TOYOHASHI UNIVERSITY of TECHNOLOGY
Tempaku-cho, Toyohashi, Aichi, 441-8580 Japan
PHONE: +81-532-44-6577 FAX: +81-532-44-6557
E-mail: press@office.tut.ac.jp

PRESS RELEASE

Source: Toyohashi University of Technology, Japan, Committee for Public Relations

Release Title: Microfluidic chip technology enables rapid multiplex diagnosis of plant viral diseases
Release Subtitle: Early detection of diseases of crops at a gene level and protection of food safety

Overview

A research group composed of Professor Takayuki Shibata and his colleagues at the Department of Mechanical Engineering, Toyohashi University of Technology has applied a microfluidic chip technology to develop a multiplex genetic diagnostic device for the early detection and prevention of crop diseases. The group conducted a gene amplification experiment using four kinds of cucumber viruses on the palm-size diagnostic device, and successfully demonstrated that the rapid multiplex diagnosis can be performed within 1 hour of testing. This diagnostic device is a highly versatile technology that can be used for genetic diagnosis not only in viral diseases of crops, but also in various areas including human infections (e.g., the agriculture/livestock/fisheries industries, the food industry, and health/medical care).

Details

With increased food demand due to world population growth and decreased production due to abnormal weather as a backdrop, the “steady supply of safe and high quality agricultural, forest, and fishery products and food” has become a pressing issue common to all humankind in order to actualize a sustainable world (Sustainable Development Goals, SDGs). This research aims at developing a diagnostic technology to support the effective and stable production of high quality crops. By utilizing this technology, even regular agricultural producers without special knowledge or skills can easily and rapidly test for plant diseases and insect pests at their farms at the genetic level.

Loop-mediated isothermal amplification (LAMP) 1) is one of methods for detecting target nucleic acids (DNA or RAN). This approach can amplify the targeted gene at a constant temperature (60–65°C for 30 minutes to 1 hour) without expensive instrumentation for high-precision temperature control in PCR assays, which is the most commonly used genetic diagnosis technique. Therefore, the LAMP method has considerable potential for providing an easy-to-use diagnostic tool and enabling on-site diagnoses. However, the conventional LAMP assay is troublesome in that it is necessary to prepare and test as many sample (the DNA or RNA targets)/reagent mixtures individually for each targeted virus. This process also requires specialized knowledge and skills.

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Here, our research team has solved this problem by employing microfluidic chip technology. We have developed a polydimethylsiloxane (PDMS)-based microfluidic device for the multiplex genetic diagnosis of plant diseases by using semiconductor manufacturing technology. The fabricated multiplex genetic diagnostic device consists of an array of five reaction chambers (3 µL in volume) and a microchannel (200 µm in width and 80 µm in height) forming a network connecting them. The device was approximately 45 mm×25 mm in size (less than 1/3 of name card). As a sample, total RNA containing viral RNA target extracted from diseased cucumber leaves collected at a farm was used. In the operating procedure for the multiplex LAMP assay, a mixture of sample and reagents were autonomously dispensed into the multiple reaction chambers, with just one operation for introducing the mixture into the inlet port of the device. Then, the device was heated in hot water (63°C for 40 minutes to 1 hour), resulting in the specific amplification of targeted nucleic acids. As shown in the figure, two kinds of RNA viruses were successfully detected simultaneously on our diagnostic device. It should be noted that the device has the ability to simultaneously diagnose up to four different kinds of plant viral diseases.

1) LAMP is an isothermal gene amplification method developed by Eiken Chemical Co., Ltd.. This is a technique to amplify a target DNA at a constant temperature (60 - 65°C) by using a set of four to six primers specially designed to recognize six to eight distinct regions on the target gene based on strand displacement reaction.

Future Outlook

We will develop a diagnostic device for enabling the simultaneous detection of a total of eight items, including four kinds of cucumber viral diseases and four kinds of insect pests, with the aim of putting the device to practical use. In principle, it is possible to freely customize the types of target viruses to meet individuals' specific needs on our diagnostic device. Therefore, looking ahead to the "life with corona" era, we will provide a platform for the rapid multiplex diagnosis of human infectious diseases (such as the novel coronavirus and the influenza viruses). We will also realize the rapid multiplex allergen testing in food production (seven specified raw material items: wheat, buckwheat, peanut, egg, milk, shrimp, and crab) as food safety technology.

Reference

Daigo Natsuhara, Keisuke Takishita, Kisuke Tanaka, Azusa Kage, Ryoji Suzuki, Yuko Mizukami, Norikuni Saka, Moeto Nagai, and Takayuki Shibata, (2020) A Microfluidic Diagnostic Device Capable of Autonomous Sample Mixing and Dispensing for the Simultaneous Genetic Detection of Multiple Plant Viruses, Micromachines, 11(6), 540. 10.3390/mi11060540.

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Further information

Toyohashi University of Technology
1-1 Hibarigaoka, Tempaku, Toyohashi, Aichi Prefecture, 441-8580, JAPAN
Inquiries: Committee for Public Relations
E-mail: press@office.tut.ac.jp

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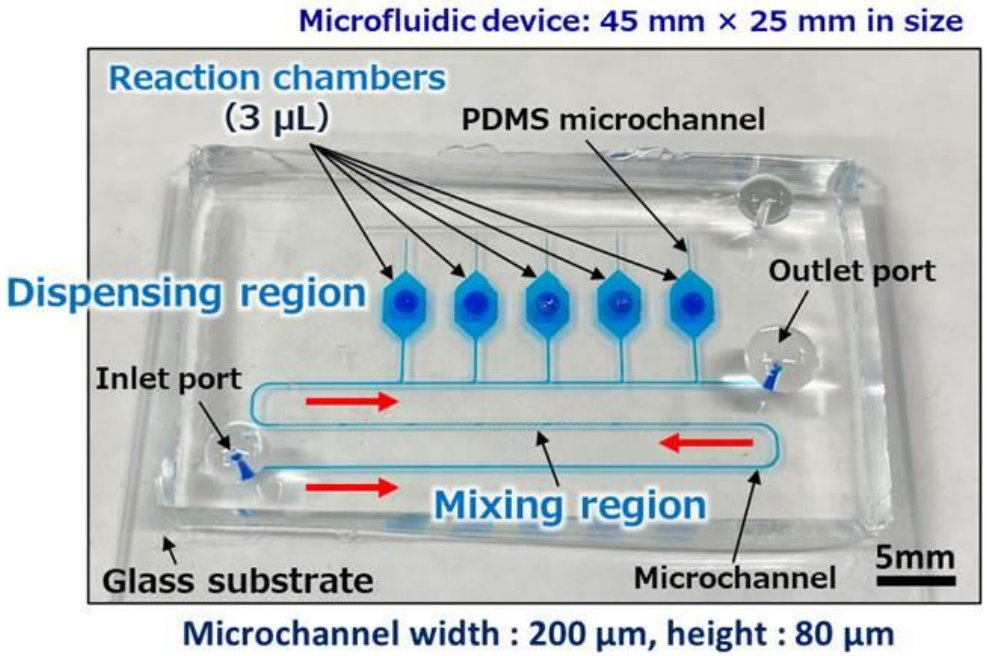
Website: https://www.tut.ac.jp/english/

Figure1:



Caption: Photograph of multiplex genetic diagnostic device

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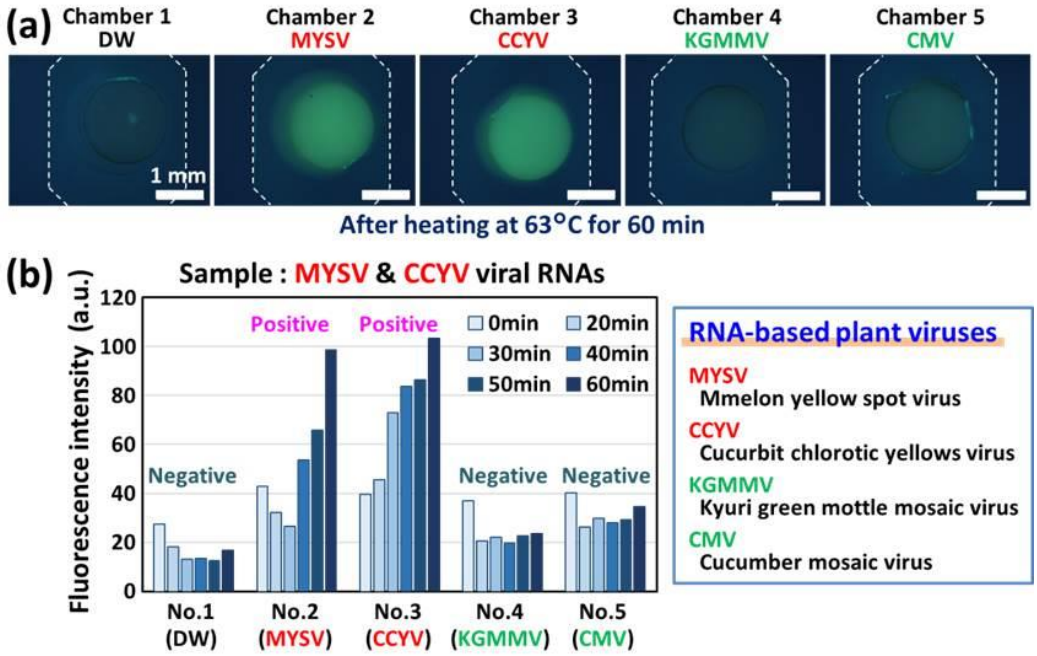
Figure2:



Caption: The simultaneous detection of multiple RNA-based plant viruses (MYSV and CCYV) (Fluorescence intensity increased only in reaction chambers No.2 and No.3 corresponding to target viruses)

Keywords: AGRICULTURE, BIOLOGY, BIOMEDICAL/ENVIRONMENTAL/CHEMICAL ENGINEERING, BIOTECHNOLOGY, BIOTECHNOLOGY, GENES, NANOTECHNOLOGY/MICROMACHINES, TECHNOLOGY/ENGINEERING/COMPUTER SCIENCE, VIROLOGY