DNA Extraction from Plant Leaves Using a Microneedle Patch

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Isolation of high-quality DNA from infected plant specimens is an essential step for the molecular detection of plant pathogens. However, DNA isolation from plant cells surrounded by rigid polysaccharide cell walls involves complicated steps and requires benchtop laboratory equipment. As a result, plant DNA extraction is currently confined to well-equipped laboratories and sample preparation has become one of the major hurdles for on-site molecular detection of plant pathogens. To overcome this hurdle, a simple DNA extraction method from plant leaf tissues has been developed. A microneedle (MN) patch made of polyvinyl alcohol (PVA) can isolate plant or pathogenic DNA from different plant species within a minute. During DNA extraction, the polymeric MN patch penetrates into plant leaf tissues and breaks rigid plant cell walls to isolate intracellular DNA. The extracted DNA is polymerase chain reaction (PCR) amplifiable without additional purification. This minimally invasive method has successfully extracted *Phytophthora infestans* DNA from infected tomato leaves. Moreover, the MN patch could be used to isolate DNA from other plant pathogens directly in the field. Thus, it has great potential to become a rapid, on-site sample preparation technique for plant pathogen detection.

Basic Protocol: Microneedle patch-based DNA extraction
Support Protocol 1: Microneedle patch fabrication
Support Protocol 2: Real-time PCR amplification of microneedle patch extracted DNA

Keywords: DNA extraction • in-field diagnostics • microneedle patch • PCR • plant pathogen • MN patch

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INTRODUCTION

Plant pathogens play a key role in worldwide agricultural productivity. On a global scale, ∼20% to 30% annual crop losses are due to plant pathogens and pests (Oerke, 2006; Savary, Ficke, Aubertot, & Hollier, 2012). To minimize the economic loss caused by plant pathogens, several diagnostic techniques, such as nucleic acid amplification (NAA) via polymerase chain reaction (PCR) or loop-mediated isothermal amplification (LAMP); enzyme-linked immunosorbent assays (ELISA); immunofluorescence (IF); and a smartphone-based volatile organic compound (VOC) sensor, have been developed for the detection of plant pathogens (Fang & Ramasamy, 2015; Li et al., 2019; Ristaino, Saville, Paul, Cooper, & Wei, 2019; Sankaran, Mishra, Ehsani, & Davis, 2010). Among these methods, NAA-based assays are most widely used for the identification of pathogen species (Haas et al., 2009; Ristaino, Groves, & Parra, 2001). The first step of the NAA assay is to isolate pathogens' genetic code (e.g., DNA or RNA) from complex plant tissues. However, the extraction of high-quality DNA from plant tissues is a complicated, multistep process due to the presence of mechanically stable polysaccharide cell walls (Current Protocols article: Leach, McSteen, & Braun, 2016; Murray & Thompson, 1980; Current Protocols article: Zhou et al., 2016). Currently, plant DNA isolation is carried out by following conventional DNA extraction protocols, such as the cetyltrimethylammonium bromide (CTAB)-based extraction protocol, in a well-equipped laboratory before running the amplification reaction for pathogen identification. As a result, NAA assays are confined to laboratory settings. To facilitate the transfer of NAA assays from laboratory to field, we have developed a rapid DNA extraction method from plant leaves using a polymeric microneedle (MN) patch (Paul et al., 2019). In this protocol, we describe the detailed steps to perform MN patch-based DNA extraction. Besides the DNA extraction procedure, we also discuss the protocols for MN patch fabrication and real-time PCR amplification of MN patch extracted DNA.

The MN extraction method involves two simple steps. First, an MN patch is pressed gently by hand on a plant leaf for a few seconds. After that, the MN patch is removed and washed with 100 μl TE buffer to collect DNA from the needle tips. Each MN patch has 225 conical MNs which penetrate through the leaf surface and break cell walls to fish out DNA. The MN patches are made of polyvinyl alcohol (PVA). PVA and its derivatives are highly water-swellable polymers (Hassan & Peppas, 2000; Kim, Lee, Kim, Lee, & Kim, 2003). As a result, these MNs rapidly swell by absorbing intracellular water molecules during the extraction process and deposit DNA on the surfaces of needle tips. The entire MN extraction process takes less than a minute and the extracted DNA is ready for PCR amplification without additional purification steps. Therefore, the MN patch could be a great tool to isolate plant or pathogenic DNA directly in the field and integrating this simple DNA extraction method with miniaturized DNA amplification devices will create an on-site sample-to-answer plant pathogen detection platform.

BASIC PROTOCOL

MICRONEEDLE PATCH-BASED DNA EXTRACTION

This protocol describes a DNA extraction procedure using an MN patch. The overall DNA extraction time for this method is less than a minute and it involves two simple steps, as shown in Figure 1. During DNA extraction, polymeric MNs break the plant cell walls and fish out intracellular DNA from a plant leaf. These MN patches are successfully used to extract genomic or pathogenic DNA from several plant species, such as potato, tomato, and pepper (Figure 2).

Materials

Plant leaves (e.g., fresh tomato, potato, or pepper leaves)
1× TE buffer (pH 8; Integrated DNA Technologies, cat. no. 11-05-01-09)

Parafilm (Thermo Fisher Scientific, cat. no. NC9751594)
Disposable gloves (Thermo Fisher Scientific, cat. no. 19-149-863B)
Microneedle (MN) patch (see Support Protocol 1)
1,000-µl micropipet (Thermo Fisher Scientific, cat. no. FBE01000)
1,250-µl pipet tip (VWR International, cat. no. 10017-030)
1.5-ml microcentrifuge tube (Thermo Fisher Scientific, cat. no. 05-408-129)
NanoDrop One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, cat. no. ND-ONE-W)

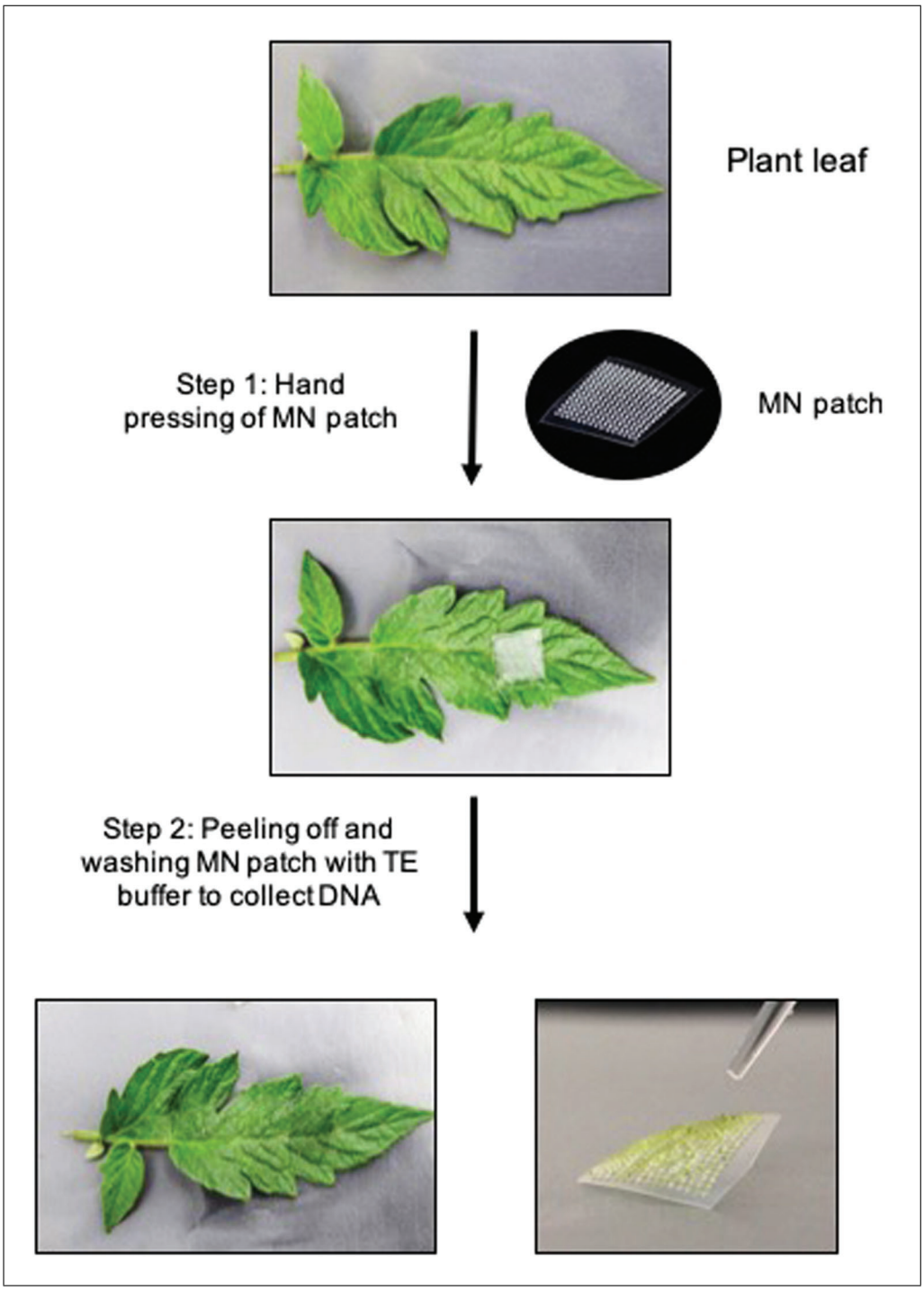

Figure 1 Steps of microneedle (MN) patch-based DNA extraction method from a fresh plant leaf.

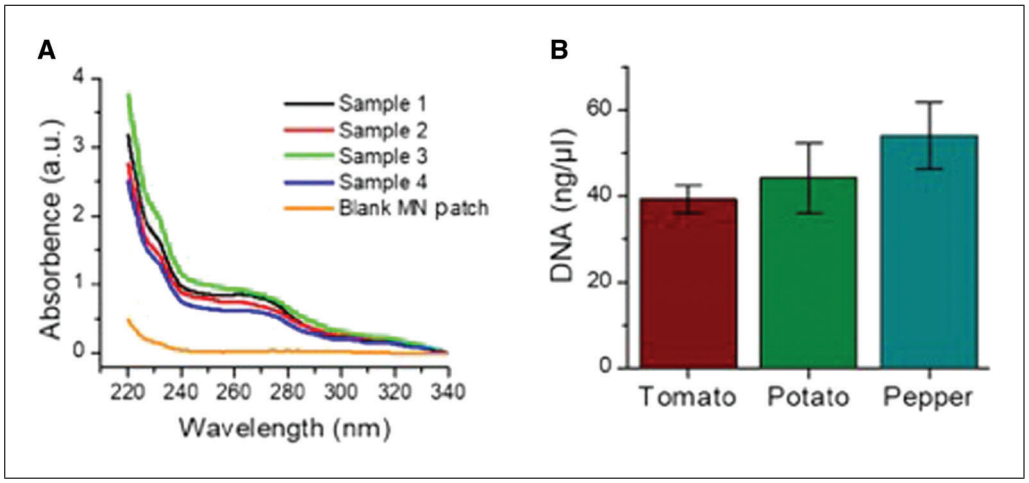

Figure 2 Plant DNA extraction using an microneedle (MN) patch. (**A**) UV absorption spectra of DNA extracted by MN patches from tomato leaves. The orange curve (negative control) represents the UV spectrum of TE buffer used for washing a blank MN patch without leaf puncturing. (**B**) DNA extraction from three different plant species using MN patches. Four different fresh leaves of each species were used for DNA extraction.

Extract DNA from plant leaves

1. Cut a small piece of Parafilm and place on a hard, smooth surface such as laboratory workbench.

   For field extraction, any portable solid and flat surface such as notebook, wood board, or the back of a cell phone could be used.

2. Place a plant leaf on Parafilm and choose a leaf area for DNA extraction.

   Carefully select the DNA extraction area for pathogen detection. A visual inspection helps to identify the infected areas in a leaf. For example, a tomato leaf infected by P. infestans shows dark lesions. For best results, gloves are recommended to prevent cross contamination. Surface disinfestation of leaves is not a requirement.

3. Take MN patch and place on top of the selected area of the leaf.

   Make sure needles are facing downward.

4. Press gently by hand 5 to 10 s for DNA extraction.

5. Peel off MN patch and place on the Parafilm.

   Make sure needles are facing upward.

6. Wash MN patch four to five times using 100 µl TE buffer (pH 8).

7. Transfer extracted DNA to a 1.5-ml microcentrifuge tube.

8. Store extracted DNA solution at −20°C.

Sample data

9. Quantify the total amount and purity of DNA extracted by an MN patch using a NanoDrop One Microvolume UV-vis spectrophotometer.

   The extracted DNA solution from plant leaf may contain polysaccharides, proteins, cell debris, and other intracellular molecules (Varma, Padh, & Shrivastava, 2007). However, among these impurities, proteins and polysaccharides are major contaminants for MN patch-based DNA extraction. The characteristic wavelengths of UV absorption for polysaccharides, DNA, and proteins are 230, 260, and 280 nm, respectively. Therefore, UV absorption (or optical density, OD) at 260 nm indicates the total DNA amount and absorption ratios of A_{260}:A_{280} and A_{260}:A_{230} inform the purity of the extracted DNA.

Figure 2A shows an average UV spectra for MN patch-based DNA extraction for four different tomato leaves, where significant UV absorption at 260 nm indicates the presence of DNA in extracted solutions. However, the washing solution of a blank MN patch without leaf punctuation (orange curve) does not show insignificant absorption at 260 nm. Furthermore, this rapid DNA extraction method is successfully applied to isolate DNA from different plant leaves, including fresh potato and pepper leaves (Figure 2B). For all three plant species, consistent extraction performance has been achieved by using MN patches.

The standard values of A_{260}:A_{280} and A_{260}:A_{230} for pure DNA solution are between 1.8-2.0 and >1.8, respectively. For the MN patch-based DNA extraction method, the average purity ratios are 1.44 (A_{260}:A_{280}) and 0.47 (A_{260}:A_{230}). Because of the absence of additional purification steps, relatively low A_{260}:A_{230} ratios have been observed for the MN patch-based method. In contrast, conventional DNA extraction methods such as the CTAB-based method involve extensive DNA purification steps for achieving high values of purity ratios (Murray & Thompson, 1980). However, the quality of MN-extracted DNA is still sufficient for PCR-based analysis without any purification steps (see Support Protocol 2).

10. If other applications require more purified DNA, purify the MN patch extracted DNA by following a standard DNA purification protocol.

MICRONEEDLE PATCH FABRICATION

SUPPORT PROTOCOL 1

This protocol describes the fabrication procedure of an MN patch using a polydimethylsiloxane (PDMS) mold. The fabrication process is very simple and inexpensive and it does not involve any special facilities such as cleanrooms. Each MN patch consists of 225 conical MNs and these strong MNs easily penetrate human skin and plant tissues without breaking (Wang et al., 2018; Wang, Ye, Hochu, Sadeghifar, & Gu, 2016; Yu et al., 2015).

Materials

10% polyvinyl alcohol (PVA) solution (see recipe)
Silica gel (Thermo Fisher Scientific, cat. no. S161-500)

Ultrasonic cleaning bath (Thermo Fisher Scientific, cat. no. 15-337-418)
Microneedle (MN) mold (Blueacre Technology)
Polycarbonate clear vacuum chamber (SP Scienceware, cat. no. 999320237)
Vacuum pump (Gast Manufacturing, cat. no. 0523-545Q-G588DX)
1,000-µl micropipet (Thermo Fisher Scientific, cat. no. FBE01000)
1,250-µl pipet tip (VWR International, cat. no. 10017-030)
Petri dish (Thermo Fisher Scientific, cat. no. FB0875714G)
Kimwipes (Thermo Fisher Scientific, cat. no. 06-666)

Clean microneedle mold

1. Place MN mold in the ultrasonic bath for 5 min.

   Sonication removes residual PVA from the cavities of the MN mold.

2. Wipe MN mold using Kimwipes to remove excess water from the surface.

Fabricate MN patch

3. Add 500 µl 10% PVA solution to the mold.
4. Place mold in the vacuum chamber containing silica gel and seal chamber.
5. Seal vacuum chamber and turn on the vacuum pump to draw the PVA solution into needle cavities.

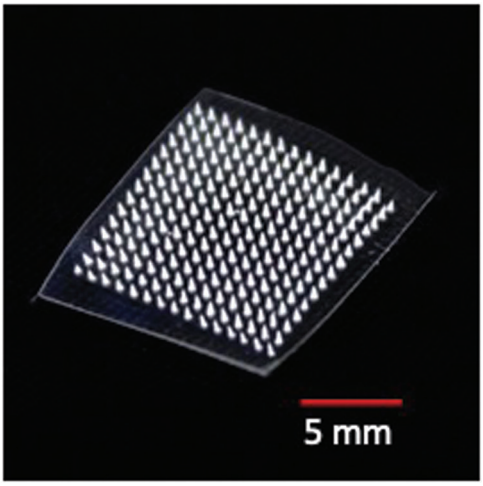

Figure 3 Photograph of a microneedle (MN) patch. It contains a 15 × 15 microneedle array. The height of each needle is 800 µm and the tip diameter and base are 10 and 300 µm, respectively.

6. Turn off vacuum after 20 min.

   Do not open the vacuum chamber immediately after turning off the pump. Wait for another 15 to 20 min for air bubbles to dissipate.

7. Transfer mold in a chemical hood and keep overnight at 25°C to dry the PVA solution.
8. After drying, carefully peel away MN patch from the mold; a well-formed MN patch exhibits a visible array of MNs without significant breakage.
9. Store MN patch at room temperature in a sealed petri dish for future use.

   Figure 3 shows a properly formed MN patch. All MNs are intact and show proper dimensions.

SUPPORT PROTOCOL 2

REAL-TIME PCR AMPLIFICATION OF MICRONEEDLE PATCH EXTRACTED DNA

This protocol describes real-time PCR amplification of DNA extracted using an MN patch. The MN patch-based DNA extraction method is a purification-free protocol. After extraction, we directly mix 1 µl extraction solution with the PCR master mix for DNA amplification. In the PCR, both plant and pathogen DNA can be amplified, depending on the choice of primers. To demonstrate the presence of plant DNA in the MN rinsing solution, rbcl_Rf (5′-GTAACTCCTCAACCTGGAGTTC-3′) and rbcl_Rb (5′-GTAAGTCCATCGGTCCATACA G-3′) primers have been used to amplify the ribulose-bisphosphate carboxylase gene (*rbcL*) of plant plastid DNA. These primers generate a 103-bp amplicon in the PCR. On the other hand, for plant pathogen detection, PINF2 (5′-CTCGCTACAATAGCAGCGTC-3′) and HERB2 (5′-CGGACCGACTGCGAGTCC-3′) are used to amplify a 100-bp region of the internal transcribed spacer (ITS) region 2 of *P. infestans* (Ristaino, Hu, & Fitt, 2013). After PCR amplification, melt curve analyses are performed to confirm the presence of target amplicons.

Materials

10× PCR buffer (Thermo Fisher Scientific, cat. no. 18067017)
50 mM magnesium chloride ($MgCl_2$; provided in *Taq* DNA Polymerase PCR buffer, Thermo Fisher Scientific, cat. no. 18067017)
dNTPs, 2 mM each (Thermo Fisher Scientific, cat. no. R0241)

20× EvaGreen (Biotium, cat. no. 31000)
50 mg/ml BSA (Thermo Fisher Scientific, cat. no. AM2616)
Taq DNA polymerase (Thermo Fisher Scientific, cat. no. EP0702)
Primers (Integrated DNA Technologies)
P. infestans detection:
Forward primer-PINF2 (5′-CTCGCTACAATAGCAGCGTC-3′)
Reverse primer-HERB2 (5′-CGCACCGACTGCGAGTCC-3′)
rbcl gene detection:
Forward primer -rbcl_Rf (5′-GTAACTCCTCAACCTGGAGTTC-3′)
Reverse primer -rbcl_Rb (5′-GTAAGTCCATCGGTCCATACA G-3′)

2-µl micropipet (Thermo Fisher Scientific, cat. no. FBE00002)
20-µl micropipet (Thermo Fisher Scientific, cat. no. FBE00020)
200-µl micropipet (Thermo Fisher Scientific, cat. no. FBE00200)
10-µl pipet tip (Thermo Fisher Scientific, cat. no. 02-682-258)
200-µl pipet tip (Thermo Fisher Scientific, cat. no. 13-811-138)
PCR tube (Bio-Rad, cat. no. TLS0801)
PCR tube cap (Bio-Rad, cat. no. TCS0803)
CFX Connect Real-Time PCR Detection System (Bio-Rad, cat. no. 1855200)

PCR amplification

1. Thaw 10× PCR buffer, dNTPs, primers, $MgCl_2$, and BSA at room temperature.
2. After thawing, quickly vortex reagents (∼5 s).

   Do not vortex DNA polymerase. Vortexing will deactivate the enzyme.

3. Briefly centrifuge (5-10 s, 100 × *g*) to collect everything at the bottom of the tube and place reagents on ice.
4. In a PCR tube, prepare 24 µl PCR master mix:

   2.5 µl 10× PCR buffer,
   1.25 µl dNTPs (2 mM each),
   1 µl 10 µM forward primer,
   1 µl 10 µM reverse primer,
   1.25 µl 20× EvaGreen,
   1.25 µl 50 mM magnesium chloride,
   0.05 µl 50 mg/ml BSA,
   0.1 µl 5 U/µl *Taq* DNA polymerase, and
   15.6 µl deionized water.

   Keep PCR tube on ice and add DNA polymerase last.

5. Add 1 µl DNA solution.
6. Close cap of the PCR tube and gently flick to mix everything.
7. Centrifuge (∼10 s, 100 × *g*) to collect reaction mixture at the bottom of the PCR tube.
8. Perform PCR on a real-time PCR thermal cycler according to the settings given in Table 1 (for *rbcl*) or Table 2 (for *P. infestans*).

Analyze melt curve

9. Perform melt curve analysis in a real-time PCR thermal cycler to confirm the presence of target amplicon in PCR (see Table 3).

Gel electrophoresis

10. Perform gel electrophoresis in 2% agarose gel to visualize amplified bands.

Table 1 Temperature Settings for PCR Amplification of the *rbcl* Gene

Cycle	Temperature	Time	
1	94°C	2 min	(initial denaturation)
2-35	94°C	15 s	(denaturation)
	56°C	30 s	(annealing)
	72°C	15 s	(extension)
		Fluorescence signal measurement	
	72°C	5 min	(final extension)

Table 2 Temperature Setting for PCR Detection of *Phytophthora infestans*

Cycle	Temperature	Time	
1	94°C	2 min	(initial denaturation)
2-35	94°C	15 s	(denaturation)
	56°C	15 s	(annealing)
	72°C	15 s	(extension)
		Fluorescence signal measurement	
	72°C	5 min	(final extension)

Table 3 Melt Curve Temperature Settings

Temperature	Time
72°-95°C (increment: 0.5°C)	5 s Fluorescence signal measurement

Sample data

During the PCR reaction, the number of duplex DNA doubles in every cycle and at the end of each cycle, amplicons are denatured to use as new templates for the next cycle. For real-time detection, EvaGreen dye is added to the reaction mixture. This dye binds to duplex DNA and emits fluorescence signals that are proportional to the concentration of amplicons (Mao, Leung, & Xin, 2007). The real-time thermal cycler reads that emitted fluorescence signal and plots as a function of cycle number.

PCR successfully amplifies both plant and pathogenic DNA from MN-extracted samples. Figure 4A and B present real-time amplification curves for the detection of the *rbcl* gene and *P. infestans,* respectively. The average threshold cycle (Ct) values are 28.45 (*rbcl* gene) and 32 (*P. infestans)*, respectively. The lower average Ct value for the *rbcl* gene indicates that the concentration of plant DNA is higher than pathogen DNA in MN patch extracted samples. After PCR amplification, the presence of single amplicons in different samples is confirmed by single melt peaks in melt curve analyses (Figure 4C and D). The melting temperature of an amplicon depends on its length and sequence. As a result, we observe two different melt temperatures for the *rbcl* gene ($T_m = 84.5$) and *P. infestans* ($T_m = 82.5$).

REAGENTS AND SOLUTIONS

Polyvinyl alcohol (PVA) solution, 10%

15 g PVA, MW ~30,000 (MilliporeSigma, cat. no. 821039)
85 g deionized water
Heat water to 60° to 70°C on a magnetic stirrer hot plate.

Gradually add PVA. Keep temperature constant and continuously stir solution until PVA is completely dissolved. Cool and store at room temperature for up to 6 months.

COMMENTARY

Background Information

The extraction of high-quality DNA from plant tissues is a complex chemical process due to the presence of mechanically stable polysaccharide cell walls and chemical heterogeneity of secondary metabolites of

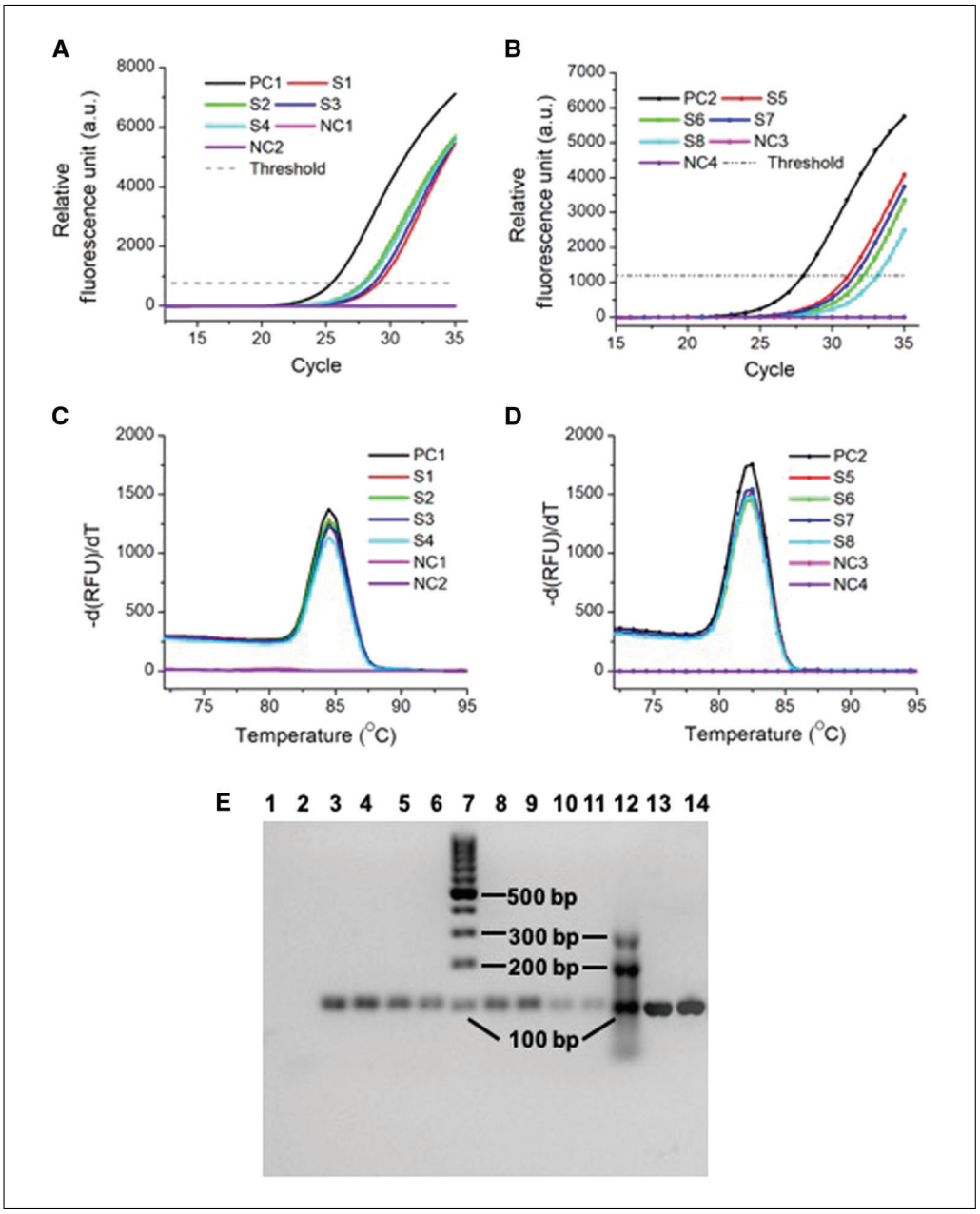

Figure 4 PCR amplification of DNA extracted by microneedle (MN) patches. (**A**, **B**) Real-time amplification curves for (A) *rbcl* gene detection and (B) *P. infestans* detection. (**C, D**) Melt curves of amplicons for (C) *rbcl* gene detection and (D) *P. infestans* detection. Black lines represent positive controls (PC1 and PC2), magenta (NC1 and NC3), and violet (NC2 and NC4) lines represent negative controls. (**E**) Gel electrophoresis showing amplified bands of the *rbcL* gene and *P. infestans*. Lanes 1 and 2: Negative controls (NC1 and NC3); Lane 3-6: Four different amplified bands of *rbcl* gene (S1, S2, S3, and S4); Lane 7: 100-bp DNA ladder; Lane 8-11: Four different amplified bands of *P. infestans* (S5, S6, S7, and S8); Lane 12: 20-bp DNA ladder; Lane 13 and 14: Positive controls (PC2 and PC1). RFU, relative fluorescence unit.

Table 4 Troubleshooting for DNA Extraction by Microneedle (MN) Patches

Problem	Possible cause	Solution
Low yield of DNA	Old leaf	Use fresh leaf if possible Extract DNA as early as possible after collecting leaves
	MN patch is not effectively pressed into the plant leaf	Press MN patch for longer time
	Microneedles are not rinsed properly	Rinse microneedles more thoroughly with TE buffer
	MN patch has broken microneedles	Use MN patch with intact needles
	MN patch is not mechanically strong	Use newly fabricated MN patch
		Store MN patches in a covered container to prevent exposure to air and absorption of moisture
Foaming when rinsing MN patch	Rinsing time too long	Decrease rinsing time
MN patches do not have properly formed microneedles	PVA solution does not flow into mold cavities	Increase vacuum time
	Mold contains broken microneedle fragments from previous fabrication	Increase sonification time
	Microneedles break when removed from the mold	Gently peel MN patches off molds
		Increase drying time before removing MN patches
		Use adhesive tape to remove MN patches
Mechanically weak MN patch	Not enough PVA used	Increase amount of PVA solution
		Increase concentration of PVA solution
No amplification in PCR reaction	Primers fail to bind to template DNA	Check primer specificity with positive control
	High annealing temperature	Optimize annealing temperature
	Low concentration of template DNA	Increase amount of DNA added to the PCR
	DNA was not stored in optimal conditions after extraction	Store DNA in the freezer at −20°C
False-positive amplification	Contamination	Use a separate lab bench for DNA extraction and PCR amplification
		Change pipet tip with each transfer
		Avoid contact with possible contaminants during extraction of DNA and making of master PCR mix

different species. As a result, current plant DNA extraction technology is a complicated multistep process and limited to laboratory settings (Current Protocols article: Leach et al., 2016; Murray & Thompson, 1980; Varma et al., 2007; Current Protocols article: Zhou et al., 2016).

The cetyltrimethylammonium (CTAB)-based DNA extraction method is considered the gold standard for plant DNA extraction and this method has been widely used to isolate DNA from complex plant specimens for more than 40 years (Murray & Thompson, 1980). The CTAB-based DNA extraction method involves mechanical grinding of specimens, chemical cell lysis, high-temperature incubation, organic phase DNA separation, salt and ethanol assisted DNA precipitation, and purification. Therefore, this method is time consuming and requires skilled lab personnel to perform these steps in a well-equipped laboratory. Moreover, the CTAB method requires toxic chemicals such as CTAB and chloroform, which are harmful to humans and the environment. Due to the complexity and involvement of health hazardous chemicals, a miniaturized version of the CTAB method has not been developed for on-site isolation of plant DNA. Besides the CTAB method, the sodium hydroxide (NaOH)-based rapid DNA extraction method is also frequently used to isolate DNA for PCR analysis. In the NaOH-based method, samples are homogenized in a NaOH solution for cell lysis, and then, to prevent the degradation of released DNA, 3 to 5 µl of cell lysate is rapidly mixed with a buffer solution to adjust the pH of the cell lysate (Wang, Qi, & Cutler, 1993). However, the DNA yield for this method is relatively low because the cell lysate is diluted to a hundred times for pH adjustment. In addition, transportation and storage of chemicals will be challenging for conventional chemical-based methods.

To overcome these barriers, a completely new method of plant DNA extraction using the MN patch is presented in this article. The MN patch-based DNA extraction method is simple to perform and does not require any chemicals for DNA isolation and purification. In the MN method, polymeric MNs break plant cells and concentrate released DNA on needle tips then DNA is collected by rinsing with TE buffer. The overall extraction process takes less than a minute and the isolated DNA is ready for DNA amplification assay. Therefore, integration of this rapid MN patch-based DNA extraction method with portable DNA amplification assays (e.g., miniaturized PCR; Jo, Gross, Shim, & Han, 2013; Julich et al., 2011) or the loop-mediated isothermal amplification (LAMP) assay (Ristaino et al., 2019) and reader devices (Kong et al., 2017) would help farmers and extension workers to screen plant pathogens directly in their fields.

Critical Parameters

During DNA extraction, the MN patch must be applied to the surface of the plant leaf with gentle pressure. The MNs may break if excessive pressure is applied due to contact with the hard surface below the leaf. Moreover, special care must be taken during the fabrication of MN patches. Vacuum time must be optimized for effective fabrication of the MN patch. A vacuum time that is too short will result in misformed MNs because the PVA solution will not flow into the cavities of the mold. The concentration of the PVA solution must also be optimized. If the concentration is too low, the MN patches will not be mechanically strong. On the other hand, for high PVA concentration, the solution will be too viscous to flow into the cavities of the mold. Another important parameter is the molecular weight of the PVA used. Increasing the molecular weight will increase the mechanical strength of the MN patches, but it will also increase the viscosity of the PVA solution.

Troubleshooting

See Table 4 to troubleshoot common problems that may arise during MN patch-based DNA extraction.

Understanding Results

We have included sample data at the end of each protocol. In summary, for each fresh plant leaf, the MN patch-based DNA extraction method usually yields 30 to 60 ng/µl DNA and the expected values of A_{260}:A_{280} and A_{260}:A_{230} are 1.45 and 0.5, respectively.

Time Considerations

DNA extraction by MN patch: MN patch-based DNA extraction from a single location of a fresh leaf usually takes 30 to 45 s.

MN patch fabrication: The overall time for MN patch fabrication is ∼24 hr.

Real-time PCR amplification: Master mix preparation for PCR should take ∼30 min and PCR thermal cycling an additional 60 to 90 min.

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