

Detection of Pigeonpea Sterility Mosaic Virus (PPSMV) by RT-PCR

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Received 16 June 2017; Accepted 19 July 2017; Published online 12 August 2017

Abstract Total RNA was isolated by triazol method from sterility mosaic diseased and healthy pigeonpea plants. From the isolated total RNA first strand cDNA was synthesized and amplified in PCR with PPSMV specific primers (Replicase and glycoprotein precursors genes). An expected band of 322bp of PPSMV replicase gene was obtained in SMD infected plant but not healthy plants in denatured agarose gel electrophoresis.

Keywords PPSMV, cDNA, RT-PCR.

Introduction

Pigeonpea (*Cajanus cajan*) a multipurpose food legume crop which is grown predominantly in the dry tropics of the Indian Subcontinent. About 90% of global pigeonpea production is in South Asia. More than 210 pathogens, 15 viruses, 3 virus like diseases and one viroid has been reported to naturally infect pigeonpea [1].

Out of all, sterility mosaic disease (SMD) is the economically most important viral disease in India, causing an estimated annual loss of more than US\$300 million [2]. SMD was first reported from Pusa (Bihar) in 1931 [3] and also from subsequent states Maharashtra [4] Tamil Nadu [5] and Chhattisgarh [6]. SMD was also reported from Bangladesh, Nepal, Thailand [7], Myanmar [8] and Sri Lanka [9].

SMD is characterized by a bushy and pale green appearance of plants, excessive vegetative growth, stunting, reduction in leaf size and mosaic of leaves. The aetiology of SMD remained a mystery for over 70 years, was resolved with the discovery of pigeonpea sterility mosaic virus as a causal agent of SMD [10] and its complete genome sequencing was done in 2014. PPSMV is a mite transmitted single stranded multipartite RNA virus belonging to *Emaravirus* genus [11]. Present investigation was under taken to detect pigeonpea sterility mosaic virus by reverse transcription PCR at Tirupati.

Materials and Methods

Young leaves were collected from both SMD infected and healthy pigeonpea plants. Total RNA was iso-

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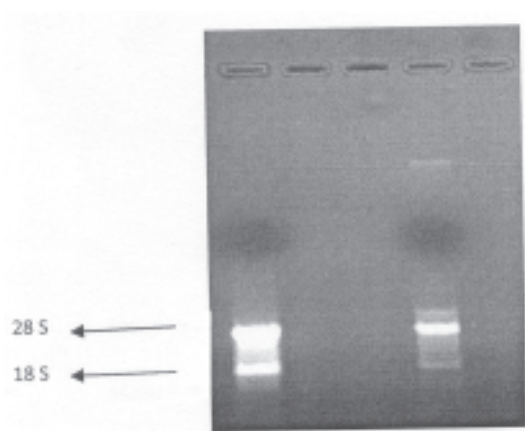


Fig. 1. Denatured agarose gel electrophoresis of total RNA in TBE buffer containing guanidine isothiocyanate.

lated from both PPSMV infected and healthy plants by Triazol method. The quality and integrity of isolated RNA was measured in Nanodrop spectrophotometer and by denatured agarose gel electrophoresis. From the total RNA first strand cDNA was synthesized with PPSMV specific primers (RNA 1/Replicase and RNA 2 / glycoprotein precursor genes) and reverse transcriptase enzyme. The synthesized cDNA was amplified in PCR. The amplified PCR products were analyzed in 1% agarose gel electrophoresis. The banding pattern was captured in Alpha image analyzer.

Results and Discussion

Total RNA isolated from infected pigeonpea plants was checked for quality and integrity in denatured agarose gel electrophoresis resulted in two sharp bands of 18S and 28S RNA indicating a very good integrity of isolated RNA (Fig. 1). The quality of isolated total RNA was measured in Nanodrop spectrophotometer which is in the range from 800-1000 ng/ μ l and purity was in the range from 1.8 to 2.0. The product of reverse transcriptase PCR shown amplification for PPSMV Replicase gene (RNA 1) at 322 bp (Fig. 2) which was observed only in pigeonpea sterility mosaic virus infected plants but not in healthy plants indicating the presence of PPSMY viral particles in the infected plants only. Kumar et al. [10] was the first to develop ELISA and RT-PCR based diagnostic method for detection of PPSMV in India. He amplified

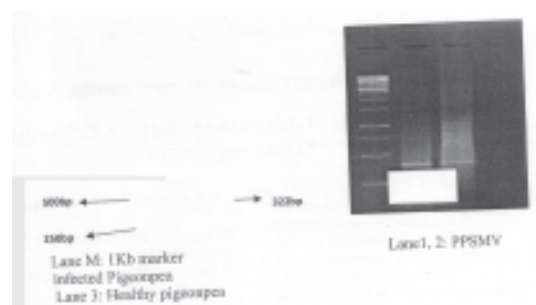


Fig. 2. Amplification of total RNA from SMD infected plants with partial replicase gene primers.

230bp product from RNA-3 which codes for coat protein. Latha and Doraiswamy [12] developed serological diagnostic methods like DAS-ELISA and DIBA to detect the presence of PPSMV from their viruliferous vector *Aceria cajani* mites using whole mite extract as an antigen. Both DAS-ELISA and DIBA were sensitive for virus detection in vector mites but DIBA is more sensitive for virus detection in vector mites when compared to DAS-ELISA. The extract from 180 mites gave a strong positive reaction and a weak positive reaction in minimum of 10 viruliferous mites by DAS-ELISA whereas the virus was detected from the extract of a minimum of 5 mites by DIBA.

References

1. Nene YL, Sheila VK, Sharma SB (1996) A world list of chickpea and pigeonpea pathogens. 5th (edn). ICRISAT Publ, pp 25.
2. Reddy MV, Raju TN, Lenne JM (1998) Diseases of pigeonpea. The pathology of food and pasture legumes. Wallingford, Oxfordshire: CAB Internat : 517—558.
3. Mitra M (1931) Report of imperial mycologist. Sci Rep of the Imp Inst Agric Res Pusa 30 : 45—71.
4. Muniyappa V, Chandrashekharaiiah SC (1980) Prevalence of sterility mosaic disease in Karnataka. Curr Sci 9 : 196.
5. Kannaiyan J, Nene YL, Reddy MV, Ryan JG, Raju TN (1984) Prevalence of pigeonpea diseases and associated crop losses in Asia, Africa, and America. Trop Pest Mgmt 30 : 62—71.
6. Singh AK, Rathi YPS, Agrawal KC (1999) Sterility mosaic of pigeonpea : a challenge of 20th century. Ind J Virol 15 : 85—92.
7. Nene YL, Reddy MV, Beniwa SPS, Mahmood M, Zote KK, Singh RN, Sivaprakasam K (1989) Multilocal testing of pigeonpea for broad based resistance to sterility mosaic in India. Ind Phytopathol. 42 : 444—448.

8. Su U (1931) Plant disease in Burma. Internat Bull Pl Prot 5 : 141—142.
9. Newton W, Peiris TWL (1953) Virus diseases of plants in Ceylon. FAO Pl Prot Bull 2 : 17—21.
10. Kumar LP, Jones AT, Sreenivasulu P, Reddy DVR (2000) Breakthrough in the identification of the causal virus of pigeonpea sterility mosaic disease. J Mycol and Pl Pathol 30 : 249.
11. Elbeaino T, Whitfield A, Sharma M, Digiario M (2013) *Emaravirus* - specific degenerate PCR primers allowed the identification of partial RNA-dependent RNA polymerase sequences of maize red stripe virus and pigeonpea sterility mosaic virus. J Virolog Methods 188 : 37—40.
12. Latha TKS, Doraiswamy S (2008) Detection of pigeonpea sterility mosaic virus, the causal agent of sterility mosaic disease of pigeonpea in viruliferous mite vector by DAS-ELISA and DIBA. Arch Phytopathol Pl Prot 41 : 537—541.