure S6: qPCR primer pair checking with synthetic target. (*a*) Fluorescence qPCR detection of known concentration of synthetic target; (*b*) Agarose gel electrophoresis of the amplified products. Lane M: $100bp^{+}$ marker; Lanes 1 to 3: 10^{10} copies/μL (10 ng/μL) of synthetic target; Lane 4: No Target Control (NTC).



Supplementary Figure S7: Validation of the assay using qPCR. Fluorescence qPCR detection for (*a*) Designated concentrations of synthetic target (10^7-10^0 copies/μL, or 10 pg/μL-1ag/μL); (*b*) Purified DNA extracted from known number of *Lxx* cells spiked in the fresh xylem sap (10^7-10^0 cells/mL). Agarose gel electrophoresis of the amplified products of (*c*) Designated concentrations of synthetic target; Where, Lane M: 100bp+ marker; Lanes 1 to 8: Serial dilutions of synthetic target (10^7-10^0 copies/μL, or 10 pg/μL-1ag/μL); Lane 9: No Target Control (NTC); (d) Purified DNA extracted from a known number of *Lxx* cells spiked in the fresh sap (10^7-10^0 cells/mL). Where, Lane M: 100bp+ marker; Lanes 1 to 8: Purified DNA extracted from known number of *Lxx* cells spiked in the fresh sap (10^7-10^0 cells/mL); Lane 9: No Target Control (NTC); Lane 10: Negative control (NC).



Supplementary Figure S8: Field sample validation with qPCR. Fluorescence qPCR detection for all the analyzed xylem sap samples collected from SRA Woodford RSD screening trials; (*a*) Heat-induced DNA isolation technique; (*b*) Commercial kit-based DNA extraction technique. Agarose gel electrophoresis of qPCR amplified products. Where, Lane M: 100bp+ marker; Lanes 1 to 10: RSD-infected xylem sap samples- CP72-2086, Ho06-537, Q232, Q253, SRA20, Q208, WSRA24, Q242, SRA26, and SRA22; Lane 11: No Target Control (NTC).