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A 3D-printed handheld device for quick citrus tissue lysis and nucleic acid extraction

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ABSTRACTS

A 3D-printed handheld device has been developed for rapid and efficient sample preparation, extracting pathogen total nucleic acids from citrus leaves for downstream molecular analysis. With its high-speed motor, knurled lysis chamber for rapid sample lysis, and quick nucleic acid extraction using cellulose paper disks (Whatman CHR1), this device can yield ready-to-use DNA and RNA in just 12 min. All components, except for the motor, wiring, and paper disks, were printed in-house using a PolyJet 3D printer with photosensitive resins. The device was optimized for maximum sample lysis by evaluating operation voltages and chamber features, as measured by DNA and RNA concentrations using a Qubit fluorometer and quantification cycle (Cq) values obtained through qPCR assays. The results showed that the lysis chamber with internal knurling and the motor operated at 7.5 V was sufficient for effective sample lysis in 1 min, achieving RNA concentrations up to 87.6 % of those obtained with mortar-and-pestle grinding. Paper disk washing and elution conditions were also optimized using a NanoDrop spectrophotometer and qPCR assays, where two 1-minute washes and a 100 μL elution volume resulted in the highest purity and lowest Cq value. The optimized handheld device was validated with citrus sources infected with *Citrus tristeza virus* (CTV) and *Spiroplasma citri* (*S. citri*), demonstrating high detection accuracy (100 %) and low assay variation (CV < 3.8 %) in qPCR-based analysis. Based on these successful results, this device is expected to be broadly applicable to similar viral and bacterial pathogens affecting plants.

1. Introduction

Plant epidemics have long been a problem affecting crop yields and the global economy, and the emergence of novel pathogenic variants and their disease synergism makes disease management even more challenging [1]. Destructive plant diseases lead to global crop yield losses of up to 40 % for maize, potato, rice, soybean, and wheat, amounting to annual financial losses of $220 billion USD [2,3]. According to the UN's 2024 Global Report on Food Crises, almost 282 million people across 59 countries/territories are now experiencing acute food insecurity, which has been increasing yearly since 2019 [4]. Crop loss due to extreme climate and plant pathogens will further exacerbate affected regions' environmental and socio-economic conditions and disproportionately affect food-insecure populations [3,5]. Take citrus, one of the most common cash crops in the world, as an example. As a mainstay of agricultural industries in many countries, the US, for example, has produced 4.9 million tons in the 2022–2023 growing season, accounting for $2.58 billion USD [6]. However, huanglongbing (HLB), a destructive citrus disease with rapid disease progression, has severely reduced citrus yields due to the early abortion of fruits from affected branches [7]. In Florida, the first state in the US to report HLB, citrus yields have been reduced by 80 %, with tens of billions of USD of economic losses over the past two decades [8,9]. Detecting diseases in their early stages is of great importance for growers to prevent disease outbreaks and successfully adopt control strategies.

Current strategies for disease management rely on sensitive and high-throughput diagnostic methods, such as high-throughput sequencing (HTS), third-generation sequencing (TGS), nanopore sequencing, and quantitative polymerase chain reaction (qPCR) to precisely identify diseased trees [10–12]. These methods are then followed

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by risk-reducing measures, including the removal of the diseased plants and subsequent vector management [1]. However, this practice is insufficient for disease control because these sensing techniques require high-quality samples and are costly, time-consuming, and require trained personnel [13]. In particular, tedious specimen collection, packaging, and submission in the field by growers prior to sample preparation in labs is usually required [14]. At the lab, the procedures include multiple steps, such as manual tissue homogenization and nucleic acid extraction, followed by downstream assays like qPCR and sequencing [15]. The entire process can take from a few days to several weeks, depending on the schedule and the specimen amount. Although this standard practice provides reliable results due to high-quality nucleic acids, the long turnaround times and labor intensiveness make it inefficient for disease control [16]. The use of high-throughput instruments mitigates some problems by offering semi-automated and large-scale sample processing [15]. Still, time-consuming sample pretreatment and high power requirements make them impractical for on-site disease diagnosis [15]. Some commercial products and lab studies, such as the QIAcard FTA PlantSaver and microneedle patch, have attempted to improve in-field sample preparation [17–19]. Additionally, serological tests, such as lateral flow immunoassays (e.g., AgriStrip, Pocket Diagnostics kits, etc.), have been proposed for in-field pre-screening of plant diseases; however, follow-up in-lab testing is still required [20,21].

To enable effective disease monitoring and crop protection on site, quick and portable sample preparation tools are highly desirable. In our previous work, we employed a commercial Omnilyse microhomogenizer for tissue lysis and paper disks for solid-phase extraction of nucleic acids from a variety of citrus species and pathogens [22]. In the current study, we devised a completely new design using 3D printing to integrate the lysis and extraction functions in a single, handheld device. With its rapid advancements in resolution, speed, materials compatibility, and affordability, 3D printing has been deployed to prototype a variety of sample preparation and molecular detection tools. These include, but not limited to, 3D microfluidic chip for extraction and isolation of nucleic acids based on water-oil interface [23], automated benchtop sample preparation and detection [24], a reactor array capable of nucleic acid extraction, concentration, and isothermal amplification [25], comb-like probes for quick nucleic acid separation [26], and an electrochemical microwell sensor [27]. Unlike these examples, the proposed device features a robust mechanical lysis mechanism to break up tough plant tissues and pathogens otherwise difficult to lyse. Specifically, a high-speed motor drives a 3D-printed blade to macerate citrus petioles and midveins in the lysis chamber, producing a crude lysate, in which paper disks are soaked for quick nucleic acid extraction, enabling downstream molecular assays (Fig. 1, right). The handheld device protocol takes 10 min for the preparation of ready-to-use paper disks, where elution of total nucleic acids takes an additional 2 min. This sample preparation is significantly faster than the benchtop protocol that takes up to an hour (Fig. 1 right).

As detailed in the Materials and method sections, *Citrus tristeza virus* (CTV), an RNA virus, and *Spiroplasma citri* (*S. citri*), a DNA bacterium, were used for characterization, optimization, and validation of the proposed handheld sample preparation device. While HLB, caused by *Candidatus* Liberibacter spp., remains the highest priority and our long-term target, its quarantine conditions and biosafety requirements make it unsuitable for the present proof-of-concept study.

This device allows farmers and growers to prepare ready-to-use

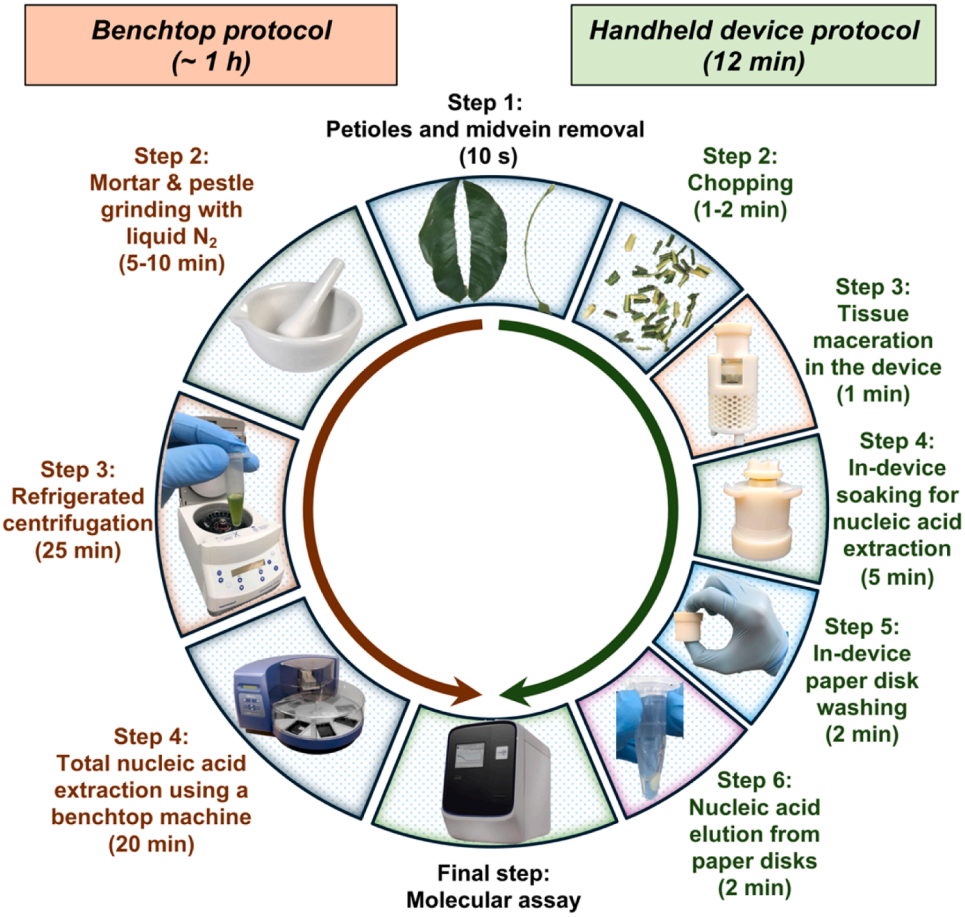

Fig. 1. Comparison between a standard benchtop sample preparation protocol (left) and the handheld device protocol proposed in this study (right), highlighting the key components for sample lysis and nucleic acid extraction.

nucleic acids straight from plants of interest in a portable manner or retain them on dried paper disks for easier storage and shipping to labs. This will allow laboratory workers to focus on analyzing the nucleic acids from the dried paper disks. The device reduces workloads and expedites the overall disease diagnosis process.

2. Materials and methods

2.1. Materials and equipment

The handheld device was designed using SolidWorks 2022 (Dassault Systèmes SOLIDWORKS Corp., France) and printed in VeroClear-RGD810 and VeroWhitePlus-RGD835 with SUP705 support in a Stratasys Objet30 3D printer (Stratasys, Ltd., Eden Prairie, MN). Total nucleic acid concentrations and purity were measured using a Qubit 4 fluorometer with RNA/dsDNA Broad Range assay kits (Cat. No. Q10210, Q32850, Life Technologies, Carlsbad, CA) and a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA), respectively. DNA/RNA extraction controls were prepared using a MagMAX Express-96 Magnetic Particle Processor with a MagMAX-96 Viral RNA isolation kit (Cat. No. AM1836) (Thermo Fisher Scientific). Refrigerated centrifugation in the benchtop protocol was performed using an Allegra X-22R centrifuge (Beckman Coulter, Hercules, CA). qPCR and RT-qPCR were performed in an Applied Biosystems QuantStudio 12 K Flex Real-Time PCR System (Thermo Fisher Scientific). The electric motor used was an RS-380PH-4045 (Mabuchi Motor Company, Japan) and powered by a DIGI 35A DC power supply (Electro Industries, Monticello, MN). Motor speed was measured with an R7050 Compact Photo Tachometer (REED Instruments, Wilmington, NC). The handheld device's shaft assembly was lubricated and sealed with silicone grease (Cat. No. 335148, Beckman Instruments, Palo Alto, CA). Temperatures inside the lysis chamber were measured using K-type thermocouples (Omega Engineering, Norwalk, CT) and an Arduino Uno Rev3 board (Arduino, Italy). ATR-FTIR spectra were obtained using Nicolet iS50 FTIR Advanced KBr Gold Spectrometer (Thermo Fisher Scientific).

2.2. Reagents and buffers

The lysis buffer used throughout the study was composed of 4 M guanidinium thiocyanate (GTC) (Sigma-Aldrich, St. Louis, MO), 0.2 M sodium acetate trihydrate (pH 5.0) (Thermo Fisher Scientific), 2 mM ethylenediaminetetraacetic acid (EDTA, Thermo Fisher Scientific), and 2.5 % polyvinylpyrrolidone (PVP, Sigma-Aldrich), where the final pH was adjusted to 5.0 with 1 N hydrochloric acid [22]. Paper disks (d = 0.25 in) were cut from untreated Whatman Grade 1 CHR chromatography paper (Cat. No. 3001–861, Sigma-Aldrich) using a hole punch. Tris–HCl solution (1 M, pH 7.5) (Cat. No. 15,567–027, Invitrogen, Waltham, MA) was diluted to 10 mM to rinse paper disks after soaking. Tris-EDTA (TE) 1X solution (pH 8.0) (Cat. No. BP2473100, Thermo Fisher Scientific) was used to resuspend nucleic acids after benchtop extraction. TE 1X solution with 100 mM sodium chloride (Thermo Fisher Scientific) added was used to elute paper disks [22]. Nuclease-free water (Milli-Q Biopak Polisher, Sigma-Aldrich) was used to prepare MasterMix of qPCR-based assays. CloroxPro Clorox Germicidal Bleach (Clorox Healthcare, Oakland, CA) was used for sterilizing the handheld device, and sodium ascorbate (Eisen-Golden Store, Dublin, CA) was used to neutralize residual bleach in the wash step.

2.3. Overview of the handheld device

2.3.1. Design and fabrication

All parts of the handheld device shown in Fig. 2 were designed using SolidWorks 2022 and were fabricated with VeroClear-RGD810 and VeroWhitePlus-RGD835 build materials and SUP705 support using a Stratasys Objet30 3D printer. Sharp tools and brushes were used to remove most of the support, followed by a 5-minute sonication in 70 % (v/v) ethanol and pure isopropanol sequentially before rinsing with water. An exploded view of the three major parts (paper-disk extraction unit, lysis chamber, and blade and clutch assembly) is depicted in Fig. S1a. The paper-disk extraction unit holds five untreated chromatography paper disks (d = 0.25 in.) cut using a hole punch (Fig. S1b, i). The untreated Whatman grade 1 chromatography paper has previously been proven effective at retaining nucleic acids from crude lysates and releasing them back into an elution buffer [22]. Cellulose paper is routinely used to extract and store DNA and RNA from various biological specimens [28–30]. The vents in the holder allow the wash buffer to flow easily back and forth, assisting in removing tissue residues (Fig. S1b, i). The disk retainer holds the paper disks in place (Fig. S1b, i). The lysis chamber is where the chopped tissues undergo 1-minute maceration with the lysis buffer. The knurling (Fig. S1b, ii) inside the chamber enhances tissue lysis. The safe lock around the chamber and the motor cap (Fig. S1b, iii) keeps the chamber fixed during operation. The blade and clutch assembly consist of a two-part clutch with the blades screwed into the upper clutch. The beveled surfaces of the clutch's teeth help it fit into its drive counterpart more easily (Fig. S1b, iv).

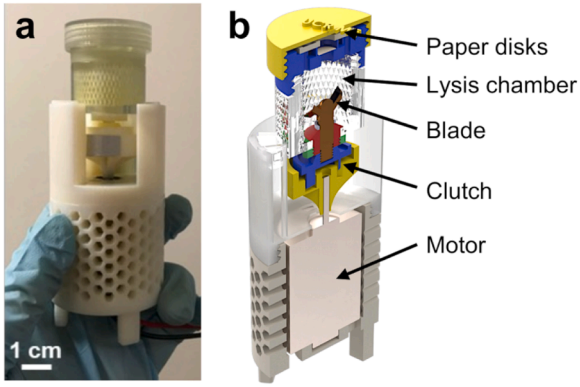

Fig. 2. Handheld sample preparation device. (a) 3D printed and assembled device in hand. (b) Cutaway drawing with key components.

2.3.2. Operation procedure

The stepwise operation procedure of the handheld device is illustrated in Fig. S2. (Step 1) 500 mg of chopped tissue (~0.25 in long) are placed in the lysis chamber with 3000 μL guanidine lysis buffer and the paper disk unit and the top lid are screwed on. (Step 2) The motor is powered on for 1 min. (Step 3) The lysis chamber is then flipped upside down for 5 min, soaking the paper disks in crude lysate. (Step 4) The lysis chamber is removed and replaced with the wash chamber containing 1 mL of wash buffer. The assembly is then mildly shaken for 1 min, ensuring that the wash buffer makes contact with the paper disks. After 1 min, the wash buffer is discarded and replaced with fresh buffer for another minute of shaking. (Step 5) The paper disks are then moved to an aluminum foil sheet using clean tweezers and allowed to air dry for 24 h before use. This simulates onsite practice where immediate elution is not available. As suggested by our previous study, the application of a drying step does not significantly impact the resulting quantification cycle (Cq) values compared to immediate elution [22]. (Step 6) The nucleic acids are then recovered from the paper disks using an elution buffer for subsequent analysis.

2.4. Plant materials and sample collection

Healthy Sour orange (*Citrus aurantium* L.), healthy Washington navel (*Citrus sinensis* L. Osbeck), and healthy Limoneira (*Citrus limon* L. Burm. f.) were collected from Agriculture Operations (AgOps) at the University of California, Riverside (UCR). Healthy Sour orange was provided by the Citrus Clonal Protection Program (CCPP) at UCR. Diseased citrus sources, Pineapple sweet orange and Sweet orange (*Citrus sinensis* L. Osbeck),

with single infection of CTV and *S. citri*, respectively, were provided by the CCPP disease bank at the UCR (Table S1). The study used these sources to evaluate the developed handheld device and the corresponding protocol. All tested leaves were evenly collected from all sides of the tree and were gently wiped with wet paper napkins to remove dust prior to processing.

2.5. Benchtop protocol

2.5.1. Preparation of crude lysates

Following the protocol previously reported [22], phloem-rich petioles and midveins from collected leaves were ground using a mortar and pestle with liquid nitrogen until finely powdered. The ground tissues were then aliquoted to 100 mg, lysed with 600 μL of guanidine lysis buffer, and vortexed for 1 min, followed by 15 min of incubation at 4 °C. The mixture was centrifuged at 14,000 × *g* at 4 °C for 25 min, where 150 μL of the supernatant was collected for subsequent nucleic acid extraction.

2.5.2. Nucleic acid extraction using benchtop instruments

Total nucleic acids in crude lysates were isolated using a pre-programmed MagMAX Express-96 Magnetic Particle Processor with a MagMAX-96 Viral RNA isolation kit. Each reaction contained 150 μL of supernatant of crude lysate, 22 μL of RNA binding bead mix (10 μL lysis/binding enhancer + 10 μL RNA binding beads + 2 μL carrier RNA), 139 μL of lysis/binding solution as recommended by the manufacturer and an extra 139 μL of 100 % isopropanol as previously reported [15]. The resulting nucleic acids were eluted in 100 μL of TE 1X solution and assessed using a NanoDrop 2000c spectrophotometer to confirm the quality. Nucleic acid concentrations (ng/μL) were determined using a Qubit 4 fluorometer with RNA/dsDNA Broad Range assay kits. These extracts were then aliquoted and stored at −80 °C for later use.

2.6. Handheld device protocol

2.6.3. Preparation of crude lysates

The handheld device was used for lysate preparation throughout the study. Petioles and midveins were collected from leaves and chopped into quarter-inch pieces with a clean razor blade to fit inside the lysis chamber. The chopped tissues were weighed out to 500 mg each, then ground and lysed with 3000 μL of guanidine lysis buffer for 1 min at the specified voltage. The lysate then underwent either a benchtop extraction (150 μL, same as benchtop protocol), as described above, or a direct extraction using paper disks.

2.6.4. Nucleic acid extraction using paper disks

The handheld device was assembled by inserting five paper disks at the beginning of the process. As previously reported [22], 5 min of paper-disk soaking is optimal for nucleic acid capture. Thus, the entire device was inverted to allow the paper disks to soak in the lysate for 5 min after homogenization. After soaking, the device was flipped back upright, and the lysis chamber was replaced with a wash chamber containing 1 mL of 10 mM Tris–HCl solution (pH 7.5), followed by 1 min of gentle shaking. This wash procedure was repeated to more thoroughly clean the paper disks. Using sterilized tweezers, the five washed disks were transferred to an aluminum foil sheet to air dry for 24 h before eluting them in 100 μL of modified TE 1X solution. Final nucleic acid concentrations (ng/μL) were determined using a Qubit 4 fluorometer with RNA/dsDNA Broad Range assay kits before storage at −80 °C.

2.7. qPCR and RT-qPCR assays

Primers and probes used in the study for singleplex qPCR/RT-qPCR assays were adapted from previous reports [31–33]. The cytochrome oxidase (COX) gene is routinely used as a reliable internal control to confirm nucleic acid integrity [31,32]. The sequences of the primers, the probes, and the corresponding nucleotide positions of the targets of interest (i.e., CTV, *S. citri*, and COX) are available in Table S2. All assays were conducted using an Applied Biosystems QuantStudio 12 K Flex Real-Time PCR System. The specific kits and protocols used for singleplex qPCR/RT-qPCR assays are detailed in Table S3.

3. Results and discussion

3.1. Characterization of the handheld device

The high-speed motor driving the handheld device, RS-380PH-4045, has a voltage range of 4.5–9.6 V with a normal working voltage of 7.2 V. To determine the correlation between rotation speed and lysis efficiency, three voltages, 6, 7.5, and 9 V, were selected for the series of assessments below. In addition, while buffer leaking from the interstice between the shaft and stabilizer was found to be an issue for practical operation, it was significantly reduced with the addition of silicone grease before each operation, as detailed in Fig. S3. Each operation's rotation speed (in RPM) under different conditions was measured using a tachometer to identify any possible differences (Fig. S4). Further characterizations included evaluating potential resin release from the printed parts during lysis, as well as heat generated during operation. These are detailed in the sections "Evaluation of resin release" and "Evaluation of heat generation", as well as Figs. S5 and S6 in the supplementary material.

3.1.1. Optimization of the handheld device

To maximize the nucleic acid release, two different lysis chambers were evaluated: one with smooth walls and one with knurled walls. Three healthy citrus species (Sour orange, Limoneira, and Washington navel) were macerated at three different voltages (6, 7.5, and 9 V) for 1 min. Nucleic acids were then extracted using a MagMAX kit and subsequently analyzed in terms of Cq values of the internal control, COX, and total DNA and RNA concentrations. Additionally, the quantitative results were compared with samples prepared with a mortar and pestle.

As shown in Fig. 3a, sample lysis with the knurled chamber always produced higher RNA concentrations than the smooth chamber, regardless of operation voltage, with up to 1.76 times higher concentrations at 7.5 V. Compared to the mortar and pestle (MP) control, the knurled chamber produced RNA yields of up to 94.6 % (9 V) and 78.4 % (7.5 V) for Limoneira and Washington navel, respectively. In contrast, the highest total RNA concentration using a smooth chamber was only 53.2 % (9 V) of its MP control. The results indicate that a knurled chamber enables much higher lysis efficiency than a smooth chamber, even when operated at a lower voltage. This is because the knurling provides a large, rough surface that scrapes off plant tissues to release more nucleic acids. The amount of nucleic acids extracted using the knurled chamber was consistent across different citrus species (Fig. 3a).

When comparing Cq values (Fig. 3b), the advantage of the knurled chamber is even more apparent, especially at 6 and 7.5 V. This can be seen in Fig. 3c. However, at 9 V, the performances of the knurled and smooth chambers are much more similar. Notably, no significant differences were found in the Cq values between Limoneira and Washington navel, either at 7.5 V or 9 V. This suggests that 7.5 V is an optimized condition, outputting equivalent yield with lower power requirements. Visualization of the sample lysis at 7.5 V can be seen in Fig. 3d, where the lysate turned green quickly within the first 10 s of operation. It is also noted that, by design, lysate can contact paper disks below the cap during the lysis step to facilitate paper disk soaking (Fig. 3d). Based on these results, a knurled chamber operated at 7.5 V was used for all subsequent tests. An additional study investigating effective sterilizing protocols for the handheld device is detailed in the section "Evaluation of device reusability" and Fig. S7 in the supplementary material.

Briefly, soaking the device in 10 % household bleach for 60 min, followed by completely neutralizing the bleach with 2.3 % sodium

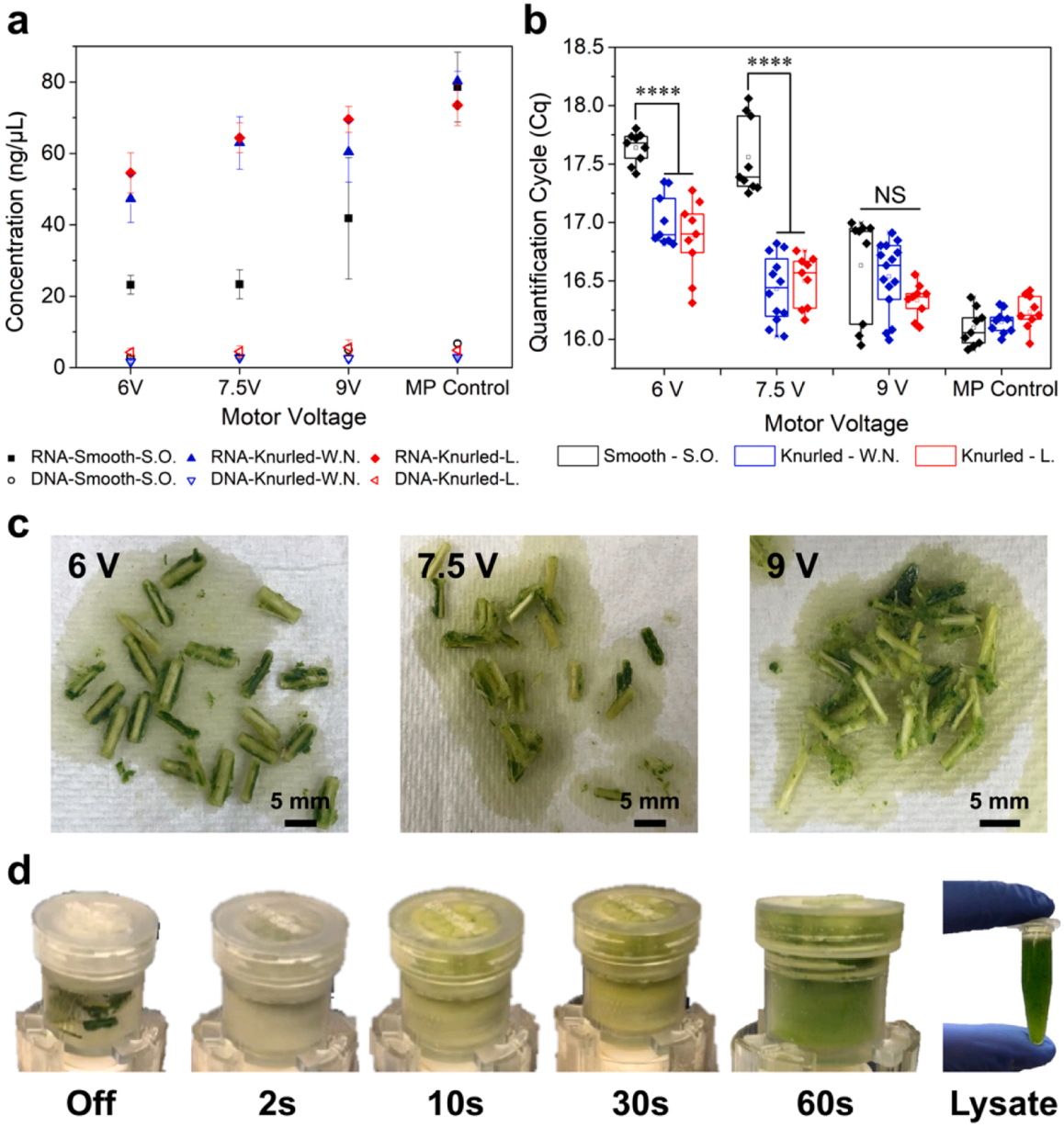

Fig. 3. Optimization of nucleic acid release in terms of motor voltages and chamber features. (a) Comparison of total RNA and DNA concentrations among different voltages and the mortar and pestle grinding control ($N = 4$ for Washington navel, and $N = 3$ for the rest). (b) Comparison of Cq values among different operation voltages and the mortar and pestle grinding benchmark ($N = 12$ for Washington navel and $N = 9$ for the rest). (c) Midvein and petioles of Limoneira after 1-minute lysis using the knurled chamber. (d) Color change of the lysate during the 1-minute lysis process at 7.5 V. **** denotes $p \leq 0.0001$, and NS (Not significant) denotes $p > 0.05$. S.O.: Sour orange; W.N.: Washington navel; and L.: Limoneira.

ascorbate, successfully eliminated any detectable genetic material from previous runs. Furthermore, this treatment did not have any impact on subsequent assays, as evidenced by the consistent Cq values. While the lysis buffer currently used is not ideal for use in the field, the byproducts of this sterilization process are non-pathogenic and non-toxic, supporting the device's potential for safe and reliable reuse for in-field applications.

3.2. *In-device sample preparation for the detection of citrus pathogens*

3.2.1. *Optimization of in-device sample preparation*

Wash and elution protocols were modified from our previously reported paper-disk extraction protocol [22] for use with the new device. Specifically, the number of wash steps and the volume of elution buffer were adjusted (Fig. 4a), while the wash/elution buffer composition remained unchanged. Both introducing a second wash step and doubling the elution buffer volume were attempted to reduce the effects of residual RT-qPCR inhibitors from the lysate. Fig. 4b illustrates how the green tints of the paper disks and wash buffer change during the extraction and wash steps. CTV was used as a proof-of-concept pathogen for these optimizations.

The purity of the resulting extracts was estimated by measuring the A260/A280 ratio of each extract, as shown in Fig. 4c. With a single wash step, extracts from the original protocol and modified protocol 1 showed low purities, ranging from 1.04 – 1.16. Similarly, false-negative RT-qPCR results for CTV and COX were also observed (Fig. 4d). It is inferred that the RT-qPCR reactions were inhibited because contaminants, such as the lysis buffer or the plants' proteins, remained in the paper disks and were not sufficiently diluted despite doubling the volume of elution buffer.

a

	Wash buffer 10 mM Tris-HCl	Elution buffer 1X TE + 0.1 M NaCl
Previously developed protocol (Original protocol)	Single wash-1mL	100 μL
Modified protocol 1	Single wash-1 mL	200 μL
Modified protocol 2	Double wash-1mL	100 μL
Modified protocol 3	Double wash-1 mL	200 μL

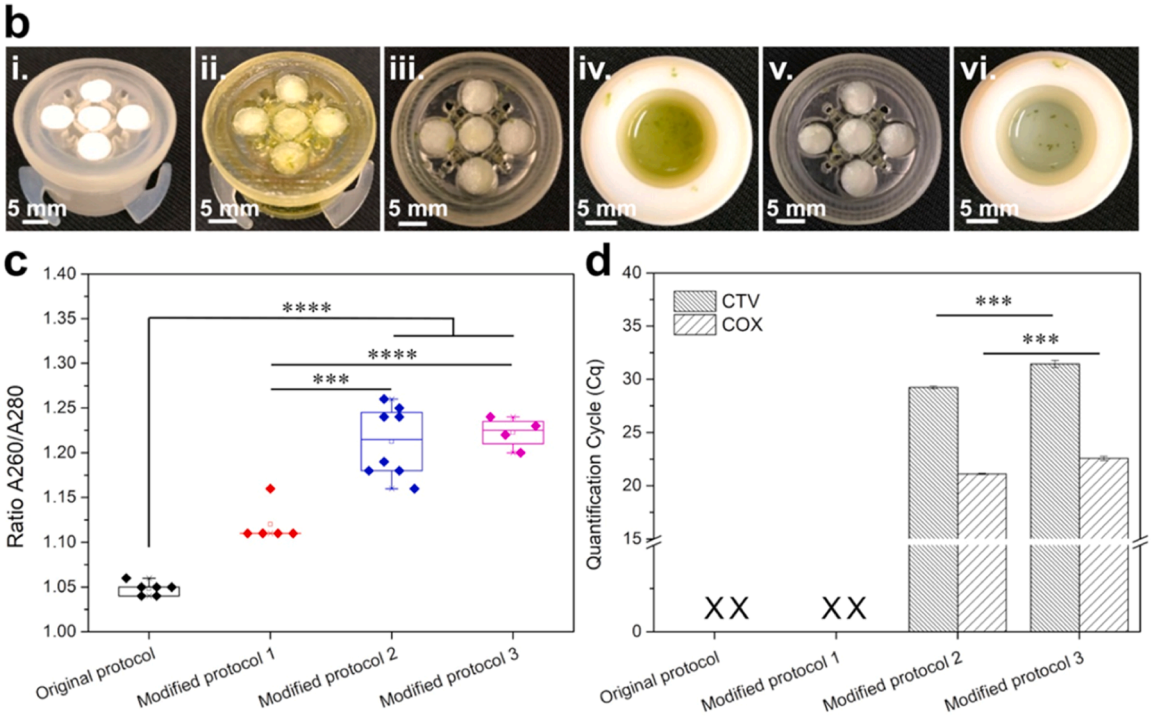

Fig. 4. Optimization of the paper disk washing and elution protocol for in-device nucleic acid extraction. (a) The four tested paper disk washing and elution protocols. (b) Paper disk washing sequence: i) Clean paper disks; ii) After soaking in crude lysate; iii) Paper disks after single wash; iv) Wash buffer after the first wash; v) Paper disks after double wash; vi) Wash buffer after the second wash. (c) Evaluation of the purity of different extracts in terms of A260/A280 ratio ($N = 4 – 8$). (d) Comparison of Cq values among the four tested protocols. Neither of the single-wash protocols resulted in a detectable signal (Mean ± 1SD, $N = 3$). *** and **** denote $p \leq 0.001$ and $p \leq 0.0001$, respectively.

In contrast, the extracts subjected to a double-wash step (i.e., modified protocols 2 and 3) had higher A260/A280 ratios, ranging from 1.18 – 1.25 (Fig. 4c) and, more importantly, positive RT-qPCR results for both CTV and COX (Fig. 4d). While the double-wash protocols were sufficient in mitigating unwanted inhibition, doubling the volume of elution buffer increased Cq values of both CTV and COX (Fig. 4d). Smaller elution buffer volumes (< 100 μL) were not explored because a minimum volume of 100 μL was required to fully immerse the paper disks in a tube and produce an adequate eluate volume, considering the amount retained by the paper disks. As such, modified protocol 2 (double wash and 100 μL elution) was adopted for all subsequent tests.

3.2.2. *Validation of the optimized in-device sample preparation with citrus pathogens*

Using the above-optimized protocols, the next step was assessing the handheld device's applicability to different citrus pathogens. To achieve this goal, CTV and *S. citri* were selected as the representative models of two major categories, viral and bacterial pathogens. MagMAX extracts directly prepared from the same pre-soak lysates served as a reference to evaluate the capability of in-device sample preparation in nucleic acid recovery.

Most importantly, all in-device extracts resulted in positive detections of the intended targets, consistent with the MagMAX extracts, demonstrating high accuracy and reliability of the proposed handheld device (Fig. 5a and 5b). Additionally, the in-device sample preparation showed low intra-assay (< 3.1 %) and inter-assay (< 3.8 %) coefficients of variation (CV), indicating excellent reproducibility. Thus, this device would be suitable for in-field uses, where detection of the pathogens, not their precise quantification, is essential.

Still, in-device extracts required more amplification cycles to be detected than the MagMAX extracts (Fig. 5c and 5d), indicating lower extraction efficiency. This is expected because the proposed paper-disk method is designed to quickly capture nucleic acids, wash off excess impurities, and elute nucleic acids, whereas MagMAX purifies and concentrates nucleic acids through sophisticated and automated particle processing. While our goal is to enable in-field nucleic acid extraction, not replace the state-of-the-art benchtop instrument for laboratory use, there is room for further improvement for paper-based nucleic acid extraction. For example, although cellulosic paper has long been used to capture and store various nucleic acid samples [19,22,28], modifying its chemical properties or using other porous membranes could significantly improve nucleic acid recovery [30,34]. Such an improvement

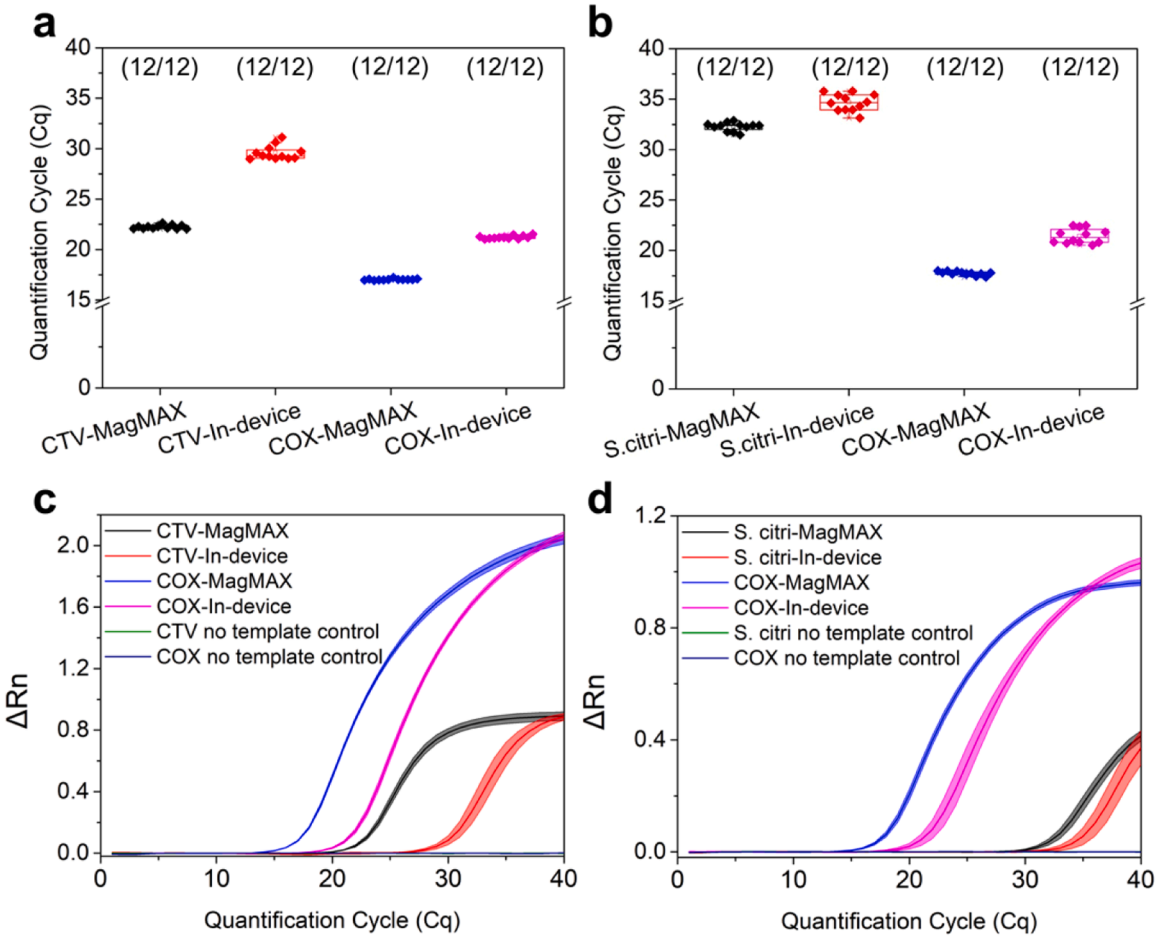

Fig. 5. Validation of the optimized in-device sample preparation with citrus pathogens. (a) Comparison of CTV and COX Cq values between MagMAX and in-device extracts ($N = 12$). (b) Comparison of *S. citri* and COX Cq values between MagMAX and in-device extracts ($N = 12$). (c) RT-qPCR curves of MagMAX and in-device extracts from CTV-infected source (Mean ± 1SD, $N = 12$). (d) qPCR curves of MagMAX and in-device extracts from *S. citri*-infected source (Mean ± 1SD, $N = 12$).

would reduce the required number of amplification cycles and also enable earlier detection of low-concentration targets.

3.3. Cost analysis

Estimated costs of sample preparation using the proposed handheld device protocol and benchtop protocol in Fig. 1 are summarized in Table S4. The reagent and consumable costs for the lysis and extraction per sample are $0.71 for the handheld device and $6.46 for the benchtop protocol, respectively. The benchtop protocol also requires high initial capital costs. However, it produces higher-quality nucleic acids and offers high-throughput extraction capacity via automated sample handling in 96-well plates, making it suitable for large-scale sample processing and subsequent molecular assays in centralized laboratories. Our handheld device, in contrast, is designed to produce nucleic acids of adequate quality for accurate detection with a low device cost. Currently, the handheld device costs $95 with the driving unit (motor + housing) at $75 and the top unit (lysis chamber + paper disk holder) at $20. With commercial-scale manufacturing (by injection molding), the device cost is expected to be further reduced, as common thermoplastics such as polypropylene, ABS, and polycarbonate, range in price for $1–4/kg vs the 3D-print resins used in this study which cost from $380–450/kg.

4. Conclusion

This study successfully developed and validated a portable sample preparation device for mechanical lysis and nucleic acid extraction for the detection of citrus pathogens. The device offers a quick and cost-effective alternative to conventional benchtop workflows, enabling on-site preparation of crude lysates and nucleic acid capture using paper disks. It yielded accurate (zero false negatives) detections with minimal variations for both RNA and DNA pathogens (CTV and *S. citri*), making it suitable for yes/no pathogen detections in the field, especially where speed and accuracy are prioritized over precise quantification. The device's top unit (lysis chamber + paper disk holder) detaches from the base driving unit and can be sterilized and neutralized for repeated reuse without carrying over leftover nucleic acids from prior use. In practice, a single user can utilize multiple top units with a single driving unit for batch processing of multiple samples in the field. The proposed in-field sample preparation is beneficial for disease detection and management, as the dried paper disks can either be shipped for lab-based analysis or be eluted immediately for on-site detection, both options contributing to a shortened turnaround time between sample and answer.

Future work will focus on improving the paper disk's nucleic acid capture efficiency, as the current DNA and RNA yields are noticeably lower than those of the benchtop instrument (i.e., MagMAX). Chemically modifying the paper surface or using other porous membranes with better nucleic acid recovery [30], such as the glass fiber pads used in spin columns, could help narrow the performance gap between the handheld device and the benchtop protocol and enable the diagnosis of low-titer, asymptomatic samples. The currently used lysis buffer, guanidine thiocyanate, is not ideal for in-field use due to its toxicity and

potential hazardous chemical incompatibility; therefore, green alternatives such as SDS/ammonium trichloroacetate should be investigated for safer adaptation [35]. Additionally, simple nucleic acid amplification tests (NAATs), such as loop-mediated isothermal amplification (LAMP) [36] and recombinase polymerase amplification (RPA), will be developed and integrated with the handheld device to enable quick pathogen detection in the field. Finally, we plan to apply the technology to other citrus and specialty crop diseases, including HLB, to establish a robust sample-to-answer plant disease diagnostics platform.

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CRediT authorship contribution statement

Chia-Wei Liu: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Brent Kalish:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Conceptualization. **Sohrab Bodaghi:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Georgios Vidalakis:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Hideaki Tsutsui:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sampre.2025.100199.

Data availability

Data will be made available on request.

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