DEVELOPMENT OF DIAGNOSTICS FOR SWEETPOTATO FEATHERY MOTTLE VIRUS

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INTRODUCTION

Sweet potato (*Ipomoea batas* L,) is an important starchy tuberous root crop after potato and cassava, that feeds millions of people in developing countries .

Productivity of sweet potato is very low in India (8 tonnes/ ha)

Constraints

Sweet potato weevil and viral diseases are the major biotic constraints

Viral Diseases : SPFMV is widely prevalent

Production of healthy quality planting material is one of major mandate of CTCRI

OBJECTIVES

- **Development of PCR based protocols for detection of SPFMV**
- **Cloning and sequencing of coat protein gene of SPFMV occurring in India**

Symptoms of feathery mottle disease

Contd..

Symptoms of feathery mottle disease

Contd..

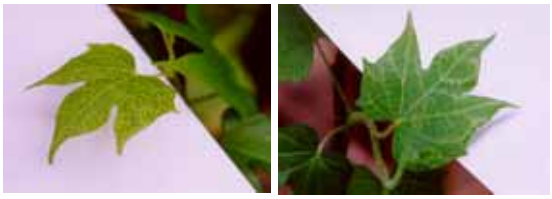
Symptoms of feathery mottle disease

Transmission to tobacco

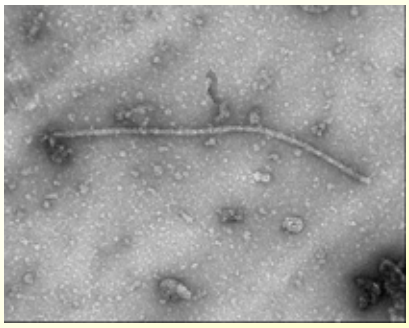
Nicotiana benthamiana *Nicotiana tabacum*

Host plants	Symptoms	Symptoms
Nicotina tabacum	8/14	Mosaic vein clearing and
Nicotiana benthamiana	4/12	Chlorotic spot and leaf distruption
Ipomoea nil	11/15	Chlorotic spot and vein clearing
Ipomoea setosa	10/15	Vein clearing ,leaf distortion, mosaic

Transmission to *Ipomoea setosa*



Graft transmission **Sap inoculation**



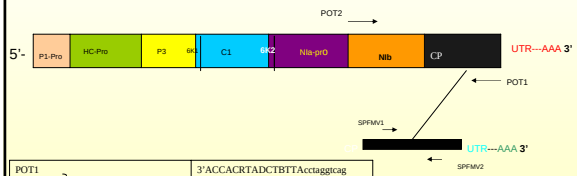
Flexuous SPFMV particles

Structural organisation of SPFMV

PROTEIN	POSSIBLE FUNCTION(S)
P1	Proteinase;
	Cell-to-cell movement (speculation).
HC-Pro	Aphid mediated transmission;
	Proteinase;
	Cell-to-cell movement (speculation).
P3	Unknown (possible role in replication)
CI	Genome replication (RNA helicase);
	Membrane attachment;
	Nucleic acid stimulated ATPase activity;
	Cell-to-cell movement (speculation).
CP	RNA encapsidation;
	Involved in vector transmission;
	Cell-to-cell movement.
NIa-VPg	Genome replication (Primer for initiation of RNA synthesis).
NIa-Pro	Major Proteinase
NIb	Genome replication (RNA-dependent RNA polymerase [RdRp]).
6K1 & 6K2	Unknown, but possible roles in: - RNA replication;
	- Regulatory function inhibiting NIa nuclear translocation;
	- Membrane anchoring of replication machinery

5' Vpg  -AAA3'

Primers for amplification SPFMV



POT1	3'ACCACRTADCTBTTAcctaggtcag
POT2 (Colinet *etal* 1998)	5',gacgaattcTGYGAYGCBGATGGYTC3'
SPFMV1	5'ATGGTGGGGCATCATCAAAGG3'
SPFMV2 (Jeong *etal* 2003)	5'CCTAAAAGTAGGCACTGCATGG3'

RT PCR

RNA	100-150ng
10x buffer	2µl
dNTP Mix	-.25µl
Cmaster RT enzyme	-.25µl
CmasterTaq enzyme	.25µl
Rnase Inhibitor	0.2µl
Nuclease free water	-
Ttotal	20µl

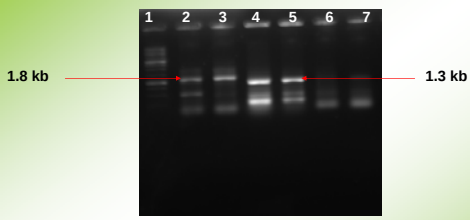
SPFMV1 & SPFMV2

42°C -45 minutes
96 °C -2 minutes
96 °C -30seconds } 40 cycles
63 °C -30 seconds
72 °C -1 minute
72 °C -7 minutes

POT 1& POT 2

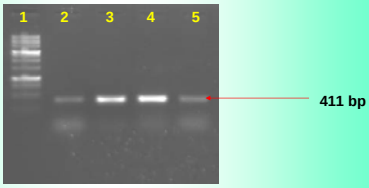
42°C -45 minutes
92 °C -2 minutes
94 °C -30seconds } 5cycles
42°C -1min
72°C - 90seconds
94-30seconds } 35 cycles
60-60 seconds
72°C -90seconds
72 °C -5 minute

Analysis of product amplified from total sweet potato RNA with Pot1,Pot 2 and oligodT primers



Lane 1,marker; lanes 2 and 3, 1.8 kb product obtained with oligodT and Pot 2 primer pair; lanes 4 and 5, 1.3 kb product obtained with Pot1 and Pot 2; lane 6 , healthy sweet potato control; lane 7, non host

Analysis of product amplified from total RNA isolated from SPFMV infected sweet potato, *I. nil* and *I. setosa* with SPFMV1 and SPFMV 2 primers.

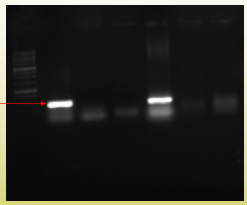


Lane 1, marker; lane 2, sweet potato(S500); lane 3, *I. nil*; lane 4, *I. setosa*; lane 5, sweet potato collected from (GOURI)Bhubaneswar

Detection of SPFMV from different parts of infected sweet potato plants with SPFMV 1 and SPFMV 2 primers

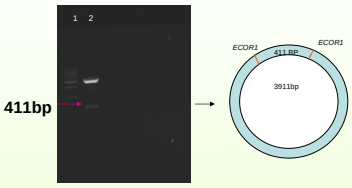
lane 1: marker
lane 2: apical bud
lane 3: middle leaf
lane 4: lower leaf

Detection of SPFMV in sweet potato by IC-RT-PCR and direct tube capture RT-PCR.



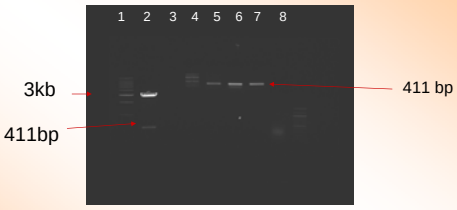
Lane 1, marker; lane 2, immuno captured SPFMV(s-500); lane 3, healthy sweet potato control; lane 4, non-host control; lane 5 direct captured SPFMV(s-500); lane 6, healthy sweet potato seedling as control; lane 7 non- host control

DIAGRAMATIC REPRESENTATION pGEM-T Partial CP



1.1Kb DNA ladder
2. partial CP restricted with *EcoR1*

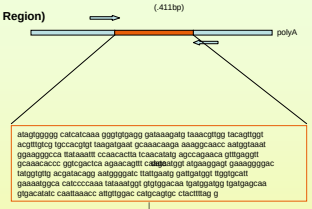
CLONE CONFIRMATION WITH RESTICTION DIGETION AND COLONY PCR



1.Kb marker 2. restriction digetion pGEMTCTIPCPpwith *EcoR1*
3. blank 4.100bp ladder 5,6,7 colony PCR positive
8 .colony PCR negative

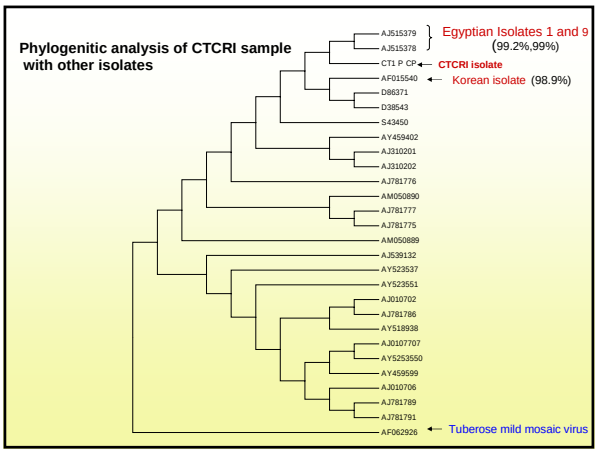
Nucleotide and amino acid sequencing data of pGEM-T Partial CP

SPFMV RNA (960 bp CP Region)



atagtgggggg catcatcaaa gggtgtgagg gataaagatg taaacgttgg tacagttggt
acgtttgtcg tgccacgtgt taagatgaat gcaaacaaga aaaggcaacc aatggtaaat
ggaagggcca ttataaattt ccaacactta tcaacatatg agccagaaca gtttgaggtt
gcaaacaccc ggtcgactca agaacagttt cagggatggt atgaaggagt gaaaggggac
tatgggtgtg acgatacagg aatggggatc ttattgaatg gattgatggt ttggtgcatt
gaaaatggca catccccaaa tataaatggt gtgtggacaa tgatggatgg tgatgagcaa
gtgacatatc caattaacc attgttggac catgcagtgc ctacttttag g

IVGASSKGVRDKDVNVGTVGTFVVPRVKMNANKKRQPMVNGRAI
INFQHLSTYEPEQFEVANTRSTQEQFQAWYEGVKGDYGVDDT
MGILLNGLMVWCIENGTSPNINGVWTMMDGDEQVTYPIKPLLD
AVPTFR(~135 amino acids)



Plants tested by PCR	POT 1&POT 2 PRIMER	SPFMV 1 & SPFMV 2 PRIMER
S-500	+	+
S-316	-	+
S-211	-	+
S-629	+	+
S1070	+	+
S-315	+	+
S-306	-	+
S-406	-	-
S-382	+	+
S-465	-	+
S-658	+	+
S-492	+	+
S-1114	-	+
S-703	-	+
S-645	+	+
S-101	-	-
S-343	-	+
I.nil	+	+
I.setosa	+	+

Detection of SPFMV in sweet potato clones by RT-PCR using Pot 1 and Pot 2 primers and SPFMV 1 and SPFMV 2 primers

Conclusion

- SPFMV could be detected using potyvirus specific degenerate(POT1&POT2) primers and gene specific SPFMV1& SPFMV2 primers
- IC RT- PCR and tube capture PCR was standardized using gene specific primers
- Partial SPFMV CP was amplified and cloned in pGEM-T vector
- Phylogenitic analysis showed that pGEM-T partial CP having 99% similarity to Egyptian isolates.


Thank you