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Extraction of Plant DNA by Microneedle Patch for Rapid Detection of Plant Diseases

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A microneedle patch-based rapid plant DNA extraction method is reported. This minimally invasive approach allows isolation of PCR amplifiable genomic DNA from hard-to-lyse plant tissues in around 1 minute without the need of bulky laboratory equipment. This method has been applied to detect low abundant *P. infestans* pathogens in both lab-inoculated and field-collected tomato leaves.

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ABSTRACT: In-field molecular diagnosis of plant diseases *via* nucleic acid amplification (NAA) is currently limited by cumbersome protocols for extracting and isolating pathogenic DNA from plant tissues. To address this challenge, a rapid plant DNA extraction method has been developed using a disposable polymeric microneedle (MN) patch. By applying MN patches on plant leaves, amplification-assay-ready DNA can be extracted within a minute from different plant species. MN-extracted DNA has been used for direct polymerase chain reaction (PCR) amplification of plant plastid DNA without purification. Furthermore, using this patch device, extraction of plant pathogen DNA (*Phytophthora infestans*) from both laboratory-inoculated and field-infected leaf samples was performed for detection of late blight disease in tomato. MN extraction achieved 100% detection rate of late blight infections for samples after 3 days of inoculation when compared to the conventional cetyltrimethylammonium bromide (CTAB)-based DNA extraction method as a gold standard, and 100% detection rate for all blind field samples tested. This simple, cell lysis-free, and purification-free DNA extraction method could be a transformative approach to facilitate rapid sample preparation for molecular diagnosis of various plant diseases directly in the field.

KEYWORDS: plant disease, DNA extraction, microneedle patch, *Phytophthora infestans*, nucleic acid amplification, point-of-care diagnostics

Global food security has become a growing concern for the international community as it directly affects our agricultural economy and sustainability.¹ The demand of food is increasing rapidly due to population growth. By 2050, the global food production needs to be increased by 100 % to fulfill the food demand of growing populations.² Additionally, the total available agricultural land area is decreasing with population growth. As a result, in the coming decades increasing agricultural productivity will be key for global food security. Plant diseases significantly affect agricultural productivity. Crop failure due to pathogen infection is a common issue in agriculture. Every year, more than 30% of crop losses occur due to plant diseases.^{3,4} Current crop protection relies on several disease diagnosis technologies, such as DNA amplification via the polymerase chain reaction (PCR), enzyme-linked immunosorbent assays (ELISA), fluorescence *in-situ* hybridization (FISH), immunofluorescence (IF), and flow cytometry (FCM).^{1,5,6} However, most of existing disease detection technologies are laboratory-based and require skilled technicians for instrument operation. Moreover, field sites are often far from centralized diagnostic laboratories or in resource-limited regions where diagnostic facilities are not readily available. As such, development of field-portable and cost-effective disease detection systems is essential for global crop protection.

Late blight is caused by the oomycete plant pathogen *Phytophthora infestans*,⁷ and is one of the major threats to global food security as it adversely affects potato production, one of the world's staple food crops. This oomycete was responsible for the famous Irish potato famine (1845-1849) ^{7–9} and most recently, the late blight pandemic of tomato in eastern USA in 2009. ^{10–12} Late blight disease spreads rapidly under favorable conditions and can destroy a crop within a few days if left untreated. Every year, the disease causes approximately 6.7 billion USD in crop losses worldwide.¹³ Molecular approaches based on nucleic acid amplification (NAA) are

currently the predominant method for diagnosis of late blight, and in particular, for genotyping different strain types of *P. infestans* with distinct fungicide susceptibility.^{14–16} However, current NAA-based diagnostics is not only limited by the cumbersome amplification assay protocols but also by the tedious sample preparation steps before the amplification assay. While significant progress has been made in improving the portability of PCR system to enable lab-on-a-chip detection of plant pathogens,^{17,18} plant sample preparation, namely the extraction of high-quality genomic DNA from plant tissues, remains a major obstacle for performing molecular diagnosis of plant diseases in remote or resource-limited settings.

Currently, the most widely used extraction protocol is the cetyltrimethylammonium bromide (CTAB)-based plant DNA extraction protocol,¹⁹ which was developed almost 40 years ago and is still considered a "gold standard" method for isolation of high-quality DNA from plant tissues. However, CTAB extraction is a complicated and time-consuming process, which involves several steps including: 1) mechanical grinding of plant tissues (*e.g.*, leaves) using mortar and pestle, 2) CTAB-based cell lysis, 3) organic phase DNA extraction, and 4) alcohol-assisted DNA precipitation and purification (**Figure 1a**). Alternatively, a NaOH-based quick plant DNA extraction protocol could be used for fast PCR analysis in the laboratory,²⁰ and plastic DNA extraction bags preloaded with extraction buffers are also available for on-site sample preparation *via* quick grinding for subsequent use with ELISA assays.²¹ FTA cards can also be used to store ground pathogen-infected plant tissue and transport them to diagnostic labs for subsequent extraction and DNA testing.^{22,23}

Development of a rapid, field-applicable, and cost-effective genomic testing platform could allow farmers and/or extension workers to perform screening assays of plant diseases directly in the field. While in recent years several lab-on-a-chip based technologies have been

developed for DNA extraction from mammalian cells or bacteria,^{24–27} these technologies are difficult to apply to plant cell lysis and DNA isolation due to the presence of rigid plant cell walls. Furthermore, plant leaves, one of the most common sample sources for plant disease screening, consist of plant cells in highly organized sandwich structures, where infected cells are usually protected by the epidermal cells and cuticle layers. Extraction of genomic DNA from infected plant cells therefore requires breakage through several plant barriers, including cell walls, epidermis, and the waxy cuticle outer layer. Despite the great progress in both laboratory- or chipbased technologies in recent years, none of the existing methods provides assay-ready plant DNA in a simple, instrument-free, and field applicable manner.

We have developed a plant DNA extraction approach that does not rely on the conventional concept of DNA isolation through tissue and cell lysis. Rather, it utilizes puncture with a microneedle (MN) patch into leaf tissue to extract intracellular DNA and other molecules without the need of cell lysis. By doing so, we were able to significantly shorten the time of plant DNA extraction from ~3-4 hours in a conventional method to around 1 minute, and to overcome the burden of using bulky and expensive equipment for plant sample preparation. We demonstrated that the MN-extracted DNA was directly applicable for subsequent molecular analysis, such as PCR. Furthermore, we showcased the application of this MN-based quick plant DNA extraction method to detect *P. infestans* in tomato leaves. The MN extraction method we developed is minimally invasive, lysis-free, purification-free, and thus has tremendous potential for applications towards rapid molecular diagnosis of plant diseases in the field.

RESULTS AND DISCUSSION

The MN patches used in the experiment are made of polyvinyl alcohol (PVA) and fabricated through a simple vacuum-based micromolding procedure.²⁸ PVA is a cost-effective water-adsorbing polymer that has a combination of excellent biocompatibility, chemical resistance, and mechanical strength. PVA gel and its derivatives are highly swellable materials that can rapidly absorb 10-30% its own weight water within minutes.²⁹⁻³¹ Swelling-driven capillary flow is considered to be one of the main force to concentrate intracellular DNA molecules around the microneedle tips. In addition, PVA-based MN patches can be conveniently fabricated in a common laboratory without the need of cleanrooms. Our patch consists of a 15 × 15 microneedle array, and each needle is 800 μ m in height, 150 μ m in base radius, and 5 μ m in tip radius (**Figure 1b&c**). The fracture force of polymeric MN patch can be up to ~1 N/needle,^{32,33} strong enough to insert into skin and plant tissues without breaking.^{34,37}

In a typical MN extraction protocol, a fresh MN patch is gently placed on the surface of the leaf of interest. Then, a punctuation force is delivered to the patch by finger pressing for a few seconds; Finally, the patch is peeled off and rinsed with 100 μ L TE buffer (**Figure 1a and Video S1**). The entire process takes less than 1 min, orders of magnitude faster and simpler than the conventional extraction protocol which usually takes ~3-4 hours minimally (**Figure 1a**). The MN patch performs two roles during the process: 1) it penetrates deep into plant tissue in a minimally invasive fashion to break hard-to-lyse plant cell walls and release encapsulated nucleic acid materials; 2) it absorbs and concentrates DNA and other molecules on the surface of MN tips during the needle retraction (**Figure 1a**). After MN retraction, plant DNA can be released from the needle tips by rinsing with elution buffer (**Figure 1a and Video S1**). The extracted samples are subsequently used for nanodrop measurement and PCR assay without further purification, or

stored at 4 °C for future use. Compared with the conventional multistep CTAB extraction protocol (**Figure 1a**), the main advantages of extraction using high-surface-area MN patches are the elimination of the need for complete tissue and cell lysis to release intracellular genomic materials. As a result, not only the tedious grinding, incubation, and cell lysis steps can be skipped, but also the exaction solutions contain much less impurifies such as cellular debris and proteins from the cell lysis steps, making MN-extracted DNA completely purification-free.



Figure 1. MN-based rapid extraction of plant DNA. (a) Schematic of conventional CTAB extraction and MN extraction. (b & c) SEM image and a photograph of MN patch, respectively

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(Scale bar: 200 µm for (b) and 5 mm for (c)); (d & e) Nanodrop UV absorption spectra of DNA solutions extracted by the MN patch (green curves), CTAB protocol (wine curves), SDS protocol (yellow green curves), and NaOH protocol (orange curves), respectively. The black curve in (c) represents rinsing solution from a blank MN patch without leaf puncturing.

The total extracted DNA and the purity of DNA obtained by different extraction methods were characterized by the NanoDrop spectrophotometer (Figures 1d&1e), a UV absorption-based nucleic acid quantification method widely used in molecular biology. The contaminants in the extracted plant DNA solutions may include proteins, polysaccharides, polyphenolics, secondary metabolites, and cell debris.³⁸ Polysaccharides are the prime interferers as they are the major components of plant cell walls; cellular proteins may also present a substantial amount in the DNA solutions, which conventionally can be removed by purification with organic solvents like phenol and chloroform. The characteristic UV absorption peaks of DNA, proteins, and polysaccharides can be found at 260 nm, 280 nm, and 230 nm, respectively. Therefore, the ratios of A260/A280 and A260/A230 are frequently used as quick indicators of the purity of DNA against proteins and polysaccharides, respectively.³⁸ From the spectral data, it is clear that the MN patch extracted significant amount of DNA from plant leaves based on the appearance of A260 absorption for all samples tested (Figure 1d, green curves). In contrast, the solution from the blank MN patch did not show any significant absorption at the 260 nm (Figure 1d, black curve). The purity of MNextracted DNA samples was compared with those obtained by conventional extraction methods, including CTAB extraction,¹⁹ sodium dodecyl sulfate (SDS) extraction,³⁹ and NaOH rapid extraction (Figure 1e & Table 1).²⁰ Among the three conventional protocols, only the NaOH method doesn't include a purification step. For pure DNA, the standard values of A260/A280 and

A260/A230 are expected to be between 1.8-2.0 and >1.8, respectively.³⁸ DNA isolated by the CTAB method demonstrated the closest nanodrop readings to the target ranges (**Table 1**); On the other hand, the A260/A230 ratio (DNA / polysaccharide) and A260/A280 ratio (DNA / protein) of DNA extracted by the MN patch reveals the presence of polysaccharides and proteins with DNA. However, the A260/A230 ratio of MN patch-extracted DNA was quite similar to the results of SDS and NaOH methods, suggesting a comparable DNA quality *via* a much simpler procedure.

Table 1. Nanodrop readings of DNA solutions extracted by different methods

	A260/A280	A260/A230
Target range	1.8-2.0	>1.8
CTAB extraction	2.14 ± 0.01	2.24 ± 0.23
SDS extraction	1.64 ± 0.09	0.63 ± 0.17
NaOH extraction	1.40 ± 0.05	0.48 ± 0.02
MN extraction	1.22 ± 0.04	0.52 ± 0.02

The total DNA extraction efficiency of the MN patch was then compared to the CTAB method. To do that, we extracted DNA from sixteen different fresh tomato leaves by applying both methods to the same regions of interest (ROI) side by side. Two different locations from each leaf were chosen for comparison, constituting a total sampling number of 32 (N = 32) for each method. As depicted in **Figure 2a** and **2b**, we first extracted DNA using a MN patch and then cut the needle

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imprinted area (~10 mm × 10 mm) for subsequent CTAB extraction. The Nanodrop UV absorption spectra from all 32 extraction locations were averaged for each method. The means of spectra were shown in solid lines in **Figure 2c** (red for CTAB extraction and green for MN extraction), whereas the 1st and 3rd quartile of the data are shown in dotted and dashed lines, respectively (**Figure 2c**). The MN method apparently extracted much less DNA than that of CTAB method based on A260 absorption (**Figure 2c**). However, such comparison does not take the difference of sampling volumes of the two methods into account. We estimated that the sample volumes for MN patch and CTAB extraction methods were 1.08 mm³ and 48.92 mm³, respectively (**Figure 2d**, purple bars). After normalization of the amount of extracted DNA by the sample volumes used in each method, the MN extraction method demonstrated a higher yield than the conventional CTAB approach (**Figure 2d**, blue bars).



Figure 2. Comparison of extraction efficiency between the MN and CTAB methods. Image of a tomato leaf (a) after punctuation by a MN patch, and (b) after cutting off the needle-imprinted areas for CTAB extraction. (c) Nanodrop UV absorption spectra of N = 32 samples for CTAB extraction (red) and MN extraction (green). Solid lines: average absorption; dotted and dashed lines: 1st and 3rd quartile of data, respectively. (d) Normalized amounts of total DNA extracted by

two extraction methods (Blue bars), revealing higher extraction efficiency of MN method. Purple bars represent the difference in sampling volumes of the two methods.

In our MN extraction approach, DNA samples were extracted from fresh leaves without using any chemicals, and as a result, the DNA was directly amplifiable without any purification. To demonstrate that, we extracted DNA by MN patches from fresh tomato and pepper leaves (**Figure 3a**). Five different leaves for each species were tested, and each time a new MN patch was used. After extraction, PCR amplification reactions were performed using 1 μ L of needle-extracted samples to amplify the 5' end of ribulose-bisphosphate carboxylase gene (rbcL) of plant plastid DNA. Next, gel electrophoresis was performed to visualize the amplified DNA bands. The MN-extracted samples were successfully amplified by the PCR reaction and the characteristic bands at around 670 bp ⁴⁰ were observed for all samples tested from both species (**Figure 3b&c**). The results confirm that the MN-extracted DNA is directly applicable for PCR amplification without the need of further purification.



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Figure 3. Direct PCR amplification of MN-extracted DNA without purification. a) DNA extraction by MN patches from tomato and pepper leaves (N = 5), indicating the applicability of the MN extraction method for different plant species. (b & c) Gel electrophoresis images showing amplified bands of rbcL gene using MN-extracted DNA from (b) tomato and (c) pepper leaves, respectively. Lane 1-5 in (b&c) represent 5 replicates using different patches; L: 100 bp DNA ladder; NC: negative control (no DNA: blank rinsing solutions).

Next, the MN patch-based DNA extraction method was tested to extract much less abundant plant pathogen DNA from infected plant leaves. Fresh tomato leaves were collected from *P. infestans*-inoculated tomato plants from the Department of Entomology and Plant Pathology (isolate NC 14-1; clonal lineage US-23). Four days after inoculation, the infected leaves were subjected to both MN and CTAB extraction. PCR amplifications were then performed using 1 μ L of extracted DNA samples and the results were characterized by gel electrophoresis (**Figure 4a&b**). To rule out the possibility that MN extraction only collects pathogen DNA from the surface of leaf samples, all leaves used in MN extraction were pre-disinfected by rinsing with a 5% bleach solution for a few seconds followed by rinsing with DI water twice. The gel electrophoresis results show that the characteristic band at around 610 bp ⁴¹ was observed for all CTAB (**Figure 4a**) and MN -extracted samples (**Figure 4b**), suggesting a successful detection of *P. infestans* in both methods.



Figure 4. Detection of *P. infestans via* **MN extraction.** (a) Schematic and gel electrophoresis showing the amplified bands of *P. infestans* from CTAB-extracted DNA. (b) Schematic and gel electrophoresis showing the amplified bands of *P. infestans* from MN-extracted DNA. The surface of the leaves was disinfested using 5% bleach solution for a few seconds before extraction. (c) Schematic and gel electrophoresis showing the amplified bands of *P. infestans* from extraction. (c) Schematic and gel electrophoresis showing the amplified bands of *P. infestans* from extraction solutions obtained by flat PVA patches (no microneedles). Lane 1-6 in (a&b): 6 replicates extracted by the CTAB and MN patch methods, respectively; Lane 1-2 in (c): flat PVA patch extraction after surface disinfestation using a 5% bleach solution; Lane 3-4 in (c): flat PVA patch extraction without bleach disinfesting; L:100 bp ladder; NC: negative control (no DNA: blank rinsing solutions); PC: positive control (purified *P. infestans* DNA). All leaves were tested 4 days after inoculation.

We further validated that MN patches indeed extract inplanta pathogen DNA by using flat PVA patches (no sharp microneedles) as a control (**Figure 4c**). When a similar surface

disinfestation procedure was applied to the flat patch extraction, no *P. infestans* bands were detected by flat patches as indicated by the gel electrophoresis (**Figure 4c**, lane 1-2); In contrast, without performing surface disinfestation, flat patches also detected the presence of *P. infestans* from the surface of infected samples (**Figure 4c**, lane 3-4). These results suggest that in order to probe pathogen DNA inside plant tissues, microneedle structures are required in order to break the leaf surface and penetrate into deep tissues.

For quantitative comparison of extracted pathogenic DNA in different methods, a real-time PCR (or quantitative PCR, qPCR) assay was developed using EvaGreen dye. Previously, several quantitative assays have been reported for P. infestans, based on either TaqMan probe ⁴² or SYBR Green dye.^{43,44} However, TaqMan probe-based assays are very expensive, and SYBR Green dye suffers several disadvantages compared to EvaGreen.⁴⁵ For instance, EvaGreen dye shows lower background fluorescence and less inhibitory effect in PCR reaction than SYBR Green.⁴⁶ Moreover, EvaGreen is more cost-effective, environmentally friendly,⁴⁷ and stable both during amplification and during storage.⁴⁸ The sensitivity of the developed qPCR assay was tested using serial dilutions of purified *P. infestans* DNA for concentrations ranging from 12 ng/ μ L to 1.2 pg/ μ L (Figure S1a). This assay was capable of successfully amplifying at levels as low as 1.2 pg/ μ L target DNA. The melt curve analysis after amplification confirmed the presence of a single amplicon (melt peak at ~82.5°C) in different samples (Figure S1b). A linear relationship between logarithmic values of P. infestans DNA concentration and threshold cycles was obtained as the calibration curve for the assay (Figure S1c). The threshold cycle for the amplification of 12 ng/ μ L target DNA was 18.7, and increased by ~3.6 cycles for each 10-fold dilution of target DNA (Figure S1c).

Using this qPCR assay, more quantitative analyses were carried out for comparing the MN extraction method and CTAB method in the detection of late blight disease from inoculated tomato

leaves (**Figure 5**). Fresh tomato leaves were inoculated by spraying 1 mL of sporangia solution (20,000 sporangia/mL) of *P. infestans* (isolate NC 14-1; clonal lineage US-23) on abaxial side of leaves (**Figure S2**). The detection sensitivity for each extraction method was quantified by using sixteen leaves at four different timepoints, which varied from 1 to 4 days after pathogen inoculation. **Figure S3** shows the images of all inoculated leaves used in the analyses before surface disinfestation. **Figure 5a** shows the images of inoculated leaves after surface disinfestation and puncturing by the MN patches. For each day after inoculation, eight different locations from four different leaves were chosen for DNA extraction using both methods. The analyses were stopped after day 4, because infection symptom became very clear and visible afterwards.

Figure 5b depicts the average amount of total DNA extracted by the two methods for different inoculation days. Although a few variations were observed for the CTAB methods, the average amount of DNA extracted by the MN patches stayed steady, suggesting a consistent extraction performance of all samples by the MN patch approach. All extracted DNA samples from these inoculated leaves were diluted to a concentration approximately 10 ng/ μ L, and 1 μ L of the diluted DNA samples were used for real-time PCR (Figure S4) followed by melt curve analyses (Figure S5). After amplification reactions, the cycle threshold (Ct) for DNA samples extracted from the same locations using different methods were compared (Figure 5c), and the difference of Ct values were calculated and shown in gray bars in Figure 5c.

During the first two days of infection, the pathogen DNA concentration was extremely low. Even the CTAB method could not extract pathogenic DNA from all sampling locations, as indicated by empty bars in **Figure 5c**. The detection rate of MN extraction was defined as follows: Detection rate % = No. of MN detection / No. of CTAB detection. Compared with the CTAB method, MN extraction showed a slightly lower detection rate for the first two days of post-

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inoculation (50% and 66.7%, respectively, **Figure 5d**). However, for days 3 and 4 postinoculation, the detection efficiency of MN approach matches up with the CTAB method, showing a 100% detection rate (**Figure 5d**). On the other side, the Ct values for both methods slightly decrease with the increase of infection time, as the amount of pathogen DNA increases over time. Moreover, the Ct values of the MN approach are about 7 cycles higher than those of CTAB method, due to the smaller sampling volume of MN approach (**Figure 5c**). Although the MNextracted DNA samples take longer time (about 10 minutes) for PCR amplification due to the smaller amount of DNA, the total assay time (including DNA extraction and amplification) of the MN-PCR combination (~1 hour total = ~1 min extraction + ~60 min PCR) is still ~4 times faster than the conventional CTAB-PCR procedure (4.3 hours total = ~3.5 hours extraction + 50 min PCR), not to mention the eliminated sample transportation time (up to several days) associated with the lab-centered diagnostics. The results suggest that by shortening the rate limiting step of plant molecular diagnostics – the sample preparation and transportation – MN patch method has the great potential to speed up plant pathogen detection in the field.



Figure 5. Application of MN extraction for detection of *P. infestans* from laboratoryinoculated samples. (a) Images of all inoculated leaves used for analyses having four different inoculation days (day 1-day 4); (b) Average amount of total DNA extracted by MN patch and CTAB methods for different inoculation days; (c) Threshold cycles (Ct) for detection of *P. infestans* from inoculated leaves for different methods; (d) Detection rate of MN extraction method compared to the CTAB method.

Finally, the MN extraction method was tested to detect late blight disease in field samples (Figure 6). Eight infected tomato leaves (Figure 6a) were collected from Haywood County, North Carolina (disease reported on August 21st, 2018). DNA was extracted from these samples first by the MN patch and then the CTAB method as usual. After extraction, real-time PCR amplifications were carried out for disease detection (Figure 6b, 6c & S6). The presence of *P. infestans* was detected by both methods in all field samples (Figure 6b&c). Again, the extracted amount of P. infestans DNA is much less by the MN patch method, as compared to the CTAB protocol (Table **S1**). This leads to slightly longer amplification reaction of the MN-extracted DNA samples. The threshold values of the MN method were ~5 cycles higher than the CTAB method for the field samples (Figure 6d & Table S1). The difference was smaller than that of lab-inoculated samples. This could be attributed to two possible reasons: First, the field samples have more visible symptoms of infection (Figure 6a) than the laboratory-inoculated samples (Figure S2&S3), indicating higher pathogen DNA, which diminishes the difference of MN and CTAB extraction; Second, surface disinfestation was not performed for field samples before DNA extraction, which also may increase the concentration of pathogen DNA. If the detection sensitivity of the MN

method is a concern due to smaller amount of DNA extracted, the same leaf samples can be potentially extracted by the MN patch multiple times to increase the total DNA amount while maintaining a relative short total assay time.



Figure 6. Application of MN extraction for detection of *P. infestans* from field-collected samples. (a) Images of infected tomato leaves collected from Mountain Research Station in Waynesville, North Carolina; red rectangles indicate DNA extraction areas for late blight disease detection. (b, c) Real-time PCR amplification curves using (b) MN-extracted and (c) CTAB-extracted DNA, respectively. (d) Comparison of Ct values between the two methods for detection of *P. infestans* in field samples.

CONCLUSIONS

In summary, we have developed a nondestructive, cell lysis-free, and purification-free MN patch-based plant DNA extraction method, which reduces sample preparation time from 3-4 hours

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of a conventional extraction method to ~1 minute. We demonstrated that MN patches made of PVA had sufficient stiffness to penetrate several different plant leaf tissues, and extracted DNA was directly usable for amplification reactions without further purification. In proof-of-concept applications, we demonstrated that MN extraction could be used to detect plant pathogen DNA by diagnosing *P. infestans* in both inoculated and field-collected tomato leaf samples. For samples collected 3 or more days after inoculation, the MN extraction approach exhibited a detection rate of 100% when compared to the CTAB method. Moreover, we demonstrated 100% coincidence between MN and CTAB methods, for detecting late blight infection in field-collected samples, and the Ct values of the qPCR assay when using MN method were only about ~5 cycles higher than the conventional CTAB method. Given its simplicity and cost-effectiveness, this rapid plant DNA extraction method could be a valuable technology to eventually achieve "sample-to-answer" nucleic acid amplification (NAA) based plant disease diagnosis in the field.

METHODS

Fabrication of Microneedle (MN) Patch. All MN patches used for DNA extraction were fabricated using polydimethylsiloxane (PDMS) molds purchased from Blueacre Technology Ltd., Ireland. These molds were fabricated by laser ablation, and the dimension of each mold is approximately 10 mm \times 10 mm which has 15 \times 15 arrays of microneedle conical cavities. The height of each cavity is 800 µm and, the diameters of the tip and base are 10 µm and 300 µm, respectively. To fabricate the microneedle patches, 0.5 mL polyvinyl alcohol (PVA, 30kDa-70kDa, 10 wt%) solution was added to each silicone mold. After that, the molds are placed in a

vacuum (600 mmHg) chamber for 20 minutes to draw the PVA solution into the cavities and achieve the desired viscosity. These molds were then kept overnight at 25°C in a chemical hood vacuum. After drying, the microneedle patches were carefully separated from the molds and stored at 25°C in a sealed petri dish.

MN Patch-based DNA extraction. There were two simple steps for MN patch-based DNA extraction from a fresh plant leaf. First, a MN patch was pressed gently onto the leaf surface by hand. The MN patch was then removed and rinsed using 100 μ L TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) for collecting the adsorbed DNA from the needle tips (**Video S1**). For each extraction, a new MN patch was used, and the concentrations of the extracted DNA in the 100 μ L solutions were measured using NanoDrop One Microvolume UV-Vis Spectrophotometer. For PCR reactions, 1 μ L of MN-extracted solutions were used each time without further purification.

CTAB-Based DNA Extraction. Leaf samples were placed in 1.5 mL microcentrifuge tubes and homogenized using disposable pestles after addition of 150 µL extraction buffer (0.35 M sorbitol, 0.1 M Tris, 0.005 M EDTA, 0.02 M sodium bisulfite, pH 7.5). Then, 150 µL of nuclei lysis buffer (0.2 M Tris, 0.05 M EDTA, 2.0 M NaCl, and 2% hexadecyltrimethylammonium bromide (CTAB), pH 7.5 and 60 µL of 5% N-lauryl sarcosine were added to the homogenized solution and vortexed to mix. The microcentrifuge tubes were incubated in a water bath at 65°C for 30 min, and mixed with one volume of chloroform:isoamyl alcohol (24:1). After chloroform extraction, the mixture was centrifuge tubes and again mixed with one volume of chloroform:isoamyl alcohol (24:1) to repeat the chloroform extraction process. After that, one volume of cold 100 % isopropanol and 0.1 volume 3M sodium acetate (pH 8.0) were mixed with the aqueous phases of each sample and kept overnight at -20°C for DNA precipitation. The samples were then centrifuged at 13000 rpm

for 5 min for precipitating DNA. The supernatants were discarded, and the precipitated DNA pellets were washed twice using 1 mL cold 70 % ethanol and air dried in a hood for 30 min. Finally, dry pellets were resuspended in 50 μ L TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0), and the concentrations of the extracted DNA were measured using a NanoDrop One Microvolume UV-Vis Spectrophotometer and diluted to ~10 ng/ μ L for PCR reactions.

Leaf Inoculation. P. infestans infected leaves were obtained from Dr. Jean Ristaino's lab in the Department of Entomology and Plant Pathology, North Carolina State University. These leaves were inoculated using P. infestans a US 23 genotype; isolate NC 14-1. To collect P. infestans sporangia, infected leaves were held vertically, and 1 mL of DI water was sprayed on the abaxial side of leaves. The runoff liquid containing sporangia was collected in a beaker, and the number of sporangia per mL in the collected solution was estimated using a hemocytometer (Hausser Scientific, USA). Then, the solution was diluted to 10,000 sporangia per mL for inoculation onto healthy leaves. For spraying, the diluted solution was transferred in a 15 mL Falcon tube with a spray cap (Container & Packaging Supply Inc., USA). All healthy leaves were inoculated under 1.5% water agar plate. The water agar helps to maintain the humidity of the plate during inoculation. Healthy leaves facing abaxial side up were placed on the non-agar side of the plate and sprayed with 2 mL of sporangia solution on each leaf. The agar sides of the plate were then placed on top of non-agar sides. The plates were sealed using parafilm and incubated at room temperature. For negative controls, healthy leaves were sprayed with 2 mL of DI water and kept under the same conditions as inoculated leaves in sealed water agar plates. After inoculation, sporangia penetrate the plant leaf tissue to gain nutrition and spread hyphae throughout the leaf.⁴⁹ After 3 to 4 days, hyphae usually come out on the surface and generate new sporangia. Figure S2

illustrates the progress of the disease in different days for an inoculated leaf after inoculation, showing lesions (water soaked, dark spots) and white growth (hyphae) on the leaf surface.

Conventional PCR Amplification. All necessary reagents and primers for conventional PCR amplification were purchased from Thermo Fisher Scientific, USA. All PCR reactions were run on a Simpliamp Thermal Cycler (Thermo Fisher Scientific, USA). For rbcl gene amplification of plant plastid DNA, Rbcla-F (5'-ATGTCACCACAAACAGAGACTAAAGC-3') and rbcLajf634R (5'-GAAACGGTCTCTCCAACGCAT-3')⁴⁰ primers were used. In a PCR reaction, these primers generate an amplicon of a length of 670 bp. The reaction master mix for rbcl gene detection consists of 1x PCR buffer, 0.1 mM dTNPs, 0.4 µM of each primer, 1.8 mM magnesium chloride, 0.1 mg/mL BSA and 0.04 U/ μ L Tag DNA polymerase. The amplification reactions were performed in 25 µL volumes, and for each PCR reaction, ~10 ng template DNA was used. For negative controls, no DNA was used in the amplification reaction for testing the contamination of the reagents. Cycling conditions were 94 °C for 2 min (initial denaturation) followed by 35 cycles of 30 s at 94 °C (denaturation), 45s at 54 °C (annealing), 45s at 72 °C (extension). After that, the temperature was set to 72 °C for 5 min for final extension followed by a hold at 4°C. For late blight disease detection, two P. infestans specific primers, PINF2 (5'-CTCGCTACAATAGCAGCGTC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') were used for amplification reaction.^{16,41} The primer pair amplifies a region of ~610 bp in the target amplicon. The reaction master mix consists of 1x PCR buffer, 0.1 mM dTNPs, 0.4 µM of each primer, 1.8 mM magnesium chloride, 0.1 mg/mL BSA and 0.02 U/µL Taq DNA polymerase. The amplification reactions were performed in 25 µL volumes by mixing 24 µL master mix and 1 μ L template DNA (concentration ~10 ng/ μ L). Thermal cycling conditions were 94 °C for 2 min (initial denaturation) followed by 35 cycles of 15 s at 94 °C (denaturation), 15s at 56 °C

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(annealing), 15s at 72 °C (extension). After that, the temperature was set to 72 °C for 5 min for final extension followed by a hold at 4°C. Either DNA extracted from healthy leaves or no DNA (sample collected from blank MN patch) were used as negative controls in PCR.

Gel Electrophoresis. After amplification, gel electrophoresis was performed to visualize the amplified PCR products. For that, agarose, SYBR safe DNA gel stain (20000X), 10x Tris-Borate-EDTA (TBE) buffer, DNA Gel loading dye, and 100 bp DNA ladder were purchased from Thermo Fisher Scientific, USA. All PCR amplified products were visualized in 2 % agarose gel. For making agarose gel, 1.2 g agarose and 6 μ L SYBR safe DNA gel stain were mixed with 60 mL of 1x TBE buffer in a glass bottle and microwaved for 1.5 min. The microwave was stopped every 30 s for suspending the undissolved agarose by gentle swirling of the glass bottle. Later, the dissolved agarose solution was kept at room temperature for cooling to 60°C before pouring to a gel casting tray (7cm×10 cm). After solidification, the gel was transferred to Sub-Cell® GT Agarose Gel Electrophoresis Systems (Bio-Rad) for gel electrophoresis. 1x TBE buffer was used for running the agarose gel, and 5 μ L PCR amplified product and 1 μ L 6x DNA loading dye were mixed for gel loading. After running the gel, the image was captured using E-GelTM Imager System with Blue Light Base (Thermo Fisher Scientific).

Quantitative PCR (qPCR) Amplification. For real-time assay of late blight disease detection, EvaGreen (20,000x) dye was purchased from Biotium, USA, and the dye was diluted to 20x in DI water before preparing the master mix. All other required reagents and primers were purchased from Thermo Fisher Scientific, USA.

For real-time detection, two primers, PINF2 (5'-CTCGCTACAATAGCAGCGTC-3') and HERB2 (5'-CGGACCGACTGCGAGTCC-3') were used, and these primers amplify a 100 bp

region of the internal transcribed spacer (ITS) region 2 of *P. infestans* in the amplification reaction.⁴²The amplification reactions were run in 25 μ L volumes consisting of 1 μ L template DNA (concentration ~10 ng/ μ L), 2.5 μ L of 10x PCR buffer, 1.25 μ L of dNTPs (2mM each), 1 μ L of 10 μ M PINF2, 1 μ L of 10 μ M HERB2, 1.25 μ L of 20x EvaGreen, 1.25 μ L of 50 mM magnesium chloride, 0.05 μ L of 50 mg/ml BSA, 0.1 μ L of 5 U/ μ L Taq DNA polymerase and 15.6 μ L of DI water. Quantitative PCR reactions were carried out on the CFX ConnectTM Real-Time System (Bio-Rad), and thermal cycling conditions were 94 °C for 2 min (initial denaturation) followed by 35 cycles of 15 s at 94 °C (denaturation), 15s at 56 °C (annealing), 15s at 72°C (extension) and, the fluoresce signal was captured for each cycle after the extension stage. After thermal cycling, the temperature was maintained at 72°C for 5 min for the final extension of products. Finally, melt curve analyses were performed by slowly raising the temperature to 95°C for finding the melt temperatures of the amplified product.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/

Quantification of *P. infestans* concentrations by a qPCR assay using EvaGreen; images of laboratory-inoculated tomato leaves; quantitative analyses of DNA extracted from laboratory-inoculated leaves at different inoculation days; melt curve and threshold cycle analyses for late blight disease detection from field

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collected tomato leaves (PDF); movie of the procedure of MN patchbased plant DNA extraction (mp4)

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