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RT-PCR Based Diagnosis of Soybean Mosaic Virus

H. V. Nandakishor[1], B. Kumaraswamy[2*], S. S. Mane[3] and G. Amrutha Veena[4]

Department of Plant Pathology, Post Graduate Institute, Dr. PDKV, Akola, 444104 Maharashtra, India

*Corresponding Author E-mail: nandakishorehv@gmail.com

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ABSTRACT

The occurrence of Soybean mosaic virus (SMV) in soybean was confirmed by symptomatology, reverse transcription-polymerase chain reaction (RT-PCR). SMV on soybean cv. JS-335 produced characteristic symptoms such as dark green colour of leaves, mosaic and mottling symptoms, crinkling, leaf puckering of leaves. The soybean mosaic virus was detected and diagnosed by applying the RT-PCR technique. The RT- PCR technique was performed using a specific primer pair CIF/CIRev and POT1/POT2. RT- PCR technique revealed presence of SMV, with a band length of 700 bp and 725 bp have been obtained from SMV infected samples.

Key words: *Soybean, Soybean mosaic virus, RT-PCR.*

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill], 'Queen of Pulses', a native of Eastern Asia belongs to the family Leguminosae, subfamily Papilionoideae and tribe Phaseolae. Soybean is considered as a 'Golden bean', 'Miracle bean', 'Agriculture's Cinderella' and Wonder crop' of the 20th Century' due to its qualities such as high protein (40%), good amount of carbohydrates (35 0/0), oil (20%) and ash (5%) content on oven dry basis. Soybean is known to be naturally infected by at least 50 viral diseases belonging to different groups[9]. In India, so far 11 viruses have been reported to occur on soybean[6]. Among the viral diseases, soybean mosaic virus (SMV)[6] seems to be much of prevalence. Clinton[3] reported. "The occurrence of soybean mosaic virus for the first time in world. In India the occurrence of soybean mosaic virus was reported from New Delhi by Nariani and Pingaley[8]. Later its occurrence was reported from Tamil Nadu[11], Uttar Pradesh[10] and Karnataka[7] Virus diseases constitute the most serious threat to soybean production in many tropical areas. The most common virus of soybean around the world is Soybean mosaic virus (SMV). SMV is a member of the genus Potyvirus[2]. SMV causes severe symptoms such as mosaic or necrosis in many soybean cultivars, and is easily transmitted by aphids in fields, thus resulting in significant reductions in soybean yield and quality. The seed borne nature of SMV possesses a serious threat to soybean cultivation. The Symptomatology studies indicated the possibility of Potyvirus infection in soybean.

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Therefore, attempt was made to identify and characterize the virus species applying reverse transcription-polymerase chain reaction (RT-PCR) based method. Results of detailed studies made on this disease are reported in this paper.

MATERIAL AND METHODS

Symptomology and collection of leaf samples

The soybean plants showing typical mosaic, mottling, curling, less pubicent, puckering symptoms were collected from the fields of the Departments of Plant Pathology, Agril Botany and Agril Entomology and from farmers field from different districts of Akola (Maharashtra). The samples were kept at -80°C in plastic bags in deep freeze with proper labels. Methodology adopted to identify and characterize the soybean mosaic virus species by applying reverse transcription-polymerase chain reaction (RT-PCR) technique is given in this chapter. The Symptomatology studies indicated the possibility of Potyvirus infection in soybean. Therefore, attempt was made to identify and characterize the virus species applying reverse transcription-polymerase chain reaction (RT-PCR) based method. Total RNA was extracted from both symptomatic and symptomless leaf samples using RNeasy Plant Mini Kit (RevertAid™, Fermentas, India) and complementary DNA (cDNA) was synthesized (Retro-script kit for cDNA). PCR assay was carried out using two sets of Potyvirus specific degenerate primers viz., CIFor/CIRev[5] and POT1/POT2[4] ("EUROFINS GENOMICS"). The cDNA - PCR amplified products of each primer were done on 1.5% gel electrophoresis and the amplified product was compared with 1kb plus DNA ladder obtained from Invitrogen.The former set was reported to amplify a ~700 bp region of cylindrical inclusion protein (CI) domain[5] and the later set was designed to amplify a ~1,300 bp region encompassing partial nuclear inclusion protein and coat protein (NIb-CP) domain[4] of Potyvirus open reading frame (ORF).

RESULTS AND DISCUSSION

PCR technique is mainly used for detection and diagnosis of soybean mosaic virus associated with symptomatic soybean samples**.** RT-PCR assay was carried out using two sets of Potyvirus specific degenerate primers viz., CIF or CIRev[5] and POT1/POT2[4]. All the symptomatic leaf samples showed virus-specific amplification of ~700 bp and ~1,300 bp in RT-PCR assay for CIF or/ CIRev and POT1/POT2, respectively. Soybean infected samples produced characteristic symptoms such as dark green colour of leaves, mosaic and mottling symptoms, crinkling, leaf puckering of leaves. RNA was isolated from both symptomatic and symptomless leaf samples using RNeasy Plant Mini Kit (RevertAid™, Fermentas, India) and RNA used as template complementary for cDNA synthesis (Retro-script kit for cDNA). Out of twenty samples of SMV, only eighteen samples showed the +ve reaction for SMV by RT-PCR (Table-1). All the symptomatic samples were showed banding pattern 700bp and 725bp by using primer pair SMV CIFor/CIRev and POT1/POT2 respectively. While no amplicon was obtained from the asymptomatic plant samples. This confirmed the presence of SMV in soybean sample.

Finally, we revalidated our findings by screening the infected samples with SMV CP specific primers SMV-CPf/SMV-CPr with findings of Wang and Ghabrial[12], Amrita banarjee *et al*[1]. The infected samples gave specific amplicon of ~460 bp same as previous report[12].

Table 1: Details of the samples collected for detection of SMV infection by RT-PCR amplification with two sets of specific primers

Sr. No	Sample	Family	Cultivar	No of samples	No of samples SMV reaction	
					+ve	-ve
1	*Glycine max*	Leguminosae	JS-335	5	5	-
2	*Vigna ungiculata*	Leguminosae	C-152	5	3	2
3	*Cucumis sativis*	Cucurbitaceae	Phule champa	5	4	1
4	*Dolichos lablab*	Leguminosae	Local	5	3	2
5	*Chenopodium amaranticolor*	Chenopodiaceae	Local	5	3	2

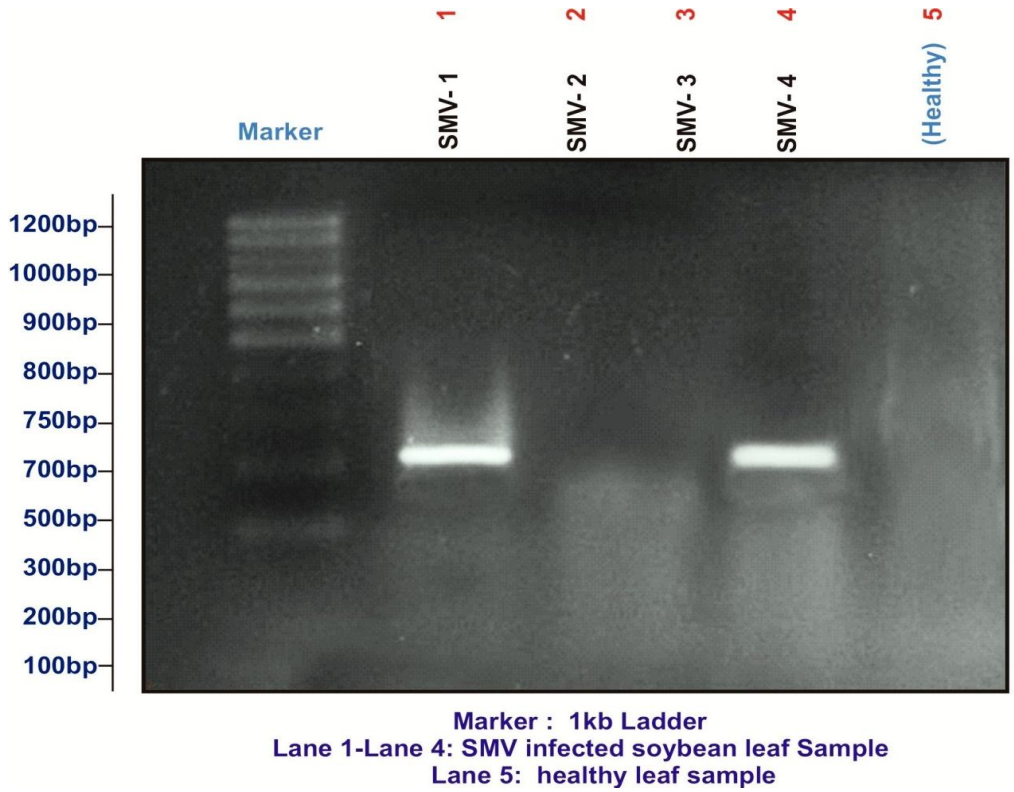

Fig. 1: Banding pattern of primer POT1-F/POT-R of SMV infected samples

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