Supporting Information

Visual detection of *Fusarium proliferatum* based on asymmetric recombinase polymerase amplification and hemin/G-quadruplex DNAzyme

Materials and Apparatus

2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and hemin were obtained from Sigma-Aldrich Co., Ltd. (USA). GeneRuler 100 bp Plus and dNTP were obtained from Thermo Fisher Scientific Ltd. (China). TwistDx/TwistAmp® Liquid Basic Kit was purchased from TwistDx™ Limited (Cambridge, UK). Ultrapure water with an electrical resistance larger than 18.2 MΩ was obtained with a ULUP- IV system (China) and used throughout the study. DNA extraction kit was purchased from TransGen Biotech Co., Ltd. (China). All other reagents were of analytical grade and used without further purification.

The primers used for *F. proliferatum* were designed based on the intergenic sequence of the ribosomal RNA gene cluster (about 230 bp) which was specific in species of *F. proliferatum*[1, 2]. The trans-complementary sequence of G-quadruplex was at the 5′-terminal of primer-F. Nucleotide sequences used for *F. proliferatum* amplification were 5′-CCCAATCCCAATCCCAATCCCTCGATTCGAGGGCCGGCCACCAGAGGATGTG-3′ **(**primer-F**)** and 5′-CAACACGAATCGCTTCCTGACGAAGACAGAAG-3′ **(**primer-R**)**. They were synthesized by Sigma-Aldrich LLC. (China). Strains of *F. proliferatum DSM62267* (F120)*, F. equiseti RD13* (F216)*, F. culmorum 3.37 dus Bomm* (F109)*, F. avenaceum borman* (F112), and maize flour infected by *F.*

proliferatum DSM62267 were kindly given by Professor Petr Karlovsky in Goettinggen University. *Ralstonia solancearum* and *Puccinia sorghi* were from lab in Tobacco Research Institute of Chinese Academy of Agricultural Sciences. Healthy maize flour was purchased in local supermarket in China.

NanoDrop 2000 (Thermo, USA) was used as absorbance measurements. The absorption spectra of the resulted solutions were monitored within wavelength ranges between 390 nm and 490 nm. Asymmetric recombinase polymerase amplification (RPA) reaction was performed in a foam box with hot water. Electrophoresis apparatus and horizontal electrophoresis trough (1645052 and Mini-Sub Cell GT Systems, Bio-Rad, USA) were used to gel electrophoresis. Gel pictures were captured by WD-9413B imaging system (China). The tubes were captured by mobile phone (Huawei, China).

NaOH-based DNA extraction

Maize flour infected by F120, healthy maize flour, and other three strains of F216, F109, and F112 grown in the solid medium were introduced in grinding bag containing 5 volumes of lysis buffer (0.5 M NaOH and 10 mM EDTA), and grinded by mouth of glass tube for 2 min. The crude mixtures were transferred to tubes successively, and 10-fold diluted by 10 mM Tris-Cl, 1 mM EDTA (pH 8.0). One μL of each supernatant of rough solution was directly used as DNA templates of asymmetric RPA. To quantify the assay, genomic DNA of F120 from solid medium was also extracted by DNA extraction kit.

Asymmetric RPA assay

Asymmetric RPA was performed in 50 μL volume bearing 25 μL 2 × reaction buffer,

2 μL 10 mM dNTP, 5 μL 10 × basic E-mix, 2.4 μL 10 μM primer-F, 2.4 μL 10 μM primer-R, 2.5 μl 20 × core reaction mix, and water up to 46.5 μL. When 1 μL DNA template and 2.5 μL 280 mM magnesium acetate were added to the solution, the reaction was started. It was performed in a foam box containing water with temperature gradient of 42-34 °C, 37 -34°C in 20 min. For comparison, constant reaction temperatures of 42°C, 37°C, 34 °C were used in other three parallel experiments. When the reaction time was ended, the resulted solution was put into hot water of about 85 °C for 3min to degenerate the enzymes and proteins in the solution.

Oxidation reaction of $ABTS^{2-}$ and H_2O_2 catalyzed by DNAzyme of G-quadruplex

Different type of monovalent cation related to the activity of the DNAzyme of G-quadruplex[3, 4]. In order to improve the sensitivity of the strategy, the effect of K^+ (buffer A, 50 mM Tris-HCl, 50 mM KCl, pH 7.9) and NH_4^+ (buffer B, 50 mM Tris-HCl, 150 mM NH_4Cl, pH 7.9) were assessed for the activity of mimicking peroxidase of hemin/G-quadruplex DNAzyme.

Hemin (0.6 μM, final concentration) was incubated with RPA products in buffer A or buffer B for 60 minutes. $ABTS^{2-}$ (2 mM, final concentration) and H_2O_2 (2 mM, final concentration) were introduced in the solution, and incubated for 30 min. The color change was observed in final solution. UV-Vis absorptions of these solutions were monitored by NanoDrop 2000.

Reaction temperature of RPA was connected to the efficiency of enzymes in the assay, and affected the intensity of colorimetric signal. RPA reaction could be performed in closed palm[5], and it was very convenient. However, only two or three samples were tested once a time. To apply the strategy in lots of samples detection in field test once and for all, a foam box with hot water was applied as reaction vessel. Hot water was put into the box, and the temperature decreased with time. Asymmetric RPA reaction was started when the temperature of water was 42℃ (a) or 37℃ (b), and the reactions were all ended at 34℃ within 20 min to simulate field circumstance. Constant reaction temperature of 42℃ (c), 37℃ (d) and 34℃ (e) were used as controls. The absorbance of resulted solution was monitored by UV–Vis absorption (Fig. S1). The data show that the absorbance from resulted solution with 42℃-34℃ gradient and 37℃-34℃gradient were similar with that of constant temperature of 42℃, 37℃ and 34℃, indicating RPA reaction could be performed in water without constant temperature. It indicates that the strategy could be applied out of lab. Foam box and hot water were enough for the assay, and did not need heating instrument. In this case, water with temperature gradient of 42℃-34 ℃ was chosen in the following assay.

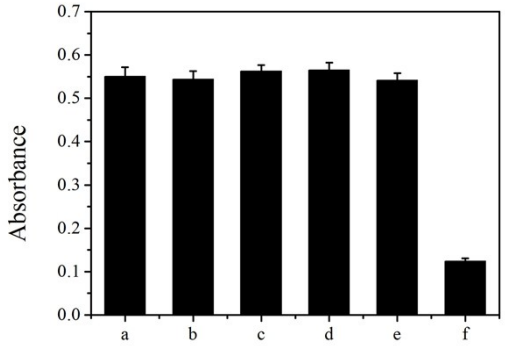

Fig. S1. Relationship between absorbance and incubation temperature of RPA.

Asymmetric RPA reaction was an isothermal amplification with excessive amount of one of the two primers. Primer-F and primer-R were used to produce dsDNA at the initial stage of RPA reaction. With the time going on, one primer was exhausted, and ssDNA became the main product with the other primer. Longer reaction times, more ssDNAs were produced. Our results show that the absorbance increased along with the reaction time within 5-30 min (Fig. S2). The colorimetric signals reached a plateau when reaction time was longer than 20 min. So, reaction time of 20 min was used in the follow assay.

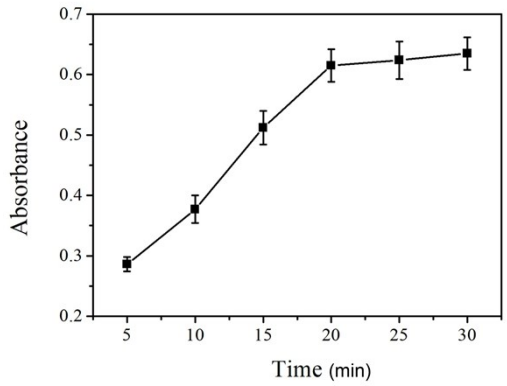

Fig. S2. Relationship between absorbance and time for RPA reaction.

Hemin/G-quadruplex DNAzyme has parallel conformer, antiparallel conformer or coexistence of parallel and antiparallel conformers in the presence of different monovalent cations[6, 7]. Conformer of hemin/G-quadruplex DNAzyme affects its peroxidase mimicking activity, and then affects the reaction time and absorbance of the results solution in the assay. In order to improve the sensitivity and shorten reaction time, effects of K^+ and NH_4^+ were assessed during the redox reaction of H_2O_2 and $ABTS^{2-}$ (Fig. S3). Test 1 and 2 were performed in buffer A containing K^+. Tests 3 and 4 were done in buffer B containing NH_4^+. Test 1 and 3 were done in the absence of target DNA, which were the blank controls of the method. Test 2 and 4 were done in the presence of target DNA. As shown in Figure 2C, the absorbance of final solution containing NH_4^+ was obviously higher than that of K^+, showing NH_4^+ was more beneficial to our strategy than that of K^+. Therefore, buffer B containing NH_4^+ was used in the following assay.

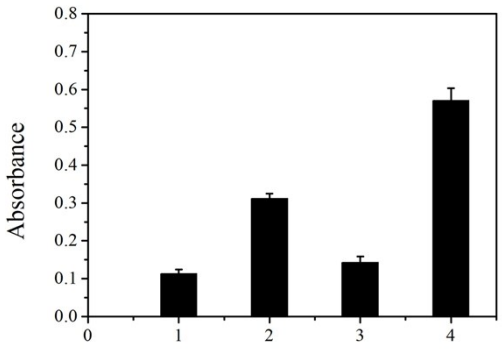

Fig. S3 Effects of K^+ and NH_4^+ on absorbance of the assay in final solution.

Different amounts of genomic DNA of *F. proliferatum* were used to verify the reproducibility of RPA reaction by 1.5% agarose gel electrophoresis, including 50 ng (8), 10 ng (1), 1 ng (2), 0.1 ng (3), 0.01 ng (4), 10^{-3} ng (5), 10^{-4} ng (6), 0 ng (7). As shown in Fig. S4, yield of RPA products was increased when RPA was completed from 10 min to 30 min. It shows that final yield of RPA reaction was related to reaction time for a certain amount of template. In the same reaction time of 10 min, yield of RPA products was similar in the case of the same amount of DNA template. Moreover, the bands were brighter with the increase of amount of target DNA. The data indicate that RPA reaction had satisfied reproducibility. Our data was also consistent with that of Wang zhao-Hua et al.[8] and M.M. Gumaa et al.[9] reports. The strips were all smearing, and it may be resulted of denatured proteins in the solutions.

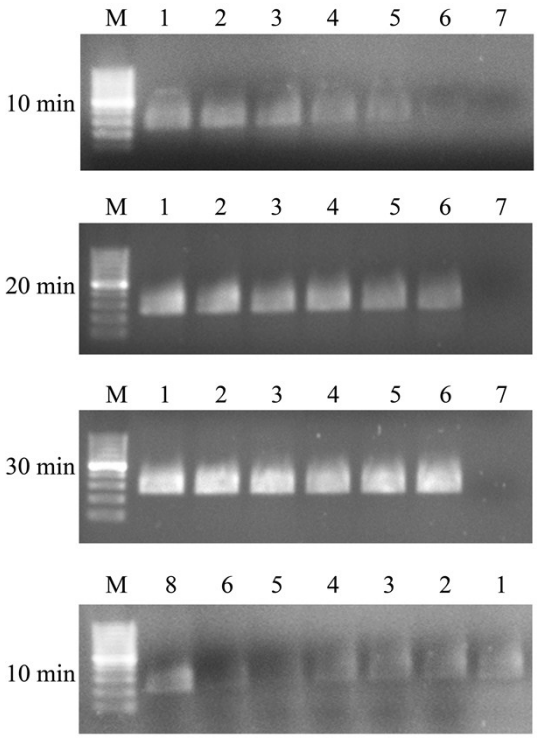

Fig. S4 Agarose gel electrophoresis (1.5%) analysis of RPA using different amounts of target DNA as template. Genomic DNA of *F. proliferatum* was 50 ng (8), 10 ng (1), 1 ng (2), 0.1 ng (3), 0.01 ng (4), 10^{-3} ng (5), 10^{-4} ng (6), 0 ng (7).

The reproducibility of the RPA-hemin/G-quadruplex-based strategy was monitored by inter-assay and intra-assay methods. The intra-assay was accomplished for each three hours one day with 10 ng genomic DNA of F120 as template, and the relative standard deviations (RSD) value was 3.7%. The inter-assay reproducibility was detected for three days by 10 ng genome of *F. proliferatum*, and the RSD value was 3.6%, which showed good reproducibilitys of the strategy. The stability of the assay was performed for parallel determination of six groups with 10 ng genome of F120. The RSD value was 3.90%, indicating favorable stability of the RPA coupled with hemin/G-quadruplex assay.

Supplemental table 1. Comparison of RPA-based methods of colorimetric and lateral flow analysis.

Analytical Method	Amplification device	LOD	T(℃)	Ref.
Colorimetric and electrochemical detection of Mycobacterium Tuberculosis	Incubator	Single cell	38	[10]
Detection of tumor-derived exosomes in nasopharyngeal carcinoma by RPA and TMA	Incubator	10^2 particles/mL	37	[11]
Paper chip detection for detection of multiple foodborne pathogens by single-step RPA	Paper and incubator	10^2 cfu /mL	37	[12]
Detection of food pathogens based on RPA with a PNA-based lateral flow device	Thermal cycler	4 cfu /mL	38	[13]
On-site screening of GTS 40-3-2 soybean based on body-heat triggered RPA	Body heat		~36.5	[5]
Nucleic Acid Test to diagnose *cryptosporidiosis* by RPA and lateral flow analysis	Incubator	10^2 -10^3 oocysts/mL	37	[14]
Visual detection via asymmetric RPA and hemin/G-quadruplex DNAzyme	Foam box with hot water	0.01ng	34-37	This study

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