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Studies on cassava mosaic disease with special reference to detection and virus variability

By

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OBJECTIVES

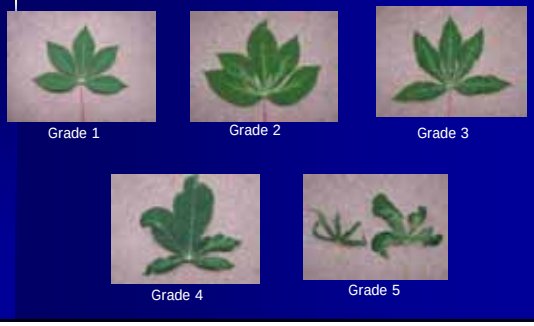
To map the disease incidence in the major cassava growing districts of Tamil Nadu

Detection and Variability among cassava infecting geminiviruses in Tamil Nadu using ELISA and PCR

SELECTION OF AREA FOR SURVEY AND SAMPLING

- The major cassava growing districts selected for surveying and sampling namely Salem, Dharmapuri, Namakkal.
- Each and every field was observed with 50 – 100 plants to record disease incidence (Fargette,1985)
- Plants selected from two sides and along a diagonal across the field in a 'Z' configuration (Otim-Nape *et al.*, 1998)
- Disease severity and type of infection (Cutting borne or Whitefly borne) (Sseruwagi *et al.,* 2004)

1 – 5 DISEASE GRADE CHART FOR CALCULATING DISEASE SEVERITY (Hahn *et al*, 1980)



Recording disease incidence and disease severity

S.No.	Name of the place	Disease incidence in percentage	Disease severity* (Based on 1-5 scale by Hahn *et al*., 1994).
1.	Ettikkuttai medu	95	3.16
2.	Konganapuram	96.3	3.03
3.	Edappadi	94	2.96
4.	Murungappatti	95.23	2.35
5.	Siddhar koyil	97.43	3.89
6.	Elampillai	90	2.91
7.	Alavappampalayam	94	2.96
8.	M.N.Patti	92	3.04
9.	Tharamangalam	98	3.2
10.	Kamandapatti	92	2.85
11.	Sankagiri	92	4.0
12.	Pullipalayam	98	3.53
13.	Paramathi vellore	95	2.8
14.	Mallur	93.3	3.0
15.	Pappirettippatti	90	2.9
16.	Neringipet	92	2.5
17.	Musiri(Trichy)	95	2.7
18.	Namagiripet (Rasipuram)	70	2.39
19.	Kattukkottai (Attur)	91	2.5
20.	Manivizhundhan colony	90	3.3
21.	Coimbatore	98	3.9

TYPE OF INFECTION

S.No.	Name of the Place	Type of Infection (%)		Age of the crop in months
		Cutting borne	Whitefly borne	
1.	Ettikkuttai medu	59	41	5
2.	Konganapuram	79	21	6
3.	Edappadi	100	0	3
4.	Murungappatti	90	10	5
5.	Siddhar koyil	94	6	5
6.	Elampillai	90	10	5
7.	Alavappampalayam	92	8	6
8.	M.N.Patti	90	10	4
9.	Tharamangalam	100	0	3
10.	Kamandapatti	100	0	3
11.	Sankagiri	100	0	3
12.	Pullipalayam	98	2	3
13.	Paramathi vellore	90	10	6
14.	Mallur	94	6	6
15.	Pappirettipatti	90	10	7
16.	Neringipet (Bhavani)	96	4	4
17.	Musiri(Trichy)	88	12	7
18.	Namagiripet (Rasipuram)	75	25	6
19.	Kattukkottai (Attur)	90	10	6
20.	Manivizhundhan colony (Attur)	90	10	6
21.	Coimbatore	94	6	3

No. OF WHITEFLIES PER PLANT

No.	Name of the place	Whitefly population per plant		
		3 months after planting	6 months after planting	10 month after planting
1.	Konganapuram	14	13	01
2.	Tharamangalam	17	15	0
3.	Omalur	13	12	0
4.	Elampillai	18	12	01
5.	Sankagiri	16	16	02
6.	Attur	15	14	01
7.	Thiruchengodu	14	13	01
8.	Rasipuram	14	13	01
9.	Pappirettippatti	15	17	01
10.	Palacode	16	15	01

SCREENING OF DIFFERENT MONOCLONAL ANTIBODIES AGAINST THE VIRUS

The following MAbs were used,

From Scottish Crop Research Institute

SCR 55, 60, 62, 66 (Raised against ICMV)
SCR 12, 14, 16, 18, 21, 22, 25, 29, 32 (Raised against ACMV)

From DSMZ, Germany

AS – 0424/1 (Raised against ICMV)
AS – 0546/2 (Raised against ACMV)
AS – 0617/1 (Raised against EACMV)

SCREENING OF MONOCLONAL ANTIBODIES AGAINST THE CMD AFFECTED CASSAVA SAMPLES

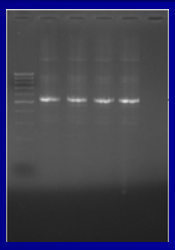
S.No.	Name of the MAb used	Raised against	Reaction
1.	SCR 12, 14, 16, 21, 22, 25, 29, 32	ACMV	-ve
2.	SCR 55, 60, 66	ICMV	+ve
3.	AS – 0424/1	ICMV	+ve
4.	AS – 0546/2	ACMV	-ve
5.	AS – 0617/1	EACMV	-ve

DETECTION OF VIRUS FROM VARIOUS PARTS OF CMD AFFECTED CASSAVA PLANTS

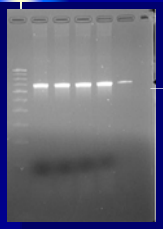
The plant parts used for test	OD Value at 405nm	Reaction to TAS - ELISA
young leaves	2.413	+ve
petiole	2.410	+ve
stem bark	0.053	-ve
rose coloured inner peel of the tuber	0.035	-ve
Tuber	0.051	-ve
immature fruits (capsules)	0.038	-ve
immature seeds	0.0124	-ve
matured seeds	0.013	-ve

Detection of cassava mosaic virus by Polymerase Chain Reaction (PCR) using degenerate Primer (Deng *et al.*, 1994)

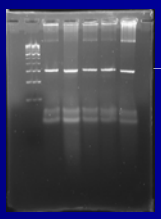
- 560 bp of DNA A amplified from CMD affected cassava DNA by Deng's primer
- confirms CMD caused by a group of geminivirus



DETECTION OF ICMV / SLCMV USING SPECIFIC PRIMERS



ICMV A



ICMV A1

Screening of virus specific primers against the virus and Differentiation of ICMV and SLCMV infection by multiplex PCR

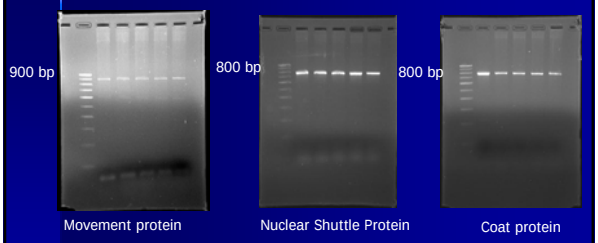
- The primer consist of 2 different forward primers and common reverse primer
- 904 bp of DNA A – ICMV and 599 bp - SLCMV
- Almost all of the field collected sample from TN, detected with SLCMV than ICMV
- 2 samples only detected with ICMV
- mixed infection was not found

DIFFERENTIATION OF ICMV AND SLCMV

AMPLIFICATION OF VIRAL GENES

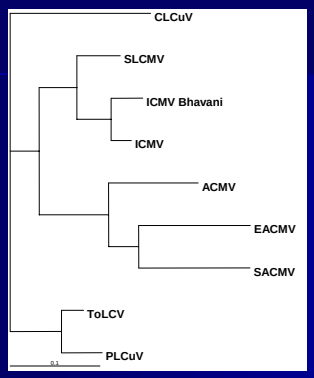
- Coat protein gene (AV1) – 800 bp amplified
- Nuclear Shuttle Protein (BV1) – 800 bp amplified
- Movement protein (BC1) – 900 bp amplified

AMPLIFICATION OF VIRAL GENES



Cloning and sequencing of 599 bp fragment amplified from mosaic affected samples by multiplex PCR

- The 599 bp fragment believed to be the SLCMV
- But sample from Bhavani amplified 629 bp fragment
- This amplified fragment shown more similarity with ICMV (Mah) than SLCMV
- But sample from Coimbatore detected with SLCMV based on sequence of the 599 bp fragment in an another study.
- This show greater variability in Tamil Nadu



Conclusion



Thank You