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PRINT CAPTURE RT-PCR TO DETECT GROUNDNUT BUD NECROSIS VIRUS CAUSE OF POTATO STEM NECROSIS DISEASE

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ABSTRACT: Potato stem necrosis disease (PSND) is a problem in early planted potato crop, caused by Groundnut bud necrosis virus (GBNV) vectored by thrips. Diagnosis of GBNV in leaf samples and thrips tissues was carried out using a simple technique *i.e.* print capture reverse transcriptase polymerase chain reaction (PC-RT-PCR). This technique involves spotting of samples onto NCMs, eluting viral RNA from the NCM in sterile distilled water (20 µl) at 95°C for 10 min, cDNA synthesis followed by PCR amplification and analysis of the PCR product on 1.6% agarose gel. GBNV was detected in the leaf samples and thrips tissues which amplify at 800 bp in the first planted crop while, samples from later planted potato crop did not correspond to the results like the first crop.

INTRODUCTION

Potato stem necrosis disease (PSND) is a serious problem in the early planted potato crop in western (Rajasthan and Gujrat) and central (Madhya Pradesh) parts of India including plateau regions, when high ambient temperatures (30-35°C) prevail (8). The disease is systemic and is characterized by stem/petiole necrosis, foliar spotting/deformation/necrosis and stunting of the plant that results in heavy losses in early planted potatoes (7). A tospovirus was reported as the causal agent of PSND in India in 1989 (6) and its serological relationship with serogroup IV tospoviruses was reported by Jain *et al.* (1, 2, 5). Subsequently, the virus was identified as a strain of Groundnut bud necrosis virus (GBNV) in the genus Tospovirus (4). GBNV was first reported in groundnut in 1968 by Reddy *et al.* (12) and is vectored by the thrips (*Thrips palmi*) in circulative and propagative manner but is not transmitted through potato tubers (8). High temperatures (30-35°C) and dry weather during September-October favour thrips activity to the maximum extent and thus potentially cause higher incidence of the disease (PSND) (13).

A simple diagnostic technique is important for the detection of GBNV in infected plant samples and thrips tissues. Serological and molecular methods such as enzyme linked immunosorbent assay (ELISA), nucleic acid spot hybridization (NASH) and polymerase chain reaction (PCR) are in use for the detection of different virus(es) (10). Additionally, a simple technique namely 'print capture PCR' was first described by Olmos *et al.* (11) using virus specific IgG to capture Plum pox potyvirus on a nitrocellulose membrane and improved by Nagata *et al.* (9), without the need for trapping using IgG. It offers the possibility of cost effective sample processing. Unlike the costly RNA extraction technique involved in the simple PCR method, it employs elution of RNA directly from the membrane followed by PCR technique as usual.

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The present study was conducted to demonstrate the implementation and validation of 'print capture reverse transcription PCR' (PC-RT-PCR) for the diagnosis of GBNV in infected potato leaf samples and vector thrips tissues.

MATERIALS AND METHODS

Print capture and isolation of viral RNA

Squares (11 cm) were made on nitro-cellulose membrane (NCM) (Hybond N+, Pharmacia biotech, USA) and spotted with different lots of leaf samples and thrips tissues, separately at Central Potato Research Station, Gwalior. These samples/tissues were collected for spotting from the potato crops planted at 5 different planting date (5 × 5 October, 15 October, 25 october, 4 November and 14 November) at 70 days after planting. The NCMs were dried at 37°C for 1 h and cut into fine pieces and transferred to 1.5 ml Eppendorf tubes containing 20 µl of sterile distilled water followed by heating at 95°C for 10 min to elute the viral RNA. The eluted viral RNA from infected leaf and thrip tissue were used for cDNA synthesis followed by PCR amplification.

cDNA synthesis

The cDNA synthesis was carried out by reverse transcription using Revert AidTM HMinus First Strand cDNA synthensis kit (Fermentas, Burlington, Canada). The reaction mix for cDNA synthesis was prepared using (5X RT Buffer (0.5 M Tris-HCl pH 8.3, 0.75 M KCl, 0.03 M $MgCl_2$)- 4 µl, 10 mM dNTP mix- 2 µl, random primers- 2 µl, RNAsin- 1 µl, Reverse Transcriptase- 1 µl and viral RNA template- 10 µl followed by incubation at 37°C for 1 h. Subsequently the reaction was stopped at 95°C for 15 min and chilled on ice for 5 min.

PCR amplification

PCR was performed using a reaction mixture of 10X PCR buffer (100 mM Tris HCl, 500 mM KCl, 15 mM $MgCl_2$, 0.01% w/v gelatin)- 5 µl, dNTP mix (10 mM)- 1 µl, primer pair upstream (5'-ATGTCTAACGT(C/T) AAGCA (A/G)CTC-3') and downstream (5'-TTACAATTCCAGCGAAGGACC-3') 2 µl (200 ng) each, Sure-Taq DNA polymerase (Bioenzyme)- 5 U, cDNA- 5 µl and distilled water- 34 µl. Amplification was performed in a thermal cycler (Mastercycler gradient, Eppendorf, Hamburg, Germany) programmed at one cycle of initial denaturation for 5 min at 94°C followed by 35 cycles of amplification with 1 min of denaturation at 94°C, 1 min of annealing at 56°C and 1 min of extension at 72°C followed by one cycle of final extension for 10 min at 72°C. The amplified PCR products were visualized on 1.6% agarose gel stained with ethidium bromide (0.5 µg/ml).

RESULTS AND DISCUSSION

The coat protein gene of GBNV was amplified at 800 bp from both PSND infected leaf samples (**Fig. 1**) and thrips tissues (**Fig. 2**) collected from the first planted potato crop (5 October). **Fig. 1** shows the presence of GBNV in the 6 test leaf sample, whereas, **Fig. 2** shows the presence of GBNV in the 9 thrips tissues. Samples in both cases were collected at 70 days after planting when disease severity was observed in the crops. In the all later planted crops, PCR products did not correspond to leaf samples and thrips tissue (data not shown). Moreover, in later planted crops least thrips were collected for the study when temperature gets lowered/colder after 15 October and thus low incidence of disease/thrips activity was observed unlike the first planted crop. However, aim of our study was to validate the PC-RT-PCR method for the detection of GBNV in leaf samples and thrips tissue which was achieved in the first planted crop. This result supports the previous work by Somani *et al.* (13) that showed that the early crop, planted on 4 October, had the highest incidence of

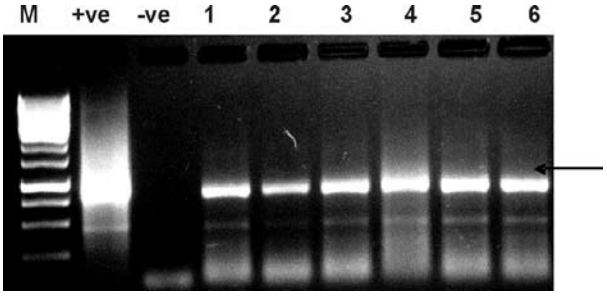

Fig. 1. Arrow indicating amplified coat protein gene of GBNV at 800 bp in infected leaf samples employing PC-RT-PCR (M: 1 Kb ladder, +ve: positive control, -ve: healthy control and test samples No. 1-6).

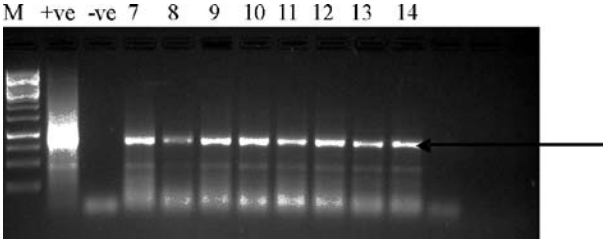

Fig. 2. Arrow indicating amplified coat protein gene of GBNV at 800 bp in vector thrips tissues by PC-RT-PCR from (M: 1 Kb ladder, +ve: positive control, -ve: healthy control and test sample No. 7-14).

PSND than the later crops. Indeed, our result shows the thrips are the potential carrier of GBNV especially in the first week of October and are of low or nil importance in the later planted crops (13). Therefore, planting after the end of October is helpful in reducing the disease incidence by avoiding crop exposure to thrips. The amplification product at 800 bp found with thrips supports evidence that GBNV is transmitted by thrips (*Thrips palmi*) and causes potato stem necrosis disease. This paper confirms that PC-RT-PCR is suitable for large scale sampling and testing of GBNV. The PC-RT-PCR method is simple and quick technique for the detection of GBNV infecting potatoes. Additionally, samples spotted on NCMs under this method have a long shelf-life and allow long distance transport to another laboratory as well for the diagnosis purpose (3).

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