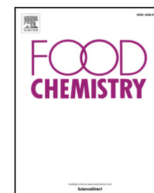
Food Chemistry 274 (2019) 822–830

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



A rapid sample-to-answer analytical detection of genetically modified papaya using loop-mediated isothermal amplification assay on lab-on-a-disc for field use

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ARTICLE INFO

Keywords:
Lab-on-a-disc
Loopmediated isothermal amplification
Sample-to-answer
Transgenic food screening
On-site detection

ABSTRACT

With genetically modified (GM) food circulating on the market, a rapid transgenic food screening method is needed to protect consumer rights. The on-site screening efficiency of GM food testing is low. We report rapid sample-to-answer detection of GM papayas with loop-mediated isothermal amplification (LAMP) and a compact, portable, integrated microfluidic platform using microfluidic lab-on-a-disc (LOAD). GM samples were differentiated from non-GM papaya, based on the detection of a specific GM (P-35S (Cauliflower mosaic virus 35S promoter)) and non-GM DNA marker (papain) in 15 min. The detection limits for DNA and juice from papaya were 10 pg/μL and 0.02 μL, respectively. Our LOAD platform is a simple and robust solution for GM screening, which is anticipated to be a foundation for on-site testing of transgenic food.

1. Introduction

Transgenic or genetically modified (GM) foods are defined by the World Health Organization as products from plants or animals, the genetic material of which has been modified by genetic engineering (World Health Organization, 2014). Such modifications introduce new traits to increase yield and resistance to disease. In the USA, 90% of crop, such as maize and soybeans, contain genetically modified organism (GMO) (United States Department of Agriculture, 2018). Since there are on-going concerns about GMO safety, labeling of foods containing GMO products is compulsory in some regions, such as the European Union, Australia and Taiwan, in order to provide additional information for consumers (Bawa & Anilakumar, 2013; Twardowski & Malyska, 2015). However, in other places, such as Hong Kong, where there is no mandatory labelling, consumers are unsure as to whether foods they purchase contain GMO products or not (Hong Kong Government, 2006). In 2015, the Hong Kong government reported that more than 60% (average 24 out of 39 samples tested annually) of imported papayas were transgenic (Agriculture, Fisheries and Conservation Department, 2015).

Papaya (Carica papaya), which originates in southern Mexico and Costa Rica, were later cultivated in other tropical and subtropical countries and are now the third largest tropical fruit crop worldwide. They have a high economic value because of their high nutrient content and multiple industrial uses (e.g. juice, dried pieces) (Evans & Ballen, 2012). A major threat to papaya plantations is the Papaya Ringspot Virus (PRSV) (Gonsalves, 2004). The first successful attempt to create and commercialize PRSV-resistant papayas was based on regulation of RPSV-resistant transgene expression by the cauliflower mosaic virus 35S promoter (P-35S) (Fitch, Manshardt, Gonsalves, Slightom, & Sanford, 1990). In the 1990s, transgenic seeds were distributed to farmers for free, which resulted in the spread of GM papayas globally, and a high occurrence rate of P-35S papaya across the market (Tecson-Mendoza, Laurena, & Botella, 2008).

Abbreviations: GM, genetically modified; GMO, genetically modified organism; LAMP, loop-mediated isothermal amplification; LOAD, lab-on-a-disc; PCB, printed circuit board; PCR, polymerase chain reaction; PDMS, polydimethylsiloxane; P-35S, cauliflower mosaic virus 35S promoter; PRSV, Papaya Ringspot Virus; RT-LAMP, realtime loopmediated isothermal amplification

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https://doi.org/10.1016/j.foodchem.2018.09.049
Received 12 December 2017; Received in revised form 1 September 2018; Accepted 9 September 2018
Available online 11 September 2018
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Traditionally, polymerase chain reaction (PCR)-based methods are employed for detection of GM products (Kim et al., 2017). P-35S is a fingerprint for GM, detected by PCR, because it is a non-plant-derived promoter for enhancing transgene expression and is used widely in GM foods (Tecson-Mendoza, Laurena, & Botella, 2008; Goda, Asano, Shibuya, Hino, & Toyoda, 2001; Guo et al., 2009; Wang, Teng, Guan, Tian, & Wang, 2015; Nakamura et al., 2016). The disadvantages of using PCR to detect GM foods include the time-consuming thermal cycling processes and the need for an expensive thermocycler, both of which hinders use for on-site detection. Recently, loop-mediated isothermal amplification (LAMP), relying on the continuous amplification of loop-formed DNA by Bst polymerase with strand-displacement activity at a single temperature, has been developed and verified with different GM/non-GM foods (Guan, Guo, Shen, Yang, & Zhang, 2010; Li et al., 2013). In LAMP reactions, four to six primers for amplification of target (GM) DNA are required to generate loop-formed DNA, which increases sensitivity and specificity when compared to more traditional PCR methods (Kim and Kim, 2018).

LAMP detection using benchtop procedures cannot readily be transferred to on-site field work because access to equipment and power are limited. Microfluidics allows the manipulation of microliters quantities of fluid through a network of channels, valves and compartments at the micrometer scale. Compared with labor-intensive traditional molecular screening of GM markers using lab-bench instruments, microfluidics reduces the size and, therefore, portability of essential equipment as well as the volumes of samples and reagents used. These advantages have enabled investigators to perform rapid and large-scale screening in the field. Shortcomings of microfluidics, however, include the need for pumps and connecting tubes for sample actuation. An alternative might be centrifugal microfluidics, also known as lab-on-a-disc (LOAD), which was introduced for automatic fluid actuation with the following advantages. In LOAD, fluid flow is controlled by tunable rotating speeds, meaning centrifugal force is available everywhere on the disc for handling of samples and reagents. No pumps, actuators, or active valves are needed, simplifying the design and work carried out on the disc. Also, a high degree of parallelization is possible, allowing multiple tests to be carried out on one disc simultaneously.

To-date, there have been a number of studies using LOAD that have benefited from the multiplex nature and automatic fluid actuation of LOAD, saving time and reagents. These studies have determined simultaneously multiple chemicals and microalgae in water as well as food-borne pathogens in milk (Hwang et al., 2013; Kim, Jeong, Kim, Kim, & Cho, 2015; Kim, Park, Kim, & Cho, 2013).

Previously, we developed a novel LOAD platform using an on-disc heater for clinical applications (Loo et al., 2017). The setup is still too complicated, especially the optic and computer communications, for on-site field tests where access to power is limited. Instead, an image-based sensing would allow detection using only analysis of color signals (Thompson, Wyckoff, Haverstick, & Landers, 2017). In this study, we developed a compact, low energy, portable LOAD platform using a smart-phone app to determine output (color). As a proof-of-concept, we investigated papaya samples from the wet markets and countryside farms in Hong Kong to determine whether these were transgenic using our sample-to-answer platform.

2. Materials and methods

2.1. Samples and DNA extraction

In our project, 27 papaya samples were selected. Among these samples, five were grown and imported from Hawaii, Malaysia and China, and the remaining 22 were grown and collected in 11 locations in Hong Kong (22° 25–30′ N, 114° 04–10′ E, 24–47 m above sea level). All the samples were stored at −80 °C until DNA extraction and downstream experiments to preserve them and ensure they had a similar degree of maturation. Specimens were also stored at School of Life Sciences, The Chinese University of Hong Kong.

A spin-column-based DNA extraction method (Wide Spectrum Plant Genomic DNA Quick Extraction Kit, Biomed, China) was applied, according to the manufacturer's instructions, to extract papaya DNA for the evaluation. Briefly, 100 mg of mesocarp of fresh or stored papaya fruit was defrosted at room temperature, and cut into cubes (approximately 5 mm^3). Samples were ground with a plastic pestle and mortar or papaya juice (50 µL) collected directly from the surface of a freshly cut section of fruit. For DNA extraction, 400 µL AP1 buffer containing 40 µg RNaseA was added to the mesocarp or juice and samples incubated at 65 °C for 10 min with occasional inverting. 130 µL AP2 buffer containing a detergent was added and the mixture was incubated at 4 °C for 5 min. After centrifugation (20,000*g* for 10 min), the supernatant was mixed with 1.5 volumes of AP3/E buffer containing ethanol for DNA precipitation and the mixture was then passed through a DNA binding column AC. The column was washed twice with 500 µL washing buffer (WB) containing ethanol. 100 µL of elution buffer (EB) containing Tris was used to elute DNA for downstream LAMP detections.

Rapid DNA extraction from papaya juice was based on a method reported previously (Kasajima et al., 2004). Briefly, 10 µL of papaya juice was collected using a pipette and diluted into 40 µL of molecular biology grade water and used directly for LAMP amplification.

2.2. Compact LOAD platform setup

The LOAD platform was designed to be compact and user-friendly, i.e. requiring as few as six buttons to adjust the speed, temperature and excitation source (Fig. 1A). This LOAD platform was composed of (1) a belt-driven motor system with feedback to provide tunable centrifugal force for fluid actuation; (2) a power coupling device made of a split-core transformer that supplies electricity to the disc during spinning; (3) a wireless data communication module for temperature feedback to ensure precise temperature regulation at 65 °C; and (4) blue LED arrays composed of 40 blue LEDs at 470 nm in parallel connected with 5 V voltage regulator (7805 IC) as a steady light source for fluorescence excitation and a long-pass (> 490 nm) 3 mm-thick PMMA filter to remove blue light for green fluorescence detection. The microfluidic disc was attached to a heater board, made of resistant heating elements (thermistors) and was connected to a printed circuit board (PCB) circuit for temperature control and wireless communication.

2.3. Microfluidic disc fabrication

The compact disposable microfluidic LOAD consisted of two layers: the top layer with channels and compartments was for screening while the bottom layer consisted of mechanical and electronic components. The disc (100 mm diameter and 3 mm in thickness) was a PDMS-based polymer, replicated from an acrylic mould that was fabricated with computer numerical control milling. PDMS was added to the milled mould, incubated at 60 °C for 2 h until solid, and sealed with a layer of optical transparent adhesive film (Life Technologies, Waltham, MA, USA). Fig. 1B shows the channels and chambers of the disc.

The different width channels in the top layer were designed in such a way that, at different spinning speeds, reagents and samples were injected into the reaction chamber for LAMP reactions to take place. LAMP detection was executed in the reaction chamber with LAMP reaction mixture, juice or DNA extracted from papaya for GM screening (Red: LAMP reaction cocktail (25 µL); blue: DNA or fruit juice (with water added up to 5 µL); yellow: molecular biology grade mineral oil (Sigma-Aldrich, St. Louis, MO, USA) (20 µL) to prevent the evaporation of the solution during heating; orange: SYBR Green (2 µL) to report LAMP products). Operation of LAMP in LOAD was set as follows: 400 rpm for 30 s; 700 rpm for 30 s; heating turned on at 65 °C and rotating at 400 rpm for up to 30 min for LAMP reaction; 1000 rpm for 30 s

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