A rapid and efficient method to evaluate the infection of major DNA viruses in sweet potato seedlings and tubers

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Methodology

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Abstract

Background

Sweet potato is an important food crop in China which is the fifth largest staple crop next to rice, wheat, maize, and soybean. Recent years the destroy infecting by DNA viruses in sweet potato was more and more serious. Three DNA viruses (sweepoviruses, *Badnavirus* and *Mastrevirus*) are major agents in viral diseases of sweet potato in China. It is necessary to establish a rapid and efficient method to evaluate the health of sweet potato seedlings and tubers, which will greatly affect the yield, quality of tubers and seeding circulation of sweet potatoes. Sweepoviruses were a group phylogenetically distinct from other begomoviruses that infects plants of the family *Convolvulaceae* including sweet potato. Sweet potato symptomless virus 1 (SPSMV-1) is the only species of *Mastrevirus* which can infect sweet potato. *Sweet potato Badnavirus B* (SPBV-B) are non-enveloped bacilliform DNA viruses with a monopartite genome which belonged to the genus *Badnavirus* of the family *Caulimoviridae* and was first found in sweet potato in 2009. As the mixed infection of these viruses is very common, rapid detection is required for etiologic diagnosis.

Results

In this study, a rapid and efficient method to evaluate the infection of major DNA viruses (sweepoviruses, *Badnavirus* and *Mastrevirus*) in sweet potato seedlings and tubers was developed and applied. A mixture of three pairs of primers was used for amplification of viral nucleic acids, yielding three different amplicons with sizes of 750 bp, 147 bp and 396 bp for sweepoviruses, *Badnavirus* and *Mastrevirus* respectively. The specificity and sensitivity of multiplex PCR were also evaluated. A total of 65 sweet potato samples with virus-like symptoms cuttings and storage roots from Henan province in China were collected between June 2019 and July 2021. They were tested for the presence of three DNA viruses by multiplex PCR which showed sweepoviruses, *Badnavirus* and *Mastrevirus* infections were 60.0%, 36.7%, and 43.3% respectively. Co-infections with three viruses, sweepoviruses and *Badnavirus*, sweepoviruses and *Mastrevirus*, *Badnavirus* and *Mastrevirus* were identified in different samples (the detection ratio of co-infections was 13.3%, 20.0%, 13.3%, and 10.0% respectively).

Conclusions

In the current situation of frequent virus mixed infection in sweet potatoes, this method is the first report on a simple assay and may be a potentially useful for a rapid and efficient method to evaluate the infection of major DNA viruses in sweet potato seedlings and tubers in China.

Background

Sweet potato, *Ipomoea batatas* (L.) Lam. (family *Convolvulaceae*), is an important food crop, is ranked third among all crops worldwide, and its calorific contribution to the human diet is ranked fifth globally [1]. Most sweet potatoes are planted in some countries of eastern Asia, and 80% of which are produced in China [2], the largest producer of sweet potatoes in the world [3]. In China, sweet potato is the fifth largest staple crop next to rice, wheat, maize, and soybean [4]. The planting area for sweet potato in China had reached 2.38×10^6 ha. and yield of 5.32×10^7 tons in 2018 [5]. Sweet potato was extensively cultivated in sixteen provinces or municipalities of China with

over 1.0×10^4 hm² which include Henan province [6, 7]. Henan province was a major production area in North China which had about 4×10^5 hectares every year.

Recent years the destroy infecting by DNA viruses in sweet potato was more and more serious. Geminiviruses cause destructive diseases in many crops worldwide recent years and seriously threaten crop production. *Begomovirus* and *Mastrevirus* of family *Geminiviridae* all can infect sweet potato plants [1]. The begomoviruses further showed a clear subdivision into four groups: New World, Old World, legumoviruses and sweepoviruses [8, 9]. Sweepoviruses were a group phylogenetically distinct from other begomoviruses that infects plants of the family *Convolvulaceae* including sweet potato [8]. Only one species of *Mastrevirus* (sweetpotato symptomless virus 1, SPSMV-1) had been reported in Peru, Tanzania, several central American countries and China [1, 10–13]. Sweet potato Badnavirus B (SPBV-B) are non-enveloped bacilliform DNA viruses with a monopartite genome containing about 7.2 to 9.2 kb of dsDNA which belonged to the genus *Badnavirus* of the family *Caulimoviridae*. SPBV-B was first found in sweet potato (*Ipomoea batatas*) plants in 2009[10].

Detection for DNA viruses was different from that for RNA viruses. As SPBV-B, SPSMV-1 and sweepoviruses compound infections were often with no specific symptoms observed, and the antibody detection had not been making and used as the diagnostic assay for the compound infections, polymerase chain reaction (PCR) became a better detection method which had the advantages of high sensitivity and high specificity. Current routine PCR assays require three separate primer sets and three separate reactions to differentiate the three viruses. In contrast, multiplex PCR made it possible to amplify multiple target sequences in a single reaction tube by using multiple primer pairs. The advantage of a multiplex PCR for the simultaneous detection and differentiation among SPBV-B, SPSMV-1 and sweepoviruses was that it combines the sensitivity and rapidity of PCR and avoids the need to test sweet potato samples separately for each virus. This paper first described the development of a multiplex PCR assay for the simultaneous detection and differentiation of SPBV-B, SPSMV-1 and sweepoviruses in sweet potato seedlings and tubers collected from farms, seedbeds and germplasm repository.

Results

Cloning of PCR amplified Sweepoviruses, SPSMV-1, and SPBV-B genes

Viral DNA extracted from the sweet potato samples was used as the template for PCR. The amplified PCR products were then analyzed by 1% agarose gel electrophoresis. The sweepoviruses coat protein regions, SPBV-B partial movement and coat protein genes, and movement protein regions of SPSMV-1 were 750 bp, 147 bp, and 396 bp respectively (Fig. 1A-a). These three target fragments were cloned into the pGEM-T Easy Vector, and the resulting plasmids were used as standardized positive-control plasmids containing cloned viral gene. The positive plants infected by different virus were determined.

The multiplex PCR was suitable for detection three viruses

To determine whether the multiplex PCR were suitable for detection of the three viruses, the positive plasmids were used as templates in multiplex PCR under the same conditions used for sweet potato plants samples. When multiplex PCR to detect the positive controls containing one virus was performed, the reactions yielded the expected size (Fig. 1A-a, Line 1–3). For the detection of two and three viruses in plasmids and DNA of sweet

potato plants samples, multiplex PCR amplifications were carried out with size-distinguishable standardized positive-controls. Positive samples of two viruses (SPSMV-1 and SPBV-B) were detected as shown by the 396 bp and the 147 bp (Fig. 1A-b). The 750 bp and the 147 bp amplified band were detected in positive plasmids and sweet potato plants containing sweepovirus and SPBV-B (Fig. 1A-c). The positive samples harboring three viruses were detected as shown by the 750 bp, 396 bp and 147 bp amplified band (Fig. 1A-d). Negative control was not detected. The minimum number of copies of SPLCV, SPBV-B and SPSMV-1 were 1.02×10^6 copies/uL, 1.57×10^4 copies/uL and 2.54×10^3 copies/uL, which was a sensitive detection method.

Detection of three viruses in sweet potato samples collected from the farms

Total thirty positive samples were detected from 65 sweet potato samples, which including 22 sweet potato cuttings and 8 sweet potato storage roots (Table 2). These sweet potato samples varied in variety (Table 2). Among the thirty positive samples, eighteen sweet potato samples were found to be SPLCV (a species of sweepoviruses) positive (18/30, 60.0%) (Table 2, Fig. 2). Eleven sweetpotato samples were found to be SPBV-B positive (11/30, 36.7%) (Table 2). Thirteen SPSMV-1-positive samples were found from three farms of the north area of Henan province (13/30, 43.3%) (Table 2). SPBV-B was not detected in any sweet potato storage roots. All samples with leaf curl symptoms were SPLCV positive (Table 2, Fig. 2A). Many samples showed the compound infection of two (12/30, 40.0%) or three viruses (4/30, 13.3%). The symptoms of sweet potato infected by the mixed viruses were diverse and irregular (Table 2, Fig. 2B).

Table 1

Collection information and detection results of 30 positive samples from 65 samples for testing in this study

Collecting sites(City)	Variety of sweet potato	Sample types	Virus	Symptoms
Huixian	Zhenghong22	Cuttings	SPLCV; SPBV; SPSMV-1	Leaf curl; leaf shriveling
		Storage roots	SPLCV; SPSMV-1	Asymptomatic
	Hongxiangjiao	Cuttings	SPLCV; SPBV; SPSMV-1	Leaf curl; vein enation
		Storage roots	SPSMV-1	Asymptomatic
Fengqiu	Shangshu19	Cuttings	SPLCV; SPBV;	Leaf curl;
		Storage roots	SPSMV-1	Asymptomatic
	Xinxiang	Cuttings	SPBV; SPSMV-1	Fan-shaped leaves; vein enation
		Cuttings	SPLCV; SPBV	Leaf curl
Nanyang	Longshu9	Cuttings	SPSMV-1	Fan-shaped leaves
	Yushu12	Cuttings	SPSMV-1; SPBV;	Mosaic
	Sushu	Cuttings	SPLCV; SPBV	Leaf curl;
Anyang	Longshu9	Cuttings	SPBV;	Leaf curl; vein enation
	Jishu4	Cuttings	SPBV; SPSMV-1	Stunting; vein yellowing
	Shangshu19	Cuttings	SPLCV; SPBV	Leaf curl
	Ziluolan	Cuttings	SPSMV-1	Chlorosis; stunting
Kaifeng	Yushu12	Cuttings	SPLCV; SPSMV-1	Leaf malformation
	Shangshu19	Cuttings	SPLCV; SPBV;	Leaf curl; vein yellowing
	Longshu9	Cuttings	SPSMV-1	Leaf curl; Fan-shaped leaves
	Jishu26	Cuttings	SPLCV	Leaf curl
Shangqiu	Shangshu19	Cuttings	SPLCV; SPSMV-1	Leaf curl
	Yushu12	Cuttings	SPLCV	Leaf curl;
	Xinxiang	Cuttings	SPLCV; SPSMV-1; SPBV	Stunting; Chlorosis
Luoyang	Yushu8	Cuttings	SPLCV; SPBV	Leaf curl;
		Storage roots	SPLCV	Asymptomatic

Full name of virus species and abbreviations: *sweet potato leaf curl virus* (SPLCV, a common species of sweepovirus); sweet potato Badnavirus B(SPBV-B); sweet potato symptomless virus 1(SPSMV-1)

Collecting sites(City)	Variety of sweet potato	Sample types	Virus	Symptoms
	Longshu9	Cuttings	SPSMV-1	Chlorosis; stunting
		Storage roots	SPSMV-1	Asymptomatic
	Xinxiang	Cuttings	SPLCV; SPSMV-1; SPBV	Leaf curl; vein yellowing
		Storage roots	SPLCV	Asymptomatic
Xuchang	Xushu18	Storage roots	SPLCV	malformation
	Yanshu25	Storage roots	SPSMV-1	Surface uneven
Full name of virus species and abbreviations: <i>sweet potato leaf curl virus</i> (SPLCV, a common species of sweepovirus); sweet potato Badnavirus B (SPBV-B); sweet potato symptomless virus 1 (SPSMV-1)				

Table 2
Primers used to detect three genus of DNA viruses infecting sweet potato in China

Primer name	Primer sequence (5'-3')	Size of PCR products (bp)	Primer universality	Virus (Genus)
MA292 MA293	CCY TAG GGT TCG AGC TVT GTT CGG TTT ATT AAT TDT TRT GCG AAT C	750	Sweepovirus (CP gene region)	Begomovirus
rt-badB- F rt- badB-R	TCGGCAGTAACAGACTACTTGG TCTGCTTATCATCTCCGTTGG	147	Sweet potato Badnavirus B	Badnavirus;
MastvKF MastvsR	GACAGACCCCTAGGGTGA ACTGCATATAGTACATGCCACA	396	Sweet potato symptomless virus 1	<i>Mastrevirus</i>
Y = C, T; V = A, C, G; D = A, G, T; R = A or G				

Discussion

DNA viruses infecting sweet potato were detected successively in recent years which caused more attentions in China. In this study, sweepoviruses had the most detection frequency in the sweet potato plants collected from farms of Henan province which were all identified as SPLCV species. Thus, it can be seen that SPLCV was a dominant species of sweepoviruses in sweet potato planting area of Henan province which was similar to the situation all over the country in China[14]. The frequency of SPSMV-1 and SPBV-B were all more than 35% among the positive samples and often mixed infected with SPLCV in a same sweet potato plant.

As the mixed infection of the DNA viruses was found frequently in sweet potato seedlings, plants and storage roots, strict quarantine based on fast and reliable detection method in sweet potato transport was very important. Multiplex PCR is an efficient detection method which has been reported widely in many research fields [15–17]. This method had been used appropriately for the simultaneous detection of the above three viruses although it was not considered novel. Multiplex PCR was an efficient and convenient detection method which only need to extract DNA of sweet potato samples and amplified in a proper reaction system. The selection of primers and their

proportion used in the multiplex PCR system were the key in detecting virus genes. Different proportion of primers were compared and the optimum ratio 4:3:3 of three pairs of primers (MA292 and MA293, rt-badB-F and rt-badB-R, MastvKF and MastvKR) was identified finally. The expected bands 750 bp, 396bp and 147 bp were specific and size-distinguishable in this identified multiplex PCR system.

Among the symptoms of positive sweet potato samples infected by SPLCV, SPSMV-1, SPBV-B, and mixed infection of two or three viruses, leaf curl was the most common symptom (Table 2). Liu et al. reported that leaf curl symptom may be an important diagnosis basis for sweet potato infected by sweepoviruses [14]. We can infer those symptoms caused by SPLCV maybe masked those of the other two viruses. The symptoms can not reflect the necessary connection among different virus infections. Most storage roots infected by DNA viruses showed asymptomatic which remind us the importance of testing viruses in the trade and transport of sweet potato storage roots.

Conclusion

This method may be a potentially useful for rapid, sensitive, and cost-effective etiological diagnostic tool for compound infection of three frequent DNA viruses infecting sweet potato.

Methods

Viral DNA preparation and single PCR reaction

Sweet potato cuttings with serious virus disease were obtained from different farms and seedbed. The varieties of sweet potato were Shangshu19, Xushu22 and Xinxiang respectively. DNA was extracted from these sweet potato cuttings using the universal genomic DNA extraction kit (TaKaRa, China) following the protocol of the manufacturer. These DNA extractions were frozen at -80°C until analysis.

Single PCR reactions were conducted using three specific primer sets for PCR assay to amplify the three DNA viruses respectively: sweepovirus, SPBV-B and SPSMV-1 (Table 2). The primers MA292 and MA293, located in the CP region, were used to detect highly specific for sweepovirus [8]. The primers rt-badB-F and rt-badB-R (Table 2), corresponding to movement and coat protein genes, were used for detecting SPBV-B [12]. The primers MastvKF and MastvsR (Table 2), located in the V1 gene region, were used to amplify movement protein of SPSMV-1[11]. All of the amplified PCR products were ligated to pMD19-T vector (TaKaRa, China). The ligated product was transformed into Escherichia coli (DH5α) competent cells, and the positive clones were sent to Sangon Biotech (Shanghai, China) for sequencing.

Multiplex PCR reaction detecting three viruses

Each multiplex PCR mixture contained 100ng/uL DNA template which was extracted from the prepared sweet potato plants infected by one, two, and three viruses, 0.4 mmol/L each of sweepovirus primers (MA292 and MA293), 0.3 mmol/L each of SPBV-B primers (rt-badB-F and rt-badB-R), 0.3mmol/L each of SPSMV-1 primers (Mastv KF and Mastv KR), 0.2 mmol/L each of dNTP, 10×PCR buffer (Takara, China), 1.0 mmol/L MgCl₂ and 5 U of Taq DNA Polymerase (Takara, China). The following thermocycling profile was used for three virus multiplex PCR: 94°C for 3 m (1 cycle); 94°C for 45 s, 51°C for 50 s, 72°C for 50 s (35 cycles), and then 72°C thereafter. PCR products were separated in 1% agarose gels, stained with ethidium bromide and visualized by ultraviolet light.

To assess the specificity of the multiplex PCR assay, recombinant plasmids of three virus partial genome sequence were used as templates to amplified respectively under the conditions described above. Various amounts of recombinant plasmid DNAs (105 ~ 101 copies) of the virus were used as templates to determine the sensitivity of the PCR assay. To evaluate the efficiency of detecting co-infection with three viruses, serial dilutions (105 ~ 101 copies) of every virus were co-amplified with a fixed copy number (104 copies) of the other viral DNA samples. To determine the sensitivity of the multiplex PCR assay, various amounts of three recombinant plasmids were prepared.

Application to sweet potato samples collected from farms, seedbeds and germplasm repository

To determine the extent of the above sweet potato viral infections in Henan province, a total of 65 sweet potato samples including forty seedlings with virus-like symptoms and twenty-five storage roots were obtained randomly from the farms of twenty different cities in Henan province of China. The multiplex PCR assay was used to simultaneously detect the three viruses. The expected PCR products (750-, 147-bp and 396 fragments) were confirmed by sequencing.

Abbreviations

SPSMV-1

sweetpotato symptomless virus 1

SPBV-B

sweet potato Badnavirus B

SPLCV

sweet potato leaf curl virus

Declarations

-Ethics approval and consent to participate

The research project and this study have been approved by an ethics committee which found them conform to all national and international guidelines for conservation of endangered species. The plant materials (sweet potato seedlings and tubers collecting from different regions) used are common varieties which are not endangered.

-Consent for publication

Not applicable.

-Availability of data and material

All data generated or analyzed during this study are included in this published article and its supplementary information files. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

-Competing interests

The authors declare that they have no competing interests.

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-Authors' contributions

QL designed and performed the research and wrote the paper; F and PW performed the research; YQ revised the manuscript critically. All authors read and approved the final manuscript.

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Figures

Figure 1

Specificity and sensitivity of multiplex PCR for detection of three DNA viruses infecting sweet potato M^{DL}2000 DNA marker

A: Specificity and sensitivity of multiplex PCR A-a: Lane 1: Sweet potato Badnavirus B; Lane 2: Sweet potato symptomless virus 1; Lane 3: Sweepoviruses infecting sweet potato; Lane 4 Mixed plasmids of three DNA viruses; A-b: Lane 1-4: Mixed plasmids of Sweet potato Badnavirus B and Sweet potato symptomless virus 1; A-c: Lane 1: Mixed plasmids of sweepoviruses and Sweet potato Badnavirus B; A-d: Lane 1-3: Mixed plasmids of three DNA viruses. B: Sensitivity of multiplex PCR Lane 1-6: 108¹107¹106¹105¹104¹103 copies/ μ L

A

B

Figure 2

Symptoms on sweet potato samples and multiplex PCR results for detection of three DNA viruses A: Symptoms on sweet potato samples a and b: leaf curl; c: vein enation; d: leaf malformation and chlorosis; e: fan-shaped leaves; f: chlorotic spots mosaic and leaf malformation; g: stunting and vein yellowing; h: fan-shaped leaves; i: surface uneven; j: asymptomatic B: Multiplex PCR for detection of three DNA viruses from different samples a-d: Viruses detected in the multiplex PCR using different sweet potato DNA samples (repeat 2-3 times per sample).