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Views & Comments

Rapid Authentication of the Poisonous Plant *Gelsemium elegans* by Combining Filter-Paper-Based DNA Extraction and RPA–LFD Detection

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Numerous plants and animals are edible and officinal, but some can be poisonous. There is sometimes confusion between poisonous and non-poisonous materials because of similarities in their morphologies. Consequently, the unwitting intake of poisonous plant or animal material has resulted in poisoning cases and sometimes in death, especially for situation in the wild. Rapid and accurate authentication of toxic species is essential for establishing and adopting optimal and urgent treatment for patients in such cases, and can be life-saving or can at least minimize the damage to health. Unfortunately, most of the current species authentication methods, including DNA barcoding, loop-mediated isothermal amplification (LAMP), chromatography technologies, and other methods, depend on professional equipment and a specialist laboratory, which are impracticable for real-time application in the field. It is therefore crucial to develop a rapid, accurate, and specific authentication method for poisonous species that does not require any equipment.

Recombinase polymerase amplification (RPA) is a unique isothermal DNA amplification technology developed by Pipenburg et al. [1]. This method achieves rapid DNA amplification of a target gene fragment at a constant temperature of 35–40 °C, which is close to human surface temperature, in a relatively short period of 15–25 min, with or without a thermocycler. The resulting RPA reaction products can be visualized in multiple ways, such as agarose gel electrophoresis, probe-based fluorescence monitoring, and lateral flow dipstick (LFD). Among these, LFD is a portable diagnostic immunoassay device whose result can be determined by direct observation of the color reaction on it. For the combination of RPA–LFD, an essential onsite rapid assay can only be realized when DNA extraction can be performed without any equipment. In 2017, Zou et al. [2] reported a reliable protocol of swift nucleic acid capture based on filter paper, which is equipment-free. In the present study, we take advantage of this fast and simple DNA extraction process and combine it with RPA–LFD to establish a protocol for rapid authentication of the highly toxic plant *Gelsemium elegans* (*G. elegans*).

G. elegans, also known as Duanchangcao in China, is a poisonous flowering plant that is widely distributed in southern China and Southeast Asia. *G. elegans* contains a variety of indole alkaloids, including sarpagine-type, koumine-type, humantenine-type, gelsemicine-type, and gelsenicine-type alkaloids [3]. These alkaloids not only possess strong pharmacological effects, but also exhibit violent toxicities. Oral consumption of *G. elegans* or its aqueous extract leads to severe toxic reactions in the digestive, circulatory, and respiratory systems, and even causes death due to heart or respiratory failure. Unfortunately, *G. elegans* is easily misidentified as the common medicinal herb *Lonicera japonica* (*L. japonica*) because the two plants are sometimes entangled in the wild and have very similar bud shapes. Consequently, this confusion causes accidental poisoning and even death.

To establish a rapid and feasible method for identifying *G. elegans* in the field, *G. elegans* and *L. japonica* were collected and their *psbA-trnH* sequences were obtained by normal polymerase chain reaction (PCR) amplification and Sanger sequencing. Based on these sequences, species-specific primers and probes were designed against *G. elegans* for the RPA–LFD assay (Table S1 in Appendix A). After a series of methodology investigations, we proposed a rapid protocol for the onsite authentication of *G. elegans*. This protocol is illustrated in Fig. 1(a), and is briefly described in the Section 1 of Appendix A. First, fresh leaf of the test sample is cut into small pieces and subjected to fast DNA extraction using filter paper, as described by Zou et al. [2]. Second, the filter paper strip carrying the sample DNA is dipped into a tube containing the RPA reaction system and species-specific primers and probe, and magnesium acetate is then added. Next, the RPA reaction is incubated by holding the reaction tube in a human palm for at least 20 min. Finally, a small amount of diluted RPA reaction product is loaded into the LFD stick, which is then dipped into a PCR tube containing running buffer and held still. After 1–2 min, one or two color bands are observed on the LFD stick. For *G. elegans*, two color bands should emerge on the LFD stick, while for non-*G. elegans* samples, only the control color band will be observed (Fig. 1(b)).

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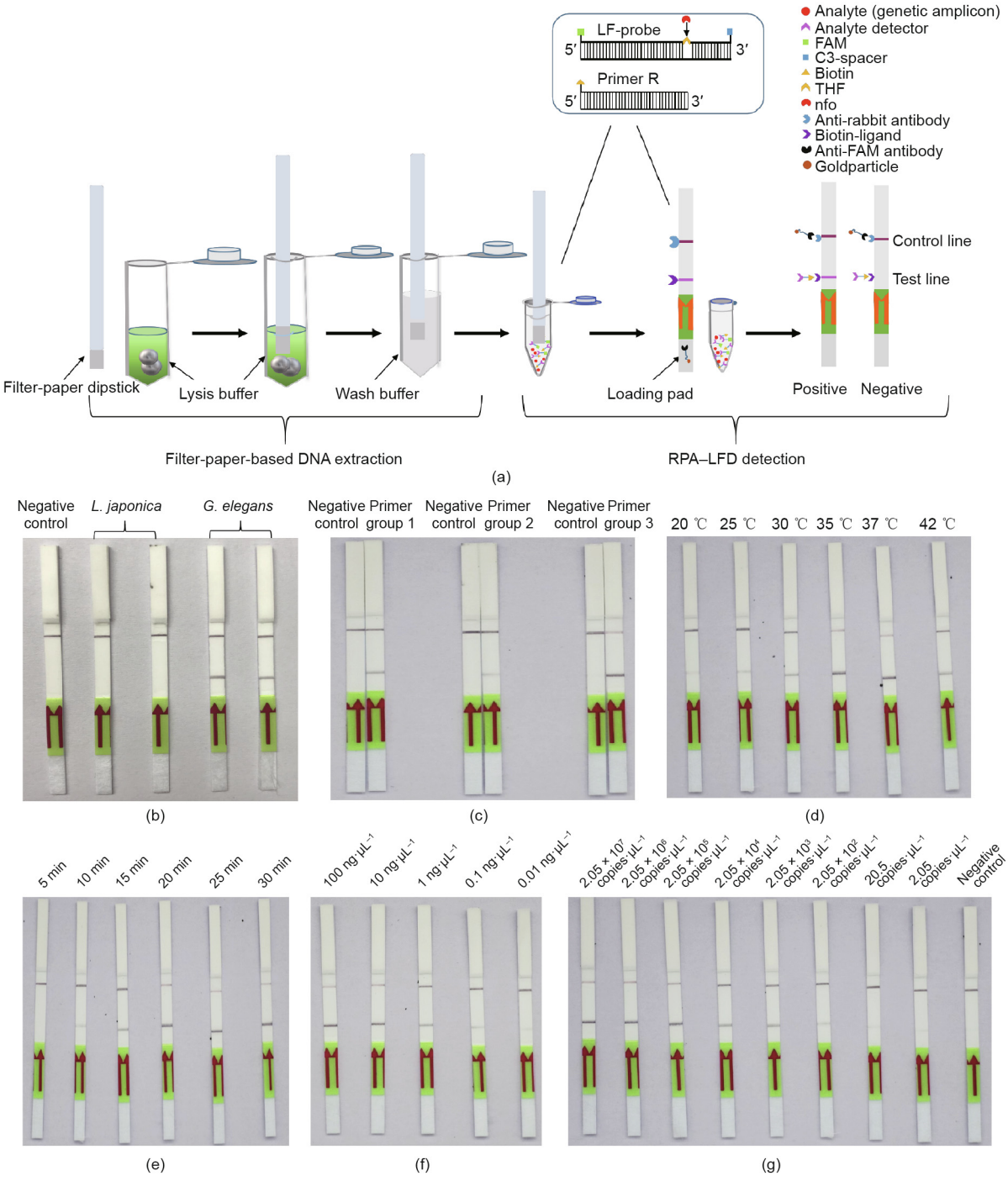

Fig. 1. Workflow of the rapid authentication protocol and methodology investigation. (a) Workflow of the proposed protocol. (b) Species authentication of *G. elegans* and *L. japonica* using the proposed protocol. (c) Specificity and amplification rate investigation on three groups of primers and probe. The number on the top of the LFD strip indicates the group number. (d) Investigation of RPA incubation temperature. (e) Investigation of incubation time in hand. (f) Sensitivity examination for dilution of genomic DNA of *G. elegans*. (g) Sensitivity investigation for dilution of plasmid pEasy-*psbA-trnH* of *G. elegans*. LF: lateral-flow; FAM: fluorescein; THF: tetrahydrofuran.

During the methodology investigations, several experiments were performed (see Section 2 of Appendix A for more detail). These included tests on the specificity and amplification rate of the primers and probe (Fig. 1(c)), RPA incubation temperature (Fig. 1(d)), incubation time (Fig. 1(e)), and reaction sensitivity. The incubation temperature test indicated that a reasonable temperature range suitable for the RPA reaction would be from 30 to 42 °C. Optimal results were observed at 37 °C, which is very close to human surface temperature. In the reaction sensitivity test, the minimal detection limit was observed at 0.01 ng gDNA (Fig. 1(f)) or 20.5 copies of *psbA-trnH* fragments (Fig. 1(g)), respectively.

In conclusion, we provide a protocol for rapid differentiation between the toxic plant *G. elegans* and the medicinal plant *L. japonica* by combining filter-paper-based DNA extraction and RPA–LFD detection. A minimum of 0.01 ng gDNA or 20.5 copies of *psbA-trnH* fragments is required for this protocol, which can produce a reliable result within 30 min. Crucially, this easily performed protocol requires no professional equipment; thus, it is suitable for rapid onsite species identification of *G. elegans*. In addition, commercial

kits could be generated for specific species using this protocol. Furthermore, this study inspires new thoughts on the rapid onsite identification of other species, including herbal and animal materials, whether toxic or not.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eng.2020.02.012.

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