

Supplementary information

A DNA Adsorption-Based Biosensor for Rapid Detection of Ratoon Stunting Disease in Sugarcane

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Supplementary Table and Figures

Table S1. Details of the probe and primer sets designed for developing *Lxx*-specific EC and qPCR assay.

Synthetic Target and Primers Name	Sequence (5'-3')	Length (nt)	GC (%)
<i>Lxx</i> _EC_STS	GCTCGAACTTAGTACGCCTGCTTGCAGGAAGGAACAGTTCGG ACCGGGGAGCCTCGCACATGCACGCTGTTGGGTCTGAGGGA CCGGACCTCATCGCTGTGTCTTCAAGACGCTGAGATGAGAAC CGAATCC	133	58.6
<i>Lxx</i> CP1	GGATTTCGGTTCTCATCTCAGCGTCTTGAAGACAC/Bio/	34	50
<i>Lxx</i> _EC_FP	GCTCGAACTTAGTACGCCTG	20	55
<i>Lxx</i> _EC_RP	GGATTTCGGTTCTCATCTC	18	50

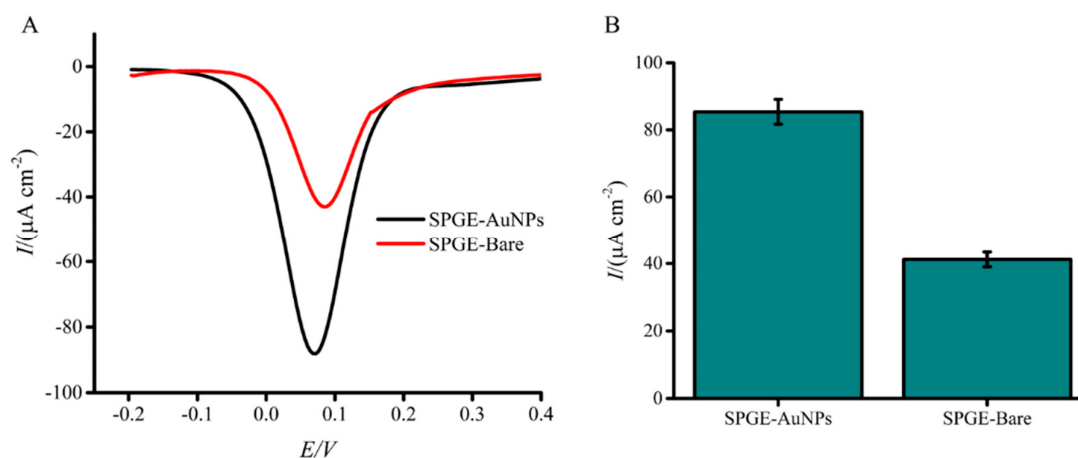


Figure S1. Optimization of AuNP adsorption on SPGEs. (A) DPVs comparing the electrochemical response of a bare electrode (without AuNPs) and an AuNP-modified electrode. (B) Average

percentage change in current response between the bare and AuNP-modified electrodes, indicating enhanced signal upon AuNP modification. Error bars represent the standard deviation from three independent measurements.

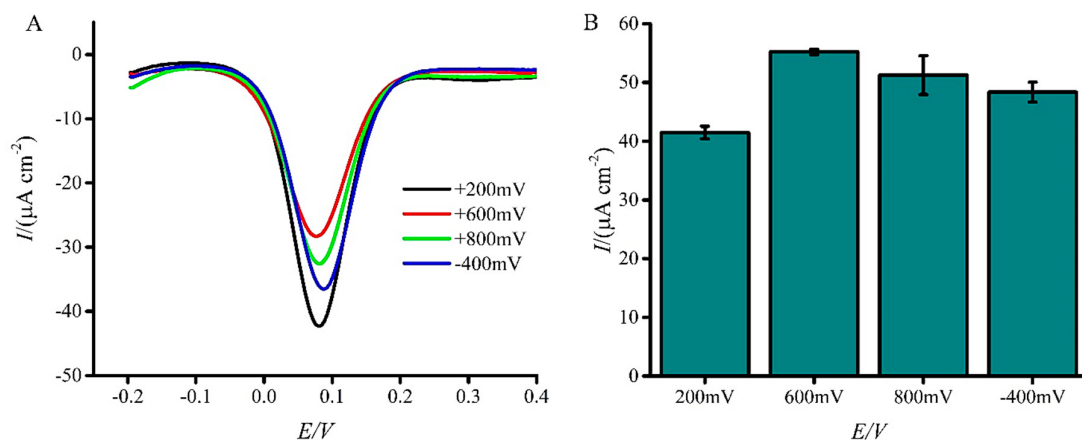


Figure S2. Optimization of the adsorption potential for DNA immobilization on gold-modified electrodes. (A) DPVs showing the electrochemical response of electrodes following DNA adsorption at four different potentials: +200 mV, +600 mV, +800 mV, and -400 mV (vs. Ag/AgCl). (B) Average percentage change in current response corresponding to each adsorption potential, highlighting the influence of potential on DNA-gold interaction efficiency. Error bars represent the standard deviation from three independent experiments.

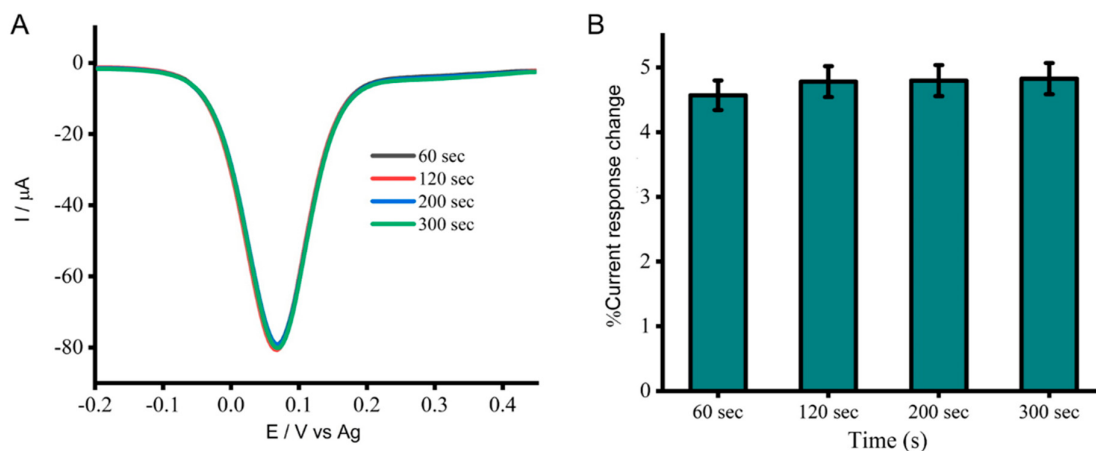


Figure S3. Optimization of DNA adsorption time on gold-modified electrodes.

(A) DPVs recorded after DNA immobilization on gold-modified electrodes for varying durations: 60, 120, 200, and 300 seconds. (B) Average percentage change in current response corresponding to each adsorption time, illustrating the effect of incubation time on DNA-gold binding efficiency. Error bars represent the standard deviation from three independent experiments.

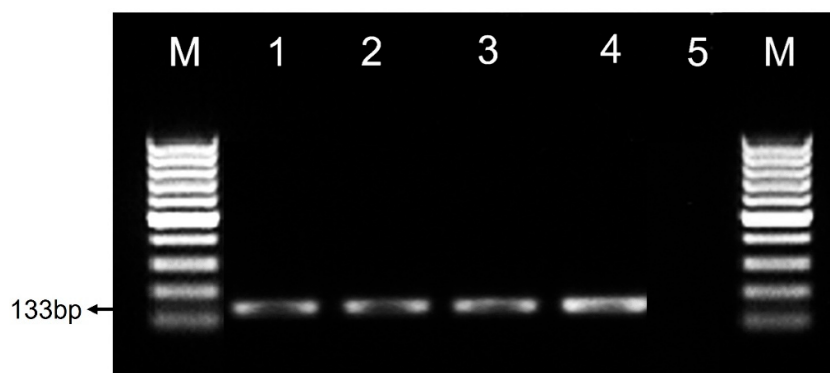


Figure S4. Agarose gel electrophoresis of qPCR-amplified products from varying concentrations of *Lxx* cells. Lane M: 100 bp DNA ladder (marker); Lanes 1–4: qPCR products obtained from samples containing 10^2 to 10^5 *Lxx* cells/ μ L, respectively; Lane 5: no target control (NTC), showing absence of amplification in the negative control.

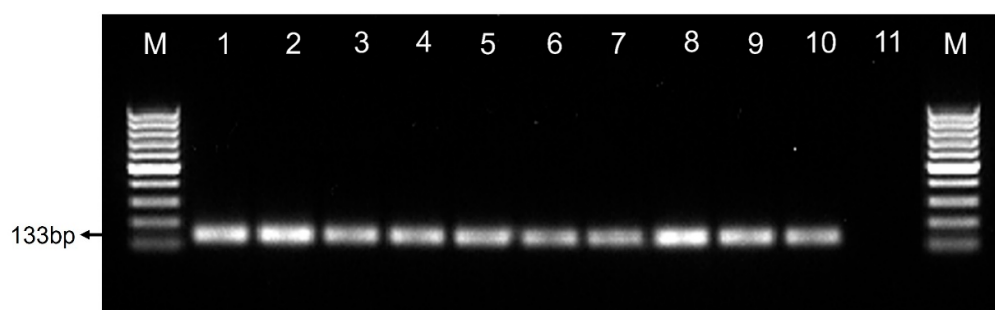


Figure S5. Detection of *Lxx* in ratoon-stunting disease-infected sugarcane samples using qPCR and agarose gel electrophoresis. Lane M: 100 bp DNA ladder (marker); Lanes 1–10: qPCR-amplified products from xylem sap of RSD-infected sugarcane cultivars—Ho06-537, CP72-2086, Q208, WSR24, SRA22, Q242, SRA26, Q232, SRA20, and Q253, respectively; Lane 11: no target control (NTC), confirming the absence of non-specific amplification.