Evaluation and Application of an Efficient Plant DNA Extraction Protocol in Laboratory and Field Testing

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Abstract

Background

CTAB has been considered as the standard protocol for DNA extraction. But the complex and time-consuming procedures can't meet the needs of rapid molecular identification. The method of using cellulose filter paper strips to transfer the DNA in the plant tissue lysate to the nucleic acid amplification system shortening the DNA extraction time to 30 s. However, cellulose filter paper strips have some shortcomings that cannot be put into widespread use. And the data supporting the rapidly purification of DNA by cellulose filter paper is not sufficient.

Results

In the study, the published filter paper strip was modified by sticking the filter paper on the PVC sheet. This modified method is named EZ-D, for easy DNA extraction. Compared with the original method, the DNA extracted by EZ-D is more efficient in PCR amplification. We also came up with a new DNA extraction buffer, which exhibited higher DNA extraction efficiency. When compared with classic CTAB, EZ-D also showed great advantages for higher efficiency, easier protocol and lower cost. PCR analyses showed that DNA extracted from several types of plants by EZ-D were appropriate for specific identification of biological samples. PCR using DNA extracted by EZ-D was sensitive enough to detect 0.1 ng/μL. Evaluation of the EZ-D showed that the DNA extracts can be successfully amplified by PCR reaction for the DNA fragments up to 3000 bp in length and up to 80% in GC content. EZ-D was successfully used for DNA extraction from a variety of plant species and plant tissues. Moreover, when EZ-D was combined with the loop-mediated isothermal amplification (LAMP) method, DNA identification of biological samples could be achieved in an equipment-free way.

Conclusion

Combined with DNA amplification technology, EZ-D protocol is a rapid, specific and sensitive method for molecular identification of plant samples. In addition, EZ-D method is the first application of cellulose filter paper in the identification of genetically modified crops and traditional Chinese medicine ultra-fine powder. In terms of practicability, EZ-D has realized the popularization of cellulose filter paper for rapid DNA purification in laboratories and markets.

Background

Nucleic acid-based molecular diagnosis assays have increased in use in many fields in recent years, due to outstanding sensitivity, specificity and speed (1). An essential first step for all subsequent analyses is obtaining DNA at high molecular weight and purity (2). For DNA extraction from plants, the Cetyltrimethylammonium bromide (CTAB) method is one standard way to isolate high quality DNA (3). The CTAB extraction protocol has been continuously optimized to reduce the polyphenols and polysaccharides present in plant tissues (2, 4, 5). However, it is still difficult to extract high quality DNA from plant materials that are rich in secondary metabolites or have a high degree of DNA degradation via the CTAB method (6). Moreover, the CTAB method requires high-end instruments, strict and time-consuming procedures, and well-trained personnel. The CTAB DNA extraction method is time-consuming and cannot facilitate rapid molecular detection when results are urgently needed.

The current rapid extraction methods include alkaline lysis and direct-PCR, both of which shorten the DNA extraction to about 10 minutes. But these methods still require controlled heating, centrifugation and other operations (7, 8). Another rapid method uses a disposable polymeric microneedle patch to puncture leaf tissue to extract intracellular DNA, which is a good way for rapid molecular diagnosis of plant diseases but not suitable for molecular identification of the plant's genetic material (9). A cellulose filter paper purification method, developed by Zou et. al (2017), was reported for the extraction of DNA from plant tissues for rapid molecular detection(10). The method uses a cellulose filter paper-based stick that consists of a DNA binding zone and a zone impervious to water, prepared by dipping half of a cellulose filter paper into molten wax. While these cellulose filter paper-based sticks (abbreviated as Z-Dipstick in the following text) can absorb DNA efficiently, we noted that when immersed in the extraction buffer or PCR reaction system the filter paper at its DNA-binding zone is very easy to become loose and soft. When this loosen filter paper was immersed in the PCR solution, a large amount of solution was sucked away, resulting in a decline of PCR

efficiency. This situation is even more serious when a large number of samples need to be processed at the same time to make these test strips to be retained in the extraction or PCR system for a longer time.

Here, we present a modified Z-Dipstick DNA extraction method, named EZ-D (EASY DNA) for plant DNA extraction. Instead of imbedding the handle of the test strip with paraplast, a PVC material is used as the handle of the dipstick as well as the support of the DNA binding zone. EZ-D was successfully used for DNA extraction from a variety of plant species and plant tissues and the DNA is suitable for wide range of downstream applications including polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP). Our results demonstrated that the EZ-D combining with nucleic acid amplification techniques provided a rapid, specific and sensitive way for the molecular identification of plant samples. The method has a great application prospect in the field or market identification where the equipment is limited and fast results are required.

Methods

Sample materials

Six different types of transgenic plants and their corresponding non-transgenic controls were used in this study (Table 1). Transgenic plants included *Oryza sativa* T51, containing the *HPT* and *cry1Ab/Ac* transgenes (11), *Zea mays* ZC1, containing the *cry1Ab* and *g10-epsps* transgenes, *Glycine max* GC1, containing the *cry1Ab* and *cp4-epsps* transgenes, soybean SWEET15, containing the *bar* transgene (12), soybean ZUTS-33, containing the *g10-epsps* transgene (13), and *Larix gmelinii* BADH, containing the *35S* transgene (14). Corresponding non-transgenic controls included the rice line MH63, the maize line Hi, the soybean lines Wandou28, Williams82, and HC-3, and the larch line WM. In addition, four kinds of Chinese medicine, prepared as ultrafine powders from *Fritillaria* spp. in the Liliaceae family, were used, namely Zhe Beimu (ZB, prepared from *Fritillaria thunbergii*), Chuan Beimu 1 (CB1, *Fritillaria unibracteata*), CB2 (*Fritillaria przewalskii*) and CB3 (*Fritillaria delavayi*).

EZ-D stick

The Whatman cellulose filter paper No.1 (*Whatman, UK*) was cut into strips measuring 4 mm × 20 cm. Self-adhesive Polyvinyl Chloride Resin (PVC) sheet (*YouLong Biotech, China*) was cut into strips of 44 mm × 20 cm. The 4 mm adhesive tape on the bottom of the plastic sheet was peeled off manually and then attached the filter paper strip to the region. Cut the plastic sheet modified with filter paper into strips of 2 mm width to become the final products of the EZ-D sticks (Fig. 1A), which consists of a 2 × 44 mm PVC waterproof handle with a 2 × 4 mm Whatman No.1 filter paper on the end of it.

To avoid contamination, filter papers were sterilized prior the preparation. Only the PVC handle side of EZ-D sticks are touched during the preparation. The ready EZ-D sticks were further treated with UV light for 30 minutes, and then packed.

DNA extraction using EZ-D

The protocol for rapid DNA extraction from biological samples using EZ-D are presented in Fig. 1B. Twenty milligrams of young plant tissues (roots, stems, leaves or flowers) were added to a 2-mL centrifuge tube containing 300 µL of DNA extraction solution (100 mM Tris [pH 8.0], 1 M NaCl, 10 mM EDTA) along with two zirconia porcelain balls (Z2359-05, *I-Quip, USA*). Plant samples were disrupted by hand-shaking the tube for 10 seconds. For ultrafine powder samples, approximately 10 mg of powder was transferred directly by the EZ-D stick into the DNA extraction solution and stirred the solution by the stick for 10 seconds.

A TL2010S high throughput ball mill (*DHS Life Science & technology, China*) was used for grinding large number of samples or hard samples, such as needle leaves of larch. In addition, we also tested to drill soybean seeds with a miniature electric drill with a 1-mm diameter bit (P-500-3, *Slite, USA*) to obtain seed powder from the cotyledon of soybean seeds, which allows the remaining of seeds to carrying forward of the next generation (15).

After disruption, the EZ-D stick was dipped into the plant tissue lysate for 10 seconds and then transferred into 200 µL of wash buffer (10 mM Tris [pH 8.0], 0.1% Tween-20) for 5 seconds. The stick was then dipped into the DNA amplification reaction system for 5 seconds, to elute the DNA. The whole protocol takes about 30 seconds, after which the purified DNA is ready for amplification or other molecular identification.

CTAB DNA extraction method

A modified CTAB method was used as the control representing a conventional DNA extraction method. One hundred milligrams of plant sample were finely ground using liquid nitrogen. The plant powder was mixed with 700 μL of extraction buffer (2% w/v CTAB, 0.7 M NaCl, 10 mM EDTA, 50 mM Tris HCl [pH 8.0], 0.5% (v/v) β -mercaptoethanol, which was preheated at 65 °C). After 1.5 hours at 65 °C, 700 μL of chloroform were added into the mixture and rotated gently at room temperature, followed by centrifugation at 12,000 rpm for 10 minutes. Three hundred microliters of supernatant were transferred to a new tube and mixed gently with 210 μL of isopropanol. After the DNA precipitated, the flocculent DNA was removed and transferred to a new tube, washed twice with 1 mL 75% ethanol. After washing, the DNA was suspended with 100 μL ddH₂O and quantified using a NanoDrop 1000 Lite spectrophotometer (Thermo Scientific, USA).

Detecting PCR sensitivity of EZ-D

Genomic DNA samples purified by CTAB method were diluted in series by a factor of 10, with final concentrations ranging from 100 ng/ μL to 0.01 ng/ μL . For the detection of PCR sensitivity of EZ-D DNA, the dilutions of DNA were prepared by adding 1 μL of genomic DNA with diluted concentrations onto the DNA binding zone of the EZ-D sticks, followed by washing. The resulted sticks were immersed into a PCR reaction mixture for test.

Primer design and PCR reaction conditions

Specific primers for each of the genes were designed the Oligo software (Version 7.0, Molecular Biology Insights, USA). The genes, primer sequences, corresponding amplicon lengths and GC content of the amplicon used for the tests are listed in Table 2. Genes *bar*, *HPT*, *cry1Ab/Ac*, and *g10* refer to the transgenes of the bialaphos resistance, hygromycin-B-phosphotransferase gene, *Bacillus thuringiensis cry1Ab/Ac* fusing gene, and *g10-epsps* gene (13), respectively. In addition, the primer design of gene referred to the work of Wang et al (16). The primer of gene *trnL-trnF* referred to the sequence mentioned in the article of Zhao et al (17). The primer design of *ZB1* was based on the work of Li et al (18). The primer design of gene *cry1Ab* as well as *cp4-epsps* was according to the unpublished work about new transgenic plants.

PCR reactions were performed in a total volume of 20 μL in a reaction mix that contained 10 μL of 2 $\times$ Taq Master Mix kit (*CWBiotech, China*) or PrimeSTAR® GXL Premix (*TAKARA, Japan*), 0.4 μL of each primer, 8.2 μL of ddH₂O and 1 μL of template DNA (the volume of template DNA transferred from the EZ-D stick was defaulted at 1 μL). The PCR cycling parameters are as follows: initial denaturation at 95 °C for 2 min, then 40 cycles of denaturation at 95 °C for 20 s, 55–60°C for 20 s, 72°C for 20 s, followed by final extension of 72°C for 5 minutes or 98°C for 2 minutes, 40 cycles of 98°C for 20 seconds, 55–60°C for 20 seconds, 68°C for 1–3 min, followed by final extension of 68°C for 5 min.

For DNA fragments rich in GC content, 10 μL 2XGC Buffer, 3.2 μL 2.5 mM dNTPs, 0.2 μL La Taq (*TAKARA, Japan*), 0.2 μL of each primer, 6.2 μL of ddH₂O and 1 μL of template DNA (the volume of template DNA transferred from the EZ-D stick was defaulted at 1 μL). The PCR cycling parameters are initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, 60°C for 30 s, 72°C for 20 s, and final extension of 72°C for 2 minutes.

All the resulting DNA products were separated on a 1% agarose gel at 150 V for 30 min.

LAMP-HNB assay

Relevant LAMP primers were designed according to the sequences of the *g10-epsps* and *ZB1* genes using PrimerExplorer V5 (<http://primerexplorer.jp/lampv5e/index.html>) and are listed in Table 3. LAMP reactions were performed using a LAMP-HNB discoloration amplification kit (*HaiGene, China*), following the manufacturer's protocol. LAMP reactions were performed in a total volume of 25 μL in a reaction mix consisting of 12.5 μL of 2 $\times$ HNB LAMP, 2.5 μL of 10 $\times$ LAMP Primer Mix (16 μM each of FIP and BIP, 2 μM each of F3 and B3 and 8 μM of LF), 3 μL of 5 M Betain, 1 μL of Bst2.0 DNA Polymerase, 5 μL of ddH₂O and 1 μL of template DNA (the volume of template DNA transferred from the EZ-D stick was defaulted at 1 μL). The mixture was incubated at 63°C to 65°C for 60 min. The LAMP amplification products were examined by directly observing the color change of the reactions.

Hydroxy naphthol blue (HNB) is added to the LAMP amplification mixture as a colorimetric indicator. A color change from violet to sky blue indicates that the tested sample contains the gene of interest (Motoki et al., 2009).

Results

EZ-D DNA extraction stick is modified on the basis of the dipstick extraction technology

The equipment-free nucleic acid extraction dipstick methodology using untreated cellulose-based paper to capture nucleic acids was reported by (10). While “Dipstick”-based protocol has great advantages for its simple, fast, and low cost, we experienced that the filter paper at its DNA-binding zone is very easy to get loose and the loose filter paper sucked up a large amount of PCR solution, which reduce the PCR efficiency. Moreover, during the preparation of the sticker, the coated wax in the handle is very easy to break and contaminate the filter paper in the DNA binding area, which will inhibit the PCR efficiency when the binding area contacts to the PCR reaction. To solve the problems, we replaced the wax-coated filter paper handle in the Z-Dipstick with a handle with a PVC sticker on the filter paper (described in the Materials and Methods; Fig. 1A). Comparison of the PCR amplification efficiency of DNA extract from the EZ-D stick and the Z-Dipstick showed the PCR products from the EZ-D were significantly greater than that from the Z-Dipstick (Fig. 1C and 1D).

Comparison of EZ-D and CTAB DNA extraction methods

The advantages of the EZ-D method over a conventional CTAB method are listed in Table 4. Similar to the Z-Dipstick, EZ-D took 30–60 seconds to prepare amplification-ready DNA, while CTAB took 1.5-3 hours. EZ-D required less skill and no electrical devices or organic reagents. EZ-D costs, including the cost of the stick, reagent and other suppliers, 0.2 CNY (Chinese Yuan, equivalent to 0.14 US dollars) per sample, while CTAB costs 0.9 CNY per sample. Thus, EZ-D is faster, simpler and lower cost than the CTAB method for the purification of DNA from plant samples.

Amplifiability of the EZ-D extracted DNA

To assess the quality of DNA extracted by EZ-D, the DNA samples were subjected to PCR amplifications of 8 DNA fragments of 6 different genes. DNA was extracted from leaves of transgenic plants or the corresponding non-transgenic control plants using the EZ-D method as described in Fig. 1. Genomic DNA of the transgenic plants extracted by CTAB was diluted to 10 ng/μL as the positive controls. The amplified DNAs from EZ-D DNA templates were as clear and bright as the bands obtained from the CTAB-extracted genomic DNA samples (Fig. 2A). There were no detectable non-specific bands among the PCR amplicons using the EZ-D DNA samples as templates, indicating that the DNA purified by EZ-D had excellent PCR specificity.

To determine whether the DNA captured by the EZ-D sticks can be used for multiple times of PCR assays, a single ED-stick soaked the genomic DNA from transgenic soybean ZUTS-33 were dipped into 16 PCR tubes, containing water only, one after another. The 16 tubes were grouped into two and used to amplify the transgene *g10* and the housekeeping gene *GmActin* by adding the rest of the corresponding components of the PCR reactions. Result showed that the DNA content in a single EZ-D stick can be used for at least 16 PCR reactions (Supplementary Figure S1).

To further examine the amplifiability of the EZ-D extracted DNA, a DNA fragment of *OsBHLH156* (16) with 80% of GC content in average was tested. Results showed that the target fragment was successfully amplified (Supplementary Figure S2A, S2B). And the sequencing of the PCR product showed that the target gene was amplified precisely (Supplementary Figure S2C). Thus, the EZ-D based PCR method can be used for the gene amplification in wild range of GC content.

EZ-D sticks can purify DNA at concentration as low as 0.1 ng/μL

To evaluate the sensitivity of EZ-D sticks, genomic DNA samples were extracted by CTAB methods from five different transgenic plants. One microgram of the diluted DNA with final concentrations ranging from 100 ng/μL to 0.01 ng/μL was added into the EZ-D sticks and then applied to PCR reaction. As shown in Fig. 2B, the first four concentrations of DNA could be amplified, while the 0.01 ng/μL DNA samples from both protocols could not be amplified. Therefore, the PCR detection limit for the DNA samples prepared by EZ-D method is considered as same as those prepared by the CTAB method, which is 0.1 ng/μL.

DNAs from the EZ-D had good integrity

To evaluate the integrity of DNA extracted by EZ-D, the EZ-D extracted DNAs were PCR- amplified using primer pairs targeting the soybean *GmActin* and *g10-epsps* genes to produce amplicons ranging from 500 bp to 3000 bp in length (Table 2). Six DNA fragments of the *GmActin* gene and *g10-epsps* gene, ranging from 500 bp to 3000 bp, were successfully amplified using the DNA extracted by EZ-D (Fig. 2C). This indicated that EZ-D can extract long fragments of DNA and that the length of amplification products can be as long as 3000 bp (Fig. 2C).

EZ-D can be used for a variety of plant samples

To determine if EZ-D is suitable for DNA extraction from various herbaceous plant tissues, roots, stems, leaves, flowers at flowering stage, and dry seeds were collected from transgenic soybean line ZUTS-33 and subjected to the EZ-D and CTAB DNA extraction protocols. The fragments of transgene *g10* and the housekeeping gene *GmActin* were amplified. Results showed that both fragments were successfully amplified from the EZ-D DNAs from all five soybean tissues with amplification efficiency similar to those using CTAB DNA (Fig. 3A). To test if the EZ-D method can be used for DNA extraction from woody plants, needles of 11 putative transgenic larch accessions, possibly containing the transgene driven by *35S* promoter, were sampled. A chloroplast intergenic region, *trnL-F*, was chosen as an internal control (Zhao et al., 2019). The PCR results using DNA extracted by EZ-D showed that none of the tested plants contained the transgene region (data not shown), while the *trnL-F* internal control fragment was successfully amplified (Fig. 3B). Thus, EZ-D can be used for DNA detection in woody plants and can also detect DNA from organelles.

Many Chinese medicines produced from plant materials are prepared as an ultrafine powder (19). Rapid and accurate molecular identification of these ultrafine powders is important for quality control (20). The EZ-D protocol was assessed for its ability to extract DNA from ultrafine powder of the Chinese medicine Beimu. Beimu is derived from various *Fritillaria* spp. The market value of Sichuan Beimu (Chuan Beimu, CB) is about 300–500 times more expensive than that of Zhejiang Beimu (Zhe Beimu, ZB). ZB is often used as a counterfeit CB (21). Species within the genus *Fritillaria* used for various Beimu preparations include *Fritillaria thunbergii* (Zhe Beimu, ZB), *F. unibracteata* (CB1), *F. przewalskii* (another CB, labeled CB2 herein) and *F. delavayi* (CB3). The ultrafine powder samples of four medicinal preparations (ZB, CB1, CB2, and CB3) were subjected to the EZ-D method for DNA extraction. For DNA molecular identification, PCR primers were designed to amplify a DNA fragment that is present only in ZB (*F. thunbergii*) (Table 2). The DNA fragment, named *ZB1*, is a molecular marker of ZB (Li et al., 2012; 2014). Result showed that the amplicon was only obtained from the DNA extracted from ZB, but not from the other three species (Fig. 3C), indicating that the EZ-D protocol could be used for molecular identification of ultrafine powder samples. To further determine the sensitivity of the PCR reaction using the DNA extracted by the EZ-D protocol, CB powder mixtures with different percentages of ZB ultrafine powder were prepared. As shown in Fig. 3C, ZB could be detected when as low as 5% of the mixture.

Combination of EZ-D with LAMP can detect DNA in the field

LAMP is one of the most powerful isothermal amplification techniques. It uses a strand-displacement polymerase combined with four to six primers and *Bacillus stearothermophilus* (*Bst*) DNA polymerase. The LAMP method does not require thermocycling since the amplification is performed at a constant temperature between 60 °C and 65 °C (Notomi et al., 2000; Tomita et al., 2008; Zhang et al., 2014), thus it has been increasingly used outside of a laboratory for molecular determination of transgenic crops (Kiddle et al., 2012; Lan et al., 2019; Li et al., 2016). To tested whether the combination of EZ-D DNA extraction and the LAMP-HNB assay could rapidly obtain reliable results for genetic analysis, tissues from the transgenic soybean line ZUTS-33 and its non-transgenic control were tested. Result showed that the negative sample appeared violet, while the samples containing the transgene showed sky blue (Fig. 4A). Thus, the combination of EZ-D with the LAMP-HNB protocol could be used for the molecular identification in the field where instrumentation is not available.

Furthermore, we also tested whether the LAMP-HNB technique could be used for identification of Chinese medicines as described in last section. While no visible color change in the negative control or pure CB samples, CB powders mixed with as little as 5% ZB turned blue in the test tube and can be identified (Fig. 4B). Therefore, combining the EZ-D method and the LAMP technique offers a novel and swift means for the authentication of Chinese medicine and can be used for quality control in the market.

Discussion

In the study, we optimized the cellulose filter paper purification method based on the published protocol by Zou et al., (2017). The major modification of our EZ-D protocol uses a PVC waterproof handle replaces the wax-coated handle in Zou's protocol. This modified EZ-D stick overcomes the shortcomings that the original Z-stick is easy to loosen in the DNA binding area and the parafilm used to seal the filter paper in the handle easily drop and contaminate the DNA sample, which makes the EZ-D stick handier and yields DNA with better amplifiability (Fig. 1). In addition, we found that the extraction buffer #1 used in Zou's work was not efficient enough for the DNA extraction of soybean. When we increased the NaCl concentration of the extraction buffer from 150 mM, gradually bright amplified bands were observed (Supplementary Figure S4). Thus, we came up with a new DNA extraction buffer, with which DNA of soybean was stably extracted (Fig. 3A).

The quality of DNA extracted by EZ-D for PCR amplification were evaluated. Results showed that the EZ-D based genomic DNA can be amplified for DNA fragment up (not limited) to 3000 bp with high specificity and sensitivity (Fig. 2A, 2C). The protocol can be employed for different tissues of herbaceous plant species, leaf of woody plants and ultrafine powder of Chinese medicine (Fig. 3). Among the samples tested, soybean seed contains a great deal of polysaccharides, polyphenols, proteins and esters, which hinder DNA from purification (Ren et al., 2012). Thus, soybean seed is considered to be difficult to employ a fast protocol for DNA extraction (Wu et al., 2015; Yu et al., 2016). In our study, we optimized the EZ-D and realized the rapid DNA extraction for soybean seeds (Fig. 3A). In woody plant larch leaves, the high contents of secondary metabolites, such as polysaccharides, polyphenols, and turpentine often bind with its genomic DNA. These compounds are not readily removed, which will inhibit PCR amplification reaction (22). The EZ-D method uses the filter paper to absorb DNA. After washing, the inhibitors of PCR reaction in samples from woody plants are greatly reduced so that the resulting DNA can be amplified efficiently (Fig. 3B). Thus, EZ-D is suitable for DNA extraction identification of wide range of plant species and plant samples.

Safety assessment of genetically modified (GM) plants and production in the food market and field is one of the most important tasks for molecular identification (Liu et al., 2020). Our data demonstrated that EZ-D can simplify molecular diagnosis of the existence of transgenes. By EZ-D combined with LAMP method, GM products can be identified in the field or market where specific instrument is not available (Fig. 4A). Similarly, the combination of EZ-D and LAMP can be used for the quality control of high value market products, such as ultrafine powder of Chinese medicine (Fig. 4B).

Conclusion

In this study, we have developed a new filter paper stick, EZ-D stick, for rapid identification of transgenic plants and Chinese medicine ultrafine powder based on the published method. The EZ-D stick overcomes the two disadvantages of Z-Dipstick, including the complicated production process, easy contamination and instability in DNA absorbing as well as releasing. We also provided a new DNA extraction solution, which is more efficient than the solution reported by Zou et al (2017). The EZ-D method can extract DNA from a variety of plants and their tissues. The sensitivity of PCR based on the EZ-D extraction method can reach 0.1 ng/ul, the length of the amplified fragment up to 3000 bp and the GC content up to 80%. EZ-D method offers more sufficient data support for the rapid extraction of plant DNA using cellulose filter paper within 30 seconds. Though the purity and quality of DNA is not good, it does not affect the amplification of DNA fragments of different length and GC content. Thus, EZ-D deserves the widely use in the lab as well as in the field.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Author contributions

QW and HS designed the study and wrote the manuscript. QW, XS, TQ, WW and ZW performed the experiments and interpreted the results.

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Tables

Table 1. Plant materials used in this study

Plant species	Materials		Gene information
<i>Oryza sativa</i>	T51	Transgenic rice	<i>35S::cry1Ab/Ac; ; 35S:HPT</i>
	MH63	Non-transgenic control	none
<i>Zea mays</i>	ZC1	Transgenic maize	<i>35S::cry1Ab; 35S::g10-epsps</i>
	Hi	Non-transgenic control	none
<i>Glycine max</i>	GC1	Transgenic soybean	<i>35S::cry1Ab; 35S::cp4-epsps</i>
	Wandou28	Non-transgenic control	none
<i>Glycine max</i>	SWEET15	Transgenic soybean	<i>35S::bar</i>
	Williams82	Non-transgenic control	none
<i>Glycine max</i>	ZUTS-33	Transgenic soybean	<i>35S::g10-epsps</i>
	HC-3	Non-transgenic control	none
<i>Fritillaria unibracteata</i>	CB1	Chinese medicine	without <i>ZB1</i> gene
<i>Fritillaria przewalskii</i>	CB2	Chinese medicine	without <i>ZB1</i> gene
<i>Fritillaria delavayi</i>	CB3	Chinese medicine	without <i>ZB1</i> gene
<i>Fritillaria thunbergii</i>	ZB	Chinese medicine	with <i>ZB1</i> gene

Table 2. Primers used in the PCR amplification reactions.

Genes	Primer name	Primer Sequence5'→3'	GC content(%)	Amplicon lengthbp
<i>bar</i>	bar-F	GTCAACCACTACATCGAGACAAGC	68	260
	bar-R	AGCAGGTGGGTGTAGAGCGT		
<i>cry1Ab/Ac</i>	cry1Ab/Ac-F	GGCCATACAACCTGCTTGAGT	49	1000
	cry1Ab/Ac-R	GCGTTTCCCATAGTTCCATA		
<i>cry1Ab</i>	cry1Ab-F	GACAACAACCCCAACATCAACGAGTG	65	583
	cry1Ab-R	GGTCGTTGTAGCGGCTGTTGATGGT		
<i>cp4-epsps</i>	cp4-epsps-F	TGATCCTCACGCTTCAGGAGATGGGAGC	63	488
	cp4-epsps-R	CAGATCCATGAACTCTGGGAAGCTCGTC		
<i>g10-1</i>	g10-1-F	TCTAAGGTCATCGGAGGCAGTAGCT	54	604
	g10-1-R	TAGAGCACAGCCATCCAAGAACTAC		
<i>g10-2</i>	g10-2-F	TCTCACCTCTGGCACCCTTTTCG	70	703
	g10-2-R	CCACGTTCTCCAGGTGGTGT		
<i>g10-3</i>	g10-3-F	TTTGATGAGCCTTTGTACACAGC	39	468
	g10-3-R	AAGCAATAAAATAAAATGAAGACAAGGT		
<i>g10-epsps</i>	33-F	TAATTCGGGGGATCTGGATTTTAGTA	\	\
	33-R1	CTCTTAGACTCAAGGAATGCGATAGA	44	500
	33-R2	AGTACGCTTCGGCCCTTATGTTCTC	48	1000
	33-R3	CTCTTCAGCTACCTTCGACGTTATC	50	1500
	33-R4	GATGTGATATCTCCACTGACGTAAGG	48	2000
	33-R5	TCAAAAGGACAGTAGAAAAGGAAGGT	48	2500
	33-R6	GGGTTTTCCAGTCACGACGTTGTAA	48	3000
<i>Gm-ACTIN</i>	ACTIN-F	TTTGATGAGCCTTTGTACACAGC	\	\
	ACTIN-R1	AAGCAATAAAATAAAATGAAGACAAGGT	41	500
	ACTIN-R2	ACCATCCTTAACATTATTTATGT	37	1000
	ACTIN-R3	AGCATCGTCACCAGCAAATCCTG	37	1500
	ACTIN-R4	ATAGATTGGTACAGTGTGACTCA	39	2000
	ACTIN-R5	GGTGGAGCTACAACCTTAATCTT	40	2500
	ACTIN-R6	TACAAAAGAAAATGGAGGAAAAGAGATC	40	3000
	ACTIN-F7	TCAATGTGCCTGCCATGTATGTG	44	246
	ACTIN-R7	CCAGCGAGATCCAAACGAAG		
<i>HPT</i>	HPT-F	GAAGTGCTTGACATTGGGGAGT	58	472
	HPT-R	AGATGTTGGCGACCTCGTATT		

<i>OsbHLH156</i>	156-F	GGTCGCCGCGCTCGAGGAGGC	80	360
	156-R	GCCACGACGCGGTCCGGCGAG		
<i>trnL-trnF</i>	trnL-trnF-F	GGGGATAGAGGGACTTGAAC	38	540
	trnL-trnF-R	CGAAATCGGTAGACGCTACG		
<i>ZB1</i>	ZB1-F	GCCCTTGAAAGATTCTGGTA	43	207
	ZB1-R	AGTTGGTGGACTCACTGG		
<i>35S</i>	35S-F	GCTCCTACA AATGCCATCA	50	195
	35S-R	GATAGTGGG ATTGTGCGTCA		
g10-1g10-2g10-3 are three different fragments of <i>g10-epsps</i>				

Table 3. LAMP species-specific primers

Gene	Primer type	Gene location (nt)	Sequence (5' to 3')	Samples for detection
<i>g10-epsps</i>	F3	718-735	AAGAGGAGCGTGGGACTT	Transgenic soybean ZUTS-33
	B3	926-944	GACCTCAGGGTGACCTTCT	
	Forward inner primer	742-761/TTTT/784-805	CGCTTCCGCCCTTATGTTCTCTTTTACCGGTGAGTCTAAGTTCGA	
	Backward inner primer	853-874/TTTT/896-915	TGGACCGGAAACGGAGATAGGGTTTTGAAAGACTTGGTGCTTGGGT	
	Loop Primer F	763-863	GGTCCTCTTCTTCCTGACGGA	
<i>ZB1</i>	F3	446-465	GCCCTTGAAAGATTCTGGTA	CB1, CB2, CB3, ZB and five CB mixtures containing different percentages of ZB
	B3	635-652	AGTTGGTGGACTCACTGG	
	Forward inner primer	466-485/T/507-526	ATTGCTACACGGTGATGCCCTATCAACCGTTGAAAAGTTGC	
	Backward inner primer	533-556/T/596-614	CTAAGGGAGTAGTGAAACTGGTGTTTTTTGGATGCCTGTTTCAG	

Table 4. Comparison of EZ-D and CTAB DNA extraction methods

Advantages	EZ-D	CTAB
Total time cost	30-60 seconds	1.5-3 hours
Personal skill	low	high
Organic reagents	no	yes
Electrical devices	no	yes
Cost per sample (CNY)	0.2	0.9

Figures

Figure 1

EZ-D DNA extraction stick is modified from the dipstick extraction technology reported by Zou et al. (2017). (A) An EZ-D stick is made of a PVC plastic handle with a Whatman No.1 cellulose filter paper attached to the bottom of it. (B) The flowchart of the EZ-D method. (C) and (D) Comparison of PCR efficiency of the fragment 3 of transgene *g10*-epsps was using the DNA extracts from EZ-D and the Dipstick (Zou et al., 2017). Four replicates for each extraction method. M, DL1000 marker; B, blank control. *** indicates P value < 0.01.

Figure 2

Molecular identification of DNA components using EZ-D DNA. PCR efficiency (A) and sensitivity (B) using DNA extracted by the CTAB and the EZ-D methods. Plant samples were from several independent transgenic plants including rice line T51, maize line ZC1, soybean line GC1, SWEET15 and ZUTS-33 with their corresponding non-transgenic controls of MH63 rice, Hi II maize, and soybean lines Wandou 28, Williams 82, and HC-3; g10-1 and g10-2 are two fragments of the g10-epsps gene ; Details of transgenic genes were listed in the Table 1; The labels of 100, 10, 1, 0.1 and 0.01 at the top of the electropherogram of B are the DNA concentrations (ng/μl). (C) DNA extracted by EZ-D was amplified for DNA fragments ranging from 500 bp to 3000 bp in length. Up, GmActin; Down, g10-epsps. 1-6, PCR products for amplification of DNA fragments of 500 bp, 1000 bp, 1500 bp, 2000 bp, 2500 bp and 3000 bp, respectively. M, DL2000 marker; B, blank control; C and E, DNA extracted by CTAB and EZ-D, respectively. N, non-transgenic control.

Figure 3

Application of EZ-D for a variety of plant samples. (A) Electropherogram of PCR using DNA extracted from different soybean tissues by EZ-D or CTAB methods. g10-epsps and GmActin are two genes used for the assay; g10-3 is fragment 3 of g10-epsps (Table2). M, DL1000 marker; N, non-transgenic control; B, blank control; C, DNA extracted by CTAB; E, DNA extracted by EZ-D with 3 replicates. (B) Electropherogram of PCR using DNA extracted from larch needles by EZ-D. P, positive control; Sample 1-11, 11 larch leaf samples. (C) Application of EZ-D for molecular identification of ultrafine powdered Chinese medicines. ZB, Zhe Beimu, Fritillaria thunbergii; CB1, Chuan Beimu 1, Fritillaria unibracteata; CB2, Chuan Beimu 2, Fritillaria przewalskii; CB3, Chuan Beimu 3, Fritillaria delavayi; and T1-T5, different percentages of ZB ultrafine powder mixed into CB powder at 5%, 10%, 15%, 20%, or 25%, respectively.

Figure 4

Combination of the EZ-D and LAMP methods for DNA detection of transgenic soybean (A) and ultrafine powder samples of Chinese medicines (B) samples in field conditions. P, positive control; B, blank control; N, non-transgenic control; Root, Stem, Leaf, Flower and Seed samples are taken from transgenic soybean line ZUTS-33; ZB, Zhe Beimu, *Fritillaria thunbergii*; B, blank control; CB1, Chuan Beimu 1, *Fritillaria unibracteata*; CB2, Chuan Beimu 2, *Fritillaria przewalskii*; CB3, Chuan Beimu 3, *Fritillaria delavayi*; T1, T2, T3, T4 and T5, are ultrafine powder of CB containing 5, 10, 15, 20 and 25% of ZB, respectively.

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