Phytopathology Research

RESEARCH

Open Access

A field survey of eight common potato pathogens in China based on integrated-loop-mediated isothermal amplification assays

Xinjie Zhang[1,2], Han Chen[1,2], Jinbin Wu[1,2], Xiao Wang[1], Shuaishuai Wang[1], Jingqi Chen[1], Qianpeng Yu[1], Zhenxin Zhang[3], Xiaobo Zheng[1,2], Juan Zeng[4] and Suomeng Dong[1,2*]

Abstract

Various pathogens from oomycete, fungi, and bacteria kingdoms can infect potato and significantly reduce potato yield. The early diagnosis of potato pathogens is important for tracing disease epidemics and the subsequent disease management. Loop-mediated isothermal amplification (LAMP) is a critical technique for pathogen detection, but available LAMP assays do not effectively meet the requirement of field diagnoses due to complexities including co-infection of different pathogens. Hence, this study aims to develop integrated-LAMP assays (iLAMPs) for simultaneous detection of eight common potato pathogens and apply iLAMPs to pathogen detection in field samples from the four main potato-growing regions of China in 2023. Therefore, eight sets of primers showing gene- and genus-specificity were designed and used for iLAMPs to determine their specificity, sensitivity, and visualization. Subsequently, iLAMPs-mediated pathogen detection revealed that 72.82% of 206 diseased leaves and 84.94% of 239 diseased tubers carry more than one pathogen. The detection rate for each pathogen significantly varies from 1.94 to 65.53% in diseased leaves, and ranges from 26.78 to 52.72% in diseased tubers, respectively. In addition, the detection rate of *Phytophthora infestans* and *Alternaria solani* positively correlates in both leaves and tubers, especially for those samples from the southwestern and southern regions. Taken together, iLAMPs developed in this study enables simultaneous detection of eight common potato pathogens from field samples and may have broad applications in early management of potato diseases.

Keywords Potato, Pathogen detection, Loop-mediated isothermal amplification, *Phytophthora infestans*, *Alternaria solani*

*Correspondence:
Suomeng Dong
smdong@njau.edu.cn
Full list of author information is available at the end of the article

Background

Potato is characterized by its high yield, strong adaptability, and comprehensive nutrition, making potato the world's fourth most important crop ranking after rice, wheat, and maize (Naumann et al. 2020). In China, potato-growing regions and potato production have continuously increased in recent years and were ranked first in the world in 2019 (FAOSTAT 2020). However, various pathogens cause different potato diseases, which significantly decrease harvests in fields and storage (Horne 2010). Hence, detecting potato pathogens is vital for securing potato production (Jansky et al. 2009; Sankaran et al. 2010).

During potato production, various pathogens spanning different kingdoms can infect potato plants. Known pathogens causing devastating diseases of potatoes include mainly oomycete, fungal, and bacterial pathogens (Horne 2010). The oomycete pathogen *Phytophthora infestans* causes late blight disease, which destroys potato leaves, stems, and tubers and was the cause of the Irish potato famine (Fry et al. 2015; Yuen 2021). The fungal pathogen *Alternaria solani* infects potato leaves, stems, and tubers to cause early blight disease, which reduces potato yield by up to 50% (Yadav and Pathak 2011; Xue et al. 2019). Similarly, *Fusarium graminearum* causes dry rot disease, which leads to sunken and wrinkled brown patches on tubers (Goswami and Kistler 2004; Xia et al. 2017; Tiwari et al. 2020). The soil-borne fungal pathogen *Rhizoctonia solani* causes black spot disease, which leads to the development of seedling damping-off, stem canker, and root rots (Andika et al. 2017; Abdoulaye et al. 2019). *Spongospora subterranea* causes powdery scab disease, which leads to potato spoilage due to the formation of scab-like lesions on tubers (Merz 2008; Gau et al. 2013). In addition, the bacterium *Ralstonia solanacearum* causes bacterial wilt disease, which leads to stunting, root and stem rots, vascular discoloration, and wilting (Chen et al. 2012). *Pectobacterium atrosepticum* causes black shank disease, which exhibits black spots and stem rots resulting in plant decline, wilting, and death (Mansfield et al. 2012). Likewise, *Streptomyces scabies* causes potato scab disease, which develops scab lesions on the surface of tubers and ultimately reduces potato quality (Hill and Lazarovits 2005; Wang and Lazarovits 2005). In short, these eight common potato pathogens cause the majority of diseases that severely reduce yield and marketability. As a wide range of pathogens simultaneously infect potato plants, rapid detection of each pathogen is important for the on-site diagnosis and treatment of potato diseases (Cunniffe et al. 2015; Susi et al. 2015).

Various methods have been used to detect potato pathogens (Nagamine et al. 2002; Daher et al. 2016; Tameh et al. 2020). Traditional methods rely on the isolation and purification of pathogens, which can be time-consuming and require prior experience (Perez-Sierra et al. 2022). With the development of molecular techniques, amplification of genes through conventional polymerase chain reaction (PCR), nested PCR, and quantitative real-time PCR has been used for timely and accurate detection of pathogens (Smith and Osborn 2009; Green and Sambrook 2018, 2019). So far, these molecular strategies have been reported to detect *P. infestans*, *Alternaria* spp., *Fusarium* spp., *R. solani*, *R. solanacearum*, *P. atrosepticum*, *Streptomyces* spp., and *Spongospora* spp. on potato (Niepold and SchoberButin 1995; Bulman and Marshall 1998; Pastrik and Maiss 2000; Jurado et al. 2005; Okubara et al. 2008; Kumar et al. 2013; Xu et al. 2016). However, PCR-based detection of pathogens requires laboratory equipment and well-trained staff, which makes it unsuitable for on-site field pathogen detection. Alternatively, recombinase polymerase amplification (RPA) is an effective detection technique that uses recombinase and coenzymes with primers to amplify targeted genes (Piepenburg et al. 2006). Recently, CRISPR/Cas12a has been reported to be a highly sensitive and programmable tool for pathogen detection (Chen et al. 2018). However, both RPA and CRISPR/Cas12a are difficult to promote in fields due to their high expense. Therefore, it is crucial to establish a straightforward, rapid, sensitive, and precise method to detect pathogens in fields.

Loop-mediated isothermal amplification (LAMP) has shown to be more straightforward, cheaper, and rapid than PCR-based detection of pathogens (Nagamine et al. 2002; Caipang et al. 2004). LAMP uses a set of four to six primers and *Bacillus stearothermophilus* DNA polymerase to amplify targeted genes with high specificity under a isothermal condition, which ranges from 60 to 65 °C (Notomi et al. 2000; Begum et al. 2010). In addition, LAMP is easily visualized using SYBR Green I after gene amplification (Feng et al. 2021). These characteristics make LAMP applicable for on-site pathogen detection (Shu et al. 2003). To date, LAMP has been employed to detect the oomycete *P. infestans*, the fungi *A. solani* and *Fusarium* spp., and the bacteria *R. solanacearum* and *P. atrosepticum* (Niessen and Vogel 2010; Pan et al. 2011; Lu et al. 2015b; Hu et al. 2016; Khan et al. 2017; Lees et al. 2019; Jiang et al. 2021). However, LAMP primers in these reports only target a certain pathogen, which make them unfeasible for detecting complex infection of potato pathogens in fields.

In this study, we aimed to develop integrated-LAMP assays (iLAMPs) for simultaneously detecting eight common potato pathogens and applied it for pathogen detection in potato samples from the four main potato-growing regions of China in 2023. Therefore, LAMP primers targeting eight genes were designed to detect

their corresponding pathogens. In addition, the specificity of iLAMPs were tested for different pathogens as well as isolates, and iLAMPs could sensitively detect gDNA with concentration ranging from 10 fg/μL to 10 pg/μL. Subsequently, infected potato samples prepared under laboratory conditions were used to validate the ability of iLAMPs for pathogen detection. Finally, iLAMPs-mediated pathogen detection in field samples revealed that *P. infestans* and *A. solani* are the two main potato pathogens, and are frequently co-detected in diseased leaves and tubers. Overall, iLAMPs developed in this study can simultaneously detect multiple potato pathogens, which can facilitate early prevention and management of potato diseases.

Results

Design of LAMP primers

To monitor and further prevent outbreaks of potato diseases, this study aimed to develop a toolkit employing LAMP assays to simultaneously detect eight common potato pathogens, including *P. infestans*, *A. solani*, *F. graminearum*, *R. solani*, *R. solanacearum*, *P. atrosepticum*, *S. scabies*, and *S. subterranea*, by using eight sets of primers, which individually targets a specific gene for each pathogen. The specific target genes were aligned with their corresponding homologs for the design of primers with the advantage of stronger specificity, higher sensitivity, and easier visualization (Fig. 1a). Potato tissues prepared under laboratory conditions and collected from fields were used for gDNA extraction (Fig. 1b). Subsequently, gDNA was used as the template for iLAMPs, of which yellow-green and orange colors indicate the presence and absence of detected pathogens, respectively (Fig. 1c).

gDNA sequences of *P. infestans extracellular protease inhibitor 12* (*Epi 12*), *A. solani β-tubulin domain-containing protein* (*β-tubulin*), *F. graminearum translation elongation factor 1α* (*TEF 1α*), *R. solani* internal transcribed spacer (ITS), *R. solanacearum* 16S ribosomal RNA (16S rRNA), *P. atrosepticum gyrase beta* (*gyrB*), *S. scabies tryptophan synthase subunit beta* (*trpB*), and *S. subterranea* ITS were retrieved from National Center for Biotechnology Information (NCBI). In addition, these eight genes were blasted to obtain homologs of pathogens belonging to the same genus (Additional file 1: Figure S1). Each set contains four to six primers, with a pair of outer primers (forward primer F3 and backward primer B3), a pair of inner primers (forward inner primer FIP and backward inner primer BIP), and one to two loop primers (loop forward primer LF and loop backward primer LB). Outer and inner primers are used to amplify six fragments of targeted genes, while loop primers are used to accelerate gene amplification (Additional file 1: Figure S2a and Additional file 2: Table S1). Aligned sequences of these eight genes and their corresponding homologs were searched for non-conserved regions, which were subsequently used for primer design (Additional file 1: Figure S2b–i and Additional file 2: Table S1).

Specificity of iLAMPs

To determine the detection specificity of iLAMPs by using these primers, each set of genus-specific primers was challenged against the other seven pathogens and homologous isolates for unwanted crossover interactions. When primers targeting *Epi 12* of *P. infestans* were used for iLAMPs, only the gDNA of *P. infestans* displayed a yellow-green color, while the other oomycetes of *P. capsici*, *P. sojae*, *P. mirabilis*, *P. parasitica*, and *P. palmivora*, as well as the fungal and bacterial pathogens displayed an orange color (Fig. 2, Additional file 1: Figure S3, and Additional file 2: Table S2). These results indicate that *Epi 12* specifically detects *P. infestans*. Similarly, when primers targeting *β-tubulin* of *A. solani*, *TEF 1α* of *F. graminearum*, ITS of *R. solani*, 16S rRNA of *R. solanacearum*, *gyrB* of *P. atrosepticum*, *trpB* of *S. scabies*, and ITS of *S. subterranea* were used for iLAMPs, only the gDNA of each targeted pathogen displayed a yellow-green color (Fig. 2, Additional file 1: Figure S3, and Additional file 2: Table S2). Taken together, these results indicated that iLAMPs using these primers have high specificity for their corresponding targeted pathogens.

To determine whether iLAMPs using these primers could detect their corresponding isolates, four isolates of each pathogen were used. When primers targeting *Epi 12* of *P. infestans* were used for iLAMPs, gDNA of four *P. infestans* isolates displayed a yellow-green color (Fig. 2 and Additional file 2: Table S2), indicating that *Epi 12* could be used for iLAMPs to detect different *P. infestans* isolates. Similarly, when primers targeting *β-tubulin* of *A. solani*, *TEF 1α* of *F. graminearum*, ITS of *R. solani*, 16S rRNA of *R. solanacearum*, *gyrB* of *P. atrosepticum*, *trpB* of *S. scabies*, and ITS of *S. subterranea* were used for iLAMPs, gDNA of respective four isolates displayed a yellow-green color (Fig. 2 and Additional file 2: Table S2), suggesting that these genes could also be used for iLAMPs to detect different isolates of *A. solani*, *F. graminearum*, *R. solani*, *R. solanacearum*, *P. atrosepticum*, *S. scabies*, and *S. subterranea*, respectively. Taken together, these results indicated that the developed iLAMPs could detect different isolates of these eight common potato pathogens.

Sensitivity of iLAMPs

To determine the detection sensitivity of iLAMPs using these primers, the minimum amount of gDNA of these eight pathogens for iLAMPs was determined. Therefore,

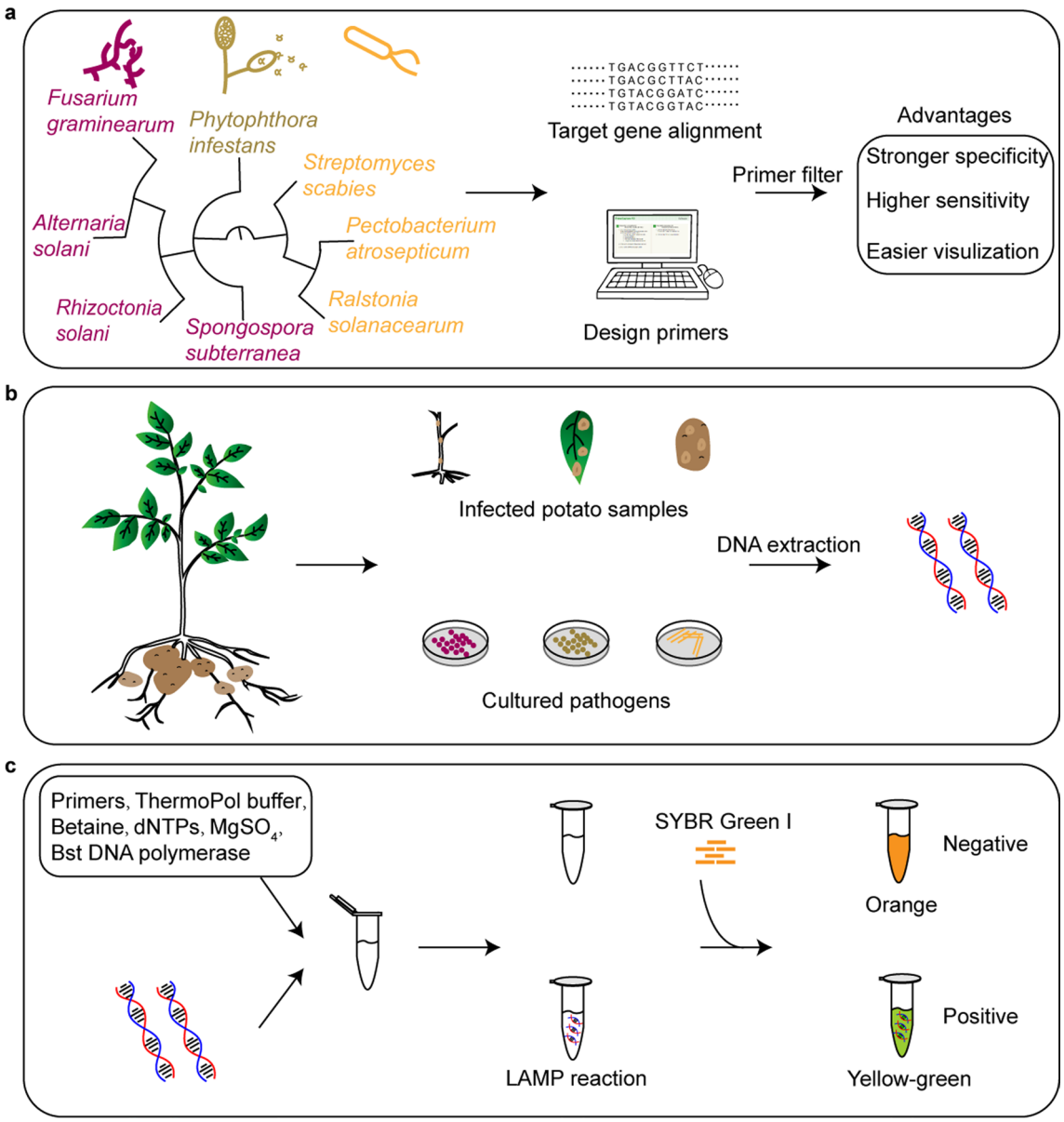

Fig. 1 A schematic view of iLAMPs for potato disease survey. **a** Primers design for various potato pathogens spanning different kingdoms. Genus-specific genes of these eight common potato pathogens were selected, and the non-conserved regions of selected genes aligned with their homologs were used for designing LAMP primers. **b** Potato tissues and cultured pathogens used for gDNA extraction. **c** gDNA was used as a template for iLAMPs, of which yellow-green and orange colors indicate the presence and absence of detected pathogens, respectively

diluted gDNA ranging from 100 ng/μL to 10 fg/μL with a ten-times serial dilution was used as templates for iLAMPs. The minimum limit of detection was 100 fg/μL for *A. solani*, *R. solanacearum*, *P. atrosepticum*, and *S. scabies*, 10 pg/μL for *P. infestans*, *R. solani*, and *S. subterranea*, and 1 pg/μL for *F. graminearum* (Fig. 3). These results indicate that designed primers could effectively amplify targeted genes with a gDNA concentration down to the level between 100 fg/μL and 10 pg/μL, and that iLAMPs with these sets of primers could be used to detect low abundance of pathogens from diseased samples.

iLAMPs-mediated pathogen detection in different potato tissues prepared under laboratory conditions

To test whether iLAMPs could detect these eight pathogens under laboratory conditions, leaves, stems, and tubers infected by eight pathogens were used for gDNA

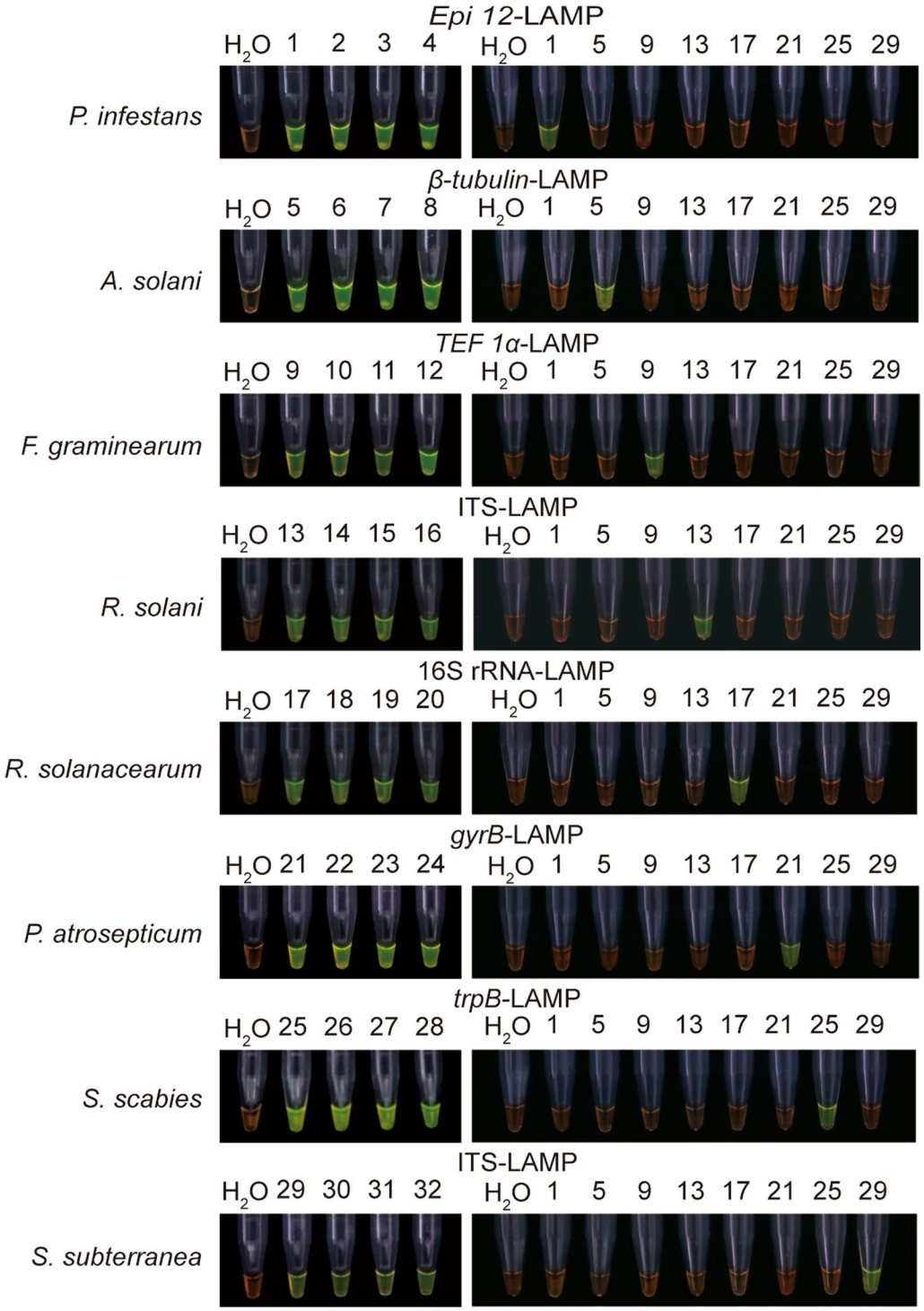

Fig. 2 Specificity of iLAMPs using gDNA of different pathogens. H_2O was used as a negative control and numbers indicate gDNA of different pathogen isolates. The numbers of 1–4 indicate *P. infestans*, 5–8 indicate *A. solani*, 9–12 indicate *F. graminearum*, 13–16 indicate *R. solani*, 17–20 indicate *R. solanacearum*, 21–24 indicate *P. atrosepticum*, 25–28 indicate *S. scabies*, and 29–32 indicate *S. subterranea*. Representative pictures of at least three experiments with similar results were shown

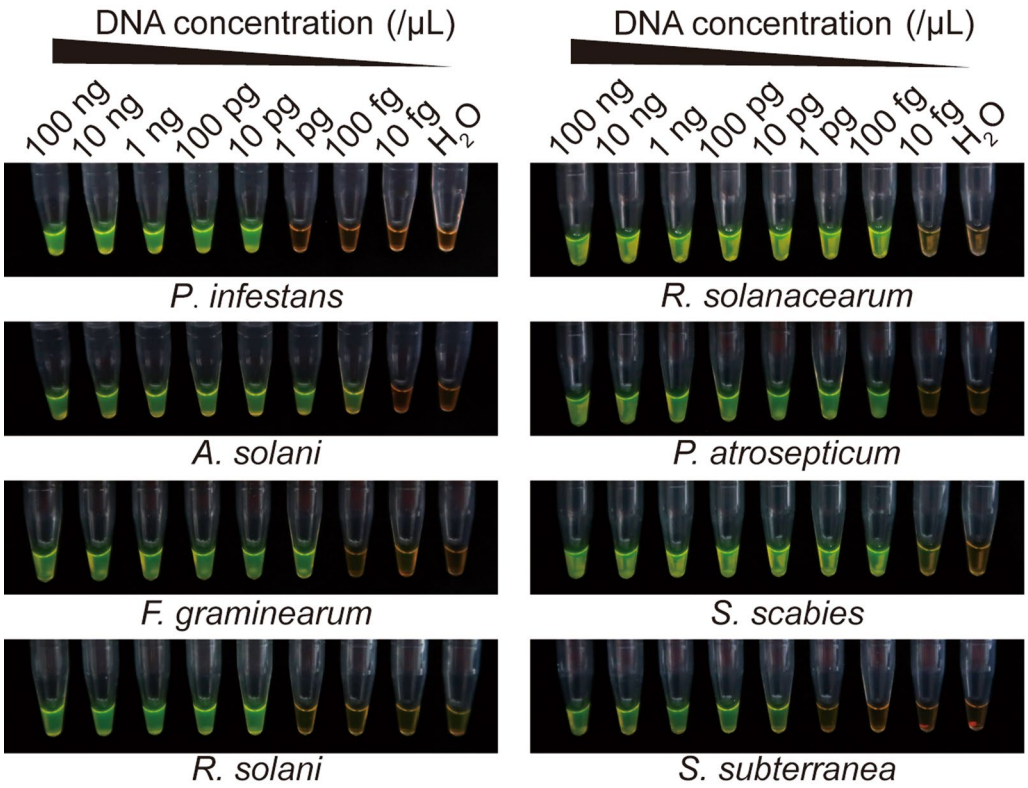

Fig. 3 Sensitivity of iLAMPs using diluted gDNA of different pathogens. Diluted gDNA of eight pathogens was used for iLAMPs, and H_2O was used as a negative control. Representative pictures of at least three experiments with similar results were shown

isolation. In addition, healthy leaves were used for the negative control of *P. infestans*, *A. solani*, *F. graminearum*, and *R. solani*, healthy stems were used for the negative control of *R. solanacearum* and *P. atrosepticum*, and healthy tubers were used for the negative control of *S. scabies* and *S. subterranea*. iLAMPs with primers of *Epi 12*, *β-tubulin*, *TEF 1α*, ITS, 16S rRNA, *gyrB*, *trpB*, and ITS only detected the presence of *P. infestans*, *A. solani*, *F. graminearum*, *R. solani*, *R. solanacearum*, *P. atrosepticum*, *S. scabies*, and *S. subterranea*, respectively, and did not show false-positive results when used for other pathogens, H_2O, and healthy potato tissues (Fig. 4). These results suggested that iLAMPs could be used to detect the presence of these eight pathogens in diseased potato leaves, stems, and tubers.

iLAMPs-mediated pathogen detection in field samples

To evaluate whether iLAMPs could detect these eight pathogens from field samples, which were collected during the potato production season in 2023 from the four main potato-growing regions of China (Jansky et al. 2009), a total of 206 diseased leaves and 239 diseased tubers were collected (Fig. 5a and Additional file 2: Table S3) and used for gDNA isolation prepared for iLAMPs.

When diseased leaves were used for iLAMPs, *P. infestans*, *A. solani*, *F. graminearum*, *R. solani*, *R. solanacearum*, and *P. atrosepticum* were detected from 47.57%, 65.53%, 21.84%, 25.24%, 35.92%, and 32.04% of all samples, respectively, while *S. scabies* and *S. subterranea* were detected from 1.94% and 5.34% (Fig. 5b, Additional file 2: Table S4 and Table S5). When sorted by each potato-growing region, in the northern region, *P. infestans*, *A. solani*, *F. graminearum*, *R. solani*, *R. solanacearum*, and *P. atrosepticum* were detected from 32.61%, 76.09%, 30.43%, 41.30%, 23.91%, and 19.57% of samples, respectively, while *S. scabies* and *S. subterranea* were detected from 4.35% and 8.70% (Fig. 5b, Additional file 2: Table S4 and Table S5). Likewise, in the central region, *P. infestans*, *A. solani*, *F. graminearum*, *R. solani*, *R. solanacearum*, and *P. atrosepticum* were detected from 25.00%, 37.50%, 16.67%, 12.50%, 45.83%, and 45.83% of samples, respectively, while *S. scabies* and *S. subterranea* were not detected (Fig. 5b, Additional file 2: Table S4 and Table S5). Similarly, in the southwestern region, *P. infestans*, *A. solani*, *F. graminearum*, *R. solani*, *R. solanacearum*, and *P. atrosepticum* were detected from

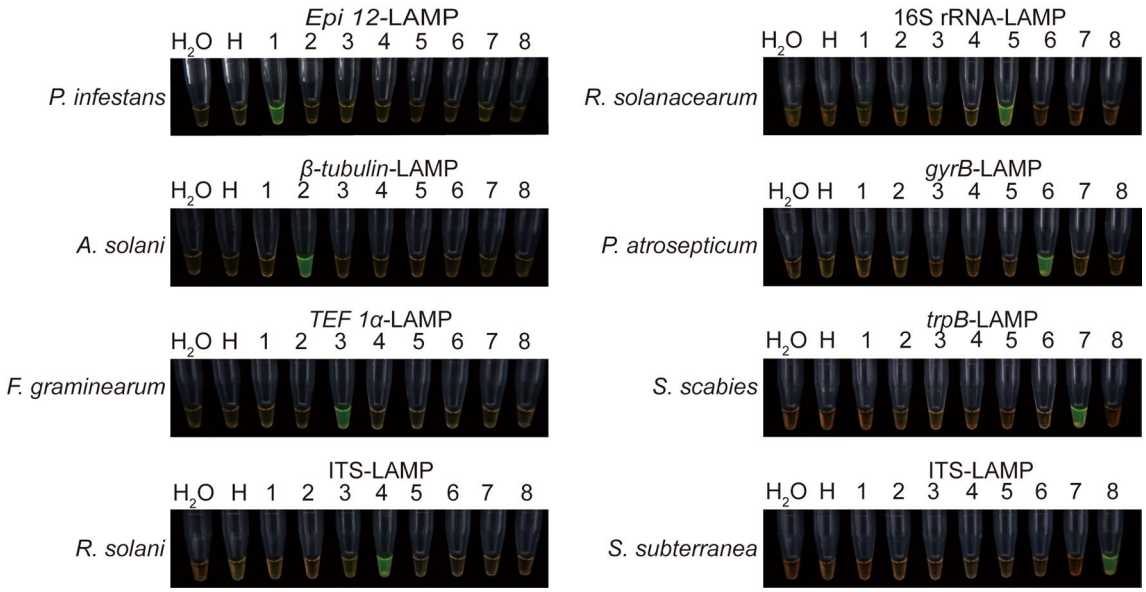

Fig. 4 iLAMPs specifically detect pathogens on potato grown under laboratory conditions. gDNA of infected potatoes growing under laboratory conditions was used for iLAMPs. H_2O and healthy potato (H) were used as negative controls, and the numbers of 1–8 indicate the gDNA of potatoes infected by *P. infestans*, *A. solani*, *F. graminearum*, *R. solani*, *R. solanacearum*, *P. atrosepticum*, *S. scabies*, and *S. subterranea*, respectively. The numbers of 1–4 indicate gDNA isolated from potato leaves, 5–6 indicate gDNA isolated from potato stems, and 7–8 indicate gDNA isolated from potato tubers. Representative pictures of at least three experiments with similar results were shown

56.15%, 66.92%, 19.23%, 23.08%, 38.46%, and 33.85% of samples, respectively, while *S. scabies* and *S. subterranea* were detected from 1.54% and 5.38% (Fig. 5b, Additional file 2: Table S4 and Table S5). Additionally, in the southern region, *P. infestans*, *A. solani*, *F. graminearum*, *R. solanacearum*, and *P. atrosepticum* were detected from 66.67%, 66.67%, 33.33%, 33.33%, and 33.33% of samples, respectively, while *R. solani*, *S. scabies*, and *S. subterranea* were not detected (Fig. 5b, Additional file 2: Table S4 and Table S5).

When diseased tubers were used for iLAMPs, *P. infestans*, *A. solani*, *F. graminearum*, *R. solani*, *R. solanacearum*, *P. atrosepticum*, *S. scabies*, and *S. subterranea* were detected from 26.78%, 38.08%, 52.30%, 46.03%, 52.72%, 41.00%, 33.05%, and 40.59% of all samples, respectively (Fig. 5c, Additional file 2: Table S4 and Table S6). In the northern region, *P. infestans*, *A. solani*, *F. graminearum*, *R. solani*, *R. solanacearum*, *P. atrosepticum*, *S. scabies*, and *S. subterranea* were detected from 26.36%, 32.73%, 46.36%, 31.82%, 43.64%, 40.00%, 30.00%, and 40.00% of samples, respectively (Fig. 5c, Additional file 2: Table S4 and Table S6). Likewise, in the central region, *P. infestans*, *A. solani*, *F. graminearum*, *R. solani*, *R. solanacearum*, *P. atrosepticum*, *S. scabies*, and *S. subterranea* were detected from 17.54%, 61.40%, 85.96%, 45.61%, 52.63%, 59.65%, 33.33%, and 38.60% of samples, respectively (Fig. 5c, Additional file 2: Table S4 and Table S6). Similarly, in the southwestern region, *P. infestans*, *A. solani*, *F. graminearum*, *R. solani*, *R. solanacearum*, *P. atrosepticum*, *S. scabies*, and *S. subterranea* were detected from 36.84%, 29.82%, 40.35%, 63.16%, 66.67%, 22.81%, 35.09%, and 42.11% of samples, respectively (Fig. 5c, Additional file 2: Table S4 and Table S6). Additionally, in the southern regions, *P. infestans*, *A. solani*, *F. graminearum*, *R. solani*, *R. solanacearum*, *P. atrosepticum*, *S. scabies*, and *S. subterranea* were detected from 26.67%, 20.00%, 13.33%, 86.67%, 66.67%, 46.67%, 46.67%, and 46.67% of sample, respectively (Fig. 5c, Additional file 2: Table S4 and Table S6). Taken together, these results indicated that iLAMPs could detect these eight pathogens in diseased potato tissues collected from field samples.

Co-existence of *P. infestans* and *A. solani* in both leaf and tuber samples

Plants are constantly infected by a variety of pathogens and complex infection of *Fusarium* spp. and *R. solani* as well as *P. sojae* in soybean has been reported (Ye et al. 2020; Wang et al. 2023). Since various pathogens simultaneously infect potatoes in fields and that 72.82% diseased leaves and 84.94% diseased tubers carry more than one pathogen, iLAMPs detection results were analyzed for the complex infection of pathogens in diseased leaves and tubers.

When diseased leaves were used, 90.78% of them contained one to five pathogens, 0.49% contained six

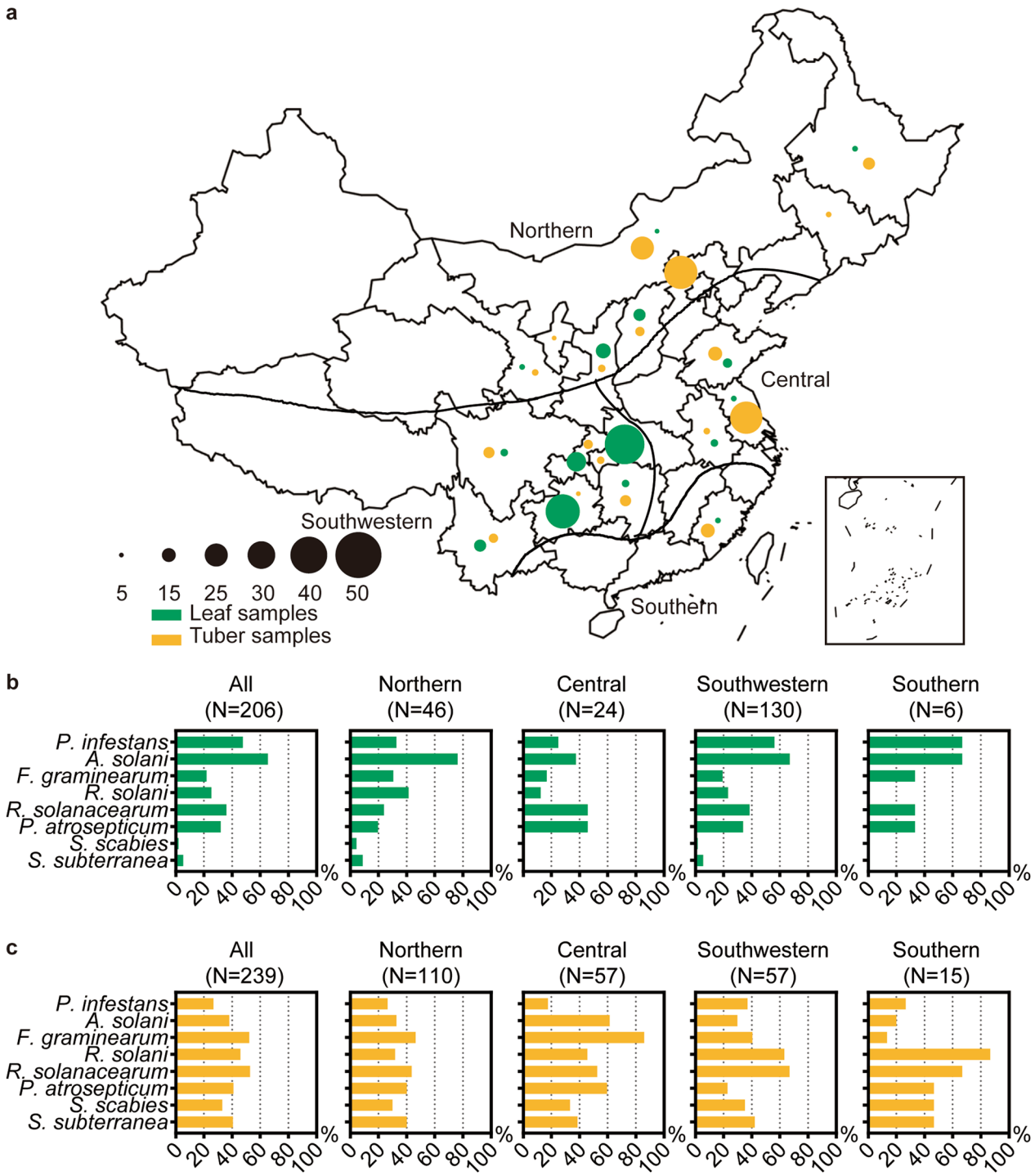

Fig. 5 iLAMPs specifically detect pathogens from field potato samples. **a** A illustration for sample collection sites from the four main potato-growing regions. Green represents diseased leaves, orange represents diseased tubers, and circle size represents the number of collected samples. **b**, **c** Percentage of detected pathogens from diseased leaves and tubers described in the four potato-growing regions

pathogens while 8.74% were free of these eight pathogens (Fig. 6a). In addition, 31.55% and 20.39% of them contain two and three pathogens, respectively (Fig. 6a). Likewise, when diseased tubers were used, 6.69% were free of these eight pathogens, 8.37% of them contained only one pathogen while 2.51% and 0.42% contained seven and eight

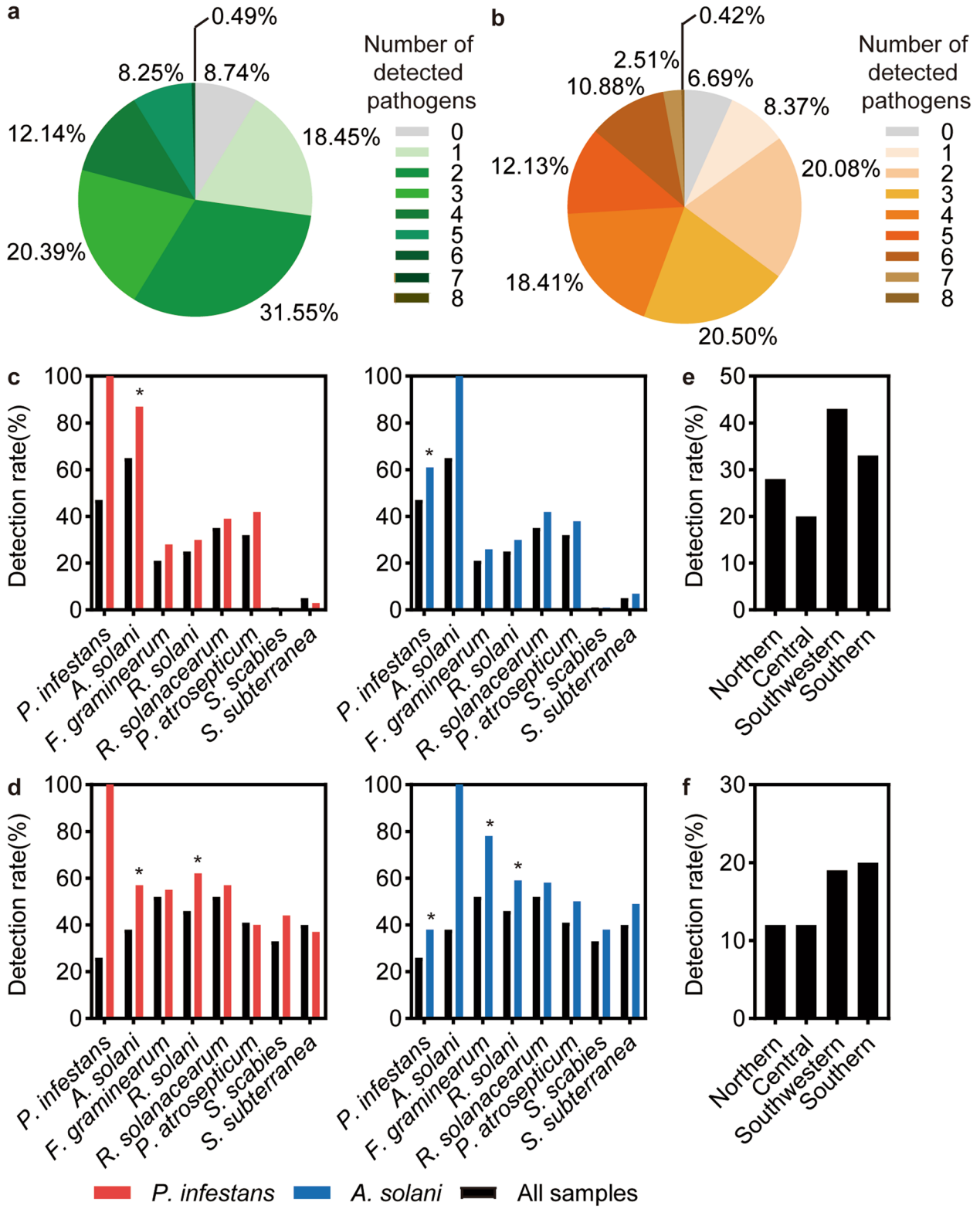

Fig. 6 Co-existence of *P. infestans* and *A. solani* in both diseased leaves and tubers. **a**, **b** Frequencies of LAMP-detected potato pathogens in diseased leaves and tubers, respectively. **c**, **d** Detection rates of indicated species in all samples and in selected diseased leaves and tubers containing *P. infestans* and *A. solani*, respectively. Asterisks indicate a significantly higher detection rate of the indicated species in samples associated with a particular pathogen species compared with that in all samples (chi-square test, * $p < 0.05$). **e**, **f** *P. infestans*-*A. solani* was preferentially concurrent in diseased leaves and tubers, respectively, from the four main potato-growing regions

pathogens, respectively (Fig. 6b). In addition, two to six pathogens were detected in 82.01% of diseased tubers, in which 20.08% and 20.50% contained two and three pathogens, respectively (Fig. 6b). Taken together, these observations revealed that the majority of diseased leaves and tubers are likely to simultaneously contain two to three pathogens.

To determine the co-existed pathogens in potato leaves and tubers, the frequency of simultaneous detection of multiple species was calculated. In diseased leaves, *P. infestans* was frequently concurrent with *A. solani*, which was found in 87.50% of *P. infestans*-positive samples and was significantly higher than those of other pathogens (Fig. 6c and Additional file 2: Table S7). Likewise, *A. solani* was frequently concurrent with *P. infestans*, which was found in 61.11% of *A. solani*-positive samples and was significantly higher than those of other pathogens (Fig. 6c and Additional file 2: Table S7). In addition, combinations such as *F. graminearum-R. solani* and *R. solanacearum-P. atrosepticum* were also frequently concurrent in diseased leaves, but their correlation rates were not as high as the correlation rate between *P. infestans* and *A. solani* (Additional file 1: Figure S4a and Additional file 2: Table S7). In diseased tubers, *P. infestans* was frequently concurrent with *A. solani* and *R. solani*, which were found in 57.38% and 62.30% of *P. infestans*-positive samples, respectively (Fig. 6d and Additional file 2: Table S8). Likewise, *A. solani* was frequently concurrent with *P. infestans*, which was found in 38.46% of *A. solani*-positive samples (Fig. 6d and Additional file 2: Table S8). Moreover, combinations such as *A. solani-F. graminearum*, *A. solani-R. solani*, *F. graminearum-P. atrosepticum*, *R. solani-R. solanacearum*, and *S. scabies-S. subterranea* were also frequently concurrent in diseased tubers (Additional file 1: Figure S4b and Additional file 2: Table S8). Furthermore, when sorted by each potato-growing region, the combination of *P. infestans-A. solani* was higher in both southern and southwestern regions than other regions (Fig. 6e, f). Taken together, diseased leaves and tubers from the four main potato-growing regions of China mainly contained two to three pathogens, and frequent occurrence of co-existence of *P. infestans* and *A. solani* was observed.

Discussion

Potato production is severely affected by various pathogens, and detection of these pathogens is vital for identification and control of potato diseases. However, current methods do not meet the requirement for simultaneous detection of multiple potato pathogens. In this study, a toolkit termed iLAMPs was developed to provide a fast and accurate detection system for monitoring up to eight common potato pathogens simultaneously.

Currently, *P. infestans* has been targeted for LAMP assays using the genes of ITS-II (Hansen et al. 2016), ITS-I (Verma et al. 2019), *ras-related protein* (Khan et al. 2017), and *P. infestans specific multiple copy* (*PiSMC*) (Kong et al. 2020). In this study, *Epi 12* was selected for iLAMPs with a stronger sensitivity than ITS and a comparable sensitivity to *PiSMC*, which makes *Epi 12* suitable for detecting *P. infestans*. As for *A. solani*, *histidine kinase 1*, which is a genetic marker, *alternaria allergen 1*, and *β-tubulin* were used for PCR-based detection of *A. solani* (Pavon et al. 2010; Kumar et al. 2013; Khan et al. 2018). In this study, *β-tubulin* was used to detect *A. solani* by iLAMPs, enabling easy-visualization of detection results and making it more convenient than traditional PCR. Similarly, ITS of *S. scabies* and *S. subterranea* has been used for PCR to detect these two pathogens (Bulman and Marshall 1998; Xu et al. 2016), and in this study, *trpB* and ITS were used for iLAMPs-mediated detection of *S. scabies* and *S. subterranea*, respectively, which provides a simpler way to detect these two pathogens when compared with PCR methods. Additionally, *cytochrome P450 51C* (*CYP51C*) of *F. graminearum* has been used for LAMP-mediated detection of this pathogen with a sensitivity of 100 pg/μL (Lu et al. 2015b). In this study, *TEF 1α* of *F. graminearum* was selected for iLAMPs-mediated detection of this pathogen and had a higher sensitivity of 1 pg/μL when compared to *CYP51C*. Hence, primers targeting specific regions of ITS, 16S rRNA, and *gyrB* were redesigned for iLAMPs detecting *R. solani*, *R. solanacearum*, and *P. atrosepticum*, respectively (Lenarcic et al. 2014; Hu et al. 2016; Huang et al. 2017). Taken together, this study provides eight pairs of primers, which could be used for iLAMPs-mediated detection of corresponding potato pathogens.

The diagnose of potato diseases in fields is difficult because of simultaneous infections by various pathogens and a lack of visible symptoms at early infection stage. Thus, a rapid and sensitive on-site detection technique is important for diagnosing potato diseases. To meet this requirement, LAMP has been used for the detection of *P. infestans*, *F. graminearum*, and *R. solani* with sensitivities of 1 pg/μL, 100 pg/μL, and 10 pg/μL, respectively (Lu et al. 2015a, b; Kong et al. 2020). In this study, iLAMPs-mediated detection of *P. infestans*, *F. graminearum*, and *R. solani* had a sensitivity of 10 pg/μL, 1 pg/μL, and 10 pg/μL, respectively, which has a comparable sensitivity for the detection of these pathogens. In addition, iLAMPs-mediated detection of *A. solani*, *R. solanacearum*, *P. atrosepticum*, and *S. scabies* has a sensitivity down to 100 fg/μL, and of *S. subterranea* has a sensitivity of 10 pg/μL. Overall, iLAMPs using primers designed in this study were found to be precise and sensitive for accurate detection of these eight potato pathogens.

In this study, *P. infestans* and *A. solani* were found to be the two main pathogens in diseased leaves, which is consistent with the fact that these are the two main potato pathogens and aligns with survey data showing these two pathogens are widely presented in areas where samples were collected (Zhang et al. 2012, 2017; Meno et al. 2021; Yuen 2021). In addition, the combination of *P. infestans-A. solani* was prevalently detected in diseased leaves and tubers collected from the southern and southwestern potato-growing regions. Since co-infection of *P. infestans* and *A. solani* enhances infection in potatoes (Brouwer et al. 2023), the high detection rate of *P. infestans-A. solani* suggests that measurements are needed to prevent the outbreaks of diseases caused by these two pathogens. Although it remains unknown whether combinations other than *P. infestans-A. solani* might enhance infection or not, the observation that pathogens with low detection rates also co-exist should raise the alarm to monitor the abundance of certain pathogen populations in order to prevent outbreaks of potato diseases.

Conclusions

This study developed a toolkit termed iLAMPs for simultaneous detection of eight common potato pathogens in field samples. In short, eight sets of primers showing gene- and genus-specificity were designed to effectively amplify targeted genes. In addition, iLAMPs could simultaneously, efficiently, and accurately detect multiple common pathogens. Furthermore, iLAMPs-mediated pathogen detection revealed that *P. infestans* and *A. solani* are the two main potato pathogens and these two pathogens tend to co-exist in both diseased leaves and tubers from the four main potato-growing regions of China.

Methods

Strain sources

Strains of *Streptomyces coelicolor*, *S. recifensis*, *S. graminifolii*, *S. xiangtanensis*, *S. lutosisoli*, *S. camponoticapitis*, *S. rubellomurinus*, *S. sporoverrucosus*, *S. xiangtanensis*, *S. hygroscopicus*, *Agrobacterium pusense*, *Kitasatospora acidiphila*, and *R. solanacearum* were provided by Prof. Zhong Wei (Nanjing Agricultural University). *P. atrosepticum* was provided by Prof. Jiaqin Fan (Nanjing Agricultural University), *A. solani* was provided by Prof. Qinghe Chen (Fujian Academy of Agricultural Sciences), *R. solani* was provided by Prof. Xuehong Wu (China Agricultural University), and *S. scabies* was provided by Prof. Bo Zhou (Shandong Agricultural University). Other pathogens used in this study are self-maintained in laboratory. The information relating to origin, host, and pathogen quantity used in this study are listed in Additional file 2: Table S2.

Culture conditions

P. infestans was grown on rye-sucrose plates at 18°C for 8 d in the dark. *A. solani*, *F. graminearum*, and *R. solani* were grown on potato dextrose agar (PDA) plates at 28°C for 5 d in the dark. *R. solanacearum* was grown on nutrient agar (NA) plates at 28°C for 2 d in the dark. *P. atrosepticum* was grown on luria bertani (LB) plates at 28°C for 1 d in the dark. *S. scabies* was grown on oatmeal agar plates at 28°C for 2 d in the dark. *S. subterranea* was stored on potato tubers.

To prepare pathogens for gDNA isolation, 20 mycelium plugs, which have the size around 1 mm^3, of *P. infestans* were cultured in green bean liquid medium at 18°C, 220 rpm in the dark and mycelia were collected after 5 d. Similarly, 20 mycelium plugs, which have the size around 1 mm^3, of *A. solani*, *F. graminearum*, and *R. solani* were grown in PDA liquid medium at 28°C, 220 rpm in the dark and mycelia were collected after 3 d. In addition, *R. solanacearum* was cultured in NA liquid medium at 28°C, 220 rpm for 2 d, *P. atrosepticum* was amplified in LB liquid medium at 28°C, 220 rpm for 1 d, and *S. scabies* was amplified in oatmeal liquid medium at 28°C, 220 rpm for 2 d. Moreover, *S. subterranea* was used to infect potato tubers, and reproduced *S. subterranea* was harvested from diseased tissues. Harvested mycelia and pathogens were used for further gDNA isolation.

Infected potato samples and field potato samples

To generate samples for iLAMPs under laboratory conditions, mycelium plugs of *P. infestans* were attached to detached potato leaves with 10 μL H_2O added to the gap between plugs and leaves, which were subsequently stored in an incubator with 8 h in the dark and 16 h in the light at 18°C. Samples infected by *A. solani*, *F. graminearum*, and *R. solani* were similarly treated like *P. infestans* except that the temperature was 28°C. After 5 d, diseased potato leaves infected by these four pathogens were collected. As for *R. solanacearum*, *P. atrosepticum*, and *S. scabies*, these three pathogens were grown in liquid medium. Cultured medium was used to water potato roots and diseased stems or roots were collected after 7 d. As for the biotrophic pathogen *S. subterranea*, *S. subterranea*-infected potato tubers were mixed with healthy tubers and infected tubers were collected after two months.

As for field samples for iLAMPs, diseased leaves and tubers were separately collected from the four main potato-growing regions, namely the northern, central, southwestern, and southern regions, in China. Diseased leaves and tubers were washed with H_2O for three times. Next, washed leaves and tubers were dried by papers. Subsequently, around 1 g leaf and tuber tissues with

diseased symptom were collected for further gDNA isolation.

DNA extraction

Total gDNA was isolated by DNA secure Plant Kit (Tiangen Biotech) and quantified by NanoDrop One (Thermo Fisher Scientific). As for assays determining iLAMPs sensitivity, DNA was serially diluted in a ten-times dilution using RNA-free water to a range of 100 ng/μL to 10 fg/μL. For the rest assays, gDNA of pathogens, diseased leaves and tubers was 100 ng/μL. Diluted gDNA was used for further iLAMPs and stored at − 20°C.

Selection of targeted genes and designing of primers for iLAMPs

Genus-specific genes of these eight common potato pathogens were selected, and the non-conserved regions of selected genes following alignment with their homologs were used for designing LAMP primers. Target genes were found in the literature for each pathogen including *Epi 12* (AY586284.1) of *P. infestans*, *β-tubulin* (MK388240.1) of *A. solani*, *TEF 1α* (GQ848544.1) of *F. graminearum*, ITS (DQ356412.1) of *R. solani*, 16S rRNA (AH004176.2) of *R. solanacearum*, *gyrB* (JF311589.1) of *P. atrosepticum*, *trpB* (NC013929.1) of *S. scabies*, and ITS (MT116436.1) of *S. subterranea*. In addition, homologous genes were obtained from NCBI (https://www.ncbi.nlm.nih.gov/) and aligned by Bioedit V7. Finally, non-conserved regions of targeted genes were selected for primer design using PrimerExplorer V5 program (http://primerexplorer.jp/lampv5e/index.html). For each gene, a set of four to six primers was designed with high genus-specificity and sensitivity.

Optimization of iLAMPs

Each LAMP assay was performed in a PCR tube with a 25 μL reaction mixture, which consisted of 0.8 μM FIP and BIP (Sangon Biotech), 0.1 μM F3 and B3 (Sangon Biotech), 0.1 μM LF and LB (Sangon Biotech), 2.5 μL 10 × ThermoPol buffer (New England Biolabs), 0.8 M Betaine (Sigma), 1.4 mM dNTPs (Takara Bio), 6 mM $MgSO_4$ (New England Biolabs), 3 μL H_2O, 8 U Bst DNA polymerase (New England Biolabs), and 4 μL diluted gDNA. In the absence of LF or LB, H_2O was added to the final volume of 25 μL. iLAMPs were incubated in T100 Thermal Cycler (Bio-Rad) at 62°C for 80 min. Once finished, each reaction mixture was mixed with 0.25 μL SYBR Green I (Guyu Shengwu Company) for visualization in the fume hood. Yellow-green or orange color indicates the presence or absence of targeted pathogens, respectively.

Statistical analysis

Statistical analyses for co-existence were conducted by using the chi-square calculator for 2 × 2 contingency table (https://www.socscistatistics.com/tests/chisquare/default2.aspx/).

Abbreviations

CYP51C	*Cytochrome P450 51C*
Epi 12	*Extracellular protease inhibitor 12*
FIP/BIP	Forward/backward inner primer
F3/B3	Forward/backward primer
gyrB	*Gyrase beta*
iLAMPs	Integrated-LAMP assays
ITS	Internal transcribed spacer
LAMP	Loop-mediated isothermal amplification
LB/LF	Loop backward/forward primer
NA	Nutrient agar
PDA	Potato dextrose agar
PiSMC	*P. infestans* specific multiple copy
TEF 1α	*Translation elongation factor 1α*
trpB	*Tryptophan synthase subunit beta*
16S rRNA	16S ribosomal RNA

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-024-00284-3.

Additional file 1: Figure S1. Phylogenetic tree of the eight common potato pathogens and their homolog pathogens from the same genus. **Figure S2.** Targeted regions of primers used for iLAMPs. **Figure S3.** iLAMPs with specific primers only detect their corresponding pathogens. **Figure S4.** Some pathogens tend to co-exist with specific pathogens.

Additional file 2: Table S1. Primers used in this study. **Table S2.** Oomycete, fungal, and bacterial isolates used to test the specificity of iLAMPs. **Table S3.** Location and number of collected potato samples. **Table S4.** Detection rates of indicated pathogens in diseased leaves and tubers from the four potato-growing regions. **Table S5.** Results of iLAMPs in diseased leaves. **Table S6.** Results of iLAMPs in diseased tubers. **Table S7.** Detection rates of indicated species in diseased leaves and selected samples containing different pathogens. **Table S8.** Detection rates of indicated species in diseased tubers and selected samples containing different pathogens.

Acknowledgements

We thank Philip J Kear (International Potato Center) for critical suggestions for this manuscript. We thank Prof. Zhong Wei (Nanjing Agricultural University) for providing different *Streptomyces* isolates, *A. pusense*, *K. acidiphila*, and *R. solanacearum* isolates, Prof. Jiaqin Fan (Nanjing Agricultural University) for providing *P. atrosepticum*, Prof. Qinghe Chen (Fujian Academy of Agricultural Sciences) for providing *A. solani*, Prof. Xuehong Wu (China Agricultural University) for providing *R. solani*, Prof. Bo Zhou (Shandong Agricultural University) for providing *S. scabies*, and Fan Lu (Nanjing Agricultural University) for providing different bacteria isolates.

Author contributions

SD, HC, and XZ designed the project; XZ, JW, XW, SW, and JC performed the experiment; HC, QY, and JZ collected filed samples; XZ, JW, and HC wrote the manuscript; All authors have read and approved the final manuscript.

Funding

This research was supported by grants from the National Key Research and Development Program (2022YFD1100202, 2022YFD1400400) and earmarked funds from the China Agriculture Research System-potato (CARS-09-P20).

Availability of data and materials

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

Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China. [2]The Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education, Nanjing Agricultural University, Nanjing 210095, China. [3]International Potato Center (CIP), CIP China Center Asia Pacific, Beijing 100081, China. [4]National Agro-Tech Extension and Service Center, Beijing 100125, China.

Received: 6 June 2024 Accepted: 1 September 2024
Published online: 10 December 2024

References

Abdoulaye AH, Foda MF, Kotta-Loizou I. Viruses infecting the plant pathogenic fungus *Rhizoctonia solani*. Viruses. 2019;11:1113.
Andika IB, Wei S, Cao CM, Salaipeth L, Kondo H, Sun LY. Phytopathogenic fungus hosts a plant virus: a naturally occurring cross-kingdom viral infection. Proc Natl Acad Sci USA. 2017;114:12267–72.
Begum MM, Sariah M, Puteh AB, Abidin MAZ, Rahman MA, Siddiqui Y. Field performance of bio-primed seeds to suppress colletotrichum truncatum causing damping-off and seedling stand of soybean. Biol Control. 2010;53:18–23.
Brouwer SM, Wolters PJ, Andreasson E, Liljeroth E, Vleeshouwers VGAA, Grenville-Briggs LJ. Double trouble: co-infection of potato with the causal agents of late and early blight. Plant Pathol. 2023;72:1365–79.
Bulman SR, Marshall JW. Detection of *Spongospora subterranea* in potato tuber lesions using the polymerase chain reaction (PCR). Plant Pathol. 1998;47:759–66.
Caipang CMA, Haraguchi I, Ohira T, Hirono I, Aoki T. Rapid detection of a fish iridovirus using loop-mediated isothermal amplification (LAMP). J Virol Methods. 2004;121:155–61.
Chen YJ, Lin YS, Chung WH. Bacterial wilt of sweet potato caused by *Ralstonia solanacearum* in Taiwan. J Gen Plant Pathol. 2012;78:80–4.
Chen JS, Ma EB, Harrington LB, Da Costa M, Tian XR, Palefsky JM, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science. 2018;360:436–9.
Cunniffe NJ, Koskella B, Metcalf CJE, Parnell S, Gottwald TR, Gilligan CA. Thirteen challenges in modelling plant diseases. Epidemics. 2015;10:6–10.
Daher RK, Stewart G, Boissinot M, Bergeron MG. Recombinase polymerase amplification for diagnostic applications. Clin Chem. 2016;62:947–58.
FAOSTAT. Food and agriculture organization of the united nations. http://www.fao.org/faostat/en/#data/QC.; 2020.
Feng H, Ye WW, Liu ZY, Wang Y, Chen JJ, Wang YC, et al. Development of LAMP assays using a novel target gene for specific detection of *Pythium terrestris*, *Pythium spinosum*, and '*Candidatus Pythium huanghuaiense*'. Plant Dis. 2021;105:2880–9.
Fry WE, Birch PRJ, Judelson HS, Grunwald NJ, Danies G, Everts KL, et al. Five reasons to consider *Phytophthora infestans* a reemerging pathogen. Phytopathology. 2015;105:966–81.
Gau RD, Merz U, Falloon RE, Brunner PC. Global genetics and invasion history of the potato powdery scab pathogen, *Spongospora subterranea f.sp subterranea*. PLoS ONE. 2013;8:e67944.
Goswami RS, Kistler HC. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol Plant Pathol. 2004;5:515–25.
Green MR, Sambrook J. The basic polymerase chain reaction (PCR). Cold Spring Harb Protoc. 2018;5:338–45.
Green MR, Sambrook J. Nested polymerase chain reaction (PCR). Cold Spring Harb Protoc. 2019;2:175–9.
Hansen ZR, Knaus BJ, Tabima JF, Press CM, Judelson HS, Grunwald NJ, et al. Loop-mediated isothermal amplification for detection of the tomato and potato late blight pathogen, *Phytophthora infestans*. J Appl Microbiol. 2016;120:1010–20.
Hill J, Lazarovits G. A mail survey of growers to estimate potato common scab prevalence and economic loss in Canada. Can J Plant Pathol. 2005;27:46–52.
Horne AS. Potato diseases. Ann Appl Biol. 2010;1:183–203.
Hu LX, Yang ZH, Zhang D, Zhao DM, Zhu JH. Sensitive and rapid detection of *Pectobacterium atrosepticum* by targeting the *gyrB* gene using a real-time loop-mediated isothermal amplification assay. Lett Appl Microbiol. 2016;63:289–96.
Huang W, Zhang H, Xu JS, Wang S, Kong XJ, Ding W, et al. Loop-mediated isothermal amplification method for the rapid detection of *Ralstonia solanacearum* phylotype I mulberry strains in China. Front Plant Sci. 2017;8:325.
Jansky SH, Jin LP, Xie KY, Xie CH, Spooner DM. Potato production and breeding in China. Potato Res. 2009;52:57–65.
Jiang HB, Wu N, Jin SM, Ahmed T, Wang H, Li B, et al. Identification of rice seed-derived *Fusarium* spp. and development of LAMP assay against *Fusarium fujikuroi*. Pathogens. 2021;10:1.
Jurado M, Vazquez C, Patino B, Gonzalez-Jaen MT. PCR detection assays for the trichothecene-producing species *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium poae*, *Fusarium equiseti* and *Fusarium sporotrichioides*. Syst Appl Microbiol. 2005;28:562–8.
Khan MR, Li BJ, Jiang Y, Weng QY, Chen QH. Evaluation of different PCR-based assays and LAMP method for rapid detection of *Phytophthora infestans* by targeting the *Ypt1* Gene. Front Microbiol. 2017;8:1920.
Khan MR, Wang RB, Li BJ, Liu PQ, Weng QY, Chen QH. Comparative evaluation of the LAMP assay and PCR-based assays for the rapid detection of *Altemaria solani*. Front Microbiol. 2018;9:2089.
Kong L, Wang HB, Wang SS, Xu PP, Zhang RF, Dong SM, et al. Rapid detection of potato late blight using a loop-mediated isothermal amplification assay. J Integr Agric. 2020;19:1274–82.
Kumar S, Singh R, Kashyap PL, Srivastava AK. Rapid detection and quantification of *Alternaria solani* in tomato. Sci Hortic. 2013;151:184–9.
Lees AK, Roberts DM, Lynott J, Sullivan L, Brierley JL. Real-time PCR and LAMP Assays for the detection of spores of *Alternaria solani* and sporangia of *Phytophthora infestans* to inform disease risk forecasting. Plant Dis. 2019;103:3172–80.
Lenarcic R, Morisset D, Pirc M, Llop P, Ravnikar M, Dreo T. Loop-mediated isothermal amplification of specific endoglucanase gene sequence for detection of the bacterial wilt pathogen *Ralstonia solanacearum*. PLoS ONE. 2014;9:e96027.
Lu CC, Song B, Zhang HF, Wang YC, Zheng XB. Rapid diagnosis of soybean seedling blight caused by *Rhizoctonia solani* and soybean charcoal rot caused by *Macrophomina phaseolina* using LAMP assays. Phytopathology. 2015a;105:1612–7.
Lu CC, Zhang H, Wang Y, Zheng X. Rapid diagnosis of *Fusarium* root rot in soybean caused by *Fusarium equiseti* or *Fusarium graminearum* using loop-mediated isothermal amplification (LAMP) assays. Australas Plant Path. 2015b;44:437–43.
Mansfield J, Genin S, Magori S, Citovsky V, Sriariyanum M, Ronald P, et al. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol. 2012;13:614–29.
Meno L, Escuredo O, Rodriguez-Flores MS, Seijo MC. Looking for a sustainable potato crop. Field assessment of early blight management. Agric Forest Meteorol. 2021;308:108617.
Merz U. Powdery scab of potato-occurrence, life cycle and epidemiology. Am J Potato Res. 2008;85:241–6.
Nagamine K, Hase T, Notomi T. Accelerated reaction by loop-mediated isothermal amplification using loop primers. Mol Cell Probe. 2002;16:223–9.
Naumann M, Koch M, Thiel H, Gransee A, Pawelzik E. The importance of nutrient management for potato production part II: plant nutrition and tuber quality. Potato Res. 2020;63:121–37.
Niepold F, SchoberButin B. Application of the PCR technique to detect *Phytophthora infestans* in potato tubers and leaves. Microbiol Res. 1995;150:379–85.
Niessen L, Vogel RF. Detection of *Fusarium graminearum* DNA using a loop-mediated isothermal amplification (LAMP) assay. Int J Food Microbiol. 2010;140:183–91.

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. Loop-mediated isothermal amplification of DNA. Nucl Acids Res. 2000;28:e63.
Okubara PA, Schroeder KL, Paulitz TC. Identification and quantification of *Rhizoctonia solani* and *R. oryzae* using real-time polymerase chain reaction. Phytopathology. 2008;98:837–47.
Pan W, Wang JY, Shen HY, Zhao MQ, Ju CM, Dong XY, et al. Development and application of the novel visual loop-mediated isothermal amplification of *Omp25* sequence for rapid detection of *Brucella* sp. J Anim Vet Adv. 2011;10:2120–6.
Pastrik KH, Maiss E. Detection of *Ralstonia solanacearum* in potato tubers by polymerase chain reaction. J Phytopathol. 2000;148:619–26.
Pavon MA, Gonzalez I, Pegels N, Martin R, Garcia T. PCR detection and identification of *Alternaria* species-groups in processed foods based on the genetic marker *Alt a1*. Food Control. 2010;21:1745–56.
Perez-Sierra A, Jung MH, Jung T. Survey and monitoring of *Phytophthora* species in natural ecosystems: Methods for sampling, isolation, purification, storage, and pathogenicity tests. Methods Mol Biol. 2022;2536:13–49.
Piepenburg O, Williams CH, Stemple DL, Armes NA. DNA detection using recombination proteins. PLoS Biol. 2006;4:1115–21.
Sankaran S, Mishra A, Ehsani R, Davis C. A review of advanced techniques for detecting plant diseases. Comput Electron Agr. 2010;72:1–13.
Shu PY, Chang SF, Kuo YC, Yueh YY, Chien LJ, Sue CL, et al. Development of group- and serotype-specific one-step SYBR green I-based real-time reverse transcription-PCR assay for dengue virus. J Clin Microbiol. 2003;41:2408–16.
Smith CJ, Osborn AM. Advantages and limitations of quantitative PCR (qPCR) based approaches in microbial ecology. Fems Microbiol Ecol. 2009;67:6–20.
Susi H, Barrès B, Vale PF, Laine AL. Co-infection alters population dynamics of infectious disease. Nat Commun. 2015;6:5975.
Tameh MH, Primiceri E, Chiriaco MS, Poltronieri P, Bahar M, Maruccio G. *Pectobacterium atrosepticum* biosensor for monitoring blackleg and soft rot disease of potato. Biosensors. 2020;10:64.
Tiwari RK, Kumar R, Sharma S, Sagar V, Aggarwal R, Naga KC, et al. Potato dry rot disease: current status, pathogenomics and management. 3 Biotech. 2020;10:503.
Verma G, Sharma S, Raigond B, Pathania S, Naga K, Chakrabarti SK. Development and application of fluorescent loop mediated isothermal amplification technique to detect *Phytophthora infestans* from potato tubers targeting ITS-I region. 3 Biotech. 2019;9:345.
Wang AX, Lazarovits G. Role of seed tubers in the spread of plant pathogenic *Streptomyces* and initiating potato common scab disease. Am J Potato Res. 2005;82:221–30.
Wang SC, Zhang XY, Zhang ZC, Chen Y, Tian Q, Zeng DD, et al. *Fusarium*-produced vitamin B_6 promotes the evasion of soybean resistance by *Phytophthora sojae*. J Integr Plant Biol. 2023;65:2204–17.
Xia Y, Fei BH, He JY, Zhou ML, Zhang DH, Pan LX, et al. Transcriptome analysis reveals the host selection fitness mechanisms of the *Rhizoctonia solani* AG1IA pathogen. Sci Rep. 2017;7:10120.
Xu R, Falardeau J, Avis TJ, Tambong JT. HybProbes-based real-time PCR assay for specific identification of *Streptomyces scabies* and *Streptomyces europaeiscabiei*, the potato common scab pathogens. Lett Appl Microbiol. 2016;62:153–9.
Xue WY, Haynes KG, Qu XS. Characterization of early blight resistance in potato cultivars. Plant Dis. 2019;103:629–37.
Yadav R, Pathak SP. Variability among *Alternaria solani* isolates causing early blight of potato. Plant Arch. 2011;11:953–6.
Ye WW, Zeng DD, Xu M, Yang J, Ma JX, Wang YC, et al. A LAMP-assay-based specific microbiota analysis reveals community dynamics and potential interactions of 13 major soybean root pathogens. J Integr Agr. 2020;19:2056–63.
Yuen J. Pathogens which threaten food security: *Phytophthora infestans*, the potato late blight pathogen. Food Secur. 2021;13:247–53.
Zhang J, Cheng Y, Gong X, Si HA, Sun Q, Miru DU, et al. Occurrence, distribution and damage of diseases and pests in one-crop potato region of North China. Chin Potato J. 2012;26:30–5 (**in Chinese**).
Zhang K, Wang XZ, Sun J, Yang Q, Sun HT. Identification of pathogens from rotted potato tubers in ventilated storage house of huade county, Inner Mongolia during the storage period. Agric Biotech. 2017;5:63–7.