

**MOLECULAR CHARACTERIZATION, DETECTION, ECONOMIC
IMPACT, RESISTANCE MECHANISM AND ELIMINATION OF
SUGARCANE MOSAIC POTYVIRUS IN SUGARCANE**

*Thesis submitted in part fulfillment of the requirements for the award of the degree of
DOCTOR OF PHILOSOPHY (AGRICULTURE) in PLANT PATHOLOGY to the
Tamil Nadu Agricultural University, Coimbatore 641 003*

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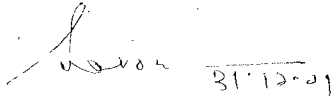
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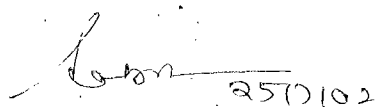
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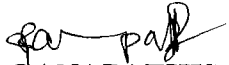
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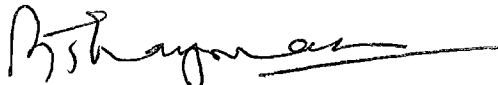
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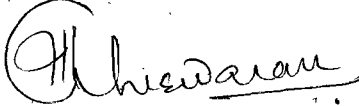

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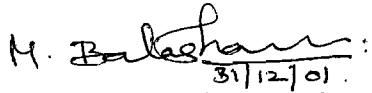
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31/12/01
(M. BALAMURALIKRISHNAN)

ABSTRACT

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By

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The sugarcane clones surveyed for the sugarcane mosaic virus (SCMV) incidence in major sugarcane belts of Tamil Nadu exhibited the symptom as mild or severe forms and practically none of the variety was free from the disease. The SCMV symptoms were broadly classified into streak type, chlorotic patches with green islands, and yellow blotches with systemic yellowing. The symptom severity was high in genotype collections as compared to the canes cultivated in the field. In majority of sugarcane areas the symptoms of chlorotic patches with green islands were commonly noticed. Reactions of SCMV in sorghum and sugarcane differential hosts as well as serological reactions revealed the presence of mixture of SCMV strains in many sugarcane varieties. The strains identified were SCMV-A, B, D, E, maize dwarf mosaic virus-B (MDMV-B) and sorghum mosaic virus-H (SrMV-H) and there were no reaction with the antisera of SCMV- Sabi, YU, BC and Johnson grass mosaic virus (JGMV). The samples from SCMV infected varieties reacted strongly with the polyclonal antiserum. Among the different sugarcane varieties, Co 740 and CoC 671 recorded the maximum number of SCMV strains, followed by UP 95-30 and CoJn 86141. Among the different strains, SCMV-B and SCMV-D were widely prevalent in most of the tested sugarcane varieties.

The SCMV was purified from the infected sorghum 'cv 'Rio'. The SCMV was separated after sucrose gradient centrifugation and the virus particles appeared as long flexuous rods and measured between 690 and 740 nm. The purified SCMV preparation produced typical symptoms on sorghum cultivar 'Rio' and serologically reacted with SCMV- A, D, MDMV-B and SrMV-H. The UV absorbance ratio of 260/280 nm was 1.2. The molecular weight of the coat protein was 40.1 kDa. The viral RNA genome was confirmed after RNase treatment in agarose gel. The viral genome was amplified in RT-PCR method with SCMV specific primer and the size of amplified product was 870 bp. The polyclonal antisera were raised against SCMV by administering single and multiple immunizations in rabbits. The early bleed serum from single immunization was very specific and not reacted with SrMV. However, serum from multiple immunization was not specific. In both the immunization methods, the titre of late bleed antisera was high. The antisera collected from both the immunization methods, reacted with the SCMV infected tissues tested in DAC-ELISA, electroblot immunoassay, dot blot immunoassay and immunosorbant electron microscopy. Among the different sugarcane tissues tested by DAC-ELISA, except the roots, all the other tissues *viz.*, leaf lamina, midrib, buds and leaf sheath reacted positively to SCMV and stronger reaction was observed from young leaves. The SCMV genome was amplified by Reverse transcriptase polymerase chain reaction (RT-PCR) method from the SCMV infected leaf lamina, buds, midrib and leaf sheath, with SCMV specific primer, but not from roots and pith region. The amplified DNA was 870 bp in size. By using potyvirus specific primer also, the viral genome was detected from the mosaic infected sugarcane tissues and the molecular weight of the amplified DNA was 750 bp and matched with the cloned SCMV cDNA.

The younger sugarcane plants up to two months were found to be highly susceptible to SCMV upon mechanical inoculation in both Co 740 and CoC 671 varieties, but infection rate was very low in five to six months aged plants. The SCMV infection affected the different growth parameters namely germination, tillering, cane thickness and number of millable canes, in Co 740 variety, in both the plant crop and

ratoon crop. Whereas, the impact on these parameters was noticed only in ratoon crop of CoC 671. The net photosynthesis, sucrose, brix, purity and sugar yield were affected in plant and ratoon crop of these two varieties. SCMV infection reduced the cane yield between 27.5 and 36.1 per cent in the plant crop and 35.6 and 41.0 per cent in ratoon crop in Co 740. In ratoon crop of CoC 671 variety the loss ranged from 7.9 to 11.3 per cent. The loss in sugar yield was between 3 and 11 per cent in plant crop and between 8 and 22 per cent in ratoon crop. The foliar application of bion and salicylic acid and seed treatment with *Pseudomonas fluorescens* exhibited induced systemic resistance (ISR) and significantly slowed down the initial SCMV infection in sorghum plants during the earlier growth phase. Among the different sugarcane genotypes and inter-specific hybrids tested for SCMV resistance, the inter-specific hybrids Co 740 and CoC 671 were highly susceptible. The *Erianthus bengalense* clone IND 81-47 and *Saccharum spontaneum* clone IND 81-161 were highly resistant, followed by *S. spontaneum* clones IND 81-80 and IND 81-142. The enhanced induction of total phenols, phenylalanine ammonia lyase, peroxidase and polyphenol oxidase might have contributed for the SCMV resistance in the resistant genotypes and also in ISR triggered by various biotic and abiotic inducers. The meristem tip culture combined with antiviral chemotherapy was effective in SCMV elimination. Amending the antiviral chemotherapeutant, ribavirin at 50 ppm with MS medium increased the SCMV elimination up to 95 per cent. The serial hot water treatment partially reduced SCMV concentration however it affected the sett germination severely.

CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
I	INTRODUCTION	01
II	REVIEW OF LITERATURE	08
III	MATERIALS AND METHODS	27
IV	RESULTS	54
V	DISCUSSION	196
VI	SUMMARY	218
	REFERENCES	

LIST OF TABLES

Table No.	Title	Page No.
1.	SCMV Survey in various sugarcane growing areas of Tamil Nadu	55
2.	Differentiation of SCMV stains by sorghum and sugarcane differential host reactions	83
3.	Serological reactions of SCMV from different sugarcane varieties with various strain specific antisera and polyclonal antisera by DAC-ELISA	88
4.	Serological relationship of the purified SCMV	94
5.	Serological reactions of SCMV antigen and antisera in DAC-ELISA (early bleed-single immunization)	100
6.	Serological reactions of SCMV antigen and antisera in DAC ELISA (late bleed single immunization)	100
7.	Serological reactions of SCMV antigen and antisera in DAC ELISA (early bleed multiple immunization)	102
8.	Serological reactions of SCMV antigen and antisera in DAC ELISA (late bleed multiple immunization)	102
9.	Serological reactions between SCMV antisera and three potyviruses in DAC ELISA	103
10.	Determination of SCMV concentration in different sugarcane tissues by DAC-ELISA method	109
11.	Influence of age of sugarcane plant on SCMV infection (Co 740 variety)	114
12.	Influence of age of sugarcane plant on SCMV infection (CoC 671 variety)	115
13.	Effect of SCMV on growth parameters of sugarcane - germination, tillering and plant height Plant crop Experiment I at Coimbatore)	118
14.	Effect of SCMV on growth parameters of sugarcane – cane thickness and NMC (Plant crop experiment I at Coimbatore)	119
15.	Effect of SCMV on growth parameters of sugarcane – germination, tillering and plant height (Plant crop experiment II at Coimbatore)	120

Table No.	Title	Page No.
16.	Effect of SCMV on growth parameters of sugarcane - cane thickness and NMC (Plant crop experiment II at Coimbatore)	125
17.	Effect of SCMV on growth parameters of sugarcane - germination, tillering and plant height (Plant crop experiment III at Pollachi)	131
18.	Effect of SCMV on growth parameters of sugarcane - cane thickness and NMC (Plant crop experiment III at Pollachi)	134
19.	Effect of SCMV on cane juice quality and sugar yield (Plant crop experiment III at Pollachi)	140
20.	Effect of SCMV on growth parameters of sugarcane - tillering and plant height (Ratoon crop experiment I at Coimbatore)	141
21.	Effect of SCMV on growth parameters of sugarcane - tillering and plant height (Ratoon crop experiment II at Coimbatore)	142
22.	Effect of application of biotic and abiotic agents on the induction of total phenols in SCMV inoculated sorghum plants	153
23.	Effect of application of biotic and abiotic agents on peroxidase activity in SCMV inoculated sorghum plants	154
24.	Effect of application of biotic and abiotic agents on polyphenol oxidase (PPO) activity in SCMV inoculated sorghum plants	159
25.	Effect of application of biotic and abiotic agents on phenyl-alanine ammonia lyase (PAL) activity in SCMV inoculated sorghum plants	162
26.	Effect of application of biotic and abiotic agents on SCMV titre in sorghum plants	164
27.	Changes in total phenol content in SCMV inoculated resistant and susceptible sugarcane genotypes	166
28.	Changes in phenylalanine ammonia lyase activity (PAL) in SCMV inoculated resistant and susceptible sugarcane genotypes	169
29.	Changes in peroxidase activity in SCMV inoculated resistant and susceptible sugarcane genotypes	171
30.	Changes in polyphenol oxidase activity (PPO) in SCMV inoculated resistant and susceptible sugarcane genotypes	177

Table No.	Title	Page No.
31.	Assessment of SCMV infection in the virus inoculated resistant and susceptible sugarcane genotypes	182
32.	Combined effect of antiviral chemotherapeutants and meristem tip culture against SCMV in sugarcane (CoC 671 variety)	189
33.	Assessment of SCMV elimination by chemotherapeutants in DAC-ELISA method	190
34.	Effect of serial hot water treatment on SCMV elimination in CoC 671 sugarcane variety	192
35.	Effect of serial hot water treatment on SCMV elimination in Co 740 sugarcane variety	194

LIST OF FIGURES

Fig. No	Title	Page No.
1.	UV absorbance profile of the purified SCMV	90
2.	Effect of SCMV on net photosynthesis of sugarcane (plant crop experiment I at Coimbatore)	127
3.	Effect of SCMV on sugarcane growth-single cane weight (plant crop experiment I at Coimbatore)	127
4.	Effect of SCMV on cane juice quality and sugar yield (plant crop experiment I at Coimbatore)	128
5.	Effect of SCMV on net photosynthesis of sugarcane (plant crop experiment II at Coimbatore)	128
6.	Effect of SCMV on sugarcane growth – single cane weight (plant crop experiment II at Coimbatore)	129
7.	Effect of SCMV on cane juice quality and sugar yield (plant crop experiment II at Coimbatore)	129
8.	Effect of SCMV on cane yield in two sugarcane varieties (plant crop experiment III at Pollachi)	135
9.	Effect of SCMV on sugarcane growth-cane thickness (ratoon crop experiment I at Coimbatore)	135
10.	Effect of SCMV on sugarcane growth-NMC (ratoon crop experiment I at Coimbatore)	145
11.	Effect of SCMV on net photosynthesis of sugarcane (ratoon crop experiment I at Coimbatore)	145
12.	Effect of SCMV on sugarcane growth – single cane weight (ratoon crop experiment I at Coimbatore)	146
13.	Effect of SCMV on cane juice quality and sugar yield (ratoon crop experiment I at Coimbatore)	146
14.	Effect of SCMV on sugarcane growth – cane thickness (ratoon crop experiment II at Coimbatore)	147

Fig. No	Title	Page No.
15.	Effect of SCMV on NMC (ratoon crop experiment II at Coimbatore)	147
16.	Effect of SCMV on net photosynthesis of sugarcane (ratoon crop experiment II at Coimbatore)	149
17.	Effect of SCMV on sugarcane growth – single cane weight (ratoon crop experiment II at Coimbatore)	149
18.	Effect of SCMV on cane juice quality and sugar yield (ratoon crop experiment II at Coimbatore)	151
19.	Effect of SCMV on cane juice quality in sugarcane (Purity)	151
20.	Changes in total phenol content in SCMV inoculated resistant and susceptible sugarcane genotypes	167
21.	Changes in phenylalanine ammonia lyase activity (PAL) in SCMV inoculated resistant and susceptible sugarcane genotypes	167
22.	Changes in peroxidase activity in SCMV inoculated resistant and susceptible sugarcane genotypes	172
23.	Changes in polyphenol oxidase activity (PPO) in SCMV inoculated resistant and susceptible sugarcane genotypes	172
24.	Combined effect of antiviral chemotherapeutants and meristem tip culture against SCMV in sugarcane (CoC 671)	188
25.	Effect of serial hot water treatment on sett germination in sugarcane	195

LIST OF PLATES

Plate No.	Title	Page No.
1	Appearance of servere SCMV symptoms in the field (CoC 92061)	57
2.	Appearance of servere SCMV symptoms in the field (CP 44-101 and Co 740)	58
3.	Closer view of SCMV symptoms (CoS 86218 and CoJ 84191) a. Chlorotic streaks (CoS 86218) b. Chlorotic specks (CoJ 84191)	59
4.	Closer view of SCMV symptoms (Co 740 and CoC 671) c. Chlorotic streaks (Co 740) d. Chlorotic patches and green islands (CoC 671)	60
5.	Closer view of SCMV symptoms (CoC 92061) a. Yellow blotches (CoC 92061) b. Systemic yellowing and marginal drying (CoC 92061)	61
6.	Comparison of healthy and SCMV infected leaves (CoC 671 and Co 740) a. CoC 671 b. Co 740	62
7.	Comparison of healthy and SCMV infected leaves (CoC 92061)	63
8.	Variation in varietal symptoms of SCMV-I	64
9.	Variation in varietal symptoms of SCMV-II	65
10.	Differentiation of SCMV strains by sorghum differential host - Atlas (Group A – SCMV B and SCMV D) a. Severe mosaic symptom b. Chlorotic and reddish stripes	69

Plate No.	Title	Page No.
11.	Differentiation of SCMV strains by sorghum differential host Atlas a. Stunting (Group A SCMV B and SCMV D) b. Severe chlorosis and necrotic stripes (Group B SCMV B and SCMV D)	70
12.	Differentiation of SCMV strains by sorghum differential host Atlas a. Chlorosis – younger and older leaves (Group C –SCMV-A) b. Chlorosis – younger and older leaves (Group D- SCMV - A and SCMV –E)	71
13.	Differentiation of SCMV strains by sorghum differential host- Rio	72
14.	Differentiation of SCMV strains by sorghum differential host – Jonhson grass a. Infected (Mild Mosaic SrMV-H/MDMV-B) b. Healthy	73
15.	Differentiation of SCMV strains by sugarcane differential host – CP 31-294 a. Severe stunting, chlorotic strips and necrosis (Group A-SCMV D) b. Severe chlorosis (Group B-SCMV B)	75
16.	Differentiation of SCMV strains by sugarcane differential host – CP 31-294 a. Chlorosis in younger leaves (Group C SCMV A) b. Scattered streaks (Group D SCMV E)	76

Plate No.	Title	Page No.
17.	Differentiation of SCMV strains by sugarcane differential host – CP 31-588 a. Severe chlorosis (Group A – SCMV - ?) b. Severe pale green and yellow patches (Group B – SCMV-B)	77
18.	Differentiation of SCMV strains by sugarcane differential host – CP 31-588 a. Mild chlorotic patches and stripes (Group C SCMV A and SCMV-D) b. Mild chlorotic streaks (Group D SCMV-D)	79
19.	Differentiation of SCMV strains by sugarcane differential host – Co 281 (Group A : SCMV-?) a. Pale green and longitudinal streaks b. Bleached apperance	80
20.	Differentiation of SCMV strains by sugarcane differential host – Co 281 (Group B SCMV A/B/D/E)	81
21	Immunosorbant electron micrograph of the purified SCMV particles	91
22.	Biological assay of the purified SCMV	92
23	Electrophoretic separation of the coat protein from purified SCMV	95
24	Determination of serological relationship of the purified SCMV by electro blot immunoassay	96
25	Separation of RNA genome from the purified SCMV by agarose gel electrophoresis	98

Plate No.	Title	Page No.
26	Amplification of viral genome from the purified SCMV by RT-PCR method (SCMV specific primer)	99
27	Polyacrylamide gel electrophoresis of the SCMV from the sugarcane (CoC 671) and sorghum (Rio) leaves	105
28	Detection of SCMV from the infected sugarcane tissues by electroblot immunoassay	106
29	Detection of SCMV particles from the infected sugarcane leaves by immunosorbant electron microscopy (ISEM)	107
30	Detection of SCMV from the infected sugarcane leaves by dot blot immunoassay (DBIA)	108
31	Detection of SCMV from the infected tissues of sugarcane by RT-PCR method (SCMV specific primer)	111
32	Detection of SCMV from the sugarcane varieties by RT-PCR method (SCMV specific primer)	112
33	Detection of SCMV from the infected leaves of sorghum and sugarcane by RT-PCR method (Potyvirus specific primer)	113
34.	Foliar symptoms of SCMV in the field (Co 740) a. Germination phase b. Fourth month	122
35.	Effect of SCMV on plant height, tillering and NMC (Co 740)	123
36.	Effect of SCMV on NMV and cane thickness (Co 740)	124

Plate No.	Title	Page No.
37.	Field view of healthy and SCMV infected canes (Co 740) a. Healthy b. Infected	132
38.	Effect of SCMV on number of millable canes (Co 740) a. Healthy b. Infected	133
39.	Effect of SCMV on cane thickness (Co 740) a. Healthy c. Infected	134
40.	Effect of SCMV on cane yield (Co 740)	138
41.	Effect of SCMV on plant height, number of millable canes and cane thickness in ratoon crop (Co 740)	144
42.	Induction of peroxidase isozymes due to application of biotic and abiotic agents against SCMV (third day)	155
43.	Induction of peroxidase isozymes due to application of biotic and abiotic agents against SCMV (sixth day)	156
44.	Induction of peroxidase isozymes due to application of biotic and abiotic agents against SCMV (ninth day)	157
45.	Induction of polyphenol oxidase isozymes due to application of biotic and abiotic agents against SCMV (third day)	161
46.	Induction of peroxidase isozymes in resistant and susceptible sugarcane genotypes against SCMV (third day)	174

Plate No.	Title	Page No.
47.	Induction of peroxidase isozymes in resistant and susceptible sugarcane genotypes against SCMV (sixth day)	175
48.	Induction of polyphenol oxidase isozymes in resistant and susceptible sugarcane genotypes against SCMV (third day)	179
49.	Induction of polyphenol oxidase isozymes in resistant and susceptible sugarcane genotypes against SCMV (sixth day)	180
50.	SCMV resistant sugarcane genotypes a. <i>Saccharum spontaneum</i> (IND 81-161) b. <i>Erianthus bengalense</i> (IND 81-47)	182
51.	Combined effect of antiviral chemotherapeutants and meristem culture on SCMV elimination a. Meristem in liquid medium b. Shoot initiation c. Emerged shoots	185
52.	Root induction of emerged sugarcane shoots in half MS solid medium a. Shoots in solid medium b. Rooting c. Rooted plants	186
53.	Hardening of tissue culture sugarcane plants a. Rooted plants in plastic cups b. Hardened plants in poly bags	187

INTRODUCTION

CHAPTER I

INTRODUCTION

Sugarcane (*Saccharum* hybrids) is one of the most important commercial crops contributing for 60 per cent sugar production in the world (FAO Report, 1999). It is an important commercial crop grown under a wide range of agro climatic conditions in more than 110 countries. Sugarcane cultivars currently planted throughout the world are inter-specific hybrids derived primarily from a few clones of four *Saccharum* spp viz., *S. spontaneum* L, *S. officinarum* L., *S. robustum* Brandes and Jesw.ex Grassl and *S. sinense* Roxb. (Arceneaux, 1967).

India stands first in sugar production with about 450 established sugar factories. The area under cultivation is 4.2 million hectares with 299 million tonnes of cane production and 18 million tonnes of white sugar (Anon., 2001). In India, it is the third largest crop in terms of value next to rice and wheat. About 100 diseases of sugarcane caused by fungi, bacteria, viruses, phytoplasma and nematodes have been reported from India and other countries (Rott *et al.*, 2000). Among all the diseases, mosaic disease caused by sugarcane mosaic poty virus (SCMV) is one of the most widespread diseases of sugarcane, having been recorded in virtually all the major sugarcane growing countries. The disease occurs in many parts of the world where susceptible varieties are grown and causes yield loss in sugarcane, sorghum and other poaceous plants (Shukla *et al.*, 1994). According to Viswanathan and Mohanraj (2001), among the viral pathogens of sugarcane, SCMV is an important virus and it occurs in almost all the sugarcane growing countries except Mauritius and Guyana. The virus belongs to the large and economically important potyvirus genus within the family potyviridae and has a linear polyadenylated single stranded RNA genome of approximately 10 kb size. The virions are flexuous filaments of approximately 750×13 nm with helical symmetry and contain a single genome molecule (Bannett *et al.*, 1995).

Experimentally, the causative virus can infect 298 species representing 109 genera in three families with the great majority of the hosts occurring in Graminae (Edwardson and Christie, 1991). The virus causes an yield loss of more than 50 per cent in South America (Costa and Muller, 1982) and up to 40 per cent in Australia (Smith *et al.*, 1992). According to Singh *et al.* (1997) many sugarcane varieties have gone out of cultivation due to yield losses of up to 50 per cent caused by SCMV. Agnihotri (1996) reported that SCMV causes an appreciable damage in susceptible varieties and even 10 to 15 per cent yield loss due to this disease is highly significant because of extensive cultivation of the crop. At Coimbatore conditions, the seedlings raised from fluff were free from the symptoms. However, three to four generations later, the clonal propagation materials exhibited the viral symptoms. Most of the sugarcane varieties under cultivation in Coimbatore show varying symptoms of the disease (Viswanathan and Mohanraj, 2001).

Number of SCMV strains have been reported from different parts of the world and were characterized based upon the differential host reactions in sorghum, sugarcane, maize and oat cultivars. Detailed studies about serology of coat protein, amino acid and nucleotide sequencing, biological properties and phylogenetic relationship of SCMV strains have led to the regrouping and currently it consists of four distinct potyviruses viz., sugarcane mosaic virus (SCMV), Johnsongrass mosaic virus (JGMV), maize dwarf mosaic virus (MDMV) and sorghum mosaic virus (SrMV) (Shukla *et al.*, 1994; Yang and Mirkov, 1997). According to Farrag (1978), SCMV strains A, B, C, D, E, F, H, I and M were present in Tamil Nadu. Whereas, Joshi (1989) reported the occurrence of SCMV strains A, E, F, H, I and K in India. Molecular characterization of the virus is necessary to determine the existing strains, their inter relationship and grouping and also for formulating the virus control measures based on biotechnological tools like pathogen derived resistance.

Standardization of diagnostic techniques especially at molecular level is essential while monitoring the exchange of germplasm materials to contain the entry of the virus and newer viral strains to a disease free area. The international exchange of

sugarcane germplasm for breeding and commercial production has played a major role in the development of sugar industry worldwide. Reports of asymptomatic plants containing virus are of considerable concern in quarantine operations, which rely on expression of symptoms for the diagnosis of disease. Both SCMV and Fiji Disease Virus (FDV) were of concern during the movement and quarantine of sugarcane germplasm into and within Australia as well as movement between and within many other countries (Smith and Van de Velde, 1994). The SCMV is an important virus among the different viral pathogens and it poses the most serious challenges in obtaining disease free seed cane and for germplasm movement (Viswanathan and Mohanraj, 2001). Hence, standardization of reliable and economical molecular diagnostic techniques against the prevailing strains of SCMV is very much required to monitor and to prevent the movement of virus within the country and also between the countries.

Disease control measures for preventing or reducing crop losses are aimed at the removal of virus sources and replacing with virus free planting stocks. Developments in plant tissue culture greatly assisted this process. The virus eradication from vegetatively propagated plants like sugarcane is comparable to virus eradication from seed propagated plants since a proportion of the progeny is expected to be virus free (Stace-Smith, 2000). Elimination of SCMV through meristem tissue culture and hot water treatment was reported (El-Nasr *et al.*, 1989). In recent years, antiviral chemicals are being exploited in combination with tissue culture for the elimination of different plant viruses. Since sugarcane is amenable for tissue culture, the meristem tip culture method can be improved in combination with other techniques like chemotherapy for the elimination of the virus in a better way.

Host plant resistance is the most important means of managing disease and crop losses caused by viruses. Chemical treatments, which are widely used against microbial pathogens of plants are either not available for diseases caused by plant viruses or are unsuitable for application at the crop level because of its expensive or phytotoxicity (Fraser, 2000). Use of biotic and abiotic means to induce host plant resistance against plant viruses is gaining momentum.

One of the key criteria of sugarcane breeding and improvement programme is to increase sugar yield. Significant improvements in sugar yield have been made since the initial development of the nobilization breeding system in the Dutch East Indies in the 1920's. It is very costly to discard agronomically elite clones late in the selection process due to susceptibility to pest or pathogens. In such circumstances, genetic engineering for the introduction of desired traits into sugarcane is an attractive option to rescue elite germplasm and avoid the loss of superior cultivars. There is good resistance to most sugarcane pathogens within the hybrid gene pool or within the larger gene pool of the '*Saccharum* Complex' (Smith and Harding, 2001). Hence it is very essential to exploit the resistance mechanisms available against SCMV among the wild forms of sugarcane, which can be further, utilized for the identification and incorporation of SCMV resistant genes and also for obtaining pathogen derived resistance.

In Tamil Nadu, after the preliminary studies on SCMV by Farrag (1978), no work related to virus purification, molecular characterization, strainal variations, virus elimination and exploitation of resistance mechanisms was carried out. Hence, work on the following objectives was carried out.

- a) Survey and assessment of SCMV incidence in major sugarcane areas of Tamil Nadu.
- b) Differentiation of existing SCMV strains by differential host studies, serological methods.
- c) Purification and molecular characterization of the widespread strain.
- d) Production of polyclonal antiserum.
- e) Standardization of molecular diagnostic techniques.
- f) Assessment of economic impact of SCMV on sugarcane and sugar yield.
- g) Effect of induced resistance against SCMV.
- h) Exploitation of resistance mechanisms against SCMV among wild forms and relatives of sugarcane.
- i) Elimination of SCMV through combination of chemotherapy and meristem tip culture.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1. Historical background and distribution of SCMV

2.1.1. History

Sugarcane mosaic disease was first recognized as an abnormality of sugarcane in Java and named it as “*gelestrepenziekte*” meaning yellow stripe disease (Musscherbroek, 1892). Subsequently, the disease was observed in Hawaii by Lyon (1908) and termed it as an “infectious chlorosis akin to the mosaic disease of tobacco”. The disease occurred in epiphytotic forms in Puerto Rico in 1916 as reported by Earle (1918) who first used the term “mosaic” for this sugarcane disease. Outbreak of this disease in Louisiana completely destroyed the sugarcane crop and adversely affected the sugar industry. This has stimulated remarkably the breeding programme for resistant varieties in USA and led to the establishment of the cane breeding station at Florida in 1918 and influenced the research programme of other breeding stations in the world especially in Java and Barbados (Barnes, 1974).

In India, after the first record by Dastur (1923) from Pusa (Bihar), Chona and Rafay (1950) studied the disease in detail. According to Bhargava (1975) as well as Rishi and Rishi (1985), SCMV occurred widely in India and strains A, B, D, E and F were more common. Kondaiah and Nayudu (1984) identified a previously unrecorded strain of SCMV-H in sugarcane cultivar Q 49 in Karnataka based on symptomatology on sugarcane and sorghum differentials.

2.1.2. Distribution

SCMV occurred in many parts of the world where susceptible varieties are grown and mosaic disease caused yield loss in sugarcane, maize, sorghum and other Poaceous plants. Gillaspie and Mock (1987) presented and compared the worldwide

distribution of different strains of SCMV occurred in Bangladesh (strains A, B, and J), Brazil (D), Mexico (A), Morocco (A), Pakistan (A), Republic of South Africa (F), Sri Lanka (A and F), Thailand (A and B), Uruguay (A and B) and Venezuela (B, D and J). Chen *et al.* (1988) observed that among the ten isolates of mosaic infected sugarcane from Guangxi and Fujian region, Taiwan, eight produced symptoms identical to those of SCMV A, one identical to SCMV D and one different strain which produced mild symptoms in all the plants tested including sugarcane and sweet sorghum and considered it as a new strain. Since 1950, sugarcane mosaic has been the most important disease in the Hualian district of Taiwan. Two strains of SCMV *viz.*, SCMV A and SCMV D were the predominant strains and the varieties NCo 310 and F177 were initially showing resistance but become susceptible to these strains later (Chen *et al.*, 1989).

According to Neen and Armitage (1988), there was a rapid increase in the incidence of sugarcane mosaic in the Natal Islands of South Africa. According to Smith *et al.* (1992), the yield loss in mosaic susceptible sugarcane cultivars in Queensland, Australia have led to the replacement of agronomically less suited but mosaic resistant cultivars. The SCMV was found to be the cause of maize common leaf mottle in Venezuela (Lastra and Tryzillo, 1976) and also in Israel (Klein *et al.*, 1973). The SCMV epidemics was recorded in countries such as Cuba, Puerto Rico, U.S.A and Brazil (Agnihotri, 1996). According to Comstock and Mirkov (1996), an outbreak of SCMV-E in Florida, U.S.A., affected the major commercial sugarcane cultivar CP72-2086. Gertel *et al.* (1997) observed that SCMV was the most prevalent virus infecting maize crop in Germany.

In India, Gopal and Reddy (1988) observed the reduction in plant height and weight of four sorghum cultivars tested and also reported that none of the cultivars were free from SCMV infection in a survey of sugarcane crops in two districts of Andhra Pradesh and Karnataka during 1986-1987. The incidence was ranging from 20 to 100 per cent. The virus was more closely related to SCMV B than to the other strains tested. The SCMV is present in all the sugarcane growing areas in India and

caused considerable economic losses (Varma, 1989). Gopal *et al.* (1991) observed that most of the sugarcane cultivars grown from Rayalaseema region of Andhra Pradesh were susceptible to SCMV-B. Singh *et al.* (1997) observed that many sugarcane varieties have gone out of cultivation in most of the sugarcane growing countries. During survey of poaceous crops in different regions of India from 1991 to 1996, the crops such as sugarcane, maize and sorghum were found severely infected with mosaic disease and it was widespread in many survey locations and caused serious damage. The incidence of infection varied from 2 to 30 per cent under field conditions (Rao *et al.*, 1998a).

2.2. Host Range

The virus infects wide range of hosts within the family Graminae with the exception of the abaca mosaic strain, which infects *Musa textiles*, a monocotyledon in the family Musaceae. Experimentally SCMV can infect 298 species in 109 genera of three families with the great majority of the hosts occurring in Graminae (Edwardson and Christie, 1991). Brandes and Klaphaak (1923) mentioned that SCMV infected cultivable crops viz., maize and sorghum. Abbott and Tippet (1964) reported that wheat, barley, rye, rice and other cultivated wild grasses were also infected by SCMV.

The SCMV caused common leaf mottle of maize in Venezuela (Ordesgoitty and Malguti, 1969) and Israel (Klein *et al.*, 1973). Rishi (1973) carried out mechanical inoculation of SCMV strains A and F in maize cv Deccan hybrid, and infection was reported to be maximum when both temperature and humidity were higher. In Australia, maize crop was mainly infected by Johnsongrass strain of SCMV and caused ring spot mottle disease (Teakle and Grylls, 1973). In Kenya, Louie (1980) reported the SCMV infection in maize in 21 districts with infection percentage ranged from 15.2 to 19.6. In maize cultivars Kalahari and Early Pearl, SCMV inhibited the germination and reduced the growth (Wechmar and Chauhan, 1984). Rishi *et al.* (1973) observed the natural infection of sorghum with SCMV for the first time in India. Benigno and Vaggara (1977) observed

that a strain of SCMV caused red stripe and mosaic in grain sorghum. Garud and Mali (1983) identified SCMV- Johnsongrass strains causing a red stripe virus disease of sorghum in India. Gopal *et al.* (1991) recorded that out of 25 species and varieties within the family of Graminae, all except four species became infected by SCMV-B when artificially inoculated.

Farrag and Kandasamy (1979) identified local lesion hosts for SCMV. Local lesions were developed on the 2nd trifoliate leaves of *Vigna radiata* plants, two days after the cotyledonary leaves had been inoculated. The lesions were irregular and purple while those developed on soybean were well defined and necrotic and appeared five days after inoculation. Seifers *et al.* (1993) found that SCMV strain MDMV infected eastern gamma grass (*Tripsacum dactyloides*) and higher incidence (12 and 72 per cent in 3 month old and 5 year old plantings, respectively) in experimental breeding nurseries in Kenya.

The SCMV also infects bajra (*Pennisetum typhoides*), Johnsongrass (*Sorghum halepense*), ragi (*Eleusine coaracana*), bamboo (*Arundinaria gigantea*) and elephant grass (*Pennisetum purpureum*) (Rishi *et al.*, 1973). Joshi and Gupta (1976) reported that *Digitaria adscendons* as another weed host of SCMV. Grasses namely *Rottboellia exaltata* in U.S.A (Gillaspie and Koike, 1973). *Dicanthium annulatum* to be in India (Sadraddin *et al.*, 1981) and 55 grasses from Mississippi were found susceptible to SCMV (Rosenkranz, 1987).

2.3. Yield Loss

The virus causes varying impact on germination, quantity and quality of sugarcane as well as white sugar. Loss of cane yield, sucrose quality and sugar recovery was reported by different workers in many countries especially in susceptible varieties. Further, susceptibility of agronomically important varieties to SCMV resulted in their removal from cultivation.

2.3.1. Germination

Sugarcane being a vegetatively propagated crop the pathogens including SCMV build upon the cane materials. When such infected materials were used for planting, a reduced germination was noticed. Ahmed *et al.* (1991) in Pakistan compared SCMV infection and further growth of sugarcane plant. They found that SCMV infection reduced the sett germination by 5 to 11 per cent and plants from such infected setts recorded about 1.34 less tillers as compared to plants from healthy setts.

2.3.2. Cane yield

Several workers have established the impact of SCMV infection on sugarcane yield from time to time. Yield reduction of 3-33 per cent and 9-17 per cent was reported in sugarcane cultivars Co 281 and Co 290 respectively depending on the strain of the SCMV infection (Summers, 1934). In Texas region of USA, cane yield reductions of 30 and 36 per cent were observed in naturally infected NCo 310 and CP 52-68 respectively. Infected plants also exhibited reduced stalk height and weight (Villalan, 1980). Severe type of mosaic disease (yellow) reduced the cane yield to a greater extent than mild type (green) on clones BO 67, BO 70, Co 975 and I-112 / 67 in Bangladesh (Rahman *et al.*, 1981). In cultivars Isd. 2154, BO 70 and Co 1148, Rahman *et al.* (1990) observed the yield reductions at the rate of 21.4, 14.5 and 34.5 per cent respectively when compared to healthy crops. More than 50 per cent yield loss had been estimated in South America (Costa and Muller, 1982). In Spain, yield loss of 0.4 - 0.5 t /ha was observed for every 1 per cent SCMV infection when the healthy sugarcane plants were planted between infected rows (Olallamercade *et al.*, 1982). Early studies in India indicated that even 100 per cent infection reduced the cane yield by only 10 per cent. Considering the vast area under sugarcane, even less than 10 per cent loss in yield is enormous (Varma, 1989). In Taiwan, Chen *et al.* (1989) estimated the cane yield loss of 24.2 and 30.8 per cent in F 177 and F 178 varieties respectively. Ahmed *et al.* (1990) in Pakistan observed 21.39 per cent decrease in tillers/plant in SCMV infected cultivar Co 975. The SCMV infection decreased the cane length, number of internodes and cane thickness at the rate of 15.63, 24.63 and 17.45 per cent

respectively. Yield loss was 14.31 and 20.61 per cent in the first year and ratoon crop respectively. Seedlings from SCMV free seeds yielded 48.51 t/ha compared to 44.50 t/ha from infected seeds. Mosaic free canes were superior to infected canes in length, number, thickness and length of internodes.

2.3.3. Cane quality

In SCMV infected cultivar Co 975, juice weight and sucrose content were decreased by 4.28 and 2.06 per cent respectively in the plant crop and 5.33 and 1.44 per cent respectively in the ratoon crop. Sugar recovery in the mosaic free cane was 11.8 per cent higher than mosaic infected crop (Ahmed *et al.*, 1990, 1991). Singh *et al.* (1997) observed general reductions in juice quality, sucrose content and purity coefficients in the infected sugarcane.

2.4. Molecular characterization of SCMV and strains

The term 'sugarcane mosaic virus' was used as a general term covering four groups of the viruses *viz.*, sugarcane mosaic virus (SCMV), maize dwarf mosaic virus (MDMV), Johnson grass mosaic virus (JGMV) and sorghum mosaic virus (SrMV). This larger group of viruses was found to include 13 strains of SCMV from the United States (SCMV A to M) and eight strains of MDMV (A to F, KS1 and 'O'). Within Australia, four viruses were originally defined, Johnson grass mosaic virus (SCMV JG, reclassified as JGMV), Sugarcane mosaic virus (SCMV-SC), Queensland blue couch grass (SCMV BC), and Sabi grass (SCMV Sabi) with further strains of SCMV (Brisbane, Isis and Bundaberg) introduced later.

Differential host reactions on sugarcane, sorghum, maize and oat cultivars as well as in Johnson grass were widely used for strain differentiation. Serological reactions and cytoplasmic inclusions were also used for the grouping of strains. However, confusion related to strainal variation, overlapping of groups *etc.* lead to molecular characterization of strains at coat protein and nucleic acid level. Combination of different characteristics leads to regrouping of SCMV strains.

Shukla *et al.* (1994) compared the strains of SCMV in detail. Based on the differential host reactions in sorghum, sugarcane and oat cultivars (Tosic *et al.*, 1990), aminoterminal serology (Shukla and Ward, 1989), cell free translation of RNA (Berger *et al.*, 1989), morphology and serology of cytoplasmic inclusions (Lesemann *et al.*, 1992), aminoacid sequence (Shukla *et al.*, 1987) and peptide profile of coat proteins (Mckern *et al.*, 1991) comparisons were made. Based on the above findings it was concluded that four distinct potyviruses namely SCMV, JGMV, MDMV and SrMV comprised the sugarcane mosaic potyvirus subgroup. The relationships among different isolates of SCMV were recently examined at a phylogeneic level using sequences derived from the NIB (replicase) and coat protein coding regions (Handley *et al.*, 1996, 1998; Yang and Mirkov, 1997).

2.4.1. Differential host reactions and strainal variation

Strains of SCMV were first differentiated by their reaction on sugarcane clones by Summers (1934). The sugarcane clones CP31-294, CP31-588, Co 281 and sweet sorghum cultivar 'Rio' were generally utilized for this purpose. A comprehensive account of strain identification was earlier discussed by various workers (Summers *et al.*, 1948; Abbott and Tippet, 1966 ; Tippet and Abbott, 1968; Zummo and Stokes, 1973; Koike and Gillaspie, 1976). Ramallo (1981) identified strains A, B and F on commercial sugarcane cultivars using symptomatology on three differential cultivars. Kondaiah and Nayudu (1984) developed the key for identification of SCMV strains based on the reactions in sugarcane (Co 281, CP 31-294 and CP 31-588) and sweet sorghum (Atlas, Rio and Johnson grass) differentials. Tosic *et al.* (1990) differentiated sugarcane, maize dwarf, Johnson grass and sorghum mosaic viruses based on reactions on a set of oat and sorghum cultivars. JGMV alone infected oats. Strains within MDMV and JGMV reacted uniformly in sorghum whereas SCMV differed in reactions on some sorghum cultivars and SrMV strains differed on two sorghum cultivars. Leon *et al.* (1991) compared Cuban isolates and type races of sugarcane mosaic virus in sorghum inbred lines under controlled conditions, by using reactions on 19 sorghum hybrid lines as differential hosts.

2.4.2. Serogrouping of SCMV strains .

Serological relationship among the Johnson grass (JG), sugarcane (SC), Queensland blue couch grass (BC) and Sabi grass (Sabi) strains was studied by using the two variations of immune electron microscopy. It was found that the Johnson grass strain (JG) was not related serologically to the other three strains whereas, BC and Sabi were very closely related to each other (Shukla and Gough, 1984). Jarjees and Uyemoto (1984) compared the serological relatedness of strains of MDMV and SCMV by micro precipitin test and ELISA. Accordingly, the strains MDMV A and SCMV J comprised one sero group; MDMV KSI, second; MDMV-B and SCMV A, B and D, third and SCMV H, I and M, fourth group. Gillaspie and Harris (1987) separated the SCMV strains into different groups based on serological relatedness. a) SCMV J, b) SCMV A, B, D, K, c) SCMV E, d) SCMV H, I and M. It was concluded that serological method couldn't be used independent of differential host tests to identify individual SCMV strains. Chen *et al.* (1987) in Taiwan observed that sugarcane mosaic virus strains A, B, D and S-1 comprised one sero group, MDMV from strain 'B' second group and SCMV isolate from *Paspalum conjugatum* third group. It was concluded that the strains A, B and D comprised one group and was different to MDMV B.

Shukla *et al.* (1989 a, b) compared 17 strains of SCMV and MDMV from Australia and United States based on their reactivity with polyclonal antibodies directed towards surface located, virus-specific N-termini of coat proteins. This led to grouping of the SCMV/MDMV strains into four distinct potyviruses rather than strains of the same virus as originally proposed. The four distinct viruses were sugarcane mosaic virus (MDMV B, SCMV A, B, D, E SC, BC and Sabi), maize dwarf mosaic virus (MDMV A, D, E, F), Johnson grass mosaic virus [JGMV (SCMV JG), MDMV O and MDMV KSI (JGMV MDKSI)] and sorghum mosaic virus (SCMV H, I, M). Hema *et al.* (1997) in Andhra Pradesh purified the potyvirus causing mosaic disease on commercial sugarcane. The purified virus reacted positively only with the heterologous antiserum of a potyvirus isolated from sorghum and not with the strains of SCMV.

Tosic *et al.* (2001) concluded that MDMV, SCMV, JGMV and SrMV and their strains were serologically overlapping with each other in different ways, but they could be differentiated by use of more specific or cross absorbed antisera. Recently, Rao *et al.* (2001) reported that SCMV isolate from Uttar Pradesh was serologically similar to SCMV A, SCMV MDB and two SCMV isolates from Thailand after the studies involving DAC-ELISA, EBIA, DIBA and ISEM.

2.4.3. Coat protein and nucleic acid

Gough and Shukla (1981) examined the molecular weight, amino acid composition and tryptic peptide map of the coat protein of four Australian sugarcane mosaic virus strains *viz.*, JG, SC, BC and Sabi. The proteins migrated as a single band with molecular weights of 33.7, 34.2, 39.1 and 40.3kDa. The BC and Sabi strains have identical aminoacid compositions and tryptic peptide maps. The SC strain has a similar aminoacid composition and tryptic peptide map but those two properties of the JG strains showed very little similarity with three other strains. Rao *et al.* (1998b) reported that the molecular weight of the capsid protein of an Indian SCMV isolate purified was 34 kDa. Shukla *et al.* (1987) observed the coat protein of the Johnson grass (JG) strain of SCMV contained 303 aminoacid residues corresponding to the molecular weight of 33.51 kDa and it was found to have additional residues at its N-terminus when compared with the coat protein of some other potyviruses (263-267 residues). Partial aminoacid sequences of the coat proteins of the other three Australian SCMV strains *viz.*, SC, BC and Sabi showed that JG coat protein was subsequently different. The sequence homology being less than 66 per cent and concluded that the JG strain should not be considered as the strain of SCMV but regarded as an independent member of the group. Frenkel *et al.* (1992) used PCR methodology to amplify cDNA to the 3' non-coding region. The primers used correspond to the 18-20 nucleotide sequence immediately downstream of the stop codon and immediately upstream from the poly (A) tail. These cDNAs were used successfully in hybridization studies to discriminate between related strains and distinct species. On comparison of coat proteins of seven strains of SCMV with different host ranges, they were shown to be very similar except

for the presence of hypervariable regions of different sizes (21-68 aminoacids) at the surface exposed N-terminal region (Frenkel *et al.*, 1991; Xiao *et al.*, 1993).

Lavastida and Leon (1993) reported that the molecular weight of the two Cuban isolates of SCMV was 3×10^6 Daltons as determined by urea and heat denaturation and was within the reported value rates for other isolates and strains of SCMV. Yang and Mirkov (1997) developed group specific primers for use in RT-PCR based RFLP analysis for rapid discrimination between strains of SCMV and SrMV. This method did not require interpretation of symptoms on differential hosts. The amino acid sequence identity of coat protein was compared for SCMV strains *viz.*, A, B, D, E, SC and MDB with MDMV A, SrMV strains *viz.*, SCM, SCH and SCI and JGMV strains *viz.*, MDO and MDSK1. Phylogenic analysis of the nucleotide sequence of the entire coat protein coding region showed that in all cases, the strains of the virus were grouped together with the exception of SCMV MDB. The amino acid sequence identity within the SCMV (96-100%) and SrMV (98-100%) groups was high with the exception of SCMV MDB, which was only 83-86 per cent identical to the other SCMV sequences.

Sequence of the entire NIB-coding region of twelve samples, nine from various locations within Australia, two isolates of SCMV-A from the United States and one from South Africa were identical between 94.9 and 99.8 per cent (Handley *et al.*, 1996, 1998). Handley *et al.* (2001) proposed a further grouping of SCMV strains. As per that, the SCMV MDB should be renamed as MDB MV and made as a type member. SCMV BC might be included in this group and this formed as the fifth virus under SCMV groups along with SCMV, MDMV, SrMV and JGMV. Likewise, SCMV Sabi and SCMV Bundaberg can be tentatively grouped together as distinct undefined group of viruses, which do not infect sugarcane.

2.5. Electron Microscopy of the SCMV

Different workers carried out electron microscopic studies about the size and structure of nucleoproteins in detail. Gold and Martin (1955) studied the virus particles associated with sugarcane mosaic virus by electron microscopy and indicated rod

shaped particles measuring 630 x 15 m μ in size. Paliwal and Raychaudhari (1966) reported that rod shaped virus measuring 339-964 nm x 27-35 nm as a strain of SCMV causing maize mosaic in India. Tosic *et al.* (1978) from Italy described the SCMV as typical potyvirus with 750 nm length. Chen (1978) from Taiwan reported that the SCMV particles were long flexuous rods mostly 750x13 nm in size. Liu (1979) reported that SCMV strains A and F in Puerto Rico, measured 720-1446 x 14-15 nm and 720- 1730 x 14-15 nm respectively. In Australia, Moghal and Francki (1981) observed 750 nm as the mean particle length of SCMV. Rishi and Rishi (1985) in India, reported the length of SCMV A and F strains between 670 and 770 nm. Giorda *et al.* (1986) observed 706 nm length of SCMV H strain isolated in Texas from grain sorghum. Teakle *et al.* (1989) reported that the SCMV particles were 750 x 13nm. Rao *et al.* (1998c) observed 700-755 nm length for the majority of SCMV and MDMV virus particles.

2.6. Physiological alterations in response to SCMV infection

The SCMV incidence affects the physiology of sugarcane in many ways. It alters the starch and chlorophyll content, sugar levels etc. Enzymes involved in various metabolisms are also altered there by reducing the energy content. Phenyl propanoid metabolism is also affected by SCMV and influencing the resistance and susceptibility.

Shukla and Joshi (1979) observed that SCMV infected plants always had a lower energy content comparable to healthy ones. The virus infection reduced the activities of pyruate and NADP-malicdehydrogenase, while those of aspartate aminotransferase were stimulated (Ghorphade and Joshi, 1982). A higher amount of reducing sugars, starch and lesser non-reducing sugars in leaves and nodes were reported for plants infected with SCMV. These changes were correlated with decreased activity of sucrose synthetase, sucrose phosphatase and high starch synthetase and acid invertase activities in the infected tissues and continued vegetative growth of the infected plant. The total chlorophyll content was less in virus infected leaves (Sreenivasulu *et al.*, 1985).

Adenosine triphosphatase (ATPase) activity was ultra structurally localized in SCMV-A infected leaves of seven cultivars of sugarcane. In the inoculated plants, chlorophylls were almost completely degenerated in the mesophyll in chlorotic lesions, and bundle sheath cells were filled with starch particles. Variation in ATPase activity was probably related to the resistance or susceptibility of sugarcane cultivars to the virus (Pan *et al.*, 1989). In the SCMV infected cultivars, the activities of the Ca^{2+} -ATP enzyme and Mg^{2+} - ATP enzymes were higher in inoculated plants than in leaves of non-inoculated plants 70 days and 100 days after SCMV infection. The total chlorophyll 'a' and 'b' contents were lower in the leaves of inoculated plants than in healthy plants 50 and 70 days after inoculation. Contents of soluble protein, K, Ca and Mg were also higher which indicated that the resistance of sugarcane plants was affected by the nutrient contents (Hong and Shi, 1991).

In studies with four wild types of sugarcane, higher phenolic contents were detected in the varieties resistant to SCMV than in the susceptible ones. The resistant varieties also had phenolic compounds, which were absent in the susceptible ones, which indicated the involvement of phenolic compounds in the mechanism of plant response to infection (Molina *et al.*, 1993). A significant decrease in photosynthetic activity and pigment content (chlorophyll a, b and carotenoids) in SCMV infected susceptible (B34-104 and CP 31-294) and the intermediately susceptible (PR-980) varieties were observed. However, there was no change observed in resistant plant MY- 53174 (Acevedo *et al.*, 1993).

There were significant alterations in the height of the SCMV infected plants and correlation was found with changes in enzymatic activities. There was an increase in the nitrate reductase activity in the susceptible varieties (PC21-294 and PR-980) immediately after inoculation. The peroxidase activity in the susceptible variety increased with time after inoculation. The resistant plants did not show changes in height and enzymatic activity (Pinon and Sanchez, 1993). According to Singh *et al.* (1997), total chlorophyll and carbohydrate content decreased and nitrogen, protein and

phenolic content increased in infected leaves. Activities of peroxidase, nitrate reductase and polyphenol oxidase were higher in the infected leaves.

2.7. Diagnosis of SCMV

Standardisation of diagnostic techniques is imperative for monitoring and management of plant viruses especially for the sett borne viruses like SCMV. Selection of virus free materials for planting is one of the basic strategies for the management of plant virus diseases. Moreover, reliable and precise diagnostic techniques form the basis in the quarantine operations. Being sett borne in nature, SCMV can spread very easily into disease free area. Antisera to SCMV had been widely used in precipitin tests, immuno diffusion tests, immunosorbant electron microscopy, ELISA and Western blott for the detection of virus.

2.7.1. Polyclonal antiserum based techniques

The sandwich type ELISA method was used by Devergne *et al.* (1982) to detect 1ng/ml of purified virus (SCMV D). The method revealed the latent infections so that the resistance against SCMV in young seedlings can be determined. Shukla *et al.* (1983) used electoblot radio immuno assay (EBRIA) to detect plant viruses and detected as low as 0.5 ng of virus. EBRIA could probe a paper containing 8 virus samples using 5 μ l antiserum. Rishi and Rishi (1985) purified SCMV- A and F and produced polyclonal antisera. The titre of the antisera produced was 1/2048. The virus was detected in leaves, rind, buds and roots of sugarcane by ELISA method. The virus concentration was maximum in youngest leaves and minimum in roots. Hewigh *et al.* (1986) used biotin conjugated antisera of 2 strains of sugarcane mosaic virus, which proved sufficiently sensitive to detect the viruses in extracts of infected plants using ELISA and electro blot immunoassays. Virus strain specificities observed for the antisera agreed with those found in electron microscopy and electro blot immunoassays. The biotin- avidin system offered several advantages over other current methods of antibody labelling notably in speed of development and versatility. Ultra micro DAS - ELISA was demonstrated by Alfonso and Peralta (1993) and claimed that

the method even detected one leaf sample in a group of 5, 15, 30 and 50 with reliability of 100 per cent and the procedure can be used for routine diagnosis. Viswanathan (1997) detected the SCMV even at an antigen dilution of 1×10^{-5} in highly susceptible varieties using an indirect-ELISA method. Polyclonal antisera developed against SCMV strain-N were also suitable for other SCMV strains. Rao *et al.* (1998b) revealed that polyclonal antibodies from an early (3-4 weeks-As) and late (6-8 weeks-As) bleeds developed against an Indian isolate of SCMV, reacted strongly only with homologous antigen and no reaction was obtained with many other potyvirus.

2.7.2. Monoclonal antibodies

Monoclonal antibodies had been produced to the MDB strain of SCMV by Hill *et al.* (1984). Peralta *et al.* (1991) obtained hybridomas secreting monoclonal antibodies to sugarcane mosaic potyvirus by fusion of splenocytes from Balb/c mice with myeloma SP₂/O cells.

2.7.3. Immunosorbant electron microscopy

Gough and Shukla (1980) studied the use of protein A in immune electron microscopy and found that pre coating the electron microscopic grids with protein A before coating them with specific antiserum enhanced the number of SCMV particles at each optimum antiserum dilution by 25.7 fold over those grids treated with specific antiserum alone. Further, the protein A coated grids can be stored for up to 6 months at 4°C but not at room temperature. Acevedo *et al.* (1987) developed a rapid method of electron immuno microscopy in the diagnosis of sugarcane mosaic virus. Diseased leaves from sugarcane cultivars B.34104 were cut and added with phosphate buffer (0.1M, pH 7.6) at a rate of 3 ml/g and incubated for 5 min for sensitization, 15 min for antigen application and 15 min for decoration at 22°C. By this technique, the sample could be prepared in 40 min.

2.7.4. Polymerase chain reaction

Smith and Van de Velde (1994) developed an RT-PCR (Reverse Transcription Polymerase Chain Reaction) for detection of sugarcane mosaic potyvirus and Fiji disease causing virus (FDV) from total nucleic acid extracts in the diseased sugarcane.

2.8. Management

Sugarcane varieties respond in a different manner for SCMV infection. Growing resistant varieties may solve the problem of the virus in the field. The SCMV epidemics on sugarcane in different sugarcane growing countries resulted in replacement of the agronomically important varieties and caused serious crisis in sugar industry. The problem necessitated formulating breeding programmes for the development of resistant varieties against SCMV. Different species of *Saccharum* complex and related wild cultivars are being exploited for incorporating SCMV resistance. In recent years, pathogen derived resistance is gaining momentum against SCMV like other plant viruses especially coat protein mediated resistance.

2.8.1. Resistant varieties

Although the varieties PR-1141, PR-1152, PR-62-285, PR-64-1548, PR-65-153, PR-65-2538, Q-68 and Soller were infected with the strains of SCMV A, B and D in Puerto Rico, no field symptoms were recorded in the varieties and were accepted for planting (Liu, 1979). Olalla Merçade *et al.* (1982) in Spain identified the cultivars CP 44-101 and Co 1148 as practically immune, while Co 62175 and NA-56/79 as sufficiently resistant for commercial production. Zhou *et al.* (1989) observed that *Saccharum spontaneum* was immune to SCMV. Among the *S. officinarum* varieties, Mintang 70/611 and Guitang 11 were resistant whereas, Fuyin 79/9, F134 and Minxian 703 were moderately resistant to SCMV strain A. The variety Fuyin 79/8 was susceptible and NCo 310 and Co 740 were highly susceptible.

In Taiwan, the sugarcane varieties F160, ROC 1, ROC 7, ROC 10 and ROC 11 were resistant to mosaic disease while NCo 310 and F177 were susceptible (Chen *et al.*, 1989). Among the different *Saccharum* species and relatives tested against SCMV-H to assess the resistance, it was found that *Erianthus*, *S. spontaneum* and *S. barberi* / *S. sinense* clones were the resistant and *S. robustum* clones were the susceptible while the interspecific hybrid and *S. officinarum* clones were intermediate. Grisham *et al.* (1992) and Lodge *et al.* (1993) observed that virus resistance from other plant species such as pokeweed may provide resistance to viral pathogens of sugarcane. Neen and Armitage (1988) formulated a programme for the control of sugarcane mosaic in South Africa. The programme involved monitoring of mosaic levels in individual fields, careful roguing of diseased plants from fields of plant cane, use of improved quality in seed cane, planting in early spring and introduction of varieties less susceptible to mosaic than those previously grown on the farm. Such practices resulted in reduced mosaic levels over a five year period.

2.8.2. Pathogen derived resistance

Smith *et al.* (1992) first demonstrated that coat protein constructs of SCMV-SC were expressed in a sugarcane protoplast cell system and these constructs were subsequently used to transform sugarcane calli. Microprojectile bombardment was used by Smith and Gambley (1993) to transform meristematic tissues and callus with plasmids containing the SCMV- coat protein (CP) gene which conferred resistance to the virus and lines regenerated from the transformed and selected calli resisted mechanical challenge inoculation (Joyce *et al.*, 1997a, b). The transformation frequency was higher in the calli and SCMV-CP gene was detected in the regenerated plantlets by polymerase chain reaction. The NIB region (known as nuclear inclusion protein b) from nine Australian SCMV-SC isolates had been cloned and sequenced (Handley *et al.*, 1996) and constructs developed for sugarcane transformation (Joyce *et al.*, 1997 a, b). According to Mirkov *et al.* (1997) sugarcane plants containing a CP construct derived from SrMV-H resisted subsequent infection by SrMV strains H, I and M but not SCMV strains A, D or E. Smith and Harding (2001) observed that CP

coding region of the potyviruses has been widely used for pathogen derived resistance as the coding region is relatively easy to clone, being the last coding region of the viral RNA genome.

2.9. Induced resistance against plant viral diseases through biotic and abiotic agents

Certain biotic and abiotic agents can induce defense mechanisms present in the host plants against fungal, bacterial and viral pathogens. The method not only makes the susceptible varieties to behave somewhat resistant but also for identifying defense genes and their related defense response mechanisms. Against plant viruses, induced resistance is gaining momentum in recent years.

White (1979) observed that salicylic acid decreased the disease development caused by tobacco mosaic virus in the tobacco cultivar Xanthi-nc. Maurhofer *et al.* (1994) reported that induced systemic resistance (ISR) by *Pseudomonas fluorescens* strain CHAO against tobacco necrosis virus (TNV) in tobacco was associated with accumulation of pathogenesis related (PR) proteins namely β -1,3 - glucanase and endochitinases. They also established that the ineffective strain P3 did not accumulate PR proteins in the induction of resistance. Induction of systemic resistance by plant growth promoting rhizobacteria (PGPR) against viral diseases was reported in cucumber and tobacco plant. Seed treatment with *P. fluorescens* strain 89B-27 and *Serratia marcescens* strain 90-166 had consistently reduced the number of cucumber mosaic virus infected plants (CMV) and delayed the development of symptoms in cucumber and tomato (Raupach *et al.*, 1996). Soil application of *P. fluorescens* strain CHAO had induced systemic protection against inoculation with tobacco necrosis virus (TNV) in tobacco. Introduction of Pch A and Pch B gene which encoded for the synthesis of salicylic acid in *P. fluorescens* strain P3, rendered this strain capable of salicylic acid production and significantly improved its ability to induce systemic resistance in tobacco against tobacco necrosis virus (Maurhofer *et al.*, 1998).

Zehnder *et al.* (2000) identified PGPR strains (*Bacillus amyloliquefaciens*, *B. pumilus* and *B. subtilis*) that protected tomato against systemic infection by cucumber mosaic virus under greenhouse and field conditions. The percentage of symptomatic plants in the most effective PGPR treatments ranged from 32 to 58 per cent when compared with 88 to 98 per cent in the non bacterized challenged disease control treatment. Murphy and Zehnder (2000) reported that up to 40 days after transplanting under natural conditions of high levels of vector-virus pressure, some PGPR treatments (*Bacillus amyloliquefaciens* 937 a, *B. subtilis* 937 b and *B. pumilus* SE 34) resulted in reduced tobacco mottle virus incidence and disease severity and in some cases a corresponding increase in fruit yield and suggested that the use of PGPR could become a component of an integrated program for the management of this virus in tomato. Benzol (1,2,3) thiadiazole derivative (Kunz *et al.*, 1997) with S- methyl benzol [1,2,3] thiadiazole-7-carbothiate (acibenzolar-s-methyl) (ASM) was the first commercial product marketed under the trade names BION, ACTIGARD™ and Boost^R. Reduction of disease symptoms under field conditions in tomato spotted wilt virus, tobacco mosaic virus, cucumber mosaic virus and potato virus Y was reported when BION^R/ ACTIGARD™ were used at 12.37G ASM per ha ((Metraux *et al.*, 1991; Oostendorp *et al.*, 2001).

2.10.Virus elimination

Elimination of plant viruses is possible through combination of different techniques. Viral inhibitors of plant origin and chemicals were used to eliminate the virus from the host plants. Later, advancements in tissue culture technique improved the chances for virus elimination. Meristem tip culture is one such technique that is being widely followed for plant virus elimination and to obtain virus free stock material. Antiviral chemicals are being used in different ways to obtain virus free plants. Heat inactivation of viruses, otherwise called thermotherapy is also one such technique for virus elimination. Combination of chemotherapy and thermotherapy along with tissue culture can improve the virus elimination. For sett borne viruses like

SCMV, standardization of virus elimination techniques will be highly useful not only for the farmers but also for the researchers since the virus free elite stocks can be exchanged for breeding programmes.

2.10.1. Plant inhibitors

Many plant extracts and the base analogues viz., azaguanine and thiouracil were found to inhibit virus biosynthesis and nucleic acid replication (Francki and Matthews, 1962). Joshi and Prakash (1978) revealed that extracts of ginger caused an average of 70 per cent inhibition against of SCMV- A, followed by turmeric, garlic, onion and potato in the indicator hosts when applied 8 hr after inoculation. *Cassia auriculata* was the most inhibitory plant extract against SCMV out of 26 plant extracts tested (Gopal and Reddy, 1988). Molina and Leon (1991) evaluated the antiviral activity of leaf extracts of *Bougainvillea spectabilis*, *Capsicum annum*, *Datura metal* and *D. stramonium* against SCMV in sorghum variety TX412. All these extracts had a highly inhibitory effect on SCMV. The best time for extract application was before or simultaneously with virus inoculation, although extracts from *D. stramonium* and *B. spectabilis* were also highly inhibitory when applied after SCMV inoculation. Molina and Sanchez (1993) recorded the effectiveness of leaf extracts of *D. stramonium* for the control of SCMV and the phytochemical analysis revealed the presence of alkaloids, proteins and steroids in leaves. Results evaluated by symptom observation and double antibody ELISA indicated that proteins played the most important role in symptom inhibition.

2.10.2. Chemical inhibitors

Shukla (1978) reported 90 and 80 per cent inhibition with tungstosalicylic acid and phosphotungstic acid both at 1mg/ml respectively. Shukla and Joshi (1979) obtained maximum inhibition against SCMV at 1000 ppm with mono-tri and polyhydroxy phenolic acids while the dihydroxy acid was inhibitory at 10 ppm and chlorogenic acid had no effect at any tested concentrations.

2.10.3. Thermootherapy

Treatment of sugarcane with aerated steam at 56 to 57°C for 2 to 5 hr effectively controlled sugarcane mosaic virus and ratoon stunting but reduced the germination in varieties L.62-96, CP 65-357, NCo 310 and L. 61-67. Field germination of treated cane was far superior to that of single eyepieces in the glasshouse (Steib and Cifuentes, 1977). Mosaic control was obtained by Benda *et al.* (1989) after following the serial hot water- treatment of infected sugarcane cultivars. The mosaic infected (strains A, D, F or H) setts were serially submerged in hot water (51.5, 59, 61 and 63°C) for 0 to 20 min at 20 to 24 hr intervals. Increasing the treatment duration or temperature increased the severity of the serial treatment. Beyond a threshold, mosaic control increased with treatment severity up to a point where the proportion of surviving plants decreased with increasing severity.

2.10.4. Tissue Culture

Hendre *et al.* (1975) obtained more than 90 per cent of SCMV free plants from the virus infected Co 740 variety after growing it aseptically on modified MS liquid medium and subsequently on modified White's medium. Explants taken from SCMV infected apical meristem and regenerated on MS medium were free from the virus. Of the 55 plants regenerated from the callus, 53 were free from the virus as indicated by lack of symptoms and bioassay on sorghum. Bioassays of spindle tissues similar to those of the explants showed a steep infectivity (Dean, 1982). Fahmy (1990) obtained sugarcane sub clones resistant to SCMV strains A, B, D, H and I from the susceptible commercial sugarcane cultivars Co 413 and 54 C- 9. According to Irvine and Benda (1985), explants developed on MS medium with cytokinin and auxin from immature leaf tissues of sugarcane cultivars CP 72-356 (*Saccharum* interspecific hybrid), healthy or SCMV infected were assayed for the presence of virus on 'Rio' sorghum and CP 31-294 and CP 31-588 sugarcane cultivars. Results indicated that 23 per cent of regenerated plantlets showed the presence of SCMV H. When leaf tissue of POJ 234

free from SCMV or infected with strains *viz.*, A, B, D, H or I was cultured, each of the strain was transmitted from donor to plantlets. About 11-88 per cent of plantlets showed symptoms depending on the infecting strain.

In China, the incidence of SCMV varied from 0 to 4.6 per cent in clones cultured from MS medium when compared to donor plants in Co 6304 variety (Xu *et al.*, 1994). Somoclonal variants resistant to sugarcane mosaic potyvirus (SCMV) were obtained from the susceptible cultivar PR 62258 through somatic embryogenesis by increasing the number of subcultures of embryogenic callus in MS medium supplemented with 3 mg 2,4-D/ litre (Orepeza *et al.*, 1995).

2.10.5. Combination of thermotherapy and tissue culture

Apical meristem tissues after treatment at 52°C for 10 or 20 min and cultured on Vacin and Went medium supplemented with 25 per cent coconut milk were used to obtain healthy plants from SCMV, ratoon stunting disease, and white leaf or chlorotic streak virus. An excised apical dome of 2-3 mm above and 0.7 to 1 mm below the growing point was effective (Lev, 1978). According to El-Nasr *et al.* (1989), the modified Van Hoof medium was suitable for the growth of sugarcane apical meristems to obtain plants free from sugarcane mosaic virus. The best results (40%) were obtained with hot water at 55 and 57°C starting two days after harvesting.

2.10.6. Combination of tissue culture and chemotherapy

Treatment with antiviral chemicals reduced or completely prevented virus replication in the growing parts of the meristem and therefore permits the development of fully virus-free tips. These tips can then be removed and be grown into whole virus free plants. The major advantages of the meristem method for virus elimination were the availability of large tips, which had an excellent survival ratio when transferred to normal medium, and the predictability of the therapeutic success, which reduced the need for time consuming and costly post-treatment virus testing (Hansen, 1989).

Ribavirin had been found to be effective against atleast 16 plant viruses. Virus free shoots can be produced on meristems by incorporation of 20 to 50 ppm of the compound into the medium, and 10 ppm was sufficient to reduce infection of protoplasts to a minimum (Hansen, 1989). The purine-base analogue, 8- Azaguanine showed a delayed replication of alfalfa mosaic virus and cucumber mosaic virus and retarded the development of systemic infection and symptom development. Azaguanine replaced three per cent of the guanine in the nucleic acid and this incorporation greatly reduced the infectivity of the virus. 8-Azaguanine was generally too phytotoxic to be considered as a chemotherapeutant, eventhough it inhibited cowpea chlorotic mottle virus, tobacco mosaic virus, prunus necrotic ringspot virus and several other viruses (Hansen, 1989).

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1. Survey and assessment of SCMV incidence in Tamil Nadu

Major sugarcane growing areas of Tamil Nadu were assessed for the SCMV incidence. Major sugarcane varieties grown in the field and maintained in different sugar factory areas were assessed for the presence of characteristic SCMV symptoms. The sugarcane varieties maintained at National Hybridisation Garden, Sugarcane Breeding Institute, Coimbatore were also surveyed during the year 1999 for the presence of mosaic infection. Based on the intensity of symptoms, SCMV incidence was categorized as mild or severe. Mild symptoms included, mild mottling and mild streaks in younger leaves preferably in the top four to five leaves. The older leaves were relatively free from the symptoms.

Severe symptoms included, strong chlorotic patches in pale green laminar surface, severe streaks and yellowing in the top six to seven leaves. In the older leaves severe chlorotic and yellow blotches were present with marginal drying. Stunting was also noticed in some plants. Following are the different areas covered during the survey.

S.No	Area covered
1	Bannari sugars, Sathiyamangalam – Coimbatore and Erode districts.
2	MRK sugars, Sethiathoppe - Cuddalore district.
3	SV sugars, Kancheepuram district.
4	EID Parry sugars, Nellikuppam – Cuddalore district.
5	New horizan sugars, Pondicherry
6	Ambika sugars, Pennadam – Cuddalore district.
7	Thiruarooran sugars, Thirumandankudi – Tanjore district.
8	Kothari sugars, Kattur – Trichy district.
9	Rajshree sugars, Vaigai dam – Theni district.
10	Amaravathi sugars, Udumalpet – Coimbatore district.
11	Sugarcane Breeding Institute, Coimbatore

3.1.1. Maintenance of sugarcane plants in glass house

Sugarcane varieties exhibiting varying SCMV symptoms were collected from different locations and maintained in the insect proof glass house. Three to four single budded setts were planted in earthen pots containing soil mixture of 1:1:1 ratio of red earth, sand and farmyard manure.

3.2. Differentiation of SCMV strains

Strain differentiation was carried out based on differential host reaction and serological studies.

3.2.1. Differential host reaction

Following sugarcane and sorghum differential hosts were used for strain differentiation.

S. No	Differential hosts
1	Sugarcane cultivars – Co 281, CP 31-294, CP 31-588
2	Sweet sorghum (<i>Sorghum bicolor</i>) cultivars - Atlas, Rio
3	Johnson grass (<i>Sorghum halepense</i>)

3.2.1.1. Maintenance of sugarcane differential hosts

Healthy sugarcane setts were obtained from Sugarcane Breeding Institute world germplasm centre, Cannanore. Three to four single budded setts were planted in earthen pots containing soil mixture of 1:1:1 ratio of red earth, sand and farm yard manure and maintained in insect proof glass house.

3.2.1.2. Inoculation of sugarcane differential hosts

Four to five weeks old sugarcane plants were used for SCMV inoculation. For each differential host, one pot was maintained as uninoculated control. The treatments were replicated thrice (each sugarcane variety collected from different locations and maintained in the glass house and exhibiting characteristic symptoms of

SCMV was considered as a treatment). Virus inoculum was prepared by grinding the youngest exposed leaf in liquid nitrogen and the sap was extracted in precooled buffer (4°C) (1 gm tissue in one ml 0.01M potassium phosphate buffer pH 7.0). Combined inoculation method of Matz (1933) and Bain (1944) was used.

In the Bain's method, the standard virus inoculum from the affected leaves was rubbed on the two youngest expanded leaves, which was previously dusted with carborundum powder (600 mesh). The treated leaves were washed with a fine jet of distilled water five min after inoculation. As per the Matz's method, a drop of virus inoculum from the affected leaves was placed into the wedge shaped opening between the expanded leaf blade and the next younger leaf and the side of the leaf spindle. A fine needle was then pressed in and out horizontally or somewhat obliquely downwards through the liquid into the submerged area of the still rolled leaf.

For SCMV inoculation, Bain's method was first used followed by Matz's method. The procedure was repeated after one week and symptom expression was recorded for the next three months

3.2.1.3. Maintenance of sorghum differential hosts

Sweet sorghum cultivars (Atlas and Rio) and Johnson grass seeds were obtained from International Crops Research Institute for Semi Arid Tropics, Patancheru, Andhrapradesh and also from Prof. Dr. M.S.Tosic, University of Belgrade, Yugoslavia. About ten seeds were planted in earthen pots containing soil mixture and maintained in insect proof glass house.

3.2.1.4. Inoculation of sorghum differential hosts

Sorghum and Johnson grass differentials were inoculated with SCMV inoculum at 3-4 leaves stage (two to three weeks after sowing). For each differential host, one pot was maintained as uninoculated control. The treatments were replicated thrice (each sugarcane variety exhibiting characteristic symptoms of SCMV was considered

as a treatment). The virus inoculum was prepared as in chapter 3.2.1.2. It was mechanically inoculated very gently on the leaves after dusting with carborundum (600 mesh) and washed with a gentle flow of distilled water after five min. The plants were observed for symptom expression after inoculation up to three months after inoculation.

3.2.1.5. Symptom expression and strain differentiation

The differential host reactions described by Kondaiah and Nayudu (1984), Gillaspie and Mock (1984) and Tasic *et al.* (1990) were used for distinguishing the symptoms and strain differentiation.

3.2.2. Serological methods for strain differentiation

Antisera from different sources were obtained to compare the serological relationship of SCMV strains in Tamil Nadu.

Antiserum type and dilution	Source
SCMV polyclonal (1:1000)	Dr. R. K.Jain, IARI, New Delhi.
SrMV H, MDMV B (1:1000), Johnson grass mosaic virus (1:1000) (all polyclonal)	Dr. G. P. Rao, Sugarcane Research Station, Gorakhpur, India.
SCMV-A (1:500), SCMV BC (1:512), SCMV D (1:512), SCMV Sabi (1:64), and SCMV YU (1:128) (all polyclonal)	Prof. Dr. M. S. Tasic, University of Belgrade, Yugoslavia.
SCMV (1:1000) (polyclonal)	Dr.Stephan Winter, DSMZ Plant Virology Laboratory, Germany.

3.2.2.1. ELISA method for strain differentiation

The DAC ELISA (Direct Antigen Coating - ELISA) method was followed (Mowat and Dawson, 1987). The protocol is given below as flow chart.

SCMV infected and healthy leaves from sugarcane and sorghum were taken
(preferably youngest exposed leaves)

↓

Powdered with liquid nitrogen and extracted in carbonate buffer (pH 9.6)

↓

100µl of the sample added to each well of the microtitre plate and incubated at
37°C for 2 hr.

↓

Plate was emptied and washed 3 times with PBS-T (Phosphate buffer saline-Tween 20)

↓

100µl of the primary antiserum (polyclonal) was added to each well and incubated at
37°C for two hr

↓

Plates emptied and washed three times with PBS-T

↓

100µl of alkaline phosphatase conjugate (Goat anti rabbit IgG) was added to each well
(dilution of 1:2500) and incubated for 2 hr at 37 °C

↓

Plates were emptied and washed 3 times with PBS-T.

↓

100µl enzyme substrate was added (*p*-nitrophenyl phosphate) to each well and
incubated at room temperature for 30 min for colour development

↓

Colour development was arrested by adding 50µl of 3M NaOH or KOH

↓

Absorbance at 405nm was taken and the readings were compared between healthy and
infected samples. Difference of two times and above was taken as positive reaction

3.3. Purification of SCMV

3.3.1. Maintenance of virus isolates

SCMV isolates from CoC 671 sugarcane variety was used for the purification. The virus was inoculated mechanically into sweet sorghum cultivar 'Rio' as in chapter 3.2.1.2. The sweet sorghum cultivar served as propagation host for SCMV. At 3-4 leaf stage (15-20 days after sowing), the sorghum leaves were sap inoculated with the SCMV inoculum extracted in 0.01M potassium phosphate buffer pH 7.0 containing 0.2 per cent 2-mercaptoethanol. Carborundum (600mesh) was slightly dusted onto the sorghum leaves and SCMV inoculum was gently pressed over the sorghum leaves and washed with distilled water after 5 min. Three weeks after the inoculation, the sorghum leaves exhibiting typical SCMV symptoms were used for the purification.

3.3.2. Extraction and clarification of the virus inoculum

The modified procedure of Rishi and Rishi (1985) was followed for virus extraction and purification. About 100 gm of SCMV infected sorghum leaves were harvested, three weeks after inoculation. Dust and other soil particles were removed from the leaves by gently washing it in tap water and dried with blotting paper, kept at -20°C for 14-16 hr. Then the sample was ground in liquid nitrogen and extracted with 0.067M potassium phosphate buffer (pH 7.0) containing 0.01M Na-DIECA (sigma) by blending the tissues for 10 min (300ml volume buffer) followed by continuous stirring for 2 hr at 4°C. Then the extract was passed through the double layer of cheesecloth and centrifuged at 3000 rpm for 10 min at 4°C. To the supernatant, equal volume of chloroform was added and mixed gently for 20 min at 4°C on magnetic stirrer and centrifuged at 3000 rpm for 20 min at 4°C. To the supernatant yellow aqueous phase, 0.5 per cent Triton X-100 (V/V) was added and stirred for 2 min followed by centrifugation at 25,000 rpm for 2 hr at 4°C. The pellet was suspended in 30 ml volume of resuspension buffer (0.05M borate buffer pH 8.2 + 0.01M EDTA + 0.5M urea). The suspension was clarified by centrifugation at 5000 rpm for 10 min.

The supernatant was centrifuged at 25,000 rpm for 2 hr at 4°C and the pellet was resuspended overnight in 30ml resuspension buffer. Then, the suspension was centrifuged at 5000 rpm for 10 min and the supernatant was subjected for sucrose gradient centrifugation.

3.3.3. Sucrose gradient centrifugation

For the sucrose gradient centrifugation, swinging rotor was used. The sucrose gradient (10,20,30 and 40 per cent) was prepared in resuspension buffer. In each concentration, 2.5ml was used for layering and kept overnight at 4°C for gradient formation. Then, 2 ml of the virus suspension was layered above and allowed for high speed centrifugation (25,000 rpm for 2 hr at 4°C) in a swing out rotor. The light scattering zone was collected and dialysed for 4 hr against resuspension buffer and diluted to 30 ml with it. The suspension was centrifuged at 25,000 rpm for 3 hr and the pellet was suspended in minimal volume of resuspension buffer (1-2ml).

3.4. Molecular characterization of the purified SCMV

3.4.1. Immunosorbant electron microscopy (ISEM)

The base of the Petri dish was lined with parafilm. About 20 μ l of SCMV antiserum at 1:1000 dilution was placed on the parafilm. The copper grids previously coated with farmvar (0.55 per cent in chloroform) were placed on the drop of antiserum in such a manner that it floated on the drop. The Petri dish was covered with the lid to prevent evaporation and incubated at 37°C. After 1hr, the grids were washed with sterile double distilled water by flooding drop wise from a Pasteur pipette by holding the grids in forceps. After removing the excess antiserum, the grids were floated on a 20 μ l of purified virus on parafilm. Care was taken to ensure that the antiserum-coated side was in contact with the virus drop. Then the grids were incubated at 37°C for 1hr, blot dried and stained with 2 per cent ammonium molybdate (pH 5.6) by slowly dropping on the grid. Then the grids were washed with sterile distilled water and dried at room temperature and were viewed under JEOL-TEM 100sx electron microscope.

3.4.2. Biological assays

Purified SCMV preparation was inoculated onto the seedlings of two weeks aged sweet sorghum CV. Rio' for symptom expression as described under 3.2.1.4.

3.4.3. Ultraviolet absorbance profile of the purified SCMV

The purified SCMV was diluted in resuspension buffer (1/5 and 1/10 dilutions with 0.05M borate buffer pH 8.2 containing 0.1M EDTA + 0.5 M urea). The diluted fractions were taken in one cuvette and resuspension buffer alone was taken in another cuvette as control. Scanning was carried out under UV spectrophotometer from 200 to 300 nm.

3.4.4. Virus yield from the purified preparation

The yield or concentration of the purified SCMV was determined as per Hollings and Brunt (1981).

Corrected O.D value = O.D at 260nm X Dilution factor X Final volume.

Where

Dilution factor = Volume diluted with resuspension buffer.

Final volume = Volume of resuspension buffer used for final virus pellet

From the corrected O.D value, virus concentration was obtained by considering

3 O.D = 1mg of the purified virus.

3.4.5. Determination of serological relationship of the purified virus

DAC-ELISA as described previously (chapter 3.2.2.1) was followed to determine the serological relationship of the purified virus. Purified virus as an antigen at 4µg / well was used. Different antisera were used as mentioned previously in chapter 3.2.2.

3.4.6. Viral coat protein

3.4.6.1. Electrophoretic separation of SCMV coat protein

The molecular weight of the purified SCMV coat protein was determined by SDS-PAGE (Laemmli, 1970) using 4 per cent stacking gel on a 12 per cent separating gel. The protein content of the sample was determined by Bradford method (Bradford, 1976). The virus suspension equivalent to 60 μ g of protein was taken and mixed with 10 μ l of sample buffer in an eppendorf tube, boiled for 4 min and incubated at 4°C for 30 min. Then the samples were loaded into the wells of polyacrylamide gels. Electrophoresis was carried out at constant voltage of 80 volts. The gel was stained overnight with 0.2 per cent Coomassie brilliant blue (R250) solution. Destaining was carried out with the solution containing, water: methanol: glacial acetic acid (5:4:1). Based on the Rf value (Relative mobility) of the migrated band, the molecular weight was calculated. The position of the marker and SCMV coat protein bands were measured from the edge of the wall to the middle part of the band and the relative mobility (Rf) was calculated by the following formula.

$$Rf = \text{distance migrated by the protein} / \text{distance migrated by dye.}$$

For estimating the molecular weight of the SCMV coat protein, the standard curve of molecular weight markers versus their Rf were plotted on a semi log graph paper and the molecular weight of SCMV coat protein was determined based on its Rf value.

3.4.6.2. Electroblood immunoassay (EBIA) for the determination of strain relationship of the purified SCMV

After SDS-PAGE, the protein from the gel was electroblotted onto 0.45 μ m nitrocellulose membrane (Sigma) as described by Gallagher *et al.* (1995). The electrophoretic transfer of proteins was carried out from the gel to the membrane in a Bio-Rad semidry-transblot-apparatus (140mA; 30 min). Later, the membrane was stained with Ponceau S stain (Sigma) for 2 min to confirm the transfer of protein and its transfer quality and resolution. Then, destained with TBST for 2 min. Later, the

membrane was blocked in TBST containing 2.5 per cent (w/v) gelatin by keeping it in a shaker at room temperature ($28 \pm 2^{\circ}\text{C}$) for overnight. Then, washed with TBST for 5 min followed by addition of primary antiserum (appropriate dilution of primary antiserum in 30 ml TBST) and kept in shaker for 4 hr and washed three times with TBST for 10 to 15 min each to remove the unbound antibody. Then the membrane was incubated in goat anti-rabbit immunoglobulin (IgG) alkaline phosphatase conjugate for 3 hr at a dilution of 1:1000. The membrane was then washed thrice with TBST and thrice with TBS for 10 to 15 min each time. Immunological reaction was visualized by soaking the membranes in BCIP/NBT ready to use substrate (Bangalore Genei, India). Immediately after colour development, the membranes were washed in distilled water and preserved after drying. Development of blue coloured bands recorded as positive reaction.

3.4.7. Viral nucleic acid

The viral genomic RNA was analysed by electrophoresis in 1 per cent agarose gel by disrupting the purified virions at 50°C for 10 min in disruption buffer (Wood and Coutts, 1975). The viral RNA was amplified by RT-PCR method as described in chapter 3.6.3.

3.5. Production of polyclonal antiserum

Polyclonal antiserum was produced in New Zealand white rabbit (150 days old) from the purified SCMV. Two different experiments were conducted by following different immunological procedures in order to find out the serological specificity of the produced antiserum.

3.5.1. Single immunization

Single intra-muscular injection was given with $500\mu\text{l}$ (1.0mg) of the purified virus emulsified with an equal volume of Freund's complete adjuvant. First bleeding was done after one week and second bleeding during the next week.

3.5.2. Multipe immunization

Four intra muscular injections were given at weekly intervals. On each occasion, 500 μ l (1.0 mg) of purified virus was emulsified with an equal volume of Freund's complete adjuvant and immunized. First bleeding was done one week after the final injection and second bleeding after one week.

3.5.3. Storage of antiserum

Blood collected from rabbit was kept in a sterile screw cap vial in a slanting position at 4°C for four to five hours for the collection of serum in the top phase. To the serum, 0.02% of sodium azide was added and stored at 4°C.

The antiserum collected in the above two experiments were assessed for their specificity with SCMV and related potyvirus using different serological assays after standardizing the concentration and other working conditions.

3.6. Molecular diagnostic techniques

3.6.1. Serological techniques

3.6.1.1. DAC-ELISA method

The DAC-ELISA procedure was followed as described in 3.2.2.1. Concentration of the antigen and antisera used were as follows.

S.No	Anitigen concentration	Antiserum concentration
1	1:10	1:10
2	1:25	1:100
3	1:50	1:100
4	1:100	1:500
5	1:500	1:1000
6	-	1:5000

The optimum concentration of antigen as well as antiserum to differentiate the healthy and SCMV infected leaf sample was determined. The minimum difference of two times and above in the ELISA reading between healthy and infected samples was taken as positive reaction.

3.6.1.2. Electroblot immunoassay (EBIA)

About one gram of healthy and infected leaves (second and third leaf from the top) was extracted with 1ml of 0.1M sodium phosphate buffer (pH 7.0) under 4°C after grinding with liquid nitrogen. The homogenate was centrifuged for 10 min at 10000 rpm at 4°C. The supernatant was used for the SDS-PAGE (Laemmli, 1970) as described in chapter 3.4.6.1. The purified SCMV was used as control to determine the presence of the virus in the samples. The method described under 3.4.6.2 was followed for EBIA for the diagnosis of infected tissues by using the SCMV antiserum produced against the purified virus.

3.6.1.3. Immunosorbant electron microscopy (ISEM)

From the infected and healthy tissues, sap was extracted and ISEM was carried out by using the antiserum produced against SCMV. The method was as described in chapter 3.4.1.

3.6.1.4. Dot blot immunoassay

In this assay, nitrocellulose membrane (NCM) was used to load the samples and most of the procedure was similar to DAC-ELISA method. About 20µl of polyclonal antiserum diluted in carbonate buffer (pH 9.6) was placed on NCM and incubated at 37°C for 2-3 hr. Then washed three times with PBS-T followed by blocking with skimmed milk powder (2%) and washed three times with PBS-T. The antigen (20µl) extracted in PBS from infected and healthy tissues were placed on NCM and incubated at 4°C overnight followed by washing three times with PBS-T. Then, alkaline phosphatase conjugated anti-rabbit IgG (20µl) produced in goat was placed (1:2500) followed by incubation at 37°C for 3 hr. Then washed with PBS-T for three times and

added with BCIP/NBT ready to use substrate. Development of blue colour indicated the positive reaction.

3.6.2. Determination of SCMV titre in various sugarcane tissues

Various sugarcane tissues *viz.*, leaf lamina (third and sixth leaf from the top), midrib, leaf sheath, buds, pith tissues and root portion from the healthy and infected tissues were used. DAC-ELISA method as described in 3.2.2.1 was followed to determine the SCMV titre from various sugarcane tissues.

3.6.3. SCMV detection by RT-PCR

From the infected sugarcane tissues, RNA was separated and RT-PCR was carried out by using the sugarcane mosaic virus primer. From different sugarcane tissues like, leaf blade, mid rib, leaf sheath, buds and root tissues, RT-PCR was carried out for the detection of SCMV. The sugarcane varieties used were from CoC 671 and Co 740.

3.6.3.1. RNA extraction

RNA was extracted as per the procedure followed by Yambao *et al.* (1998). About 100mg of leaf tissue was ground in liquid nitrogen and 1ml of TRIZOL reagent (GIBCO BRL) was added immediately. It was incubated at 15 to 30°C (room temperature) for 5 min and added with 0.2 ml of chloroform and shaken vigorously by hand for 15 seconds followed by incubation at 15 to 30°C (room temperature) for 2 to 3 min and centrifuged at 4000 rpm for 15 min at 2 to 8°C. The tubes were removed and the aqueous phase was collected. It was mixed with 0.5 ml of absolute ethanol and incubated at 15 to 30°C (room temperature) for 10 min followed by centrifugation at 4000rpm for 10 min at 2-8°C. Then the supernatant was discarded without agitation. The pellet was washed with 0.5ml 70 per cent cold ethanol, mixed with vortex, and centrifuged at 4000 rpm for 5 min at 2 to 8°C and the excess ethanol was removed with pipette. The pellet was air dried in laminar- flow hood for atleast 10 min and redissolved in 20µl RNase free water, kept at -20°C. Total RNA extracted was confirmed in agarose gel electrophoresis.

3.6.3.2. cDNA synthesis and polymerase chain reaction

RT-PCR kit (Bangalore Genei) was used for this purpose and the methodology as described by the manufacturer was followed. To a sterile RNase free tube (DEPC treated), 6 μ l of total RNA was added followed by the addition of sterile water to bring the volume to 9 μ l and added with 1 μ l of random primer. The vials were kept at 65°C for 10 min and later at room temperature for 2 min. Finally, the vials were spun for a brief and the following reagents were added in the order.

- 1 μ l RNase inhibitor
- 1 μ l 0.1M DTT
- 4 μ l Reverse Transcriptase Buffer (5x)
- 2 μ l 30 mM dNTP mix
- 0.5 μ l Reverse Transcriptase
- 1 μ l sterile water

The solution was mixed well and incubated at 42°C for 1 hr followed by incubation at 95°C for 2 min to denature RNA-cDNA hybrids, spun briefly and quickly placed on ice. The synthesized cDNA was confirmed by agarose gel electrophoresis.

3.6.3.3. PCR Amplification

The reagents were added in the following order.

- 2 μ l cDNA product
- 5 μ l 10x PCR buffer
- 1 μ l 30mM dNTP mix
- 1 μ l Forward primer
- 1 μ l Reverse primer
- 0.5 to 1Units Taq DNA Polymerase

The following primers were used (Yang and Mirkov, 1997).

Sl.No	Primer	Sequence
1	SCMV Forward Primer	TTT YCA CCA AGC TGG AA
2	SCMV Reverse Primer	AGC TGT GTG TGT CTC TGT ATT CTC

Where T=Thymine.

C=Cytosine.

A=Adenine.

G=Guanine.

Redundancy code: Y= C+T.

The following amplification procedure of Yang and Mirkov (1997) was followed.

S.No	No.of cycles	Temperature (°C)	Time (min)
1	1	94	2
2	40	94	1
		60	1
		72	1
3	1	72	7

3.6.3.4. Precipitation of amplified product

PCR amplified product was confirmed by running 1.2 per cent agarose gel electrophoresis with 1kb DNA ladder as marker. Amplified product was made up to 100µl with sterile water, 50µl chloroform and 50µl saturated phenol, mixed well and centrifuged at 5000 rpm for 5 min. To the supernatant, 1/10th volume of 3M sodium acetate (pH 5.5) and 2.5 volume of ethanol were added and kept overnight at 4°C. It was centrifuged at 10,000 rpm for 10 min. To the pellet 70 per cent ethanol was added and centrifuged at 5000 rpm for 5 min and the pellet was dissolved in 20µl of water and subsequently analysed in 1.2 per cent agarose gel electrophoresis (Sambrook *et al.*, 1989).

The samples were also sent to DSMZ plant virology laboratory, Germany for the confirmation of SCMV by RT-PCR method. The RNA isolation was carried out by using guanidium thiocyanate-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987). The following potyvirus specific primers were used for the amplification

Forward primer: 5'-TGA GGA TCC TGG TG (TC) AT (ACT) GA (GA) AA (TC) GG-3'

Reverse primer: 5'-GCG GGA TCC TTTT'TTTT'TTTT'TTTT (GCA) (AGCT)-3'

The reverse transcription was done with MMLV transcriptase at 37°C for 10 min followed by 42°C for 30 min and the following amplification procedure was carried out.

S.No	No.of cycles	Temperature (°C)	Time (min)
1	35	95	1
		53	1
		72	1.5

3.7. Economic impact of sugarcane mosaic virus

Detailed studies were undertaken in pot culture, and in the field to find out the effect of SCMV infection on different growth parameters, cane yield and quality. Two sugarcane varieties namely Co 740 and CoC 671 were used. One pot culture experiment was taken up in green house to determine the effect of age of sugarcane on SCMV infection. Two plant crop and subsequent ratoon crop experiments were carried out at Coimbatore. One extensive plant crop trial at farmer's field was also taken up near Pollachi to find out the economic impact of SCMV. The field trials were planned in randomized block design.

3.7.1.Effect of SCMV inoculation at different stages of sugarcane

Healthy and SCMV infected setts of Co 740 and CoC 671 were used. Three single budded setts were planted in earthen pots of 30cm diameter with soil mixture containing red earth, sand and farmyard manure in the ratio of 1:1:1. Mechanical inoculation of SCMV was carried out by using the combination of Bain's and Matz's method as described in chapter 3.2.1.2. on one month, two month, three month, four month, five month and six month old sugarcane plants. Planting was done in a staggered manner at monthly intervals so as to carry out the inoculation simultaneously. For each treatment, nine pots were planted and replicated thrice in a completely

randomized design. Healthy and infected setts were also planted similarly and replicated thrice, which served as the control. SCMV infection was assessed for three times at 15, 30 and 60 days after inoculation based on visual symptoms as well as based on DAC-ELISA method as described previously in chapter 3.2.2.1.

3.7.2. Effect of SCMV on cane yield and sugar yield

To find out the quantitative and qualitative impact of SCMV on sugarcane, detailed field experiments were carried out involving three plant crops and two ratoon crops. The experiment involving two plant crop and subsequent ratoon crop was carried out at Tamil Nadu Agricultural University, Coimbatore and another experiment involving one plant crop was carried out at farmer's field at Konganattanpudur, in Pollachi (Tk) of Coimbatore District. The details of the experiments are given below.

Details	Experiment I	Experiment II	Experiment III
Varieties	Co 740& CoC671	Co 740& CoC671	Co 740& CoC671
Season	Sep 1999- Aug 2001	Nov 1999- Oct 2001	Sep 2000- Aug2001
Location	TNAU,Coimbatore	TNAU,Coimbatore	Konganattanpudur, Pollachi (Tk), Coimbatore(Dt).
Field area/ treatment	Three rows of four metres each	Two pits of 1x1x0.5m each	80 rows of four metres length in 100m ²
Spacing between rows/pits	90cm	90cm	90cm
No of setts per row/pit	15 two budded setts	10 two budded setts	15 two budded setts
Design	Randomized block- design	Randomized block design	Randomized block design
No of replications	Five	Five	Five

3.7.3. Quantitative factors

3.7.3.1. Germination

The numbers of buds germinated were counted at 30days after planting in the main crop. The germinated setts were counted randomly at six different rows and pits in all the replications for each treatment and the percent germination was derived.

3.7.3.2. Tillering

Number of tillers present per clump was counted randomly at six different rows and pits for each treatment in all the replications during the second and fourth month after planting in the plant crop and in the ratoon crop.

3.7.3.3. Net photosynthesis

By using CO₂ gas analyzer, net photosynthesis of healthy and SCMV infected plants was calculated. The third fully unfurled leaf from the top was selected for the estimation and the observation time was 11.30 A.M. during which the Sun light falls straight on the crop without any shade. Ten plants for each treatment were selected randomly during the second month, fourth month, and sixth month for taking the observation. The results were expressed as $\mu \text{ mol/ m}^2/\text{s}$.

3.7.3.4. Plant height

Height of the cane was measured at 2, 4, 8 and 10 months after planting. Ten plants were selected randomly for each treatment.

3.7.3.5. Cane thickness

By using the vernier caliper, cane thickness was calculated during the sixth, eighth and twelfth month. Ten canes were selected randomly for each treatment.

3.7.3.6. Number of millable canes (NMC)

The number of matured cane present in each clump was recorded during the eighth and twelfth months. Ten clumps were selected randomly for each treatment.

3.7.3.7. Cane weight

Harvesting was done after twelve months. Single cane weight was calculated based upon the weight of the canes in ten different rows and pits in each treatment for the trials conducted at Coimbatore. For the field trial conducted at farmer's field at Pollachi, total cane weight in the field for each treatment was taken up and per hectare yield was arrived.

3.7.4. Qualitative characters

3.7.4.1. Juice parameters

Twelve months after planting, the cane stalks were cut at ground level, cleaned and crushed immediately in a three-roller power crusher giving about 60 per cent juice extraction. The juice was then strained through a muslin cloth to remove suspended impurities and used for further analysis (Rao, 1986). The juice analysis was carried out by polariscope observation as well as by automatic sucrolyser. For each treatment, forty to fifty canes were selected randomly and separated as four kg bundles. From each bundle, juice was extracted separately for further analysis.

3.7.4.2.1. Polariscope method

The brix readings were recorded in a brix hydrometer and the correction was applied using the correction table depending on the juice temperature. To assess sucrose percent, 2g of lead acetate was added to 100ml of juice and shaken well. After five min, it was passed through filter paper; filtrate taken in polariscope observation tube (200mm) and polariscope reading was recorded. The sucrose percent was determined from the brix and corresponding polariscope reading by referring to the Schmitz table.

The juice purity percent was calculated using the following formula.

$$\text{Purity} = \text{sucrose (\%)} / \text{corrected brix} \times 100$$

The commercial cane sugar (CCS) percent was calculated by using the following formula

$$\text{CCS \%} = [(S - (B - S) \times 0.4) \times 0.73]$$

Where

S = sucrose percent of juice.

B = brix percent of juice.

3.7.4.2.2. Juice analysis by sucrolyser

By using the automatic saccharimeter (Autopol 880 model), the juice analysis was carried out and total brix (%), purity (%) and sucrose (%) were arrived automatically from the lead acetate clarified juice.

3.8. Induced resistance against SCMV

The experiment was carried out in sweet sorghum cv. Rio. Ten seeds were sown in earthen pots of 30 cm diameter filled with red earth, sand and farmyard manure in the ratio of 1:1:1. The pot mixture was sterilized before filling. For each treatment, three pots were used and replicated thrice in a completely randomized block design. The treatments were as follows

- a) *Pseudomonas fluorescens* soil drenching (20 gm / litre)
- b) *Pseudomonas fluorescens* seed treatment (20 gm / litre)
- c) *Pseudomonas fluorescens* foliar spray (20 gm / litre)
- d) Salicylic acid soil drenching (1ppm)
- e) Salicylic acid foliar spray (1ppm)
- f) Salicylic acid seed treatment (1ppm)
- g) BTH (Benzodiathiazole)- (BionTM - M/S Syngenta Ltd.,)
- h) BTH 10 ppm for seed treatment
- i) BTH 10 ppm as foliar spray.
- j) SCMV inoculated control.
- k) Healthy control.

Soil drenching was carried out before sowing. *Pseudomonas fluorescens* mixed isolate (containing CHAO and Pf1 strains) was obtained from Dr.R. Viswanathan, Sugarcane Breeding Institute, Coimbatore. Seed treatment was done in a polybag by soaking the seeds for 30 min. Foliar spray was carried out just before SCMV inoculation (3-4 leaf stage *i.e* about 20 days aged plants). The virus was inoculated by sap inoculation method as described previously in chapter 3.2.1.2. In the untreated control, buffer was used for inoculation. Inoculation was done in all the expanded leaves and samples were collected at 0, 3, 6, and 9 days after SCMV inoculation for the analysis of total phenol and induced proteins *viz.*, phenylalanine ammonia-lyase, polyphenol oxidase and peroxidase by colorimetric method. Expression pattern of different isoforms of peroxidase and polyphenol oxidase was also carried out by native

anionic polyacrylamide gel electrophoresis. The virus concentration in the plants were determined by DAC-ELISA method as described previously in chapter 3.2.2.1 from the leaf samples collected on 0, 3, 6, 9, 12, 24 and 48 days after inoculation.

3.8.1. Mechanism of induced resistance

3.8.1.1. Total phenol

Leaf samples of 0.5 gm was homogenized in 5ml of 80 per cent methanol and agitated for 15 min at 70°C (Swain and Hillis, 1959). About 1ml of methanol extract was added to 50ml of distilled water and 250 μ l of Folin- Ciocalteu reagent (1N) and the solution was kept at 25°C. After 3 min, 1ml of saturated solution of sodium carbonate (2 gm / 10ml) and 1ml of distilled water were added and the reaction mixture was incubated for 1hr at 25°C. The absorption of blue colour developed was measured using a Beckman DV 64 spectrometer at 725 nm. The content of the total soluble phenols was calculated according to a standard curve obtained from the Folin- Ciocalteu reaction with catechol and expressed as catechol equivalents in μ g /g fresh weight.

3.8.1.2. Phenylalanine ammonia-lyase

About 0.5gm of leaf sample was homogenized in 2.5 ml of 0.1M sodium borate buffer, pH 7.0 at 4°C containing 0.1g insoluble polyvinyl pyrrolidine. The homogenate was centrifuged at 10,000 rpm for 30 min. The supernatant was used in the enzyme assay. Samples containing 0.4ml of enzyme extract were incubated with 0.5 ml of 0.1M borate buffer, pH 8.8 and 0.5ml of 12mM L-phenylalanine in the same buffer for 30 min at 30°C. In the control cell, 0.4ml of the enzyme extract was taken along with 1.0ml of borate buffer.

PAL activity was determined as the rate of conversion of L- phenylalanine to trans cinnamic acid at 290nm (Dickerson *et al.*, 1984) and calculated using its extinction co-efficient of 9630 $\text{m}^{-1} \text{cm}^{-1}$. Enzyme activity was expressed on fresh weight basis ($\text{nmol min}^{-1} \text{g}^{-1}$ fresh weight).

3.8.1.3. Peroxidase

About 0.5gm leaf samples were homogenized in 1ml of 0.1M phosphate buffer, pH 7.0 at 4°C. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5ml of 0.05M pyrogallol, 0.05ml of enzyme extract and 0.5ml of 1 per cent hydrogen peroxide and was incubated at room temperature ($28 \pm 1^\circ\text{C}$). The changes in absorbance at 420nm were recorded at 30 seconds intervals for 3 min. The enzyme activity was expressed as changes in the absorbance ($\text{min}^{-1} \text{mg}^{-1} \text{protein}$) (Hammerschmidt *et al.*, 1982a).

3.8.1.4. Polyphenol oxidase

The procedure of Mayer *et al.* (1965) was followed. Leaf samples (0.5gm) were homogenized in 1ml of 0.1M sodium phosphate buffer (pH 6.5) and centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was used as enzyme source. The reaction mixture consisted of 200 μl of enzyme extract and 1.5ml of 0.1M sodium phosphate buffer (pH 6.5). Reaction was initiated by adding 200 μl of 0.01M catechol and the activity was expressed as changes in absorbance at 495 nm $\text{min}^{-1} \text{mg}^{-1} \text{protein}$.

3.8.2. Activity gel electrophoresis

To study the expression pattern of various isoforms of induced peroxidases and polyphenol oxidases in different treatments, activity gel electrophoresis was carried out. For the native anionic polyacrylamide gel electrophoresis, resolving gel of 8 per cent acrylamide concentration and stacking gel of 4 per cent acrylamide concentration were prepared without sodium dodecyl sulphate and electrophoresis was carried out as described in chapter 3.4.6.1.

3.8.2.1. Peroxidase

After electrophoresis, the gel membrane was incubated in the solutions containing 0.15 per cent benzidine in 6 per cent ammonium chloride for 30 min in dark. Then, drops of 30 per cent hydrogen peroxide were added with constant shaking till the bands appeared. After staining, the gel was washed with distilled water and photographed (Sindhu *et al.*, 1984).

3.8.2.2. Polyphenol oxidase

After native electrophoresis, the gel was equilibrated for 30 min in 0.1M phosphate buffer (pH 7.0), containing 0.1 per cent p- phenylene diamine followed by 10 mM catechol in the same buffer. Gentle shaking followed the addition of catechol, which resulted in the appearance of dark brown discrete protein bands (Jayaraman *et al.*, 1987).

3.8.3. Determination of virus concentration

DAC-ELISA method as described previously in chapter 3.2.2.1 was followed to determine the reduction in virus concentration as a result of induced resistance. About 0.5gm of the leaf sample from the plants of various treatments was collected, homogenized with carbonate buffer (pH 9.6) and 1:20 concentration was used for the ELISA procedure. The samples were collected on 0, 3, 6, 9, 12, 24 and 48 days after SCMV inoculation.

3.9. Resistance mechanism against SCMV with in the *Saccharum* and related species

To exploit the resistance mechanism available against SCMV, different clones of *Saccharum spontaneum* and *Erianthus bengalense* were inoculated with SCMV and the induced proteins and build up of the virus concentration in the inoculated plants were studied and compared with the susceptible clones. The clones used were as follows.

S. No.	Species	Clones
1	<i>Saccharum spontaneum</i>	IND.81-80, IND 81-142, IND 81-161
2	<i>Erianthus bengalense</i>	IND 81-47
3	Hybrids (Susceptible)	Co 740, CoC 671

The clones of *S. spontaneum* and *E. bengalense* were obtained from the Sugarcane Breeding Institute germplasm center, Cannanore and maintained in the insect proof glass house in 30cm diameter earthen pots. SCMV was inoculated on

each clone and healthy control was also maintained in another pot. For CoC 671 and Co 740, three single budded setts were planted in each pot and replicated thrice in completely randomized block design.

SCMV was inoculated by following the combination of Bain's and Matz' method in the top three exposed leaves as described previously (chapter 3.2.2.1). In the control, buffer alone was inoculated. Leaf samples were collected at 0, 3, 6, 9 and 15 days after inoculation for studying the induction of total phenols, phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase by colorimetric method as described previously (chapter 3.8.1). Likewise, expression of peroxidase and polyphenol oxidase isoform pattern was also studied by activity gel electrophoresis as described previously (chapter 3.8.2).

Virus concentration in the SCMV inoculated plants was determined by DAC-ELISA from the leaf samples collected on 0, 3, 6, 9, 12, 24 and 48 days after inoculation as described previously (chapter 3.2.2.1).

3.10. Virus elimination from infected sugarcane

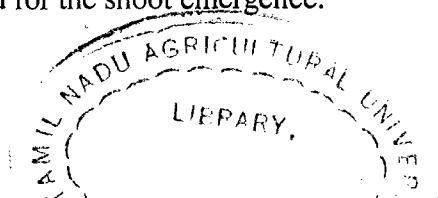
Two different experiments were conducted involving combined chemotherapy and meristem tip culture in one experiment and thermotherapy alone in another experiment were attempted to improve the existing strategies for the elimination of SCMV from the infected plants.

3.10.1. Chemotherapy and meristem tip culture

Murashige and Skoog (1962) medium with slight modification was used for the tissue culture studies.

3.10.1.1. Preparation of MS liquid medium

Along with the MS liquid medium (with out agar), coconut milk at 5 per cent (by volume) and gibberellic acid (100ppm) were added for the shoot emergence.



3.10.1.2. Addition of chemotherapeutants with the MS liquid medium

Two chemotherapeutants viz., Ribavirin (Virazole) and 8-azaguanine (Sigma chemicals, U.S.A) were used. The chemotherapeutants were added at 5,10, 25 and 50 ppm concentrations along with the MS liquid medium. Above the liquid medium in the tissue culture tubes, V-shaped whatman filter paper (Number 42) was placed and sterilized by autoclaving at 15 lb for 30 min.

3.10.1.3. Excission of meristem and shoot induction

The apical dome along with two leaf primordia was excised from the SCMV infected CoC 671 variety with the help of sharp sterile blade. The excised portions were placed in sterile distilled water to remove the dust particles and taken to the laminar flow chamber and surface sterilized with 70 per cent ethanol for 30 seconds followed by 0.1 per cent mercuric chloride for 30 seconds and washed in sterile water for 3 times. Then, the apical meristem of 0.1mm width and 0.5 to 1.0 mm length was excised by using a sterile blade and placed on the top of 'V' shaped filter paper, which was touching the liquid medium. Subsequent transfer of the growing parts to other tubes were carried out once in five days for three times for better shoot initiation.

3.10.1.4. Rooting

The developed shoots were placed in half strength solid MS medium. However, the iron stock was added in full strength and gibberellic and coconut milk was not added. Instead, 1 mg/ ml of indole butyric acid was added.

3.10.1.5. Hardening

The rooted plants were removed from the medium washed with distilled water and planted in small plastic containers filled with sieved sand, silt and composted press mud in the ratio of 1:1:1. The plants were incubated at 20-25°C for 3-4 days and transferred to polybags along with the pot mixture. The day temperature of mist chamber was 30-40°C and 18-25°C during night hours with 70-80 per cent relative humidity for 10-15 days and transferred to insect proof glass house.



3.10.1.6. Virus concentration in meristem derived plants

After shoot initiation, leaf sample (0.1 gm) was taken and DAC-ELISA was followed as described previously to determine the virus concentration. After rooting also, the leaf samples were taken and DAC-ELISA was followed. After hardening, both DAC-ELISA (chapter 3.2.2.1) as well as RT-PCR as described previously (chapter 3.2.3) was followed to determine the virus elimination. The number of plants established in each step was also observed.

3.10.2. Thermotherapy

The serial hot water treatment as described by Benda *et al.* (1989) was followed with some modifications in the temperature as below. Single budded setts of SCMV infected and disease free CoC 671 and Co 740 varieties were taken. The setts were fully submerged in hot water with the recommended temperature and agitated continuously for the specified duration. The duration of the treatment was measured from submersion to removal from the water. The temperature of the treatment tank was controlled thermostatically and monitored throughout the treatment. The variation in temperature was allowed at $\pm 0.3^{\circ}\text{C}$. The interval between the series was 20 to 24 hr. The seed pieces were kept at room temperature between the treatments. The treatment series were as follows

Treatment series	Duration (min) and corresponding temperature ($^{\circ}\text{C}$) at different days			
	First day	Second day	Third day	Fourth day
	52$^{\circ}\text{C}$	58$^{\circ}\text{C}$	60$^{\circ}\text{C}$	62$^{\circ}\text{C}$
A	20	15	5	–
B	20	15	10	
C	20	15	5	5
D	20	15	10	5
	52$^{\circ}\text{C}$	60$^{\circ}\text{C}$	62$^{\circ}\text{C}$	–
E	20	15	10	
F	20	15	15	

Treated setts were planted in polybags containing earth, sand and farmyard manure in the ratio of 1:1:1. Polybags were kept in the insect- proof glass house and watered regularly. After six to ten weeks, the plants were observed for mosaic symptoms and confirmation was done by DAC-ELISA as described previously (chapter 3.2.2.1). The plants with mosaic symptoms were discarded and mosaic free plants were replanted in earthen pots of 30cm diameter and observed for the mosaic symptoms, and subsequently confirmed by DAC-ELISA.

RESULTS

CHAPTER IV

EXPERIMENTAL RESULTS

4.1. Disease survey

In the survey conducted in major sugarcane belts of Tamil Nadu, none of the sugarcane variety was found to be free from SCMV incidence. It was present in mild or severe form (Table-1). The mild symptoms included, mild mottling and mild streaks in younger leaves preferably in the top four to five leaves. The older leaves were relatively free from the symptoms. The severe symptoms included, strong chlorotic patches in pale green laminar surface, severe streaks and yellowing in the top six to seven leaves and in the older leaves, severe chlorotic and yellow blotches were present with marginal drying. Stunting was also noticed in some plants. The various symptoms exhibited by different varieties were grouped into three major types as severely streak, chlorotic patches/green islands and yellow blotches with systemic yellowing.

4.1.1. Streak type

In this group, Co 740 variety was taken as reference (Plates-2b, 3a, 4a and 6b). Longitudinal pale green or yellowish streaks of 1 to 2mm size were present in the young leaves (two to three leaves from the top). The streaks extended up to 10mm in size in the older leaves and often merged together which led to necrotic lines in some leaves. The greenish background of the leaves was prominent, and above which, chlorotic or yellowish streaks were expressed. In some varieties, in most of the leaves, very small pale green streaks of 1 to 2 mm alone were present. In some other varieties, broad streaks were observed. Longitudinal stripes or small lines of up to 30 to 40 mm were present in some varieties. The following varieties showing typical streak type symptoms namely Co 740, Co 87023, UP 9530, CoLk 8001, Co 6806 and CoM 9217 (Plates-8 and 9).

Table.1. SCMV Survey in various sugarcane growing areas of Tamil Nadu

S.No	Area covered	Varieties with mild symptoms	Varieties with severe symptoms.
1.	Bannari Sugars, Sathyamangalam-Coimbatore and Erode districts.	Co 86032 Co 62175	Co 8021 CoC 671 / Co 97009.
2.	MRK Sugars, Sethiathope-Cuddalore district	Co 86032 CoSi 95071 CoSi 96071	CoC 90063 Co 8021
3.	SV Sugars, Kancheepuram district	CoSi 95071, Co 86032, Co 7508 and CoV 92102	Co 7805
4.	New horizon sugars, Pondichery	CoSi 95071, Co 86302, Co 8021	Co 7805 Co 86249
5.	Ambika Sugars, Pennadam, Cuddalore district	CoSi 6071, Co 86032, CoC 0063, Co 87025	CoC 671
6.	Thiruarooran Sugars, Thirumandankudi, Tanjore district	Co 7805, CoSi 96071, Co 97009, CoSi 96071, Co 86032, CoC 90063, Co 6304, Co 86032	Co 89014 Co 87025
7.	Kothari Sugars, Kattur, Trichy district	CoSi 96071, CoSi 86071, Co 86032	CoC 92061
8.	Rajshree Sugars, Vagai Dam, Theni district	Co 86032, Co 86011, CoSi 95071	CoC 671 Co 86249 CoC 92061
9.	Amaravathi Sugars, Udumalpet, Coimbatore and Erode district	Co 86032	CoC 92061 CoC 671

Table Cont...

10.	Sugarcane Breeding Institute, Coimbatore	Co 419, Co 976 Co 1307, Co 62399, Co 6904, Co 7314, Co 7910 Co 8339, Co 8340 Co 85019, Co 85036, Co 87021, CoA 71-1, CoA 8201, CoC 774, CoC 775, CoC 92061, CoH 2, CoH -2, CoH -13, CoH14, Co H -32, Co- H 7802, CoJ 64, Co J 67, CoJ 72, Co J- 75, CoJ 79, CoJ 81, CoJ 82, CoLk 7810, CoLk 9110, CoM 6615, CoM 7704, CoM 7712, CoS 778, CoS 771, CoS 797, CoS 802, BO 17, BO 43, BO 54, C 81129, C 81615, C 84070, CoS 92254, CoS 92263, CoS 93278, CoS 96260, CoS 91230, CoS 88261, CoS 87216, CoPant 90223, CoPant 92227, CoPant 94215, CoLk 9106, CoLk 91236, BO 108, BO 99, BO 91, CoSe 93232, CoSe 95427, CoS 93278, CoS 94275, CoS 96260, Co 87267, Co 87268, Co 87270, Co 87272, Co 87273, Co 86244, Co 86010, Co 86022	Co 7704, Co 8208, Co 740, Co 775, Co 88025, Co 91002, CoS 91230, CoS 95255, BO 106, BO 100, C79180, C 84136, C 84208, CoLk 8102, CoLk 8901, CoLk 9229, CoLk 9301, CoM 88021, CoM 9206, CoM 9217, CoM 9220, CoN 91132, CoPant 84212, CoPant 84214, CoPant 88219, CoPant 88220, CoPant 92224, Co 94003, CoS 510, CoS 5767, CoS 8119, CoS 8315, CoS 8407, CoS 8408, CoS 86218, UP 22, CP 44-101, CP 52-1, CP 52-68, CP 61-23, NCo 310, PoJ 2878, Q 58, Q 63 Q 65and Q 68.
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Plate:1. Appearance of severe SCMV symptoms in the field (CoC 92061)

a. Systemic yellowing and stunting



b. Yellow blotches and necrosis



**Plate:2. Appearance of severe SCMV symptoms in the field
(CP 44 101 & Co 740)**

a. Yellow (or) pale green specks (CP 44 - 101)



b. Chlorotic streaks (Co 740)



Plate:3. Closer view of SCMV symptoms (CoS 86218 & CoJ 84191)

a. Chlorotic streaks (CoS 86218)



b. Chlorotic specks (CoJ 84191)



Plate: 4. Closer view of SCMV symptoms (Co 740 & CoC 671)

a. Chlorotic streaks (Co 740)

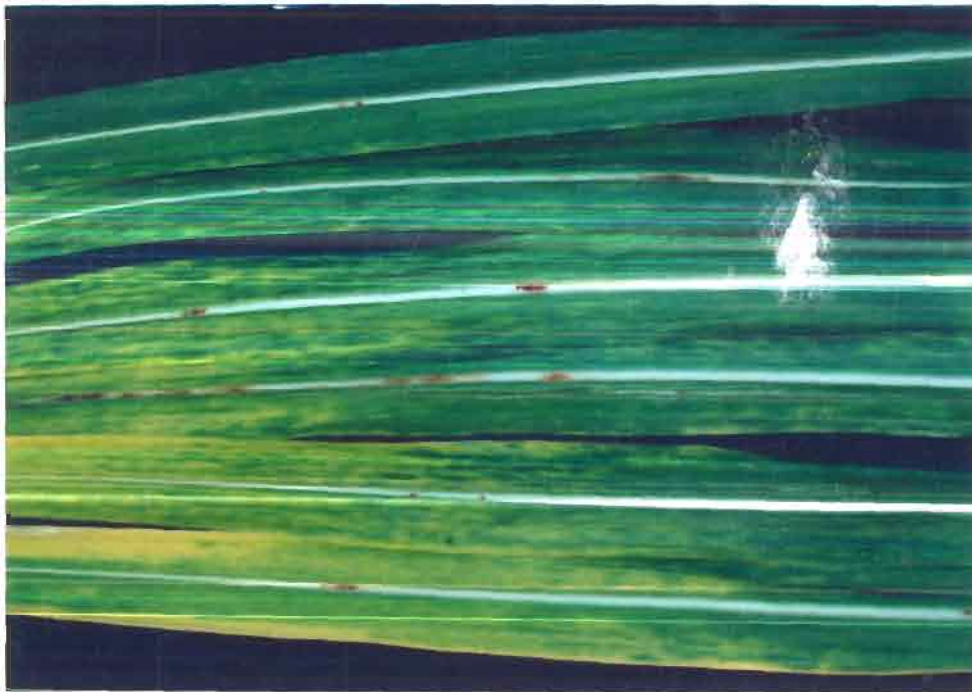


b. Chlorotic patches and green islands (CoC 671)



Plate:5. Closer view of SCMV symptoms (CoC 92061)

a. Yellow blotches (CoC 92061)



b. Systemic yellowing and marginal drying (CoC 92061)

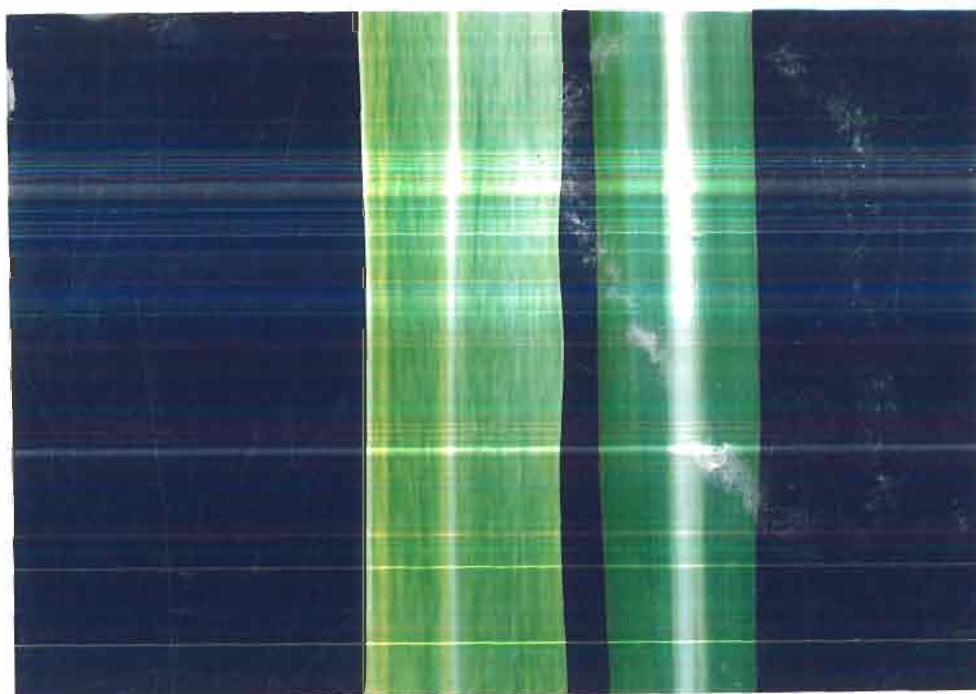


**Plate:6. Comparison of healthy and SCMV infected leaves
(CoC 671 & Co 740)**

a. CoC 671

Infected

Healthy



b. Co 740

Infected

Healthy



Plate:7. Comparison of healthy and SCMV infected leaves (CoC 92061)



Plate:8. Variation in varietal symptoms of SCMV - I

- 1.CoS 94270
- 2.CoM 88121
- 3.Co 6806
- 4.Co 740
- 5.Healthy



- 1.CoC 671
- 2.Co 87023
- 3.CoLk 8001
- 4.CoC 92061
- 5.Healthy



Plate:9. Variation in varietal symptoms of SCMV - II

- 1.UP 95-30
- 2.Q-65
- 3.CP 44-101
- 4.CoS 94210
- 5.Healthy



- 1.CoM 9217
- 2.C 84136
- 3.CoJ 84191
- 4.CoJn 86141
- 5.Healthy



4.1.2. Chlorotic patches and green islands

In the second group, the CoC 671 variety was taken as the reference (Plates 4b and 6a). The newly emerged leaves appeared pale green. However, closer examination showed green islands appearing dark green patches on the pale green surface. Variation in spread and size of green patches between the leaves in a same plant and variety to variety were noticed. Most of the varieties showed recovery of pale green to dark green lamina in the older leaves. In the older leaves, symptoms of yellowish to pale green patches on green laminar surface were also noticed. The following varieties namely CoC 671, CoM 88121, C 84136, CoJn 86141 and CoS 94210 showed these typical symptoms (Plates-8 and 9).

4.1.3. Yellow blotches and systemic yellowing

The variety, CP-44-101 was taken as the reference (Plate-2a). The symptoms included yellow or pale green specks in the younger leaves and enlarged further with the age of leaves. The yellow specks developed into spindle shaped yellow lesions, further developed as yellow blotches and covered most of the area in the older leaves. Marginal drying was also present. In some varieties, the symptoms appeared as systemic light yellowing. In few other cases, longitudinal light yellow blotches were present (Plate-1b). In some other varieties, the symptoms appeared as scattered yellow patches uniformly throughout the leaf surface with green islands in between. The stunting was common (Plate-1a). Typical examples were CoC 92601, CoS 94270, CoJ 84191, CP 44-101 and Q-65 (Plates-1b, 2a, 3b, 5a, 5b, 7, 8 and 9). Sometimes, the same variety exhibited different symptoms at different locations as well as in different seasons and different growth phases. Likewise, stunting of the plants was noticed in some varieties under all the above categories.

Among the different sugarcane varieties surveyed in different sugar factory areas of Tamil Nadu, most of the varieties expressed mild SCMV symptoms. However, in some of the clones, severe symptoms of SCMV were present. In the varieties CoC 671, CoC 92061, Co 8021, CoC 90063, Co 97009 etc., severe symptoms were

present in most of the sugarcane growing areas (Plates-8 and 9). The clones maintained at Sugarcane Breeding Institute, Coimbatore, showed SCMV symptoms to varying intensities.

4.2. Strain differentiation based on differential host reactions

The following eleven sugarcane varieties Co 740, Co 1150, CoC 671, CoS 86218, CoJ 84191, CoJn 86141, CoM 9217, CoLk 8001, CoPant 8219, UP 95-30 and CP 44-101 showing varying SCMV symptoms were maintained in the glass house (Plates-8 and 9).

4.2.1. Symptom expression by sorghum differentials

The reactions of three sorghum differentials namely Atlas, Rio and Johnson grass and sugarcane differentials namely Co 281, CP 31-294 and CP 31-588 were recorded up to three months after SCMV inoculation and compared with the reactions of individual SCMV strains described by Kondaiah and Nayudu (1984), Gillaspie and Mock (1984) and Tasic *et al.* (1990). Based upon the similarities in symptom expression, the sugarcane varieties were classified into different groups.

4.2.1.1. Symptoms in Atlas

The three sorghum differentials, namely Atlas, Rio and Johnsgrass expressed characteristic symptoms upon inoculation with different sugarcane varieties. About four groupings were made in Atlas based on the symptoms as follows.

Group A	Group B	Group C	Group D
CoC 671	CoJ 84191	CP 44-101	CoJn 86141
Co 740	CoM 9217	CoPant 85210	
CoLk 8001	CoS 86218		

In group-A, the symptoms expressed were mild or severe mosaic in the younger leaves and in the older leaves, longitudinal chlorotic streaks and flecks intermingled with reddish stripes. Presence of necrotic stripes, marginal and tip drying of older leaves as well as complete drying of leaves were also noticed. Stunting of the infected plants was common. Occasionally, the death of the plants was noticed (Plates-10a, 10b and 11a). In group-B, severe chlorosis in the younger leaves appeared, whereas the older leaves expressed necrotic stripes with severe chlorosis. Drying of leaf margin and presence of reddish spots were also noticed in the older leaves. Due to severe chlorosis, the whole plant looked pale and weak (Plate-11b). In group-C, most of the leaves expressed chlorosis especially the younger leaves. The older leaves showed partial recovery from the chlorosis and expressed mosaic symptoms (Plate-12a). However, necrosis or reddish spots were completely absent. In group-D, only the younger leaves expressed chlorotic streaks and flecks. The older leaves recovered from the chlorotic streaks and flecks (Plate-12b).

4.2.1.2. Symptoms in Rio

On Rio, all the sugarcane varieties produced almost similar symptoms. The symptoms were severe mosaic, chlorotic stripes, chlorotic flecks and mottling. Scattered necrotic spots were also present (Plate-13).

4.2.1.3. Symptoms in Johnson grass

Some of the clones viz., Co 740, Co 1158, CoC 671, CoC 92061, CP 44-101, CoJn 86141 and Q-65 produced mosaic symptoms of mild intensity as longitudinal streaks and stripes on Johnson grass (Plate-14).

4.2.2. Symptoms on sugarcane differentials

4.2.2.1. Symptoms in CP 31-294

The following five groupings were made on CP 31-294 based on the similarities of symptoms produced from different sugarcane varieties.

Plate:10. Differentiation of SCMV strains by sorghum differential host-Atlas(Group A - SCMV B and SCMV D)

a. Severe mosaic symptom



b. Chlorotic and reddish stripes



**Plate:11. Differentiation of SCMV strains by sorghum differential host
-Atlas**

a. Stunting (Group A - SCMV-B and SCMV-D)



b. Severe chlorosis and necrotic stripes (Group B - SCMV- B and SCMV-D)



**Plate:12.Differentiation of SCMV strains by sorghum differential host
-Atlas**

a.Chlorosis - Younger and older leaves (Group C- SCMV-A)



b. Chlorosis - Younger leaves (Group D- SCMV - A & SCMV-E)



**Plate:13.Differentiation of SCMV strains by sorghum differential host
-Rio**

Severe mottling



14
Plate Differentiation of SCMV strains by differential host-Johnson grass

a) Infected (Mild Mosaic- SrMV-H / MDMV-B)



b) Healthy



Group A	Group B	Group C	Group D	Group E
Co 740	CP 44-101 CoLk 8001 CoC 671	CoS 86218 UP 95-30 Co 1158 CoPant 85219	CoM 9217	CoJ 84191 CoJn 86141

The symptoms in group-A, included severe stunting, chlorotic stripes, marginal drying and tip necrosis in the leaves (Plate-15a). The symptoms observed in group-B include extreme chlorosis in emerging leaves. In the inoculated leaves, mild mottling with short streaks appeared (Plate-15b). In group-C, presence of light chlorosis in younger leaves was observed which become greener when old (Plate 16a). In group-D, the new and younger leaves expressed scattered streaks with mild chlorosis. In the inoculated and older leaves, short streaks in dense occurred (Plate-16b). There was no reaction and symptom expression in group-E.

4.2.2.2. Symptoms in CP 31-588

Based on the symptoms, the following four groupings were made.

Group A	Group B	Group C	Group D
CoM 9217 Co 1158 CoJ 84191	Co 740	CoC 671 CoPant 85219	CoJn 86141 CoS 86218 CP 44-101 CoLk 8001 UP 95-30

In group-A, the symptoms included severe chlorosis and presence of mild streaks and stripes towards the tip of the leaves (Plate-17a). In group-B, there was moderate stunting and presence of pale green mosaic patches and streaks in younger and older leaves (Plate-17b). In group-C, the symptoms include moderate chlorosis

**Plate:15. Differentiation of SCMV strains by sugarcane differential host
-CP 31-294**

a. Severe stunting, chlorotic stripes and necrosis (Group A - SCMV-D)

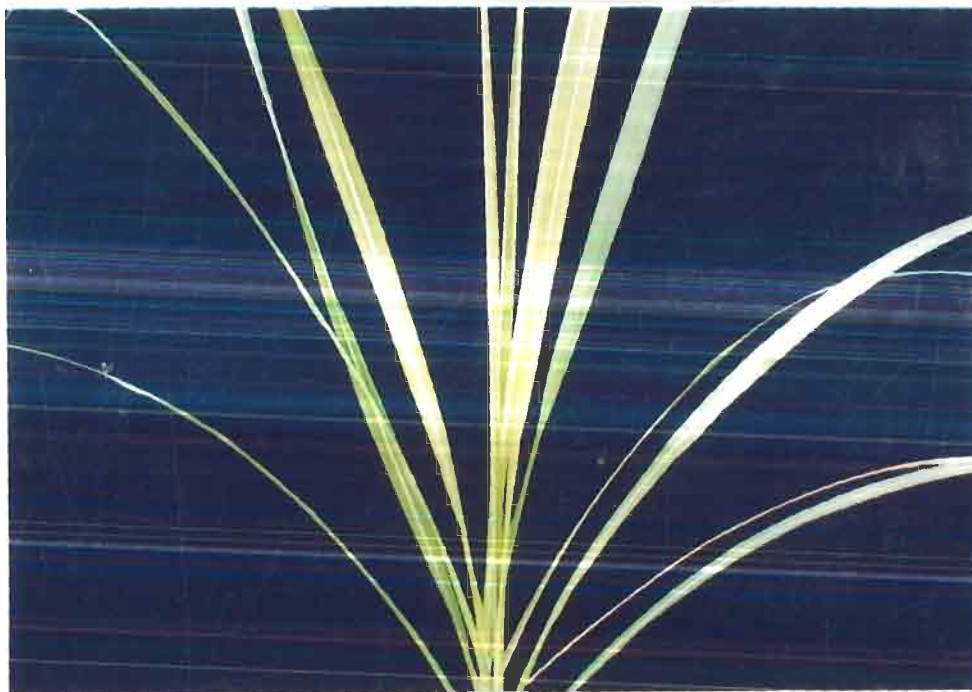


b. Severe chlorosis(Group B-SCMV-B)



**Plate:16.Differentiation of SCMV strains by sugarcane differential
host -CP 31-294**

a. Chlorosis in younger leaves (Group C - SCMV - A)



b. Scattered streaks (Group D - SCMV - E)

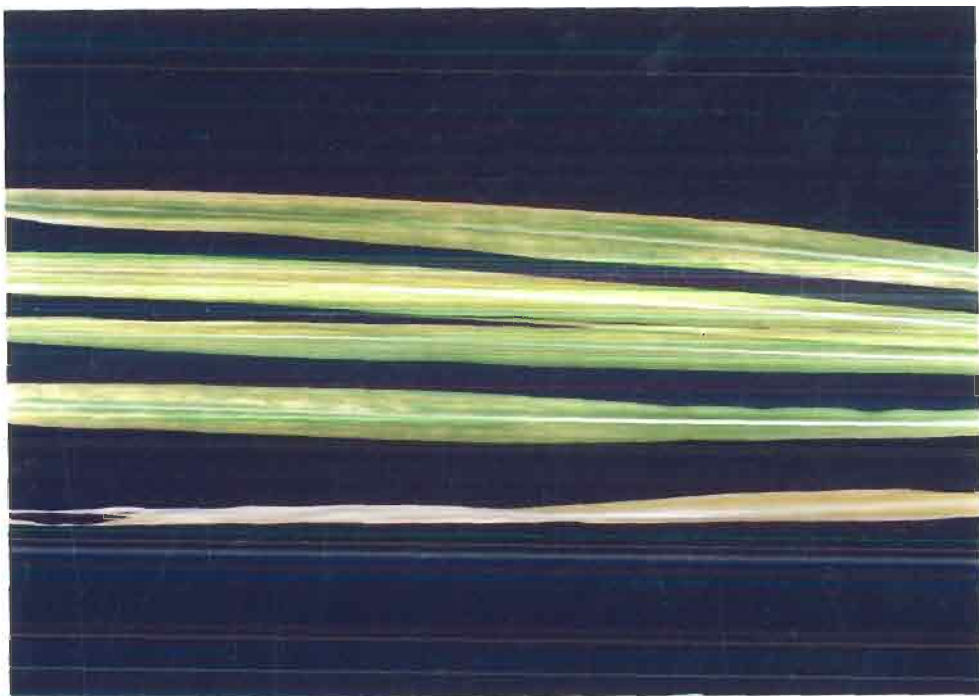


Plate:17. Differentiation of SCMV strains by sugarcane differential host
-CP 31 -588

a. Severe chlorosis (Group A - SCMV-?)



b. Severe pale green and yellow patches (Group B- SCMV-B)



with yellowing towards the base of the leaves and presence of streaks towards the tip (Plates-18a). There was presence of mild streaks and light chlorosis in group-D (Plate-18b).

4.2.2.3.Symptoms in Co 281

Three groups were formed in Co 281 variety based on the symptom similarity. They were as follows.

GroupA	GroupB	GroupC
CoM 9217, Co 1158, CoJ 84191, CoC 671	CoPant 85219	UP 95-30
CoJn 86141, CoS 86218, CP 44-101, CoLk 8001	Co 740	

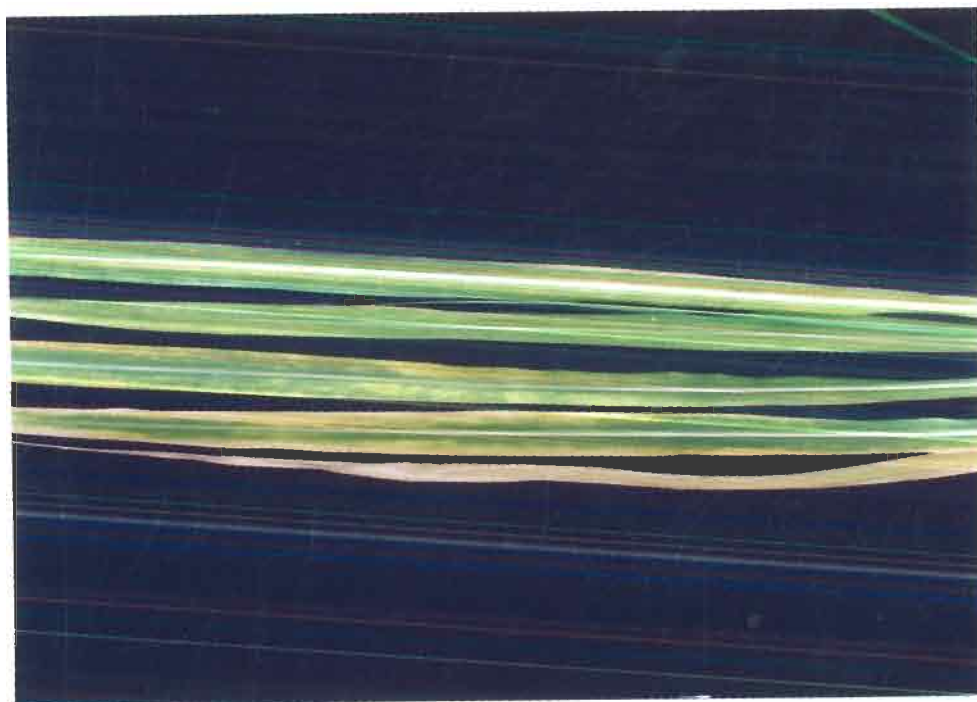
In group-A, there was severe bleached appearance towards the tip of the younger and newer leaves. In the basal portion, light green or pale yellow longitudinal streaks intermingled with green islands were observed. The intermediate leaves towards the base showed pale green streaks. In the older leaves, mild bleaching or complete absence of bleaching but light green bands appeared (Plates-19a and 19b). In group-B, in all the leaves, light green bands were present with mild bleached appearance (Plate-20). In group-C, there was no reaction and complete absence of any symptom.

4.2.3.Symptoms for the strain differentiation

Based on the symptoms in the differential hosts, the following characteristic symptoms were taken into consideration for the strain differentiation. In the present study, the symptoms produced in differential hosts were compared with the previous descriptions and presence of probable strains was identified. Since most of the sugarcane varieties might contain mixture of strains, they produced overlapping symptoms in differential host varieties.

**Plate:18. Differentiation of SCMV strains by sugarcane differential host
-CP 31- 588**

a. Mild chlorotic patches and stripes (Group C- SCMV-A and SCMV-D)



b. Mild chlorotic streaks (Group D- SCMV-D)

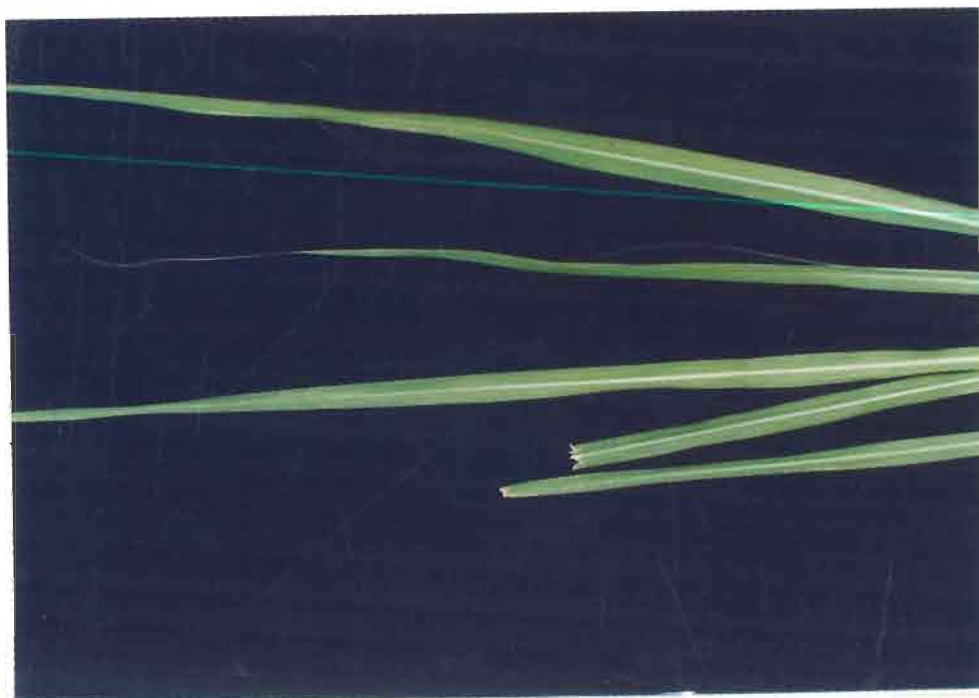
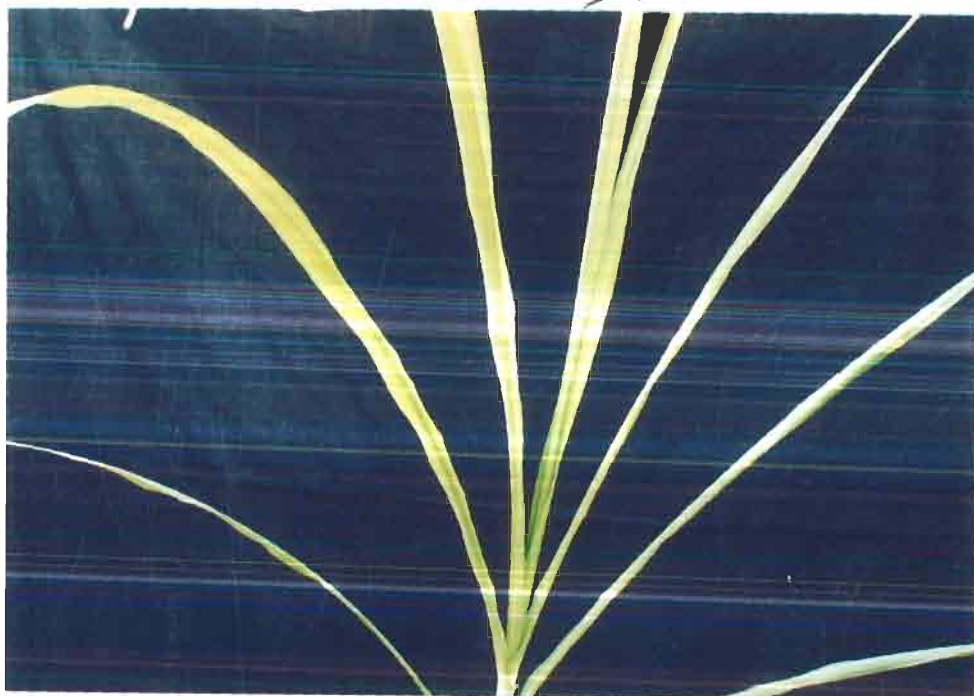


**Plate:19. Differentiation of SCMV strains by sugarcane differential host
- Co 281(Group A: SCMV-?)**

a. Pale green and longitudinal streaks



b. Bleached appearance



**Plate: 20. Differentiation of SCMV strains by sugarcane differential
host - Co 281(Group B - SCMV-A/B/D/E)**

Chlorotic bands



For SCMV strain-A, severe mosaic symptoms followed by necrotic streaks and stripes were observed only in the inoculated leaves in Atlas (Plates-12a and 12b). In CP-31-294, coarse pattern of irregular mild mottling and moderate stunting were present (Plate-16a). In CP-31-588, severe chlorosis was observed (Plate-18a). In Co 281, ordinary mosaic symptoms were noticed (Plate-20).

For SCMV-B and D, severe mosaic symptoms followed by severe necrotic streaks and stripes were present in both the inoculated and newer leaves of Atlas (Plates 10a, 10b and 11a). In CP 31-294, SCMV-B produced severe chlorotic stripes, which coalesced to give bleached appearance. Necrosis and stunting were also common (Plate 15a). For SCMV-D, severe chlorotic streaks extended to full leaves with necrosis and reddening appearance. The plants were severely stunted and the growing points were killed very often. In CP31-588, along with the chlorotic stripes, moderate stunting was also observed for strain-B whereas, for strain-D, only chlorotic stripes were observed (Plates-18a and 8b).

The SCMV-E and MDMV-B strains caused necrotic streaks and stripes only on the inoculated leaves of Atlas (Plate-12b) and SCMV-E was also identified based on necrosis and chlorotic streaks in Rio. The SCMV-E also produced scattered streaks and mild chlorosis in CP 31-294.

For SrMV-H and MDMV-B, presence of mild mosaic was reported in Johnson grass (Plate 14) whereas severe mosaic was reported for SCMV-A. In Atlas also, SrMV-I and SrMV-H caused necrosis and death but it was less pronounced for SrMV H. In Rio (Plate 13), ordinary mosaic symptoms were reported for all the strains except SCMV-B, which caused necrosis also. In Co 281 also, ordinary mosaic symptoms were reported for SCMV-A, B, D, E and SrMV-H.

4.2.3.1. Strain differentiation based on differential host reactions

From the Table-2, it was clear that each sugarcane variety expressed characteristic symptoms in each differential host similar to a particular strain.

Table-2. Differentiation of SCMV strains by sorghum and sugarcane differential host reactions

S.No.	Sugarcane variety	Sorghum differential host reactions			Sugarcane differential host reactions			Probable strains
		Atlas	Rio	Johnson grass	Co 281	CP 31-294	CP31-588	
1	CoC 671	M _(1 or 2) /Y1 C _{str} /OI R _{str} /OI N _{str} /M _{dr} T _{dr} /St _(1 or 2) D	M ₍₂₎ C _{str} , Cfl N _{sp} /OI	M ₍₁₎ C _{st} /Cfl	Ble ₍₂₎ /tip Y1 Ch _{st} /base Ch _{st} /Int. I Ble ₍₁₎ , Ch _{bd} /OI	Ch ₍₂₎ /Y1 C _{st} , mot/II	Ch _(1 or 2) St, C _{str} /tip yel. (base)	SCMV-A SCMV-B SCMV-D SCMV-E MDMV-B SrMV-H
2	Co 740	M _(1 or 2) /Y1 C _{str} /OI R _{str} /OI N _{str} /M _{dr} T _{dr} /St _(1 or 2) D	M ₍₂₎ C _{str} , Cfl N _{sp} /OI	M ₍₁₎ C _{st} , Cfl	Ch _{bd} , Ble ₍₁₎	Ch ₍₂₎ , St ₍₂₎ C _{str} M _{dr} , T _{dr}	St _(1 or 2) M _(1 or 2) St/Y1 or OI	SCMV-A SCMV-B SCMV-D SCMV-E MDMV-B SrMV-H
3	CoLk 8001	M _(1 or 2) /Y1 C _{str} /OI R _{str} /OI N _{str} /M _{dr} T _{dr} /St _(1 or 2) D	M ₍₂₎ C _{str} , Cfl N _{sp} /OI	No reaction	Ble ₍₂₎ /tip Y1 Ch _{st} /base, Y1 Ch _{st} /Int. I Ble ₍₁₎ , Ch _{bd} /OI	Ch ₍₂₎ , St ₍₂₎ C _{str} M _{dr} , T _{dr}	Ch ₍₁₎ St ₍₁₎	SCMV-E SrMV-H SCMV-B SCMV-D
4	CoJ 84191	Ch ₍₂₎ /Y1 N _{str} /OI M _{dr} /OI R _{sp} /OI	M ₍₂₎ C _{str} , Cfl N _{sp} /OI	No reaction	Ble ₍₂₎ /tip Y1 Ch _{st} /base Y1 Ch _{st} /Int. I Ble ₍₁₎ , Ch _{bd} /OI	No reaction	Ch ₍₂₎ St _{str} /tip	SCMV-B SCMV-D

Table 2. Continued

S.No.	Sugarcane variety	Sorghum differential host reactions			Sugarcane differential host reactions			Probable strains
		Atlas	Rio	Johnsons grass	Co 281	CP 31-294	CP31-588	
5	CoM 9217	Ch ₍₂₎ /YI N _{str} /OI M _{dr} /OI R _{sp} /OI	M ₍₂₎ C _{str} , Cfl N _{sp} /OI	No reaction	Ble ₍₂₎ /tip YI Ch _{st} /base YI Ch _{st} /Int. I Ble ₍₁₎ , Ch _{bd} /OI	Ch ₍₂₎ St ₍₁₎ /YI St ₍₂₎ /OI	Ch ₍₂₎ St, C _{str} /tip	SCMV-B SCMV-D
6	Co 1158	Ch ₍₂₎ /YI N _{str} /OI M _{dr} /OI R _{sp} /OI	M ₍₂₎ C _{str} , Cfl N _{sp} /OI	M ₍₁₎ C _{st} , Cfl	Ble ₍₂₎ /tip, YI Ch _{st} /base, YI Ch _{st} /Int. I Ble ₍₁₎ , Ch _{bd} /OI	Ch ₍₁₎ /YI	Ch ₍₂₎ St _{str} /tip	SCMV-B SCMV-D MDMV-B SrMV-H
7	CoS 86218	Ch ₍₂₎ /YI N _{str} /OI M _{dr} /OI R _{sp} /OI	C ₍₂₎ C _{str} , Cfl N _{sp} /OI	No reaction	Ble ₍₂₎ /tip YI Ch _{st} /base YI Ch _{st} /Int. I Ble ₍₁₎ , Ch _{bd} /OI	Ch ₍₁₎ /YI	Ch ₍₁₎ St ₍₁₎	SCMV-B SCMV-D
8	CP 44-101	Ch ₍₂₎ /YI C ₍₁₎ /OI	M ₍₂₎ C _{str} , Cfl N _{sp} /OI	M ₍₁₎ C _{st} , Cfl	Ble ₍₂₎ /tip YI Ch _{st} /base YI Ch _{st} /Int. I Ble ₍₁₎ , Ch _{bd} /OI	Ch ₍₂₎	Ch ₍₁₎ St ₍₁₎	SCMV-A SrMV-H MDMV-B
9	CoPant 85219	Ch ₍₂₎ /YI C ₍₁₎ /OI	M ₍₂₎ C _{str} , Cfl N _{sp} /OI	No reaction	Ch _{bd} Ble ₍₁₎	Ch ₍₁₎ /YI	Ch _(1 or 2) St _{str} /tip Yel / base	SCMV-A SCMV-B SCMV-D SCMV-E

Table 2. Continued

S.No.	Sugarcane variety	Sorghum differential host reactions			Sugarcane differential host reactions			Probable strains
		Atlas	Rio	Johnsons grass	Co 281	CP 31-294	CP31-588	
10	CoJn 86141	Chst / Ch- η C ₍₁₎ / Y1 / T1	M ⁽²⁾ C _{str} Cfl N _{sp} / OI	M ⁽¹⁾ , C _{st} , C η	Ble ⁽²⁾ / tip Y1 Ch _{st} / base Y1 Ch _{st} / Int. I Ble ⁽¹⁾ , Ch _{bd} / OI	No reaction	Ch ⁽¹⁾ St ⁽¹⁾	SCMV -A SCMV -E SCMV -D SrMV -H MDMV -B
11	UP 9530	Chst / Ch- η C ₍₁₎ / Y1 / T1	M ⁽²⁾ C _{str} Cfl N _{sp} / OI	No reaction	No reaction	Ch ⁽¹⁾ / Y1	Ch ⁽¹⁾ St ⁽¹⁾	SCMVA SCMV-D SCMV-E SrMV-H MDMV-B

M	-	Mosaic (1: Mild ; 2: severe)	St	-	Stunting (1 – Mild; 2- severe)		
Y1	-	Younger leaves	D	-	Death		
C _{str}	-	Chlorotic stripes	Ch	-	Chlorosis (1- mild; 2- severe)		
R _{str}	-	Reddish stripes	R.sp	-	Reddish specks		
OI	-	Older leaves	Cfl	-	Chlorotic flecks		
N _{str}	-	Necrotic stripes	Ble	-	Bleached appearance (1- mild; 2- severe)		
M _{dr}	-	Marginal drying	Tip Yel	-	Tip yellowing		
T _{dr}	-	Tip drying	Int. I	-	Intermediate leaves.		
Bd	-	Bands	Mot	-	Mottling		

In CoC 671, Co 740, and CoLk 8001, presence of mosaic, chlorotic and necrotic streaks in both the inoculated leaves and newer leaves suggested the presence of SCMV-B or SCMV-D. The mild mosaic reaction in the Johnson grass might have been due to SrMV-H or MDMV-B in CoC 671, Co 740, Co 1158, CP 44-101 and CoJn 86141. In all other varieties no reaction was observed. The virus from CoJn 86141 and UP 95-30 varieties caused chlorotic stripes and necrosis. The similar symptoms in CP 44-101 and CoPant 85219 indicating the presence of SCMV-A.

In CP 31-294, severe stunting, chlorosis and death of the growing points were observed for Co 740 variety, which strongly suggested the presence of SCMV-D. In CoC 671, CP44-101 and CoLk 8001 also above symptoms of Co 740 were observed but with lesser intensity. Moreover, the stunting was much pronounced thereby indicating the presence of SCMV-B. The CoM 9217 variety expressed characteristic scattered streaks in CP 31-294, which might be due to SCMV-E.

In CP31-588, along with the chlorotic stripes, moderate stunting was also observed with varieties namely Co 740, CoC 671, CoPant 85219, CoJn 86141, CoS 86218, CP44-101, CoLk 8001 and UP 95-30 indicating the presence of SCMV-D or SCMV-B.

From the differential reaction in Rio, it was difficult to differentiate the strains because almost all the varieties expressed similar symptoms (Plate 13). Eventhough the expressed symptoms of severe mottling and also the necrotic stripes resembled like SCMV-B, it was difficult to conclude.

In addition to the above probable symptoms, some other ungrouped and previously non-recorded symptoms were also noticed. For example in Co281, in group A, severe chlorosis with bleached appearance and presence of pale longitudinal streaks in the basal portion of the leaves were observed. It was difficult to match these symptoms with any of the reported strains. Likewise, in CP-31-588 also in group-A, extreme chlorosis without streaks was observed and did not match with any of the reported strains.

Among the eleven varieties, Co 740 and CoC 671 recorded the maximum number of strains *viz.*, SCMV-A, SCMV-B, SCMV-D, SrMV- H and MDMV – B. The Co 740 variety exhibited severe SCMV-D type of symptoms. Among the remaining varieties, CoJn 86141 and UP 95-30 recorded SCMV-A, SCMV-D, SCMV-E, SrMV-H and MDMV-B. Likewise, CoLk 8001 recorded SCMV-B, SCMV-D, SCMV E and SrMV-H and most other varieties recorded SCMV-B and SCMV- D.

4.2.3.2. Differentiation of SCMV strains based on serological reactions

As in the differential host reactions, the same eleven sugarcane varieties, which expressed characteristic SCMV symptoms, were taken up for the serological reactions with different antiserum by DAC-ELISA technique. One healthy sample was also included for comparison.

The results (Table-3) revealed that there was no positive reaction by any sugarcane variety with the antisera of JGMV, SCMV-Sabi and SCMV –Yu. Along with SCMV-BC also, except Co 740, no other varieties reacted positively. All the varieties reacted positively with SCMV A. Except CoJ 84191 all the varieties reacted positively with SCMV A. In case of SrMV–H, except Co Pant 85219 and CoS 86218, the remaining varieties reacted positively. Likewise, along with MDMV-B, except CoJ 84191 and CoS 86218, the remaining varieties reacted positively. Along with SCMV polyclonal antiserum, Co 740 recorded nine times higher absorbance when compared to healthy samples. The other varieties namely, Co 1158, CoC 671, CoJn 86141, CoS 86218, CP 55-101 and CoLk 8001 recorded five times higher absorbance when compared to healthy samples. All the sugarcane varieties reacted very strongly with SCMV – polyclonal antisera rather than the remaining strain specific antisera.

Among all the varieties, Co 740 reacted very strongly with all the antisera particularly with the SCMV D and SCMV polyclonal antisera. Along with the polyclonal antisera, nearly eight times higher absorbance was recorded when compared to healthy sample. Like wise, with SCMV-D also, nearly five times higher absorbance

Table-3. Serological reactions of SCMV from different sugarcane varieties with various strain specific antisera and polyclonal antisera by DAC-ELISA

Varieties	Absorbance at 405nm (Mean of three replications)								
	SCMV- A	SCMV- D	SCMV- BC	SCMV- Sabi	SrMV- H	MDMV- B	JGMV	SCMV- Poly	SCMV YU
Co 740	0.394 (+)	0.551 (+)	0.362 (+)	0.269	0.398 (+)	0.327 (+)	0.108	0.998 (+)	0.251
Co 1158	0.291 (+)	0.284 (+)	0.321	0.248	0.311 (+)	0.221 (+)	0.148	0.507 (+)	0.290
CoC 671	0.318 (+)	0.241 (+)	0.208	0.291	0.341 (+)	0.311 (+)	0.142	0.598 (+)	0.212
CoM 9217	0.220 (+)	0.241 (+)	0.351 (+)	0.218	0.258 (+)	0.187	0.101	0.412 (+)	0.211
Co J 84191	0.109	0.268 (+)	0.221	0.214	0.312 (+)	0.141	0.140	0.401 (+)	0.195
CoPant 85219	0.244 (+)	0.299 (+)	0.298	0.291	0.158	0.212 (+)	0.112	0.481 (+)	0.182
CoJn 86141	0.222 (+)	0.258 (+)	0.191	0.256	0.281 (+)	0.251 (+)	0.149	0.560 (+)	0.174
CoS 86218	0.251 (+)	0.223 (+)	0.213	0.241	0.143	0.181	0.181	0.581 (+)	0.181
CP 44-101	0.190	0.241 (+)	0.181	0.191	0.268 (+)	0.261 (+)	0.159	0.498 (+)	0.198
CoLk 8001	0.280 (+)	0.301 (+)	0.190	0.221	0.251 (+)	0.248 (+)	0.181	0.560 (+)	0.190
UP 95-30	0.252 (+)	0.308 (+)	0.188	0.250	0.262 (+)	0.212 (+)	0.141	0.483 (+)	0.202
Control	0.102	0.109	0.178	0.167	0.124	0.103	0.129	0.120	0.186

(+) indicates positive reaction

was observed and it was 0.551 as against 0.109 in healthy control. Along with SrMv-H and MDMV-B, nearly three times higher absorbance was recorded. Along with SCMV-A, four times higher absorbance was recorded when compared to healthy samples. Next to Co 740, the CoC 671 variety reacted with different antisera and the absorbance value against SCMV-Polyclonal antiserum was 0.598 and against SCMV – A was 0.318. Along with sorghum mosaic virus also, nearly three times higher absorbance value was recorded when compared to healthy samples.

In general, Co 740 followed by CoC 671, CoJn 86141, CoS 86218, CoLk 8001 and Co 1158 recorded the strongest reactions. The above results also suggested the possibilities of presence of mixture of strains otherwise they were closely related together and difficult to distinguish them by serological reactions.

4.3. Purification of SCMV

The SCMV particles were separated after the sucrose gradient centrifugation. The particles under UV light were visible as a scattering zone and separated out by using a syringe and dialysis was carried out. Then dissolved in resuspension buffer and used for subsequent characterization.

4.3.1. Characterization

There were number of intact, unaggregated SCMV particles in electron micrographs (Plate-21). They appeared as long, flexuous rod shaped particles measuring 690 to 740 nm in length. The UV absorbance profile of the purified SCMV from 250 to 300 nm is given in the fig. 1. The maximum absorbance of 1.49 was recorded at 268 nm. The absorbance ratio of 260/280 nm was 1.2. The purified SCMV preparation produced characteristic symptoms of severe mosaic and mottling in the sorghum cultivar 'Rio' and 'Atlas' (Plate -22). The virus yield was 5.25 mg per 100 gm of infected leaves was obtained from the SCMV infected CoC 671 variety based on the formula as described in chapter 3.4.4.

Figure.1 UV absorbance profile of the purified SCMV

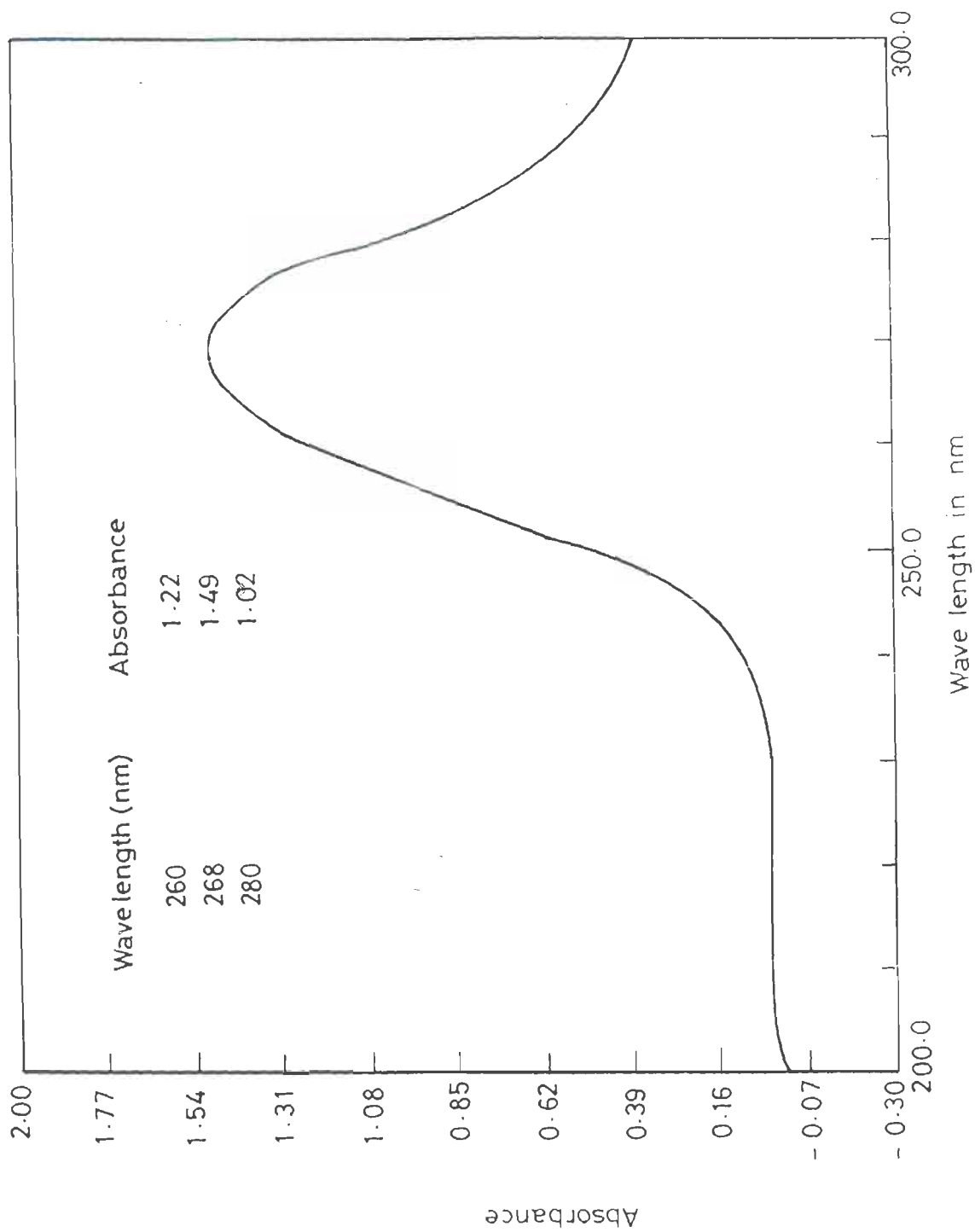


Plate 24. Immunosorbant electron micrograph of the purified SCMV particles



Plate:22. Biological assay of the purified SCMV



2. Rio - Mottling

1. Atlas - Severe mosaic

4.3.2. Serological relationship of the purified virus

The purified SCMV strongly reacted with different strain specific antisera in ELISA test. The reactions considered as positive with SCMV strains A, D, polyclonal antiserum of SCMV, MDMV-B and SrMV (Table-3). The reactions revealed the possible mixture of strains present in the purified virus preparations. Moreover, the reaction with the SCMV polyclonal antiserum was very strong when compared to the strain specific SCMV antisera. Likewise, the reaction with other related viruses namely SrMV-H and MDMV-B was weak when compared to SCMV antiserum. The purified virus did not react with SCMV strains BC, Sabi and Yu indicating the absence of these strains or the purified SCMV did not have much relationship with these strains. The JGMV antiserum did not react with the purified SCMV.

4.4. Molecular characterization of the purified SCMV

4.4.1. Electrophoretic separation of SCMV coat protein

The coat protein of the purified SCMV migrated as a single band in gel electrophoresis. The molecular weight of the purified SCMV was estimated to be 40.1kDa after comparing the R_f values of the markers and purified SCMV (Plate-23).

4.4.2. Electroblot immuno assay for the determination of strainal relationship of the purified SCMV

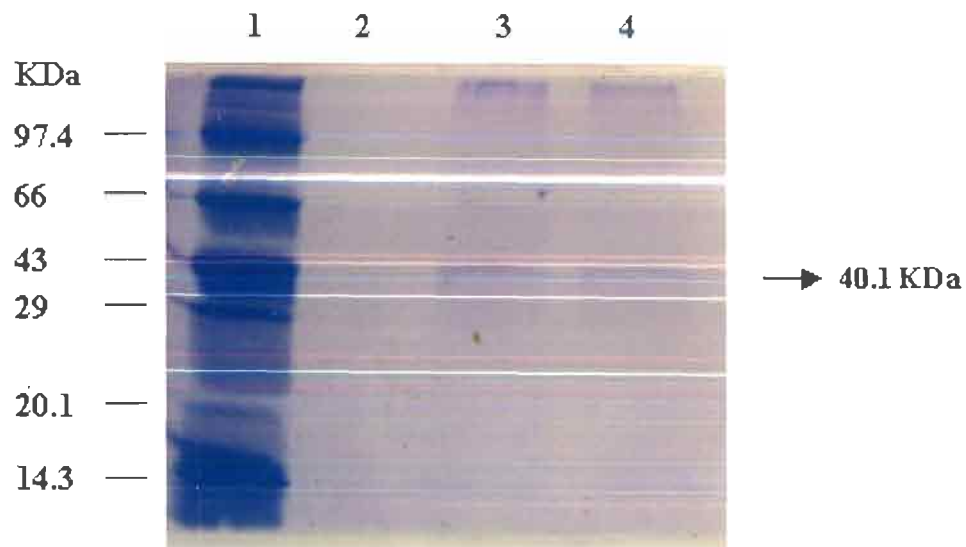
The electroblot immuno assay with different antisera was performed to determine the strainal relationship of the purified SCMV. The results (Plate-24) revealed that the purified SCMV had strongly reacted with the SCMV-polyclonal antiserum. It also reacted with the SCMV strains A, D and also with the antisera of SrMV-H. These results were in confirmation with the DAC-ELISA results except for the reaction of MDMV-B where the reaction was positive in DAC-ELISA but there was no reaction in electroblot immunoassay. Along with the other antisera, namely SCMV-BC, Sabi and Yu as well as with JGMV, there was no reaction in electroblot immunoassay. These results also revealed that, the purified SCMV might contain

Table.4. Serological relationship of the purified SCMV

Antisera of different strains	Absorbance at 405 nm (Mean of three replications)	
	Purified SCMV	Healthy control
SCMV-A	0.296 (+)	0.107
SCMV-BC	0.082	0.109
SCMV-D	0.309 (+)	0.121
SCMV-Sabi	0.080	0.111
SCMV-Yu	0.064	0.102
SrMV	0.229 (+)	0.109
MDMV-B	0.221 (+)	0.107
JGMV	0.092	0.091
SCMV- Polyclonal antiserum	0.829(+)	0.120

(+) indicates positive reactions

Plate 23 Electrophoretic separation of the coat protein from purified SCMV

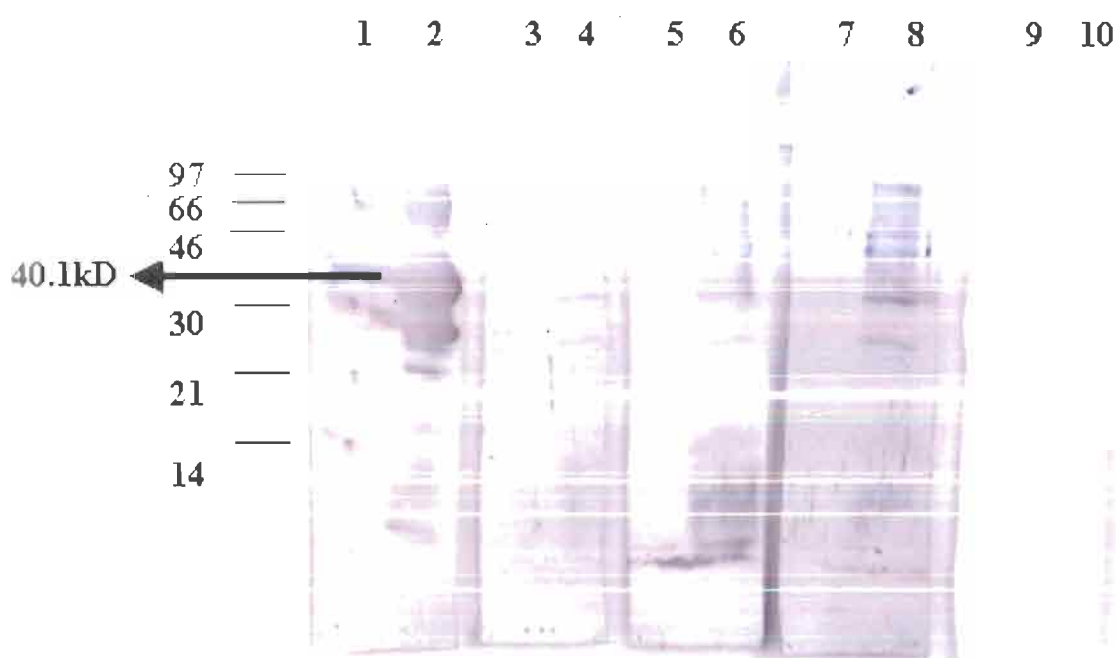


Lane 1: Molecular weight markers

Lane 2: Empty well

Lane 3&4: Coat protein of purified SCMV

Plate 24. Determination of serological relationship of the purified SCMV by electro blot immunoassay



Lane 1: Molecular weight markers

Lane 2: Sugarcane mosaic virus (SCMV) - polyclonal antiserum

Lane 3: SCMV-BC

Lane 4: SCMV-A

Lane 5: SCMV-Sabi

Lane 6: SCMV-D

Lane 7: SCMV-YU

Lane 8: Sorghum mosaic virus-H (SrMV-H)

Lane 9: Maize dwarf mosaic virus-B (MDMV-B)

Lane 10: Johnson grass mosaic virus (JGMV) - Polyclonal antiserum

mixture of strains *viz.*, SCMV-A, D and SrMV or a SCMV strain having close relation with these strains. However, the strong reaction (Plate-24) with the polyclonal antiserum and comparatively weak reaction with strain specific antiserum supports the presence of mixture of strains.

4.4.3. Virus nucleic acid

From the purified SCMV, viral RNA was separated and analysed in the gel electrophoresis, separately as well as after treating with ribonuclease enzyme (Plate-25). In the ribonuclease treated lane, there was absence of visible band whereas in the other lane a prominent band was visible indicating the presence of RNA genome.

4.4.4. RT-PCR amplification of the purified SCMV genome

The genome of the SCMV RNA from the purified SCMV got amplified with the SCMV specific primer by RT-PCR method (Plate-26). The molecular weight of the amplified DNA was nearly 870 base pairs.

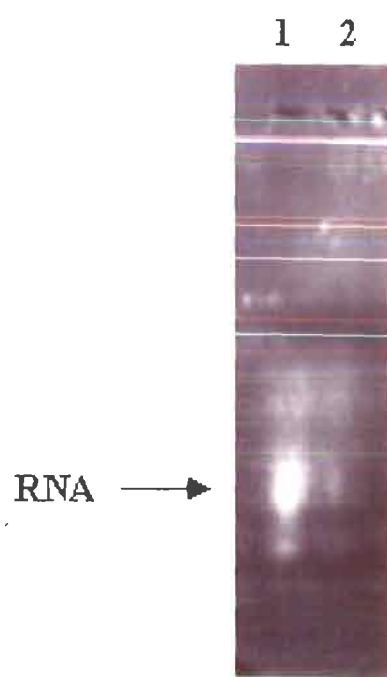
4.5. Production of polyclonal antiserum and determination of antiserum titre

Antisera were obtained by administering two different immunization methods. In the first experiment, four intra muscular injections of purified virus were administered and in the second experiment only one immunization was carried out. In both the methods, bleeding was done one week after the final injection and continued for one more time during the following week. Four different concentrations of the antisera *viz.*, 1/100, 1/500, 1/1000 and 1/5000 were used. Likewise, four different concentrations of antigen *viz.*, 1/25, 1/50, 1/100 and 1/500 were used in DAC-ELISA to study the serological reaction.

4.5.1. Reaction of antisera from single immunizaion

The antiserum was obtained one week and two weeks after single immunization. The titre of the early bleed antiserum was low and positive reaction was obtained up to 1:1000 antiserum dilution (Table-5). When the antigen dilution was increased from 1/25 to 1/50, positive serological reaction was obtained only up to 1:500 antiserum

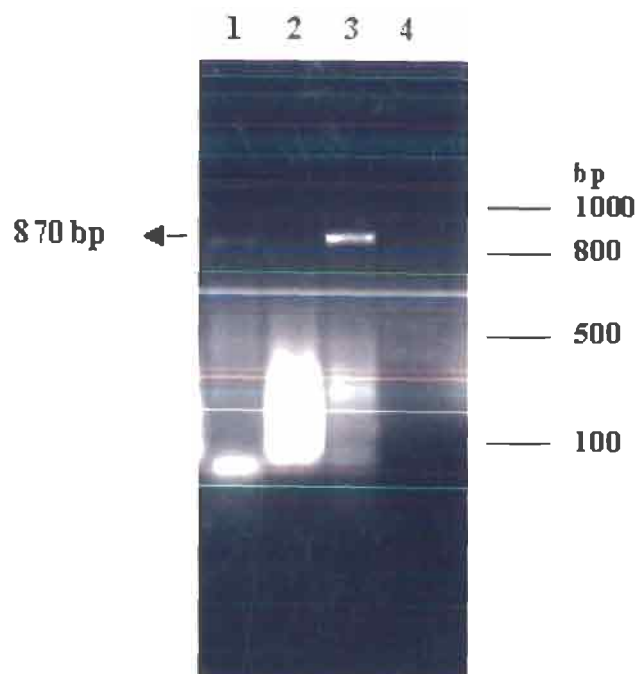
Plate 25 Separation of RNA genome from the purified SCMV by
agarose gel electrophoresis



Lane 1: SCMV RNA

Lane 2: Ribonuclease treated RNA

Plate 26 Amplification of viral genome from the purified SCMV by RT-PCR method (SCMV specific primer)



- Lane 1: SCMV infected sugarcane leaves (CoC 671)
- Lane 2: Healthy sugarcane leaves
- Lane 3: Purified SCMV
- Lane 4: 1kb DNA ladder

**Table-5. Serological reactions of SCMV antigen and antisera in DAC ELISA
(Early bleed-Single immunization)**

Antigen dilution	Absorbance at 405nm (Mean of three replications)							
	1/100		1/500		1/1000		1/5000	
	H	D	H	D	H	D	H	D
1/25	0.181	0.381 (+)	0.131	0.346 (+)	0.121	0.246 (+)	0.124	0.194
1/50	0.131	0.284 (+)	0.130	0.264 (+)	0.109	0.201	0.102	0.114
1/100	0.107	0.119	0.098	0.104	0.102	0.110	0.091	0.101
1/500	0.109	0.114	0.120	0.118	0.090	0.107	0.081	0.080

(+) indicates positive reactions

H: Healthy

D: Diseased

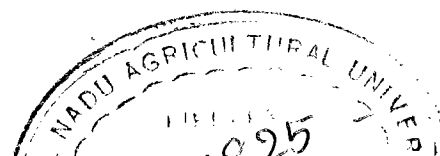
**Table-6. Serological reactions of SCMV antigen and antisera in DAC ELISA
(Late bleed-Single immunization)**

Antigen dilution	Absorbance at 405nm (Mean of three replications)							
	1/100		1/500		1/1000		1/5000	
	H	D	H	D	H	D	H	D
1/25	0.161	0.486 (+)	0.130	0.321 (+)	0.117	0.291 (+)	0.112	0.240 (+)
1/50	0.130	0.321 (+)	0.121	0.302 (+)	0.105	0.241 (+)	0.101	0.217 (+)
1/100	0.109	0.224 (+)	0.107	0.194	0.114	0.151	0.107	0.142
1/500	0.108	0.162	0.121	0.147	0.102	0.128	0.098	0.108

(+) indicates positive reactions

H: Healthy

D: Diseased



dilution. However, the titre of the late bleed antisera (Table-6) was higher and positive reactions were recorded up to 1:5000 antiserum dilution. The results revealed that, with single immunization, antiserum titre was lower in the early bleed and increased in the late bleed.

4.5.2. Reaction of antisera from multiple immunizaion

The DAC-ELISA results (Table-7) revealed that with multiple immunizations, the titre of the antiserum was increased. From the early bleed antiserum, the positive reaction with the SCMV antigen was recorded up to 1:5000 antiserum dilution. The titre of the late bleed antiserum (Table-8) was higher and positive reactions were recorded with further diluted antigen.

4.5.3. Reactions of SCMV antisera with the related potyviruses

The SCMV antisera obtained in both the above two immunization methods were subjected for serological reactions with the three related potyviruses in DAC-ELISA method. The viruses tested were, sorghum mosaic virus, banana bract mosaic virus (BBMV) and papaya ring spot virus. Constant antigen concentration of 1/25 was used and antiserum dilution was 1/500. The antisera obtained in both the immunization methods, at weekly intervals were used. The SCMV antigen was also used for the comparison. The results revealed that (Table-9), the first bleed antisera with single immunization reacted only with SCMV and did not react with the other three potyviruses. However, the late bleed antisera reacted with the SrMV also. In the second immunization method with four intra muscular injections, the antisera from the early bleed reacted positively with SrMV and BBMV infected tissues in addition to SCMV. Whereas the late bleed antisera reacted with all the potyviruses including papaya ring spot virus. With single immunization of SCMV, only low titre antiserum can be obtained but reaction was specific with SCMV and did not react with other potyviruses except SrMV. But high titre antiserum can be obtained with multiple immunizations. However, the antiserum reacted with the related potyviruses. The reaction with the SCMV antigen was very strong when compared to other three potyviruses.

**Table-7. Serological reactions of SCMV antigen and antisera in DAC ELISA
(Early bleed-Multiple immunization)**

Antigen dilution	Absorbance at 405nm (Mean of three replications)							
	1/100		1/500		1/1000		1/5000	
	H	D	H	D	H	D	H	D
1/25	0.168	0.562 (+)	0.150	0.441 (+)	0.143	0.392 (+)	0.104	0.298 (+)
1/50	0.160	0.471 (+)	0.138	0.371 (+)	0.127	0.283 (+)	0.106	0.221 (+)
1/100	0.123	0.302 (+)	0.126	0.264 (+)	0.118	0.214	0.108	0.191
1/500	0.112	0.161	0.107	0.151	0.111	0.148	0.108	0.114

(+) indicates positive reactions

H: Healthy

D: Diseased

**Table-8. Serological reactions of SCMV antigen and antisera in DAC ELISA
(Late bleed-Multiple immunization)**

Antigen dilution	Absorbance at 405nm(Mean of three replications)							
	1/100		1/500		1/1000		1/5000	
	H	D	H	D	H	D	H	D
1/25	0.169	0.739 (+)	0.161	0.656 (+)	0.145	0.501 (+)	0.138	0.301 (+)
1/50	0.154	0.598 (+)	0.150	0.451 (+)	0.128	0.320 (+)	0.121	0.252 (+)
1/100	0.138	0.407 (+)	0.113	0.307 (+)	0.120	0.251 (+)	0.117	0.198
1/500	0.127	0.298 (+)	0.121	0.244 (+)	0.114	0.198	0.108	0.162

(+) indicates positive reactions

H: Healthy

D: Diseased

Table-9. Serological reaction between SCMV antisera and related potyviruses in DAC-ELISA

SCMV Antiserum	Absorbance at 405 nm							
	Papaya ring spot		Sorghum mosaic		Banana bract mosaic		Sugarcane mosaic	
	H	D	H	D	H	D	H	D
Single immunization – early bleed	0.091	0.107	0.109	0.192	0.092	0.101	0.122	0.322 (+)
Single immunization - late bleed	0.110	0.122	0.102	0.221(+)	0.108	0.101	0.116	0.366 (+)
Multiple immunization early bleed	0.171	0.211	0.102	0.209(+)	0.101	0.212(+)	0.146	0.462 (+)
Multiple immunization-late bleed	0.140	0.289 (+)	0.147	0.302(+)	0.121	0.262(+)	0.167	0.672 (+)

(+) indicates positive reactions

H: Healthy

D: Diseased

4.6. Standardisation of serological and molecular diagnostic techniques

4.6.1. Electroblot immunoassay

The SCMV infected and healthy sugarcane tissues (CoC 671 and Co 740 varieties) were subjected for electroblot immunoassay by using the first bleed antiserum from the single and multiple immunization method (1:1000 dilution). The purified SCMV was used as control. The antiserum reacted positively with the purified SCMV and infected tissues and there was absence of positive reaction from the healthy tissues (Plate-28).

4.6.2. Immunosorbant electron microscopy (ISEM)

From the infected and healthy tissues, sap was extracted and ISEM was performed. The first bleed antiserum from the single immunization method was used (1:1000 dilution). The SCMV particles in the infected tissues were trapped by the produced antiserum. The particles were flexuous rods with 700 to 730 nm (Plate-29).

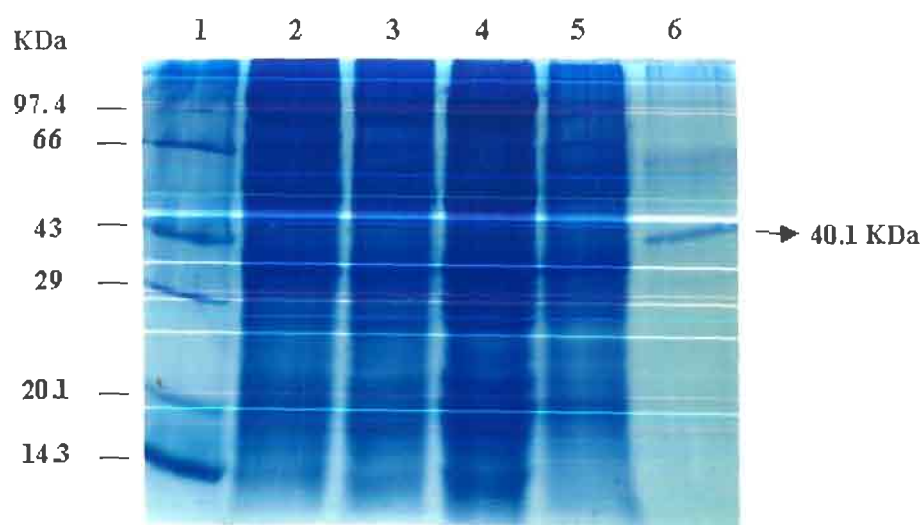
4.6.3. Dot blot immunoassay

In the nitrocellulose membrane, 1: 1000 dilution of first and second bleed antisera from both the immunization methods were used for dot blot immunoassay by using the healthy and SCMV infected sap from the sugarcane tissues. Both the antisera produced positive reaction with the sap from SCMV infected tissues (Plate-30). The antiserum from the multiple immunization method developed deep blue colour indicating a very strong reaction

4.6.4. Determination of the concentration of SCMV in various sugarcane tissues by using DAC-ELISA

The SCMV concentration from different sugarcane tissues was determined by DAC-ELISA method. The ELISA readings revealed that (Table-10), except root tissues, in all the remaining sugarcane tissues, the SCMV was present in high concentration. Among the various tissues, the concentration was higher in the top youngest leaf blade followed by third and sixth leaves. Between the mid rib and leaf

Plate 21 Polyacrylamide gel electrophoresis of the SCMV from the sugarcane (CoC 671) and sorghum (Rio) leaves



Lane 1: Molecular weight markers

Lane 2: Healthy sugarcane leaves

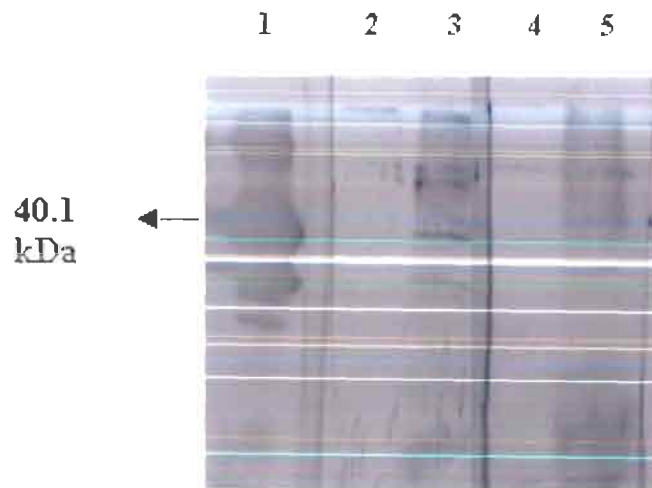
Lane 3 : SCMV infected sugarcane leaves

Lane 4 : Healthy sorghum leaves

Lane 5 : SCMV infected sorghum leaves

Lane 6 : Purified SCMV

Plate 28 Detection of SCMV from the infected sugarcane tissues
by electroblot immunoassay



Lane 1: Purified SCMV

Lane 2: Healthy leaves (CoC 671)

Lane 3: Infected leaves (CoC 671)

Lane 4: Healthy leaves (Co 740)

Lane 5: Infected leaves (Co 740)

Plate 21 Detection of SCMV particles from the infected sugarcane leaves
by immunosorbant electron microscopy (ISEM)

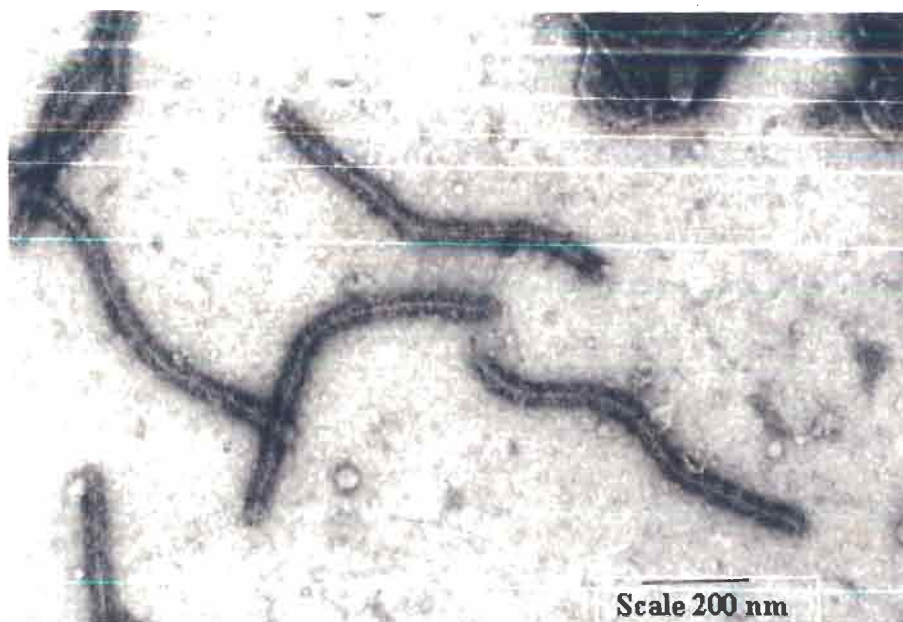
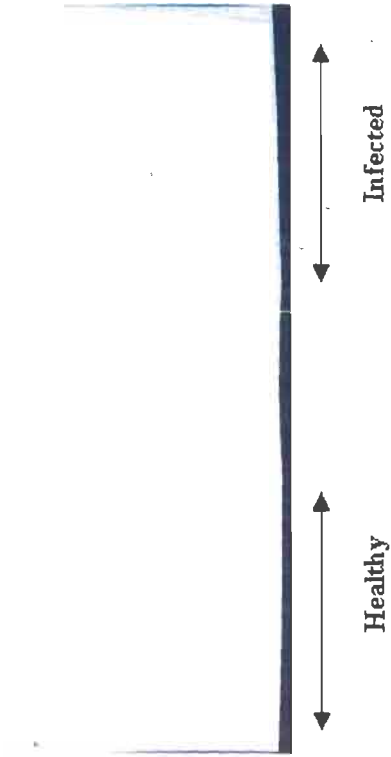
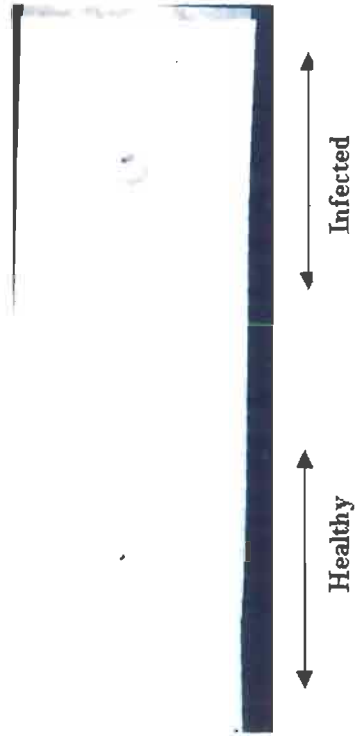


Plate 30. Detection of SCMV from the infected sugarcane leaves by dot blot immunoassay (DBIA)

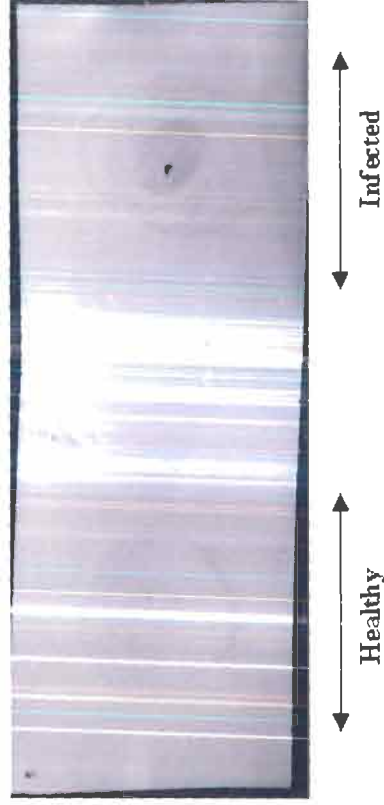
a) First bleed- antiserum with single immunization



b) Second bleed antiserum with single immunization



c) First bleed antiserum with multiple immunization



d) Second bleed antiserum with multiple immunization

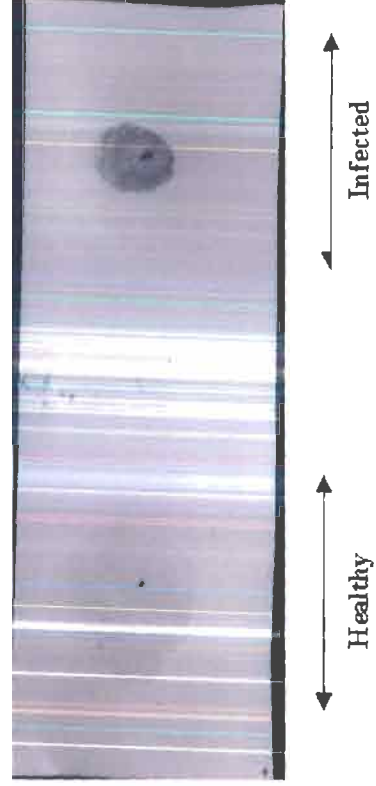


Table -10. Determination of SCMV concentration in sugarcane tissues by DAC-ELISA method

Sugarcane tissue	Absorbance at 405nm (Mean of three replications)			
	Antiserum from single immunization (Late bleed)		Antiserum from multiple immunization (Late bleed)	
	Healthy	Diseased	Healthy	Diseased
Leaf blade (top leaf)	0.189	0.616(+)	0.164	0.892(+)
Leaf blade (third leaf from top)	0.170	0.532(+)	0.159	0.712(+)
Leaf blade (sixth from top)	0.163	0.503(+)	0.166	0.701(+)
Mid rib (third leaf)	0.103	0.292(+)	0.113	0.362(+)
Mid rib (sixth leaf)	0.102	0.214(+)	0.109	0.241(+)
Leaf sheath (third leaf)	0.124	0.281(+)	0.119	0.292(+)
Leaf sheath (sixth leaf)	0.110	0.224(+)	0.124	0.251(+)
Buds	0.143	0.399(+)	0.129	0.501(+)
Roots	0.108	0.183	0.107	0.194

(+) indicates positive reactions

sheath, the virus concentration was higher in the leaf sheath. The bud region recorded highest concentration of virus next to leaf blade. Only in the root portions, no positive reaction was recorded which might be due to lesser concentration of the virus.

4.6.5. Detection of SCMV from the infected tissues by RT-PCR method

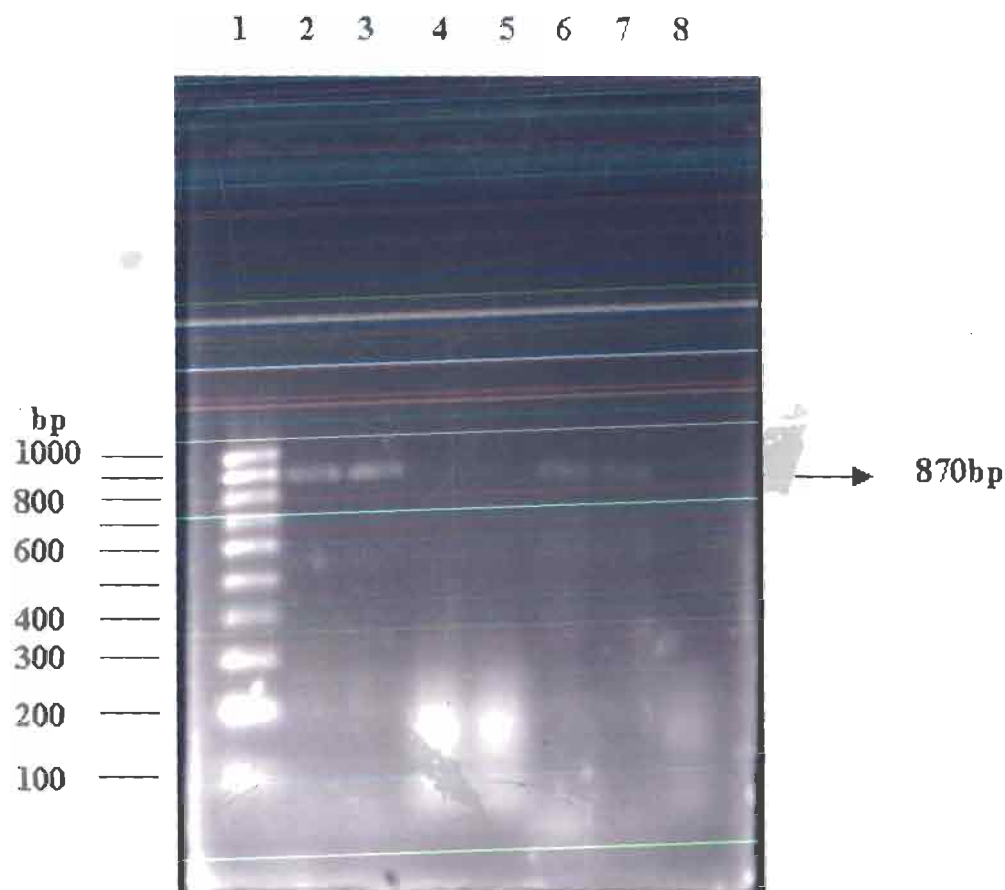
From the infected tissues, RNA was isolated and RT-PCR was performed by using the SCMV specific primers. Healthy and infected leaf samples of CoC 671 and Co 740 varieties were used for the RNA isolation and subsequently for the RT-PCR assay. (Plate-32). The amplified DNA fragment was 870 bp in size. In another experiment, RNA from different sugarcane tissues was used for the amplification with SCMV specific primer. The results revealed that there was amplification from leaf lamina, leaf sheath, bud region and midrib. No amplification was obtained from the healthy control, root and central pith region. The amplified products were nearly 870 bp in (Plate-31).

For the confirmation, the SCMV infected leaf samples of sugarcane and sorghum were sent to DSMZ plant virology lab at Germany. Potyvirus specific primer was used for the amplification. Healthy leaf samples of sorghum and sugarcane were used as control. Sugarcane mosaic virus plasmid vector PV-0368 was used as the positive control. The amplified products were 750bp in length and matched with the positive control. In the healthy control, no amplification was found (Plate-33).

4.7. Effect of SCMV inoculation at different stages of sugarcane

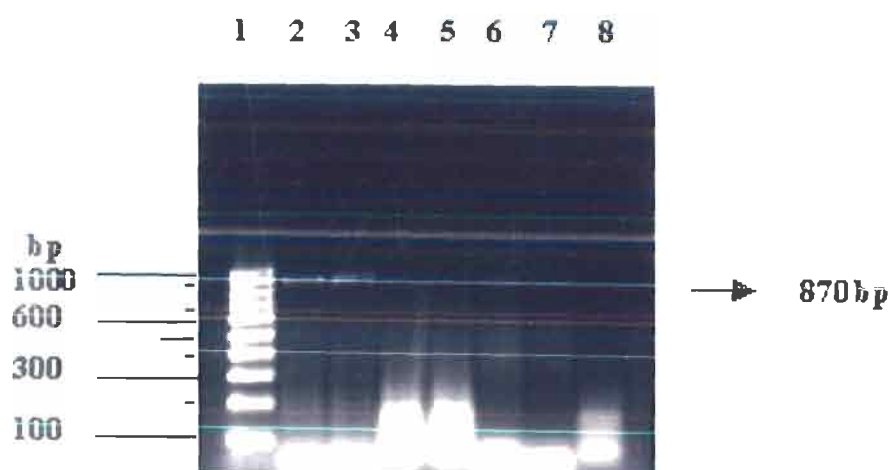
In order to find out the effect of sugarcane age on SCMV infection, one experiment was conducted involving Co 740 and CoC 671 varieties. SCMV was inoculated at different ages of sugarcane simultaneously from one to six month old plants. The experiment results revealed that, there was a significant difference in the SCMV infection when it was inoculated at different ages of sugarcane in both the varieties (Tables-11 and 12). In both the varieties, one month and two months old sugarcane were found to be highly susceptible whereas plants of five and six months were not infected and three and four months old plants were intermediate.

Plate 31 Detection of SCMV from the infected tissues of sugarcane
by RT-PCR method (SCMV specific primer)



- Lane 1: 1Kb DNA ladder
- Lane 2: SCMV infected leaf blade
- Lane 3: SCMV Infected leaf sheath
- Lane 4: SCMV infected roots
- Lane 5: SCMV infected pith
- Lane 6: SCMV infected buds
- Lane 7: SCMV infected mid rib
- Lane 8: Healthy leaves

Plate 32. Detection of SCMV from the sugarcane varieties by RT-PCR method (SCMV specific primer)



Lane 1 : 1Kb DNA ladder

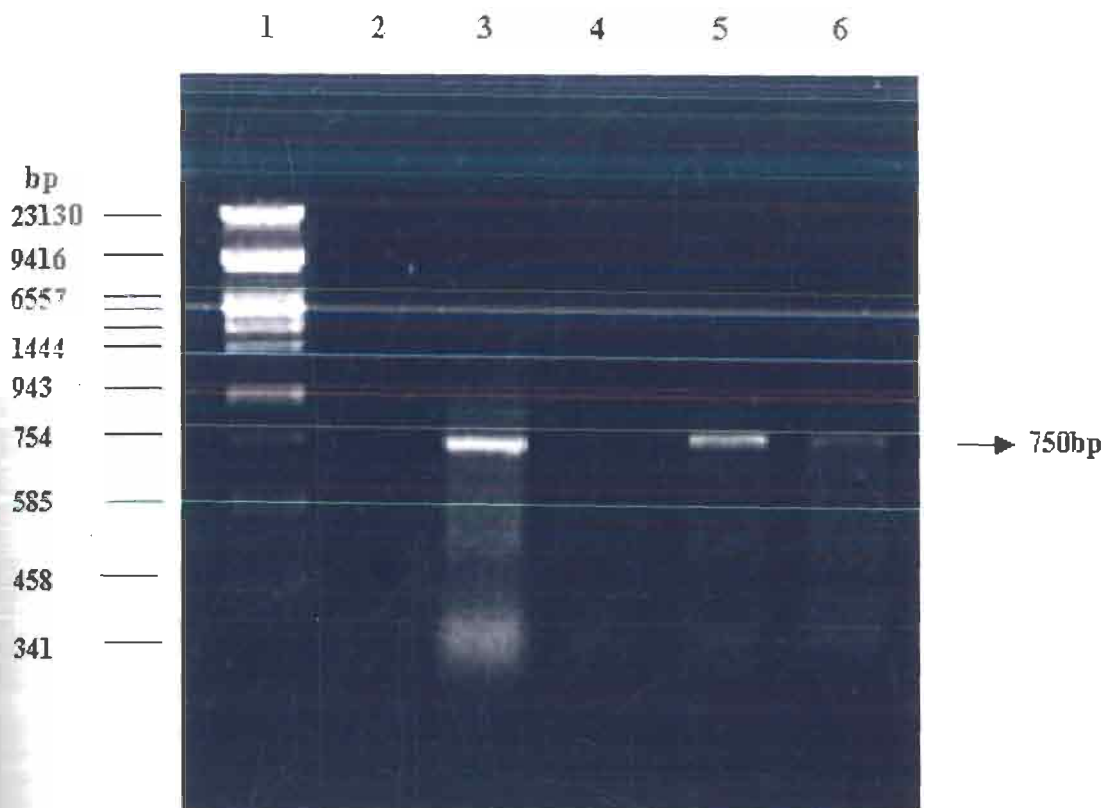
Lane 2 & 3 : SCMV infected leaves (Co 740)

Lane 4 & 5 : Healthy leaves (Co 740)

Lane 6 & 7 : SCMV infected leaves (CoC 671)

Lane 8 : Healthy leaves (CoC 671)

33.
**Plate Detection of SCMV from the infected leaves of sorghum and
sugarcane by RT-PCR method (Potyvirus specific primer)**



Lane 1: DNA ladder

Lane 2: Healthy sugarcane

Lane 3: SCMV plasmid vector (PV-0368)

Lane 4: Healthy sorghum

Lane 5: SCMV Infected sugarcane leaves

Lane 6: SCMV infected sorghum leaves

Table-11. Influence of age of sugarcane plant on SCMV infection (Co 740 variety)

Age (Months)	No. of plants inoculated	SCMV infection at different intervals (Days after inoculation)					
		15		30		60	
		Plants with visible symptoms	Plants positive to ELISA	Plants with visible symptoms	Plants positive to ELISA	Plants with visible symptoms	Plants positive to ELISA
1	27	9 ^b (3.31)	20 ^a (4.68)	25 ^a (5.20)	27 ^a (5.39)	27 ^a (5.39)	27 ^a (5.39)
2	27	4 ^c (2.43)	10 ^b (3.46)	14 ^b (3.98)	19 ^b (4.56)	15 ^b (4.11)	21 ^b (4.80)
3	27	1 ^d (1.71)	4 ^c (2.44)	8 ^c (3.15)	10 ^c (3.45)	9 ^c (3.31)	11 ^c (3.59)
4	27	0 ^d (1.41)	0 ^d (1.41)	2 ^d (1.99)	6 ^d (2.82)	4 ^d (2.44)	7 ^d (2.99)
5	27	0 ^d (1.41)	0 ^d (1.41)	0 ^e (1.41)	1 ^e (1.73)	3 ^d (2.23)	4 ^e (2.44)
6	27	0 ^d (1.41)	0 ^d (1.41)	0 ^e (1.41)	0 ^e (1.41)	0 ^e (1.41)	0 ^f (1.41)
Infected Control	27	16 ^a (4.23)	23 ^a (5.00)	27 ^a (5.39)	27 ^a (5.39)	27 ^a (5.39)	27 ^a (5.39)
Healthy Control	27	0 ^d (1.41)	0 ^d (1.41)	0 ^e (1.41)	0 ^e (1.41)	0 ^e (1.41)	0 ^e (1.41)

The values in parentheses are mean transformed (square root) values
In a column means followed by a common letter are not significantly different at 5% level.

Table-12. Influence of age of sugarcane plant on SCMV infection (CoC 671 variety)

Age (Months)	No. of plants inoculated	Number of plants positive to SCMV at different intervals (days after inoculation)					
		15		30		60	
		Plants with visible symptoms	Plants positive to ELISA	Plants with visible symptoms	Plants positive to ELISA	Plants with visible symptoms	Plants positive to ELISA
1	27	16 ^b (4.2)	22 ^b (4.86)	27 ^a (5.39)	27 ^a (5.39)	27 ^a (5.39)	27 ^a (5.39)
2	27	9 ^c (3.3)	12 ^c (3.73)	24 ^a (5.1)	26 ^a (5.29)	27 ^a (5.39)	27 ^a (5.39)
3	27	5 ^d (2.6)	8 ^d (3.13)	16 ^b (4.2)	18 ^b (4.46)	16 ^b (4.2)	20 ^b (4.69)
4	27	3 ^e (1.9)	7 ^d (3.0)	7 ^c (3.0)	10 ^c (3.45)	8 ^c (3.1)	10 ^c (3.46)
5	27	0 ^f (1.41)	0 ^e (1.41)	1 ^d (1.7)	3 ^d (2.23)	3 ^d (2.2)	6 ^d (2.82)
6	27	0 ^f (1.41)	0 ^e (1.41)	0 ^d (1.41)	0 ^e (1.41)	0 ^e (1.41)	1 ^a (1.41)
Infected Control	27	22 ^a (4.9)	27 ^a (5.39)	27 ^a (5.39)	27 ^a (5.39)	27 ^a (5.4)	27 ^a (5.39)
Healthy Control	27	0 ^f (1.41)	0 ^e (1.41)	0 ^d (1.41)	0 ^e (1.41)	0 ^e (1.41)	0 ^e (1.41)

The values in parentheses are mean transformed (square root) values

In a column means followed by a common letter are not significantly different at 5% level.

In one month old plants, infection was noticed at 15 days after inoculation in both the varieties. In Co 740, about 60 per cent of the plants produced visible symptoms and more than 80 per cent of the plants were found to be positive in ELISA test after 15 days of inoculation. Thirty days after inoculation, all the plants produced visible symptoms and found positive in ELISA test. In two months old plants, the progress of SCMV infection was reduced marginally and 33 per cent plants have shown visible symptoms and 55 per cent plants were found positive in ELISA after 15 days of inoculation. However, more than 90 per cent of the plants were found to be infected at 30 days after inoculation. The SCMV infection was found to be much slower when the SCMV inoculation was done later. The SCMV infection percent was only 30 and 26 per cent respectively in the three and four months old plants after 15 days of inoculation. Even after 60 days of inoculation, the infection percent was 74 and 37 respectively in both the three and four month old plants. In five months old plants, only 22 per cent of the plants were infected after 60 days, whereas there was almost no infection in six months old plants and only one per cent got infected even after 60 days of inoculation. After 15 days of inoculation, five and six months old plants were negative to ELISA test and also there was no visible symptom expression. Similar observations were recorded till 60 days after SCMV inoculation.

In CoC 671 variety (Table-12), the rate of infection was comparatively slower to Co 740 variety. Only 33 per cent of one month old plants produced visible symptoms, after 15 days of inoculation. After 60 days, all the plants, in the one month category produced symptoms whereas it was 78 per cent in the two months old category and remaining plants were not infected. In the three and four months old inoculated plants, the SCMV infection was only 4 and 26 per cent respectively even after 60 days of inoculation. When the inoculation was carried out on six months old plants, there was absolutely no infection even after 60 days.

4.8. Economic impact of sugarcane mosaic

Three plant crop experiments and two ratoon crop experiments were conducted to find out the effect of SCMV infection on cane yield and juice quality in Co 740 and CoC 671 varieties.

4.8.1. Plant crop experiments I and II (Coimbatore)

4.8.1.1. Effect of SCMV infection on growth parameters of sugarcane

4.8.1.1.1. Germination

The observations in the trial revealed that in Co 740 variety there was a significant difference in germination of setts. No significant difference in CoC 671 variety was observed. The plots planted with healthy setts recorded 88 per cent germination whereas it was only 78 per cent in the plots with SCMV infected setts (Table-13). In the second plant crop also, 12 per cent germination reduction in Co 740 variety was observed (Table-15).

4.8.1.1.2. Tillering

The number of tillers was counted at two different intervals. In both the intervals, in the Co 740 variety, the tillers were reduced in number in the plots with SCMV infected setts. The reduction in tillering due to SCMV infection was 30.0 and 26.0 per cent respectively at 60 and 120 days of observation (Tables-13 and 35). In the second plant crop also, similar reduction in tillering was observed in Co 740 variety due to SCMV infection (Table-15).

4.8.1.1.3. Plant height

SCMV infection significantly reduced the plant height at all stages of cane growth in both the varieties. But the impact was more in Co 740 when compared to CoC 671 variety. From the early stage onwards, the SCMV infection reduced the plant height. In Co 740 variety, 22 and 20 per cent height difference was observed during 60 and 120 days respectively. In the corresponding period, the reduction in CoC 671 variety was 12.8 and 8.3 per cent respectively. During 8th month and 12th months, the

**Table-13. Effect of SCMV on growth parameters of sugarcane - germination, tillering and plant height
(Plant crop Experiment I at Coimbatore)**

Growth parameters																	
Days	Germination (%)			L.S.D (5%)			Tillering (No/clump)				L.S.D (5%)			Plant height (cm)			L.S.D (5%)
							CoC671		Co740					CoC671		Co740	
	CoC671		Co740		CoC671		Co740		CoC671		Co740						
	H	D	H	D	H	D	H	D	H	D	H	D					
	30	84 ^b	80 ^b	88 ^c	78 ^a	3.87											
60						3.1 ^a	2.9 ^a	6.1 ^b	4.3 ^c	0.245	70 ^d	61 ^c	54 ^b	42 ^a	4.93		
120						3.6 ^a	3.4 ^a	6.6 ^c	4.9 ^b	0.267	132 ^d	121 ^c	105 ^b	84 ^a	3.32		
240											286 ^d	274 ^c	211 ^b	191 ^a	4.20		
360											332 ^d	321 ^c	280 ^b	259 ^a	3.59		

In a row, means followed by a common letter are not significantly different at 5% level by DMRT

H: Healthy

D: Diseased

Table-14. Effect of SCMV on growth parameters of sugarcane – cane thickness and NMC (Plant crop experiment I at Coimbatore)

Days	Growth parameters									
	Cane thickness (mm)				L.S.D (5%)	Number of millable canes/clump				L.S.D (5%)
	CoC671		Co740			CoC671		Co740		
	H	D	H	D		H	D	H	D	
180	15 ^a	14 ^a	18.8 ^b	14 ^a	1.360					
240	21.8 ^b	20.6 ^b	23.9 ^c	18.9 ^a	1.274	3.7 ^a	3.4 ^a	7.9 ^c	6.3 ^b	0.304
360	27.6 ^b	26.4 ^b	29.1 ^c	23.6 ^a	1.235	3.8 ^a	3.5 ^a	8.1 ^c	6.3 ^b	0.337

In a row, means followed by a common letter are not significantly different at 5% level by DMRT

H: Healthy

D: Diseased

Table-15. Effect of SCMV on growth parameters of sugarcane - germination, tillering and plant height (Plant crop experiment II at Coimbatore)

Growth parameters															
Days	Germination (%)				L.S.D (5%)	Tillering (No/clump)				L.S.D (5%)	Plant height (cm)				L.S.D (5%)
	CoC671		Co740			CoC671		Co740			CoC671		Co740		
	H	D	H	D	H	D	H	D	H	D	H	D	H	D	
	30	86 ^b	82 ^b	86 ^a	74 ^b	4.58									
60						3.3 ^a	3.0 ^a	5.7 ^a	4.0 ^b	0.345	65 ^c	59 ^{bc}	52 ^b	43 ^a	7.1
120						3.4 ^a	3.1 ^a	6.0 ^c	4.8 ^b	0.367	125 ^c	114 ^b	110 ^b	89 ^a	9.4
240											260 ^d	245 ^c	220 ^b	201 ^a	4.74
360											320 ^c	308 ^b	312 ^{bc}	289 ^a	10.45

In a row, means followed by a common letter are not significantly different at 5% level by DMRT

H: Healthy

D: Diseased

difference in height in Co 740 variety was 9.4 and 7.5 per cent respectively (Plates-34 and 35) when compared to 4.1 and 3.1 per cent respectively in CoC 671 variety (Table-13). A similar reduction in plant height due to SCMV infection in both the varieties were observed in plant crop experiment II also (Table-15).

4.8.1.1.4. Cane thickness

The data on cane thickness (Table-14) revealed that there was significant difference in Co 740 variety when compared to CoC 671 variety. Twenty five per cent reduction in cane thickness in Co 740 variety due to SCMV infection was observed after 180 days of planting and after 240 days, the reduction in thickness was 20.9 per cent. At the time of harvest, the difference in thickness between healthy and infected canes in Co 740 variety was 18.9 per cent (Plate-36). In the second plant crop experiment, up to 30.5 per cent reduction in cane thickness in Co 740 variety was observed (Table-16).

4.8.1.1.5. Number of millable canes

The data on number of millable canes (NMC) revealed that, more number of millable canes were present in healthy clumps of Co 740 variety when compared to SCMV infected clumps (Plate-35 and 36). In the healthy clumps 20 and 22 per cent more millable canes were present respectively at 240 and 360 days after planting in the Co 740 variety when compared to infected clumps (Table-14). In the second trial also, the number of millable canes were reduced in SCMV infected Co 740 variety in both the intervals (Table-16).

4.8.1.1.6. Net photosynthesis

Like various growth parameters, similar trend was observed in one of the physiological parameter namely net photosynthesis. In Co 740 variety, the impact of SCMV infection was felt very much in reducing the net photosynthesis when compared to CoC 671 variety. In all the three intervals, the net photosynthesis was less in infected

Plate:34. Foliar symptoms of SCMV in the field (Co 740)

a. Germination phase



b. Fourth month



Plate 35. Effect of SCMV on plant height, tillering and NMC (Co 740)

b) Healthy



c) Infected



Plate 36. Effect of SCMV on NMC and cane thickness (Co 740)



← Infected →

← Healthy →

Table-16. Effect of SCMV on growth parameters of sugarcane - cane thickness and NMC (Plant crop experiment II at Coimbatore)

Days	Growth parameters									
	Cane thickness (mm)				L.S.D (5%)	Number of millable canes / clump				L.S.D (5%)
	CoC671		Co740			CoC671		Co740		
	H	D	H	D		H	D	H	D	
180	20 ^b	8.8 ^b	19.9 ^b	13.83 ^a	1.899					
240	26.0 ^b	25.0 ^b	25.7 ^b	19.91 ^a	1.065	3.0 ^a	2.8 ^a	7.7 ^c	6.1 ^b	0.422
360	30.0 ^{bc}	28.9 ^b	30.74 ^{cd}	24.46 ^a	1.180	3.2 ^a	3.0 ^a	8.0 ^c	6.3 ^b	0.244

In a row, means followed by a common letter are not significantly different at 5% level by DMRT

H: Healthy

D: Diseased

canes in Co 740 variety. The reduction in net photosynthesis at 60, 120 and 180 days after planting was 18.1, 29.0 and 27.0 per cent respectively when compared to healthy canes. In CoC 671 variety also, the reduction was observed in all the three intervals (Fig. 2) in both the first and second plant crop experiments.

4.8.1.2.Cane, juice quality and sugar

After harvest, average single cane weight was calculated. The data indicated that, SCMV infection affected the cane weight in Co 740 variety significantly. The reduction in single cane weight in Co 740 variety was 36.1 per cent in plant crop experiment I (Fig. 3) and 27.5 per cent in plant crop experiment II (Fig. 6).

Regarding the juice quality parameters, there was 6.0 per cent reduction in total brix in SCMV infected Co 740 variety, whereas sucrose percent was affected in both the varieties. In Co 740 variety, the SCMV infection reduced the sucrose content considerably to 21.6 per cent whereas in CoC 671 variety the reduction was only 3.7 per cent. The other quality parameter namely the sugar purity was also affected by SCMV infection in both the varieties. In Co 740 variety, the reduction in sucrose purity was 3.6 per cent when compared to 2.53 per cent in SCMV infected Co C 671 variety. The sugar yield was also affected significantly by SCMV infection in both the varieties. The reduction in commercial cane sugar was 7.9 per cent in SCMV infected CoC 671 variety, whereas in Co 740 variety, the infection reduced it to 3.2 per cent (Fig. 4). In plant crop experiment II also, a similar reduction juice quality was recorded due to SCMV infection in both the varieties (Fig. 7).

4.8.2. Plant crop experiment III

Another plant crop experiment was carried out in farmer's field at Konganattanpudur village in Pollachi (Tk) of Coimbatore district with the healthy and infected setts of Co 740 and CoC 671 varieties.

Fig. 2. Effect of SCMV on net photosynthesis of sugarcane
(Plant crop experiment I at Coimbatore)

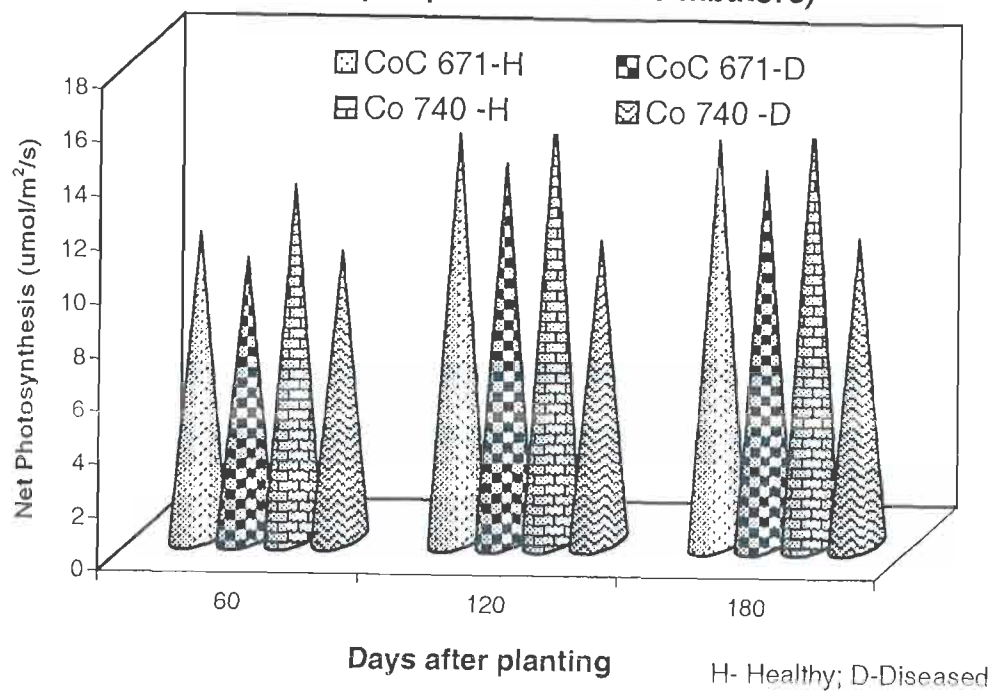


Fig. 3. Effect of SCMV on sugarcane growth - single cane weight
(Plant crop experiment I at Coimbatore)

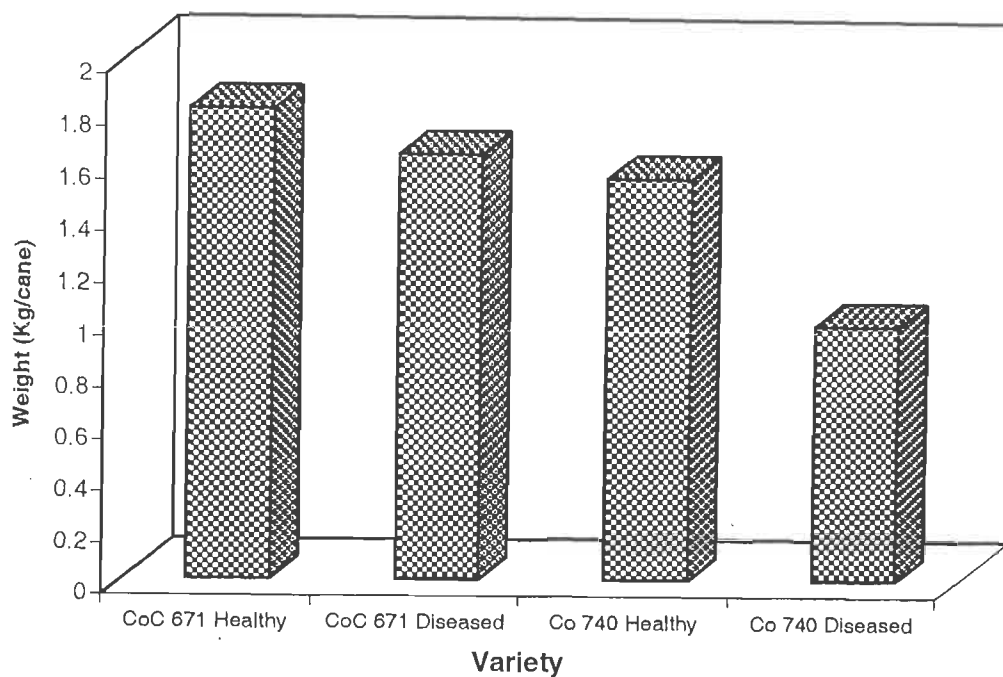


Fig. 4. Effect of SCMV on cane juice quality and sugar yield (Plant crop experiment I at Coimbatore)

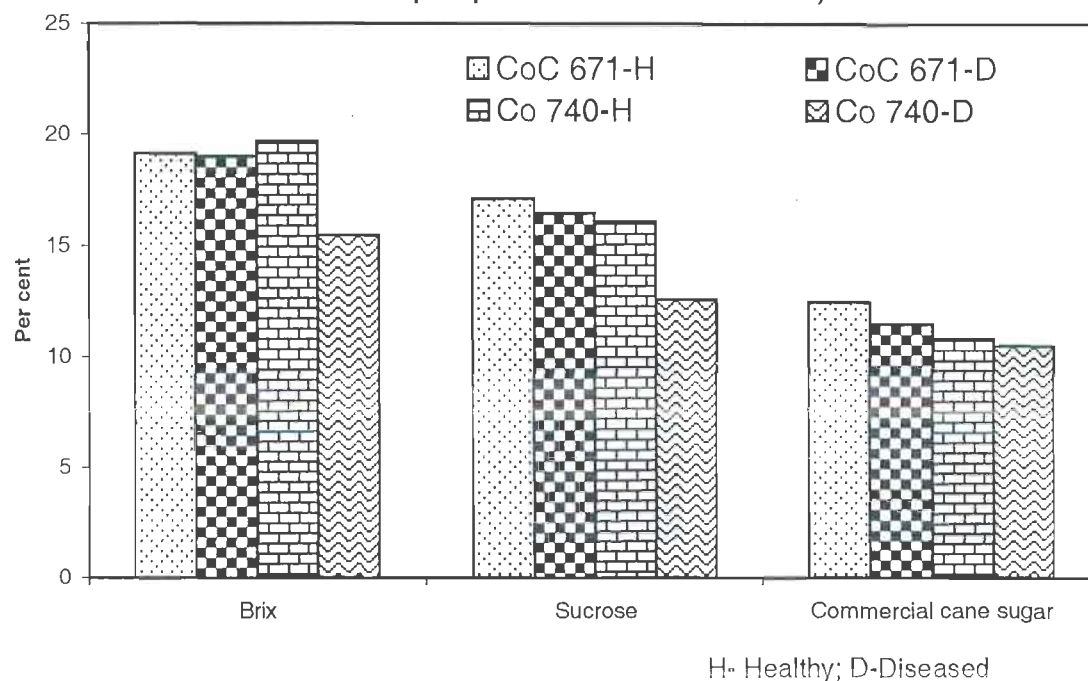


Fig. 5. Effect of SCMV on net photosynthesis of sugarcane (Plant crop experiment II at Coimbatore)

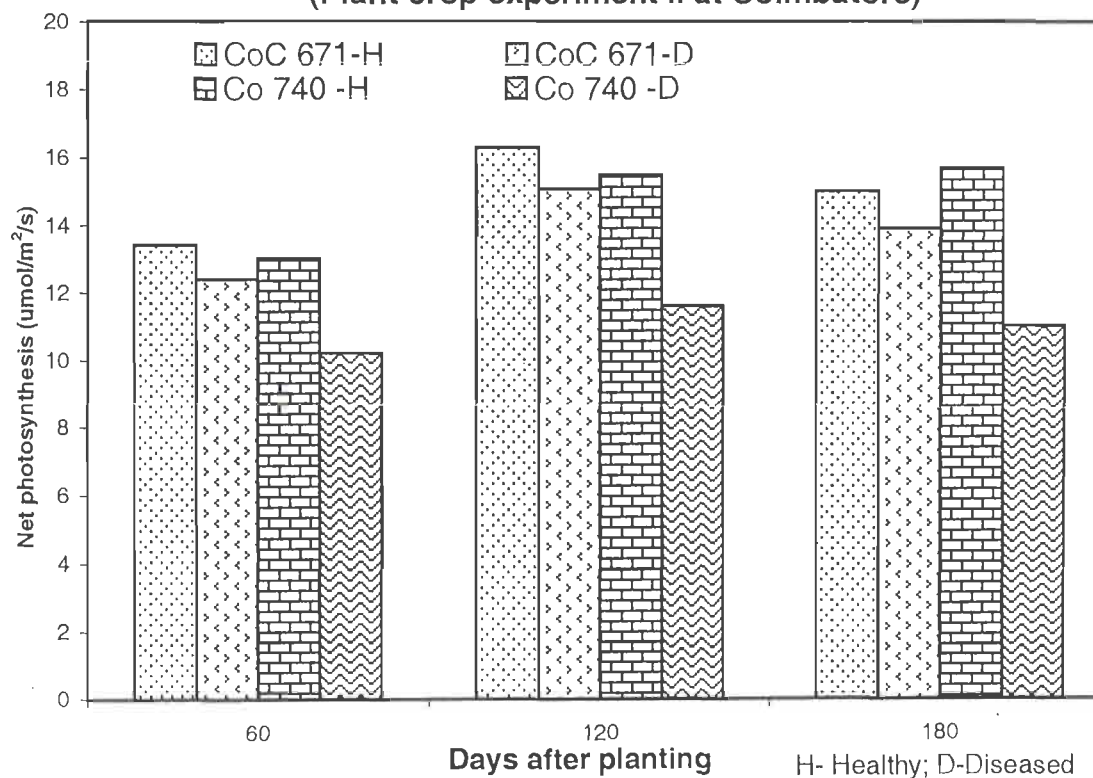


Fig. 6. Effect of SCMV on sugarcane growth - single cane weight
(Plant crop experiment II at Coimbatore)

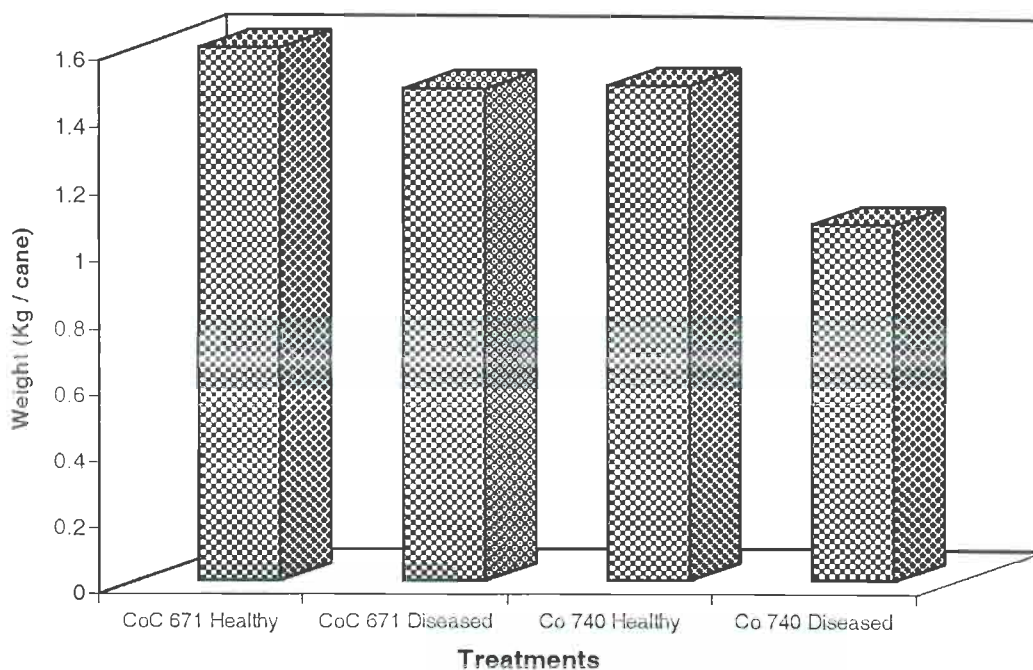
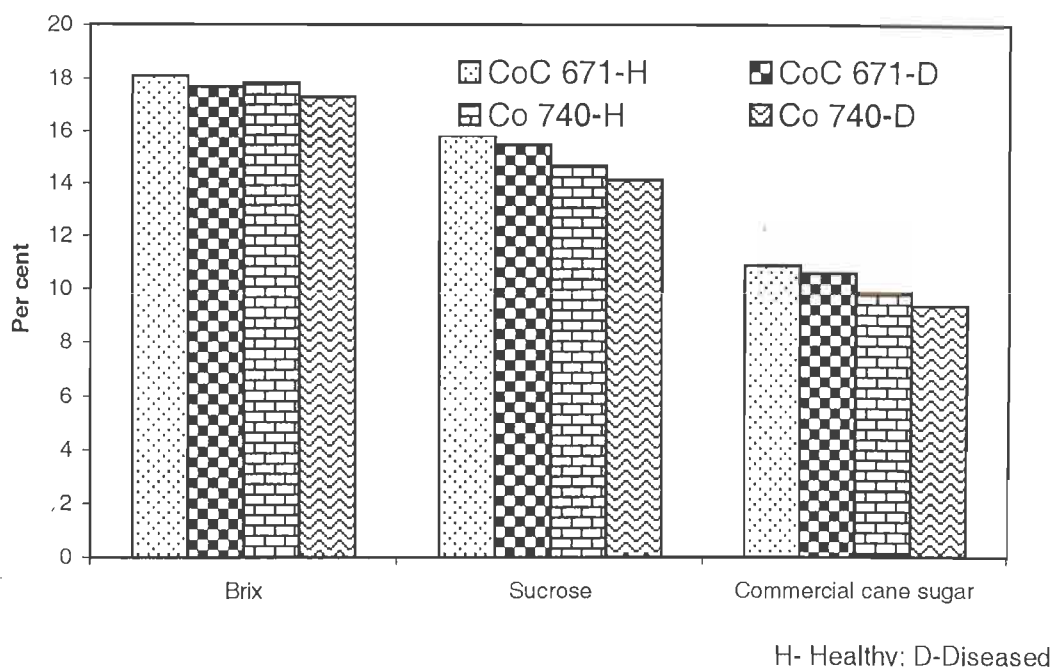


Fig. 7. Effect of SCMV on cane juice quality and sugar yield
(Plant crop experiment II at Coimbatore)



4.8.2.1.Effect on growth parameters

4.8.2.1.1.Germination

In the virus infected setts of CoC 671 variety, no significant difference in germination was observed whereas in Co 740 variety, there was 11 per cent decrease in germination (Table-17) in the SCMV infected setts, which affected the cane population in the field.

4.8.2.1.2.Tillering

At two intervals, the number of tillers present per clump was counted during 60 and 120 days after planting. In both the intervals, it was observed that SCMV infection significantly affected the number of tillers. In Co 740 variety, 25 per cent of the tillers were reduced in both the intervals (Table-17). In addition to germination, the tillering is an important factor determining the cane population in the field (Plate-37).

4.8.2.1.3.Plant height

SCMV infection significantly reduced the plant height in both the varieties. However, only in Co 740 variety, the reduction was very much pronounced at all the stages of observation. The reduction in height varied from 11.2 per cent at the time of harvest and up to 26 per cent at 120 days of planting. At 60 days, the reduction was 23 per cent (Table-17). It clearly indicated that at the early growth phase, the reduction was more pronounced when compared to the later phase. In CoC 671 variety, the reduction was 14.3 and 12.9 per cent respectively at 60 and 120 days after planting whereas the height reduction was only 6.3 and 3.4 per cent respectively during 8th and 12th months (Plate-38). It also indicated the impact of SCMV on plant height at an early growth phase.

4.8.2.1.4.Cane thickness

The difference in cane thickness was well established in the SCMV infected Co 740 variety at all the stages of observations. At 180 days, the difference in thickness was 3.8 mm between healthy and diseased (Table-18). The infected canes looked slender and weak when compared to healthy canes. At 240 days, the girth of the

Table-17. Effect of SCMV on growth parameters of sugarcane - germination, tillering and plant height (Plant crop experiment III at Pollachi)

Growth parameters															
Days	Germination (%)				L.S.D (5%)	Tillering (No/clump)				L.S.D (5%)	Plant height (cm)				L.S.D (5%)
	CoC671		Co740			CoC671		Co740			CoC671		Co740		
	H	D	H	D	H	D	H	D	H	D	H	D	H	D	
	30	91 ^c	89 ^c	82 ^b	71 ^a	2.62									
60						3.0 ^c	2.6 ^c	5.4 ^b	4.1 ^a	0.780	70 ^d	60 ^c	52 ^b	40 ^a	6.63
120						3.3 ^{ab}	3.1 ^a	6.1 ^c	4.6 ^b	1.32	139 ^c	121 ^b	118 ^b	92 ^a	7.23
240											299 ^c	280 ^c	227 ^b	205 ^a	18.17
360											350 ^d	338 ^c	324 ^b	286 ^a	11.68

In a row, means followed by a common letter are not significantly different at 5% level by DMRT

H: Healthy

D: Diseased

Plate 37. Field view of healthy and SCMV infected canes (Co 740)

a) Healthy



b) Infected



Plate 38. Effect of SCMV on number of millable canes (Co 740)

a) Healthy



b) Infected



Table-18. Effect of SCMV on growth parameters of sugarcane - cane thickness and NMC (Plant crop experiment III at Pollachi)

Days	Growth parameters									
	Cane thickness (mm)				L.S.D (5%)	Number of millable canes/clump				L.S.D (5%)
	CoC671		Co740			CoC671		Co740		
	H	D	H	D		H	D	H	D	
180	20 ^b	18.8 ^b	19.0 ^b	15.2 ^a	1.666					
240	23.6 ^c	22.7 ^{bc}	21.6 ^b	16.1 ^a	1.212	3.7 ^a	3.4 ^a	7.7 ^c	5.8 ^b	1.381
360	28.1 ^c	27.9 ^c	26.3 ^b	20.9 ^a	1.180	4.3 ^a	3.6 ^a	8.7 ^c	6.0 ^b	0.775

In a row, means followed by a common letter are not significantly different at 5% level by DMRT

H : Healthy

D : Diseased

Fig. 8. Effect of SCMV on cane yield in two sugarcane varieties (Plant crop experiment III at Pollachi)

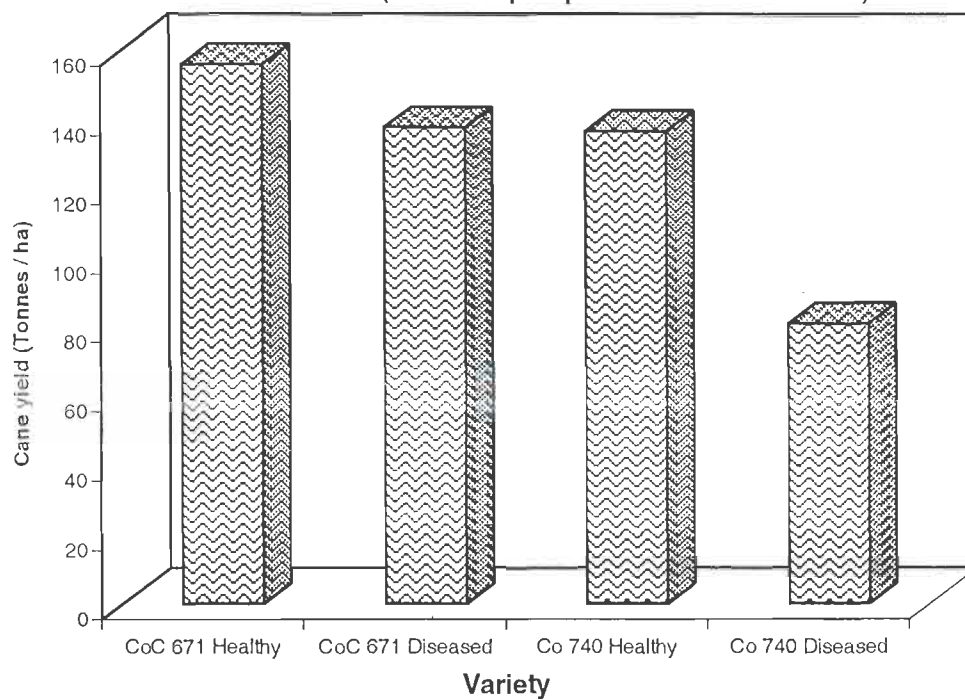
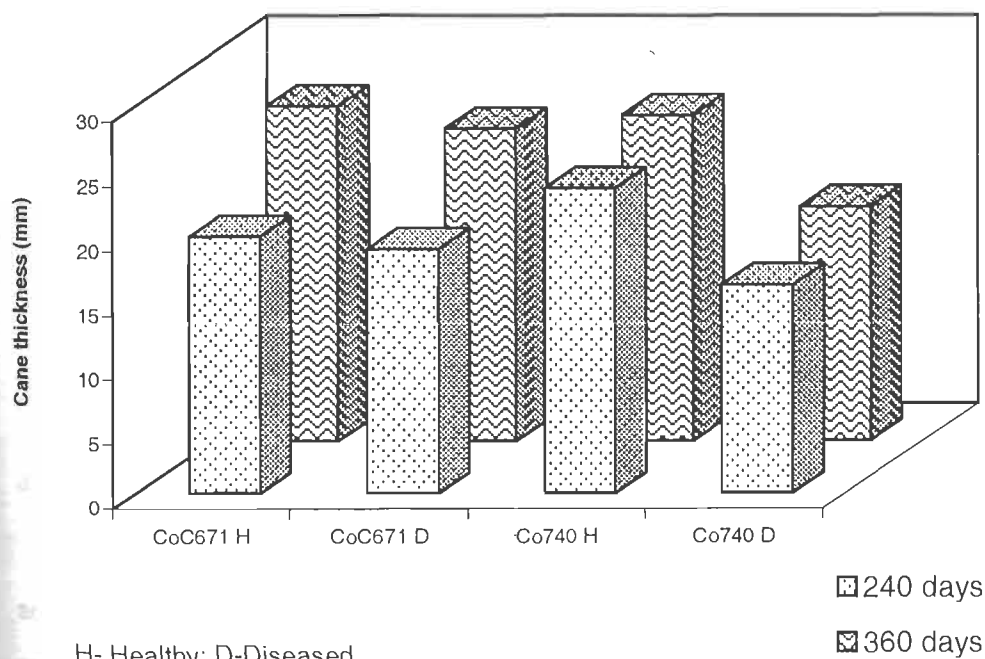


Fig. 9. Effect of SCMV on sugarcane growth-cane thickness (Ratoon crop experiment I at Coimbatore)



healthy cane was 21.6 mm whereas it was only 16.1mm in the infected canes. At the time of harvest, the difference in thickness was much pronounced. It was only 20.9 mm as against 26.3 mm in the healthy canes. The reduction was 20.5 per cent at the time of harvest (Plate-39).

4.8.2.1.5. Number of millable canes

The number of tillers at an early phase led to number of millable canes at the time of harvest. The reduction in tillers due to SCMV infection at the early phase reflected in the millable canes similarly (Plate-38). At 8th month 25 per cent reduction in millable canes was observed in SCMV infected Co 740 variety while the reduction was more pronounced at the time of harvest (Table-18). In healthy clumps, on an average 8.7 millable canes were present when compared to 6.0 canes in the SCMV infected clumps. About 31.3 per cent reduction was noted in millable canes of Co 740 variety due to SCMV infection.

4.8.2.1.6. Effect of SCMV infection on cane yield

After harvest, the cane weight in all the replications was taken and average weight per hectare was arrived. The reduction in the germination percentage, tillers, height, thickness, millable canes and net photosynthesis was clearly reflected in the cane yield also. In Co 740 variety, the reduction in cane yield was 56 tonnes per hectare (Fig. 8). In the healthy plants, the yield was 137 tonnes per hectare as against 81 tonnes per hectare in the SCMV infected plants. About 40.1 per cent reduction in cane yield was observed in Co 740 variety (Plate-40). Eventhough, there was slight reduction in the cane yield SCMV infected CoC 671 variety; it was not significantly different as like other growth parameters.

4.8.2.2. Juice quality

The effect of SCMV infection was very much felt in juice quality also in addition to the other growth parameters and cane yield. It reflected in the cane sugar out put also. The juice quality was affected in both Co 740 and CoC 671 varieties

Plate 39. Effect of SCMV on cane thickness (Co 740)

a) Healthy



b) Infected



Plate 40. Effect of SCMV on cane yield (Co 740)



(Table-19). A reduction of 7.6 and 7.5 per cent in total brix and sucrose content was observed in the SCMV infected CoC 671 variety due to SCMV infection when compared to healthy canes. The purity percent was also reduced. The commercial cane sugar out put was only 12.82 per cent in the SCMV infected CoC 671 canes as against 14.25 per cent in the healthy canes. In Co 740 variety the reduction in juice quality was felt very much due to SCMV infection. The reduction in total brix and sucrose content was 6.3 and 11.1 per cent respectively, in SCMV infected canes when compared to healthy canes. The reduction in commercial cane sugar out put in SCMV infected canes of Co 740 variety was 11.3 per cent.

4.8.3.Ratoon crop experiments I and II

4.8.3.1.Effect on growth parameters

In order to find out the effect of SCMV in the ratoon crop, different growth parameters were recorded in the ratoon crop experiments I and II at Coimbatore as like plant crop.

4.8.3.1.1.Tillering

As like plant crop, the impact on CoC 671 variety due to SCMV infection was low whereas the tillers were reduced in Co 740 variety. Thrity two per cent of the tillers were reduced at 60 days and more than 36 per cent of the tillers got reduced at 120 days. It clearly indicated the build up of virus over the period of time in the plant crop and caused more impact in the ratoon crop (Table-20). In ratoon crop II also (Table-21), a similar trend was observed and the number of tillers per clump was reduced in SCMV infected Co 740 variety whereas there was no significant difference between the healthy and infected canes of CoC 671 variety.

4.8.3.1.2.Plant height

As like tillering, the impact of SCMV infection on plant height was more in ratoon crop when compared to plant crop. Eventhough the impact was there in both varieties, it was more in Co 740 variety. At all the stages of observation, the reduction

**Table-19. Effect of SCMV on cane juice quality and sugar yield
(Plant crop experiment III at Pollachi)**

Quality parameters (%)	Varieties				L.S.D (5%)
	CoC 671		Co 740		
	H	D	H	D	
Brix	21.87 ^d	20.21 ^c	19.30 ^b	18.08 ^a	0.348
Sucrose	19.25 ^d	17.31 ^c	16.92 ^b	15.04 ^a	0.179
Purity	87.84 ^b	86.23 ^a	87.88 ^{bc}	86.38 ^b	0.446
Commercial cane Sugar (CCS)	14.25 ^d	12.82 ^a	13.92 ^c	12.33 ^b	0.170

In a row, means followed by a common letter are not significantly different at 5% level by DMRT

Table-20. Effect of SCMV on growth parameters of sugarcane – tillering and plant height (Ratoon crop experiment I at Coimbatore)

Days	Growth parameters									
	Tillering (No/clump)				L.S.D (5%)	Plant height (cm)				L.S.D (5%)
	CoC671		Co740			CoC671		Co740		
	H	D	H	D		H	D	H	D	
60	3.4 ^{ab}	2.8 ^a	5.9 ^c	4.0 ^b	1.157					
120	3.6 ^a	2.9 ^a	6.9 ^c	4.2 ^b	1.32	124 ^c	106 ^{ab}	114 ^b	97 ^a	9.07
240						284 ^d	264 ^c	230 ^b	198 ^a	6.87
360						329 ^c	309 ^b	306 ^b	262 ^a	8.39

In a row, means followed by a common letter are not significantly different at 5% level by DMRT

H: Healthy

D: Diseased

Table-21. Effect of SCMV on growth parameters of sugarcane – tillering and plant height (Ratoon crop experiment II at Coimbatore)

Days	Growth parameters									
	Tillering (No/clump)				L.S.D (5%)	Plant height (cm)				L.S.D (5%)
	CoC671		Co740			CoC671		Co740		
	H	D	H	D		H	D	H	D	
60	3.4 ^{ab}	2.8 ^a	6.9 ^c	4.4 ^b	1.157					
120	3.6 ^a	2.9 ^a	6.9 ^c	4.8 ^b	1.32	124 ^c	106 ^{ab}	114 ^b	97 ^a	9.07
240						284 ^d	264 ^c	230 ^b	198 ^a	6.87
360						334 ^c	309 ^b	306 ^b	262 ^a	8.39

In a row, means followed by a common letter are not significantly different at 5% level by DMRT

H: healthy

D: Diseased

in height was observed in Co 740 variety. During 4, 6 and 8 months, 11.5, 14.0 and 11.1 per cent height reduction over healthy plants were observed respectively. The impact was more than the plant crop in Co 740 variety (Plate-41). In CoC 671 variety also, the reduction in height ranged from 6.1 to 14.5 per cent at different intervals (Table-20). In the ratoon crop experiment II also, the impact was more in both the varieties than the plant crop (Table-21).

4.8.3.1.3.Cane thickness

The infected canes looked more slender in Co 740 variety in the ratoon crop when compared to main crop. During 8th month, 31.6 per cent reduction in cane thickness was observed in SCMV infected Co 740 variety and the reduction during harvesting was 25 per cent (Plate-41). In CoC 671 also, significant difference in cane thickness was observed in both the intervals. A reduction of 5.0 and 6.6 per cent in cane thickness was observed in both the intervals, even though there was no impact in the plant crop. In the ratoon crop experiment II, a significant difference in cane thickness was recorded in both the varieties. In the CoC 671 variety, 8.7 and 7.6 per cent reduction in cane thickness was recorded in the infected canes during 8th and 12th months respectively. In Co 740 variety, 5.2 mm difference in cane thickness between healthy and infected cane was observed during 8th month and 3.8 mm at the time of harvesting (Fig. 9 and 14)

4.8.3.1.4.Number of millable canes

The impact of SCMV on tillering was subsequently reflected in the development of millable canes. During 8th month, the reduction in NMC was 33.8 per cent in the infected canes in Co 740 variety and at the time of harvest, it was nearly 33.0 per cent (Plate-41). The impact of SCMV on NMC was more in both the ratoon crop experiments I and II. However, there was no significant impact in CoC 671 variety (Fig.10 and 15)

41.
Plate Effect of SCMV on plant height, number of millable canes and cane thickness in ratoon crop (Co 740)

Infected



Healthy

Infected



Healthy



Fig. 10. Effect of SCMV on sugarcane growth-NMC (Ratoon crop experiment I at Coimbatore)

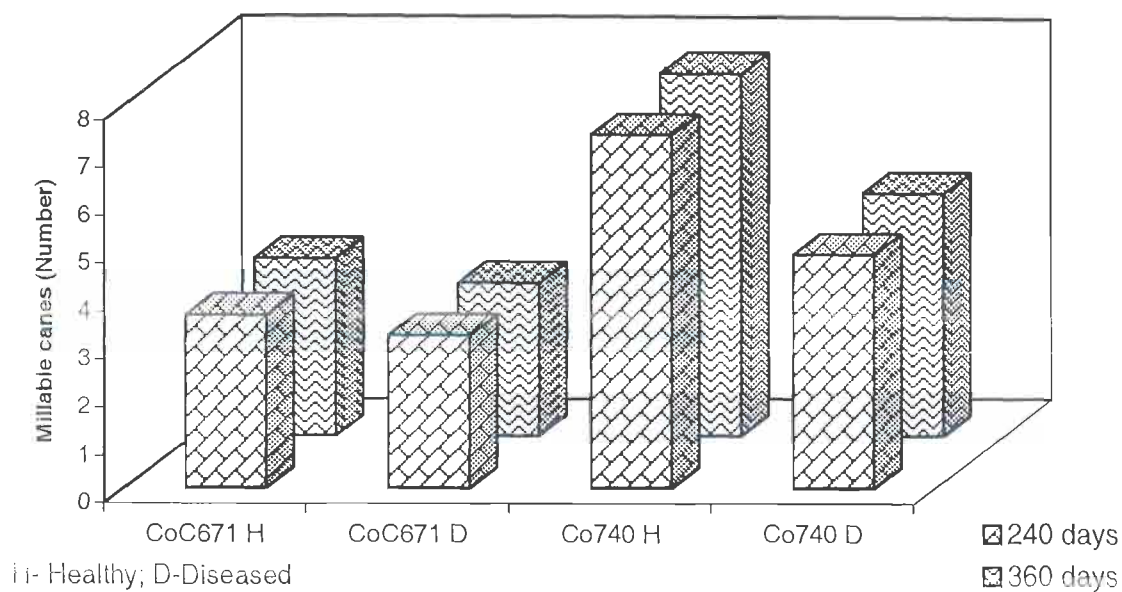


Fig. 11. Effect of SCMV on net photosynthesis of sugarcane (Ratoon crop experiment I at Coimbatore)

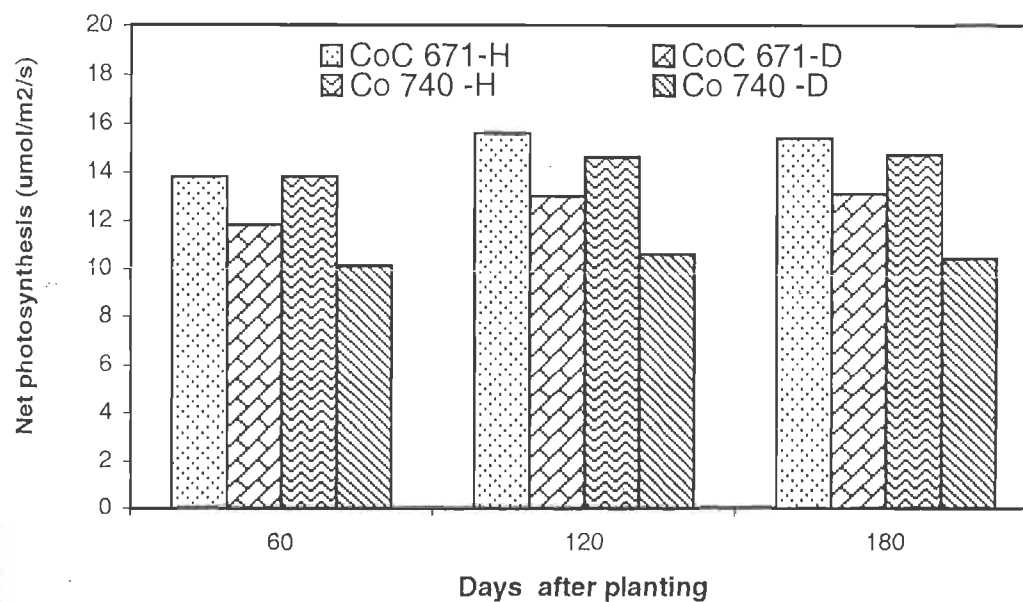


Fig. 12. Effect of SCMV on sugarcane growth - single cane weight (Ratoon crop experiment I at Coimbatore)

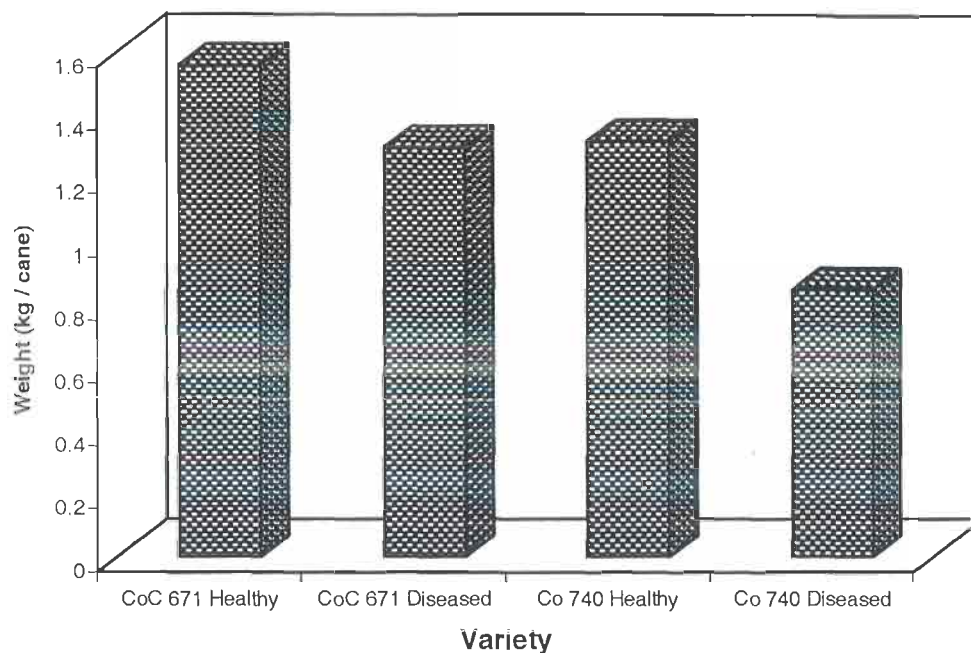


Fig. 13. Effect of SCMV on cane juice quality and sugar yield (Ratoon crop experiment I at Coimbatore)

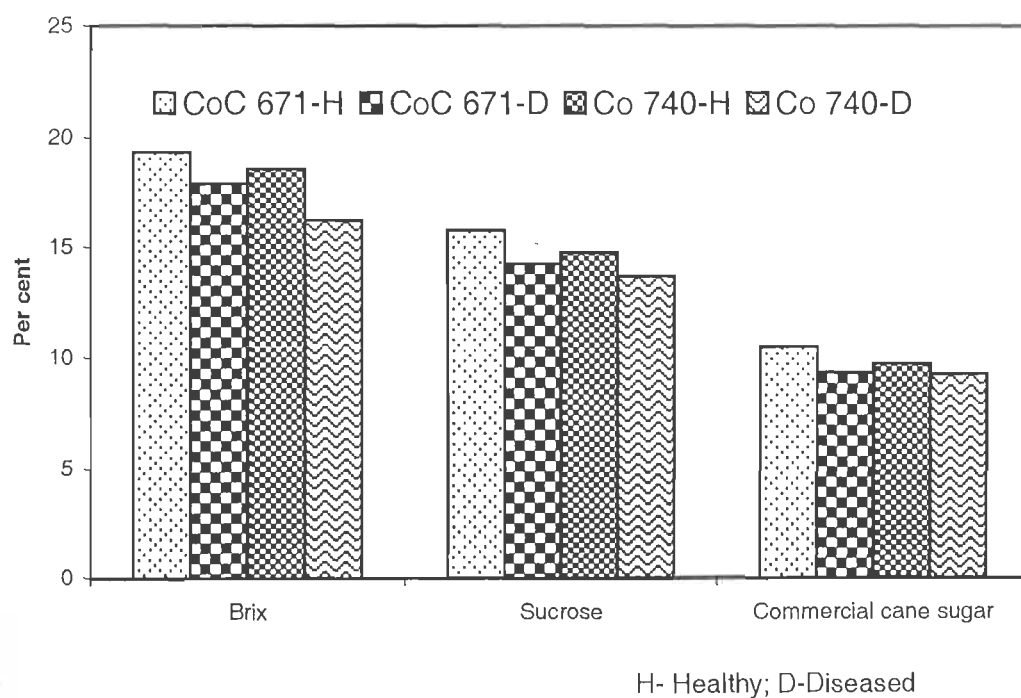


Fig. 14. Effect of SCMV on sugarcane growth- cane thickness
(Ratoon crop experiment II at Coimbatore)

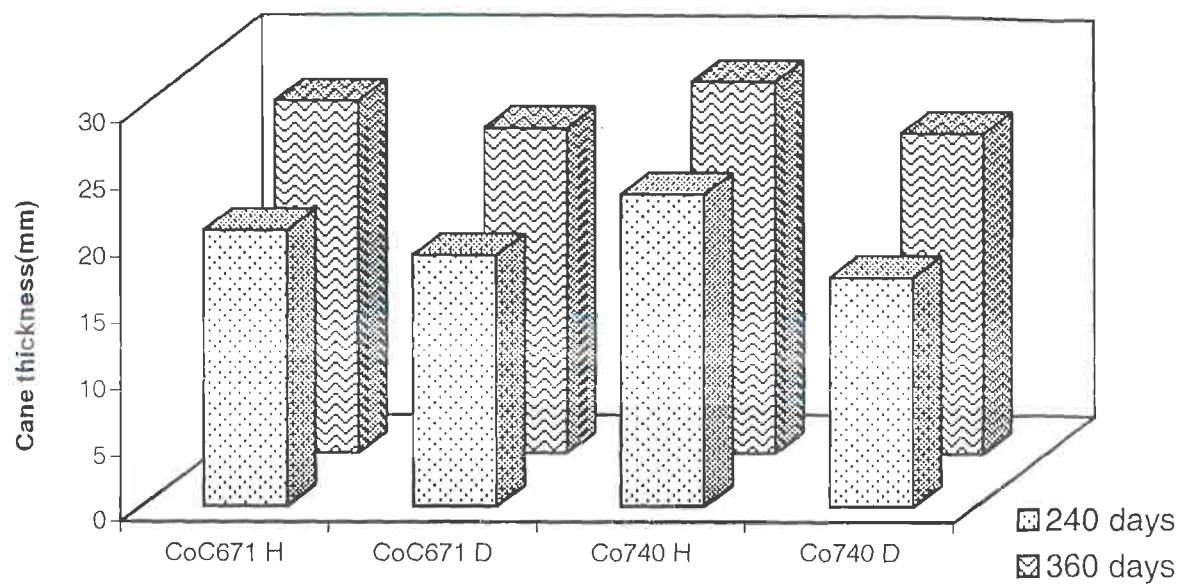
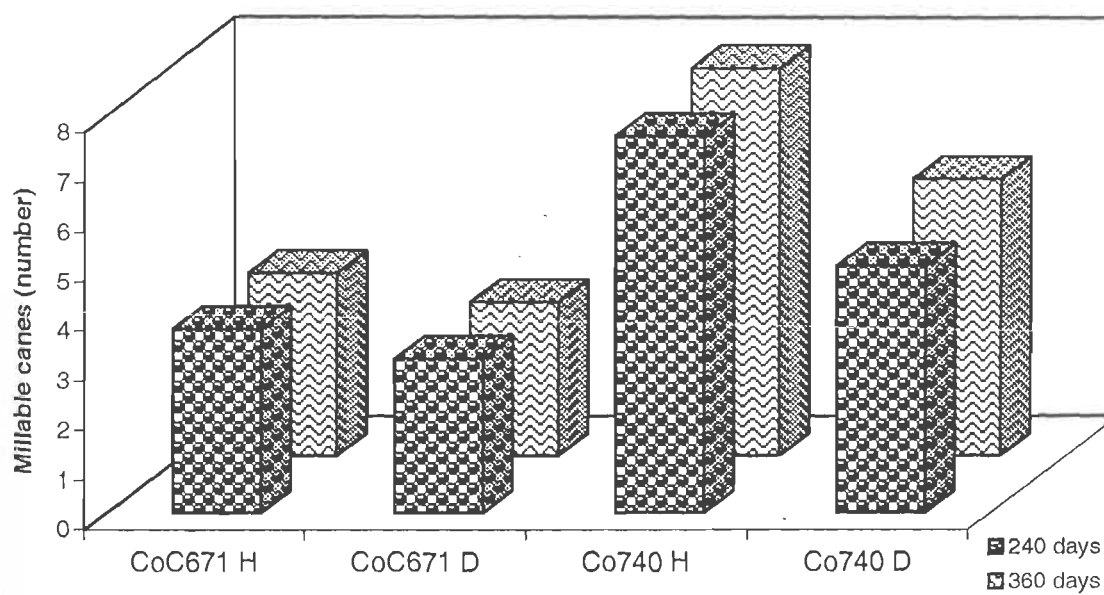


Fig. 15. Effect of SCMV on NMC
(Ratoon crop experiment II at Coimbatore)



4.8.3.1.5. Net photosynthesis

The infected plants looked pale in appearance by showing intense mosaic and yellowing when compared to main crop. It clearly reflected in the net photosynthesis. The impact was severe in the infected canes of Co 740 variety. During the second month, infected plants of Co 740 variety recorded the net photosynthesis of $14.4 \mu \text{ mol/m}^2/\text{second}$ in the diseased plants. During 120 days and 180 days, the reduction in net photosynthesis in the infected plants of Co 740 variety was 28.9 and 32.0 per cent respectively (Fig. 11). In CoC 671 variety also, the net photosynthesis was affected in the infected canes and the impact was slightly more than the plant crop. The reduction in net photosynthesis was 13.4, 14.4 and 12.9 per cent respectively during 60, 120 and 180 days of observation. A similar reduction in net photosynthesis was observed in the ratoon crop experiment II also (Fig. 16).

4.8.3.1.6. Cane weight

Even-though there was no impact of SCMV on cane weight in CoC 671 variety in the plant crop, a significant difference between healthy and infected plants was observed. A reduction of 13.0 per cent in single cane weight was recorded due to SCMV infection in the CoC 671 variety. In Co 740 variety, 35.6 per cent reduction in cane weight was recorded. The healthy canes recorded the cane yield of 1.32kg/cane when compared to 0.85kg of cane weight in the diseased (Fig. 12). In the second ratoon crop experiment, the single cane weight reduction was 11.5 and 41 per cent respectively in CoC 671 and Co 740 varieties respectively (Fig. 17).

4.8.3.2. Juice quality and sugar yield

As like other growth parameters and cane yield, the impact of SCMV on juice quality was also more in the ratoon crop when compared to plant crop. The total brix and sucrose content was reduced at the rate of 12.6 and 7.5 per cent respectively in the SCMV infected Co 740 canes whereas in CoC 671 variety, the reduction was 7.4 and 9.6 per cent respectively (Fig. 13). The reduction in sugar purity was 4.7 and 2.6 per cent respectively in

Fig. 16. Effect of SCMV on net photosynthesis of sugarcane (Ratoon crop experiment II at Coimbatore)

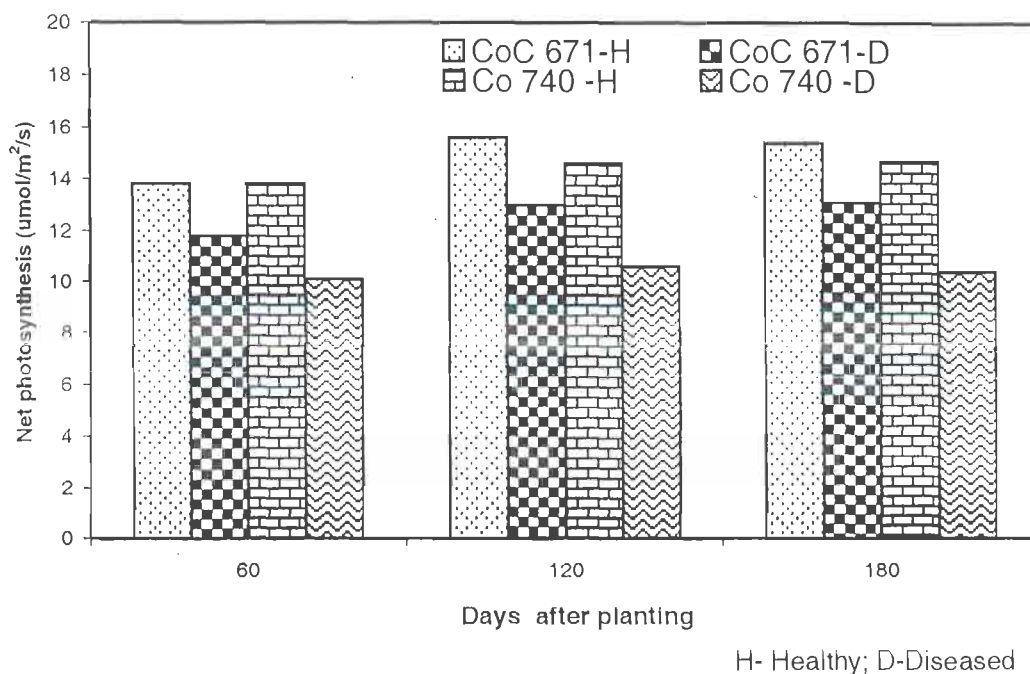
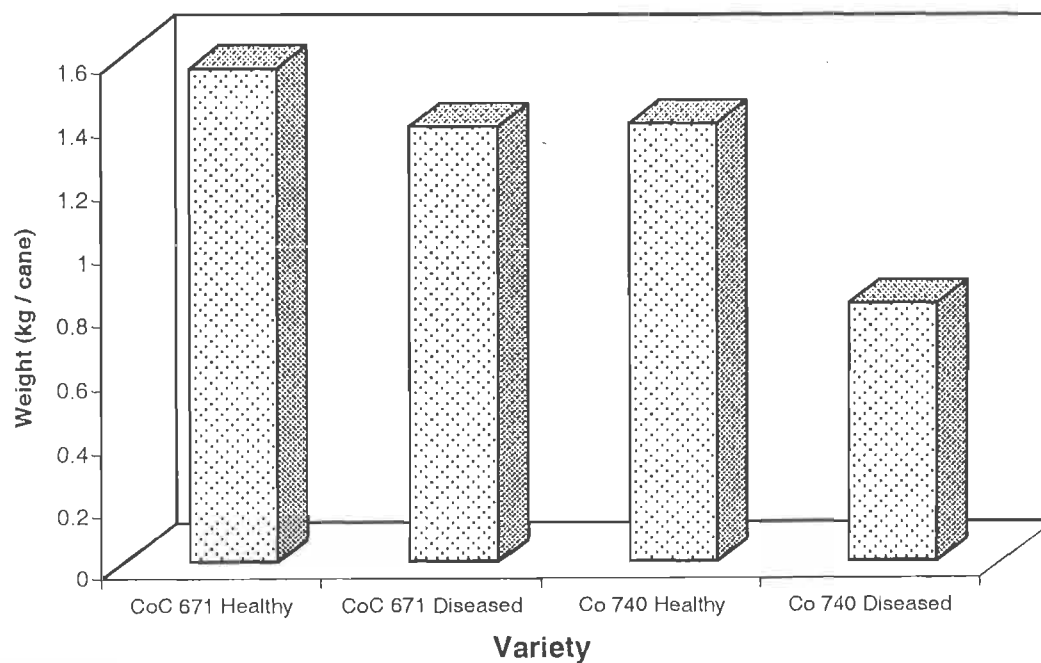


Fig. 17. Effect of SCMV on sugarcane growth - single cane weight (Ratoon crop experiment II at Coimbatore)



Co 740 and CoC 671 varieties. The impact of SCMV on juice quality was very well reflected in the sugar yield in both the varieties. In Co 740 and CoC 671 varieties, 6.8 and 8.3 per cent reduction in sugar yield were recorded respectively. A similar trend was observed in the ratoon crop experiment II also (Fig. 18). In all the plant crop and ratoon crop experiment the reduction in juice purity was observed in both the varieties due to SCMV infection (Fig. 19).

4.9. Induced resistance against SCMV

Different biotic and abiotic agents were used to find out their effect on inducing phenols and different proteins and their contribution in inducing resistance against SCMV. The chemicals used were salicylic acid and Acibenzolar-S-methyl (CGA 245704) available as bionTM. The biotic agent used was *Pseudomonas fluorescens*. The biotic and abiotic agents were applied as seed treatment, soil application and foliar spray.

4.9.1. Induction of total phenol

After three days, foliar application of salicylic acid and bion had induced the phenols in a significant level. Fifteen per cent increase in total phenols was observed by foliar application of bion and salicylic acid. All the remaining treatments recorded similar levels as in control. After six days of inoculation, in healthy control, there was no increase in total phenol content and found almost static. In bion and salicylic acid foliar applied plants, a 15 per cent further increase in total phenols was observed. It increased from 541 to 620 and 548 to 632 μg per gram of fresh tissue in salicylic acid and bion foliar applications, respectively. All the remaining treatments except healthy control were similar. The total phenol content reached maximum at six days after inoculation. Between third and six days after inoculation, there was further significant increase in phenol content in *P. fluorescens* seed treatment and soil application, bion and salicylic acid seed treatment. The increase ranged from 8 to 11 per cent. In the treatments with *P. fluorescens* foliar application, soil application of bion and salicylic acid, there was no significant increase between three and six days

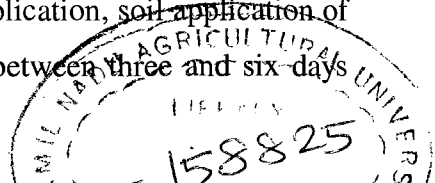


**Fig. 18. Effect of SCMV on cane juice quality and sugar yield
(Ratoon crop experiment II at Coimbatore)**

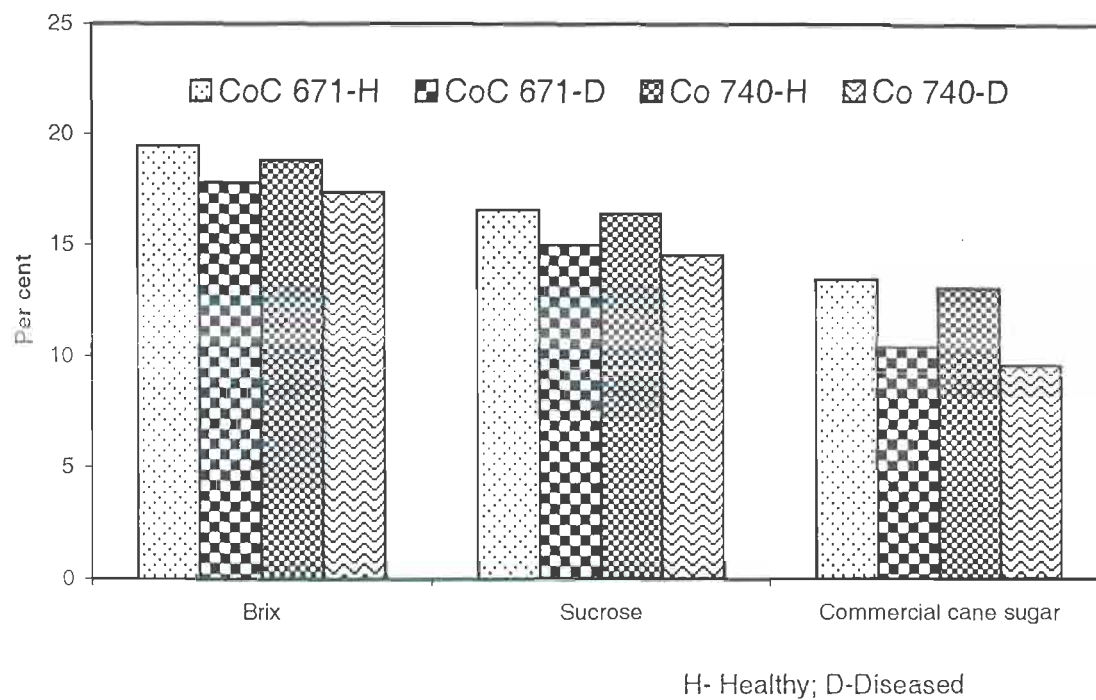
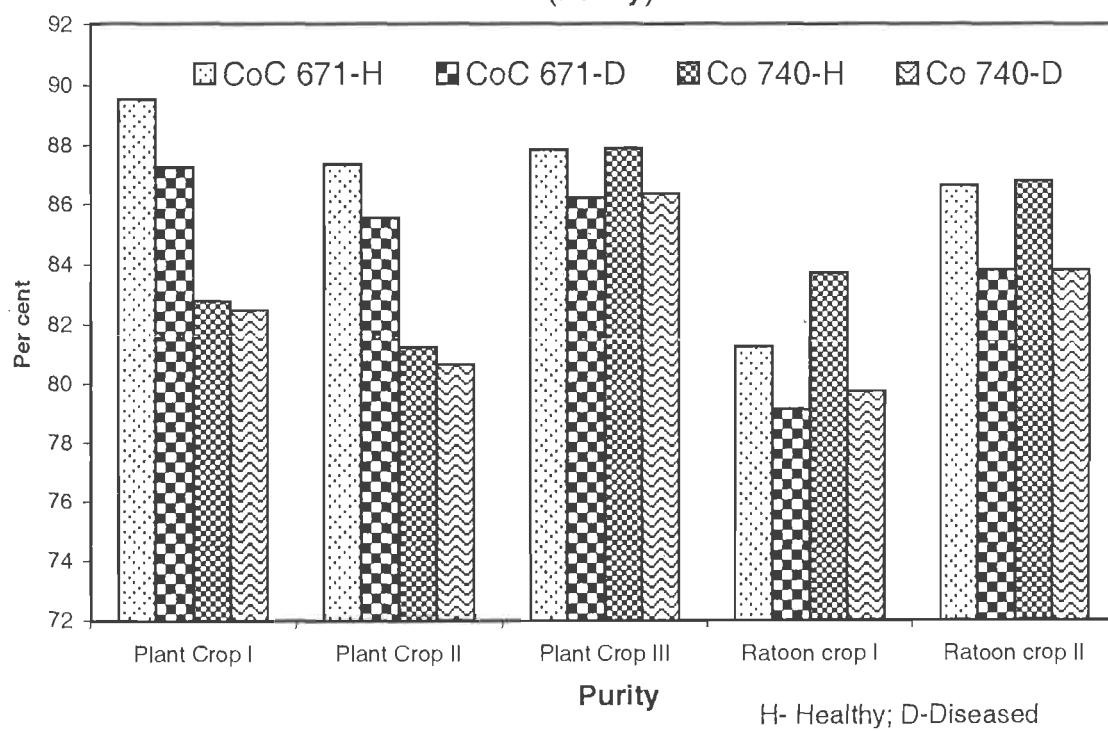


**Fig. 19. Effect of SCMV on cane juice quality in sugarcane
(Purity)**



after SCMV inoculation. After 9 days of inoculation, the total phenol content started declining slowly in all the treatments. Among the various treatments, salicylic acid and bion foliar applied plants still maintained higher induction (Table-22).

4.9.2. Peroxidase

In all the treatments, the peroxidase activity significantly increased on 3rd day except for the healthy control. Among the various treatments, foliar application of bion and salicylic acid was found to be superior. The peroxidase activity increased to nearly 3 times from 3.9 to 11.9 and from 3.7 to 11.4 respectively in the foliar applied plants with bion and salicylic acid. The seed treatments of bion, salicylic acid and *P. fluorescens* were also showing the induction of peroxidase but in a reduced level when compared to the above three treatments. Soil application treatments of bion, salicylic acid, and foliar application of *P. fluorescens* were not much effective in the induction of peroxidase and were similar to SCMV inoculated control. After six days, further increase in the peroxidase activity was recorded (Table-23). Nearly 60 per cent increase in the peroxidase activity was observed in the foliar application of bion and salicylic acid. On 9th day of estimation, the peroxidase activity has started, slightly declining but higher activity was observed in the foliar treated plants with bion and salicylic acid. The peroxidase activity reached maximum in all the treatments on 6th day after inoculation. The rate of increase was more in the bion and salicylic acid foliar applied plants. The rate of increase was slower in the SCMV inoculated control. In the healthy control, the activity was almost static. The results clearly showed that foliar spray of abiotic inducers and seed treatment with biotic inducer (*P. fluorescens*), were highly effective in inducing the defence enzymes like peroxidase.

4.9.2.1. Peroxidase isozymes

Regarding the isozyme pattern of peroxidase (Plates-42,43 and 44), variation in the intensity and number of isoforms were observed in all the three intervals. On third day, maximum of six isoforms were observed viz., PO1, PO2, PO3, PO4 and PO5 in salicylic acid foliar application as well as with bion seed treatment and foliar

Table-22. Effect of application of biotic and abiotic agents in the induction of total phenols in SCMV inoculated sorghum plants

Treatments	Total phenol content ($\mu\text{g catechol g}^{-1}$ fresh weight) at different intervals (Days after SCMV inoculation)			
	0	3	6	9
<i>P. fluorescens</i> ST	480 ^a	510 ^{bc}	552 ^b	550 ^b
<i>P. fluorescens</i> SA	469 ^a	486 ^c	531 ^b	531 ^{bc}
<i>P. fluorescens</i> FA	472 ^a	498 ^c	530 ^b	526 ^{bc}
Bion ST	483 ^a	509 ^{bc}	548 ^b	546 ^b
Bion SA	484 ^a	500 ^c	534 ^b	529 ^{bc}
Bion FA	476 ^a	548 ^a	632 ^a	630 ^a
Salicylic acid ST	479 ^a	496 ^c	551 ^b	545 ^b
Salicylic acid SA	481 ^a	504 ^{bc}	539 ^b	531 ^{cd}
Salicylic acid FA	475 ^a	540 ^{ab}	620 ^a	614 ^a
SCMV inoculated control	472 ^a	500 ^c	532 ^b	530 ^{bc}
Healthy control	470 ^a	484 ^c	469 ^c	476 ^d

L.S.D (5%): I X T=35.59

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

ST-Seed treatment; SA- Soil application; FA-Foliar application

Table-23. Effect of application of biotic and abiotic agents on peroxidase activity in SCMV inoculated sorghum plants

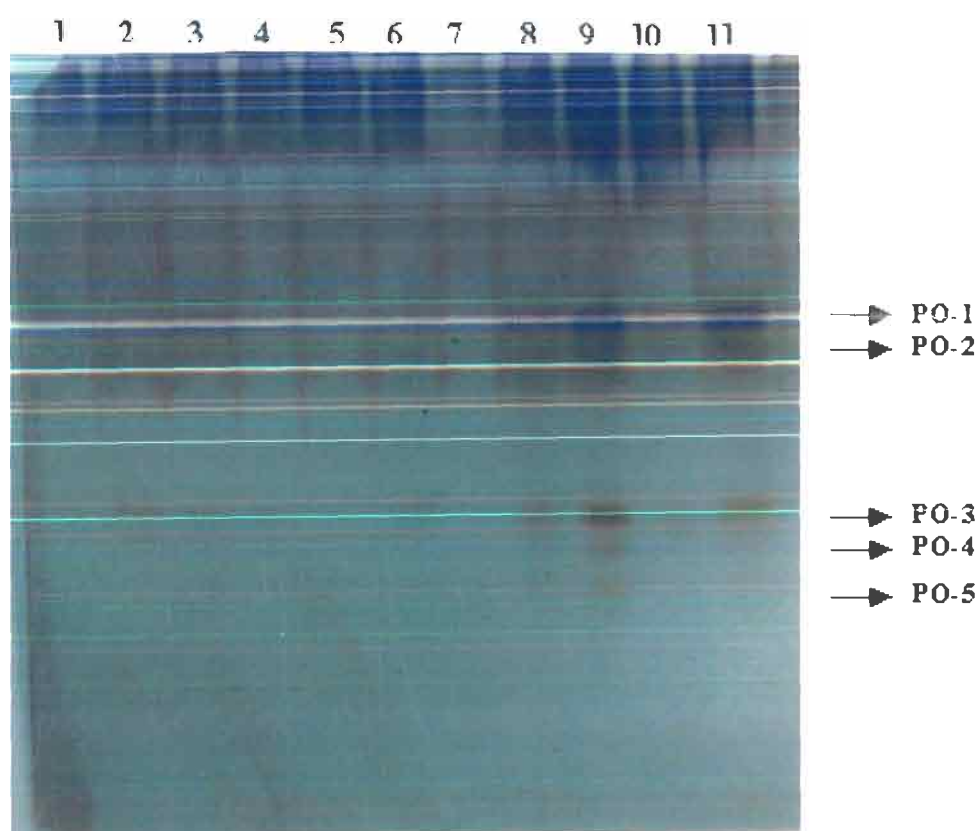
Treatments	Peroxidase activity (changes in absorbance at 420nm min ⁻¹ g ⁻¹ fresh tissue) at different intervals (Days after SCMV inoculation)			
	0	3	6	9
<i>P. fluorescens</i> ST	3.8 ^a	9.8 ^b	16.0 ^b	15.1 ^b
<i>P. fluorescens</i> SA	3.5 ^a	7.0 ^{de}	8.3 ^d	8.0 ^d
<i>P. fluorescens</i> FA	3.7 ^a	6.2 ^{de}	8.5 ^d	8.2 ^d
Bion ST	3.6 ^a	9.4 ^{bc}	15.0 ^{bc}	14.4 ^{bc}
Bion SA	4.0 ^a	6.6 ^c	7.6 ^d	7.4 ^d
Bion FA	3.9 ^a	11.9 ^a	19.0 ^a	18.4 ^a
Salicylic acid ST	4.3 ^a	8.1 ^{cd}	13.8 ^c	13.2 ^c
Salicylic acid SA	3.8 ^a	6.4 ^c	7.6 ^d	7.4 ^d
Salicylic acid FA	3.7 ^a	11.4 ^a	18.6 ^a	18.0 ^a
SCMV inoculated control	4.0 ^a	6.0 ^e	7.3 ^d	7.0 ^d
Healthy control	3.6 ^a	4.1 ^f	3.9 ^e	3.7 ^e

L.S.D (5%) : I X T=1.285

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

ST-Seed treatment; SA- Soil application; FA-Foliar application

Plate 42 Induction of peroxidase isozymes due to application of biotic and abiotic agents against SCMV (Third day)



Lane 1: Healthy

Lane 3: *P. fluorescens* seed treatment

Lane 5: *P. fluorescens* foliar application

Lane 6: Salicylic acid soil application

Lane 9: Bion seed treatment

Lane 11: Bion foliar application

Lane 2: SCMV Inoculated

Lane 4: *P. fluorescens* soil application

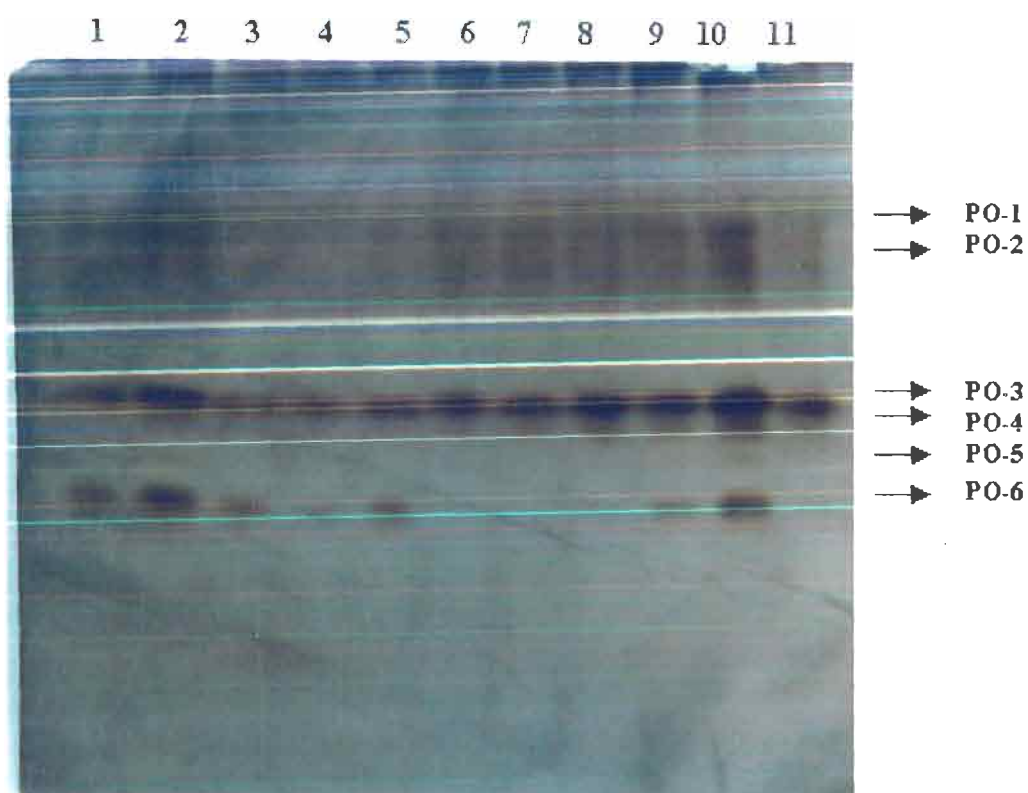
Lane 6: Salicylic acid seed treatment

Lane 8 : Salicylic acid foliar application

Lane 10: Bion soil application

43.

Plate Induction of peroxidase isozymes due to application of biotic and abiotic agents against SCMV (Sixth day)



Lane 1 : Healthy

Lane 2: *P. fluorescens* seed treatment

Lane 4: *P. fluorescens* foliar application

Lane 6 : Salicylic acid soil application

Lane 8: Bion seed treatment

Lane 10: Bion foliar application

Lane 3: *P. fluorescens* soil application

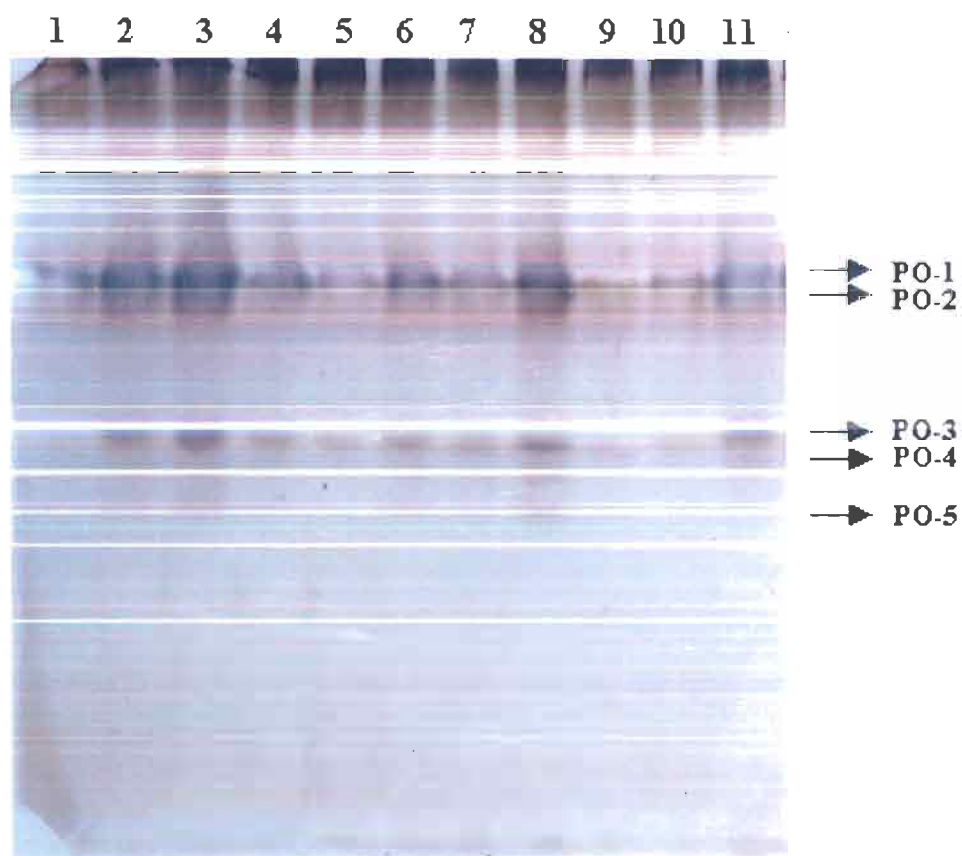
Lane 5: Salicylic acid seed treatment

Lane 7 : Salicylic acid foliar spray

Lane 9: Bion soil application

Lane 11 : SCMV Inoculated

Plate 4.4 Induction of peroxidase isozymes due to application of biotic and abiotic agents against SCMV (Nineth day)



Lane 1 : Healthy

Lane 3: *P. fluorescens* seed treatment

Lane 5: *P. fluorescens* foliar application

Lane 6: Salicylic acid soil application

Lane 9: Bion seed treatment

Lane 11: Bion foliar application

Lane 2 : SCMV Inoculated

Lane 4: *P. fluorescens* soil application

Lane 6: Salicylic acid seed treatment

Lane 8 : Salicylic acid foliar application

Lane 10: Bion soil application

application. Likewise, the induction of PO 1 was also higher in the above three treatments (lanes 8,9,11). In the healthy plants only PO1 was prominent. In most of the remaining treatments, PO1, PO2 and PO3 were present

The peroxidase isozyme pattern on the sixth day revealed that maximum of six peroxidase isoforms viz., PO1, PO2, PO 3, PO4, PO5 and PO6 was present in bion foliar application (lane 10). Likewise, the Induction of PO1, PO3 and PO6 were very high in the bion foliar applied plants (lane 10). In *P. fluorescens* and salicylic acid seed treated plants, PO 3 and PO5 induction was higher. But compared to bion foliar applied plants, the induction was less.

The peroxidase isozyme pattern on the ninth day, revealed that maximum of five isoforms viz., PO1, PO2, PO3, PO4 and PO5 was present in the treatments with *P. fluorescens* seed treatment, salicylic acid seed treatment, salicylic acid foliar application, bion seed treatment and bion foliar application. More induction of PO1 and PO3 were observed in the treatments with *P. fluorescens* seed treatment, salicylic acid seed treatment and foliar application and also in bion foliar applied plants. The results clearly showed that foliar spray of abiotic inducers and seed treatment with biotic inducer were highly effective in inducing the peroxidase isoforms that may induce the plant defense mechanism.

4.9.3. Polyphenol oxidase

The polyphenol oxidase activity increased at a higher rate in the foliar applied plants with salicylic acid and bion on 3rd day after SCMV inoculation. The activity increased to 8.9 from 2.8 and to 8.0 from 2.8 in bion and salicylic acid foliar applied plants respectively (Table-24). Bion was superior to other treatments. Seed treatment with *P. fluorescens*, bion and salicylic acid were found to be on par with each other. Nearly 2.5 times increase in polyphenol oxidase activity was recorded in these treatments when compared to nearly four times increase in bion and salicylic acid foliar sprays.

Table-24. Effect of application of biotic and abiotic agents on polyphenol oxidase (PPO) activity in SCMV inoculated sorghum

Treatments	PPO activity (changes in absorbance at 495nm min ⁻¹ g ⁻¹ fresh tissue) at different intervals (Days after SCMV inoculation)			
	0	3	6	9
<i>P. fluorescens</i> ST	2.8 ^a	7.6 ^{bc}	9.0 ^c	8.6 ^c
<i>P. fluorescens</i> SA	2.7 ^a	5.0 ^c	6.5 ^{ef}	5.8 ^g
<i>P. fluorescens</i> FA	2.6 ^a	4.8 ^c	6.4 ^c	5.9 ^{ef}
Bion ST	2.5 ^a	7.4 ^{cd}	8.4 ^d	7.8 ^d
Bion SA	2.9 ^a	4.8 ^e	6.9 ^e	6.0 ^{fg}
Bion FA	2.8 ^a	8.9 ^a	11.4 ^a	10.2 ^a
Salicylic acid ST	2.4 ^a	7.0 ^d	7.9 ^d	6.9 ^e
Salicylic acid SA	2.8 ^a	4.5 ^b	6.7 ^{ef}	6.0 ^{fg}
Salicylic acid FA	2.8 ^a	8.0 ^e	10.3 ^b	9.6 ^b
SCMV inoculated control	2.9 ^a	4.9 ^c	6.2 ^f	5.8 ^g
Healthy control	2.9 ^a	2.6 ^c	2.5 ^g	2.4 ^h

L.S.D(5%): I X T=0.565

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

ST-Seed treatment; SA- Soil application; FA-Foliar application

The maximum PPO activity was observed on the 6th day after SCMV inoculation in all the treatments. The maximum activity of 11.4 was recorded in the foliar application of bion followed by salicylic acid with the activity of 10.3 and followed by 9.0 in the case of *P. fluorescens* seed treatment.

All the soil application treatments were found to be on par with each other and also with the inoculated control. When compared to inoculated control, the increase in PPO activity in bion foliar spray, salicylic acid foliar spray and *Pseudomonas fluorescens* seed treatment were 83, 66 and 45 per cent respectively. On 9th day, the PPO activity was showing a downward trend however bion and salicylic acid foliar applications retained higher activity.

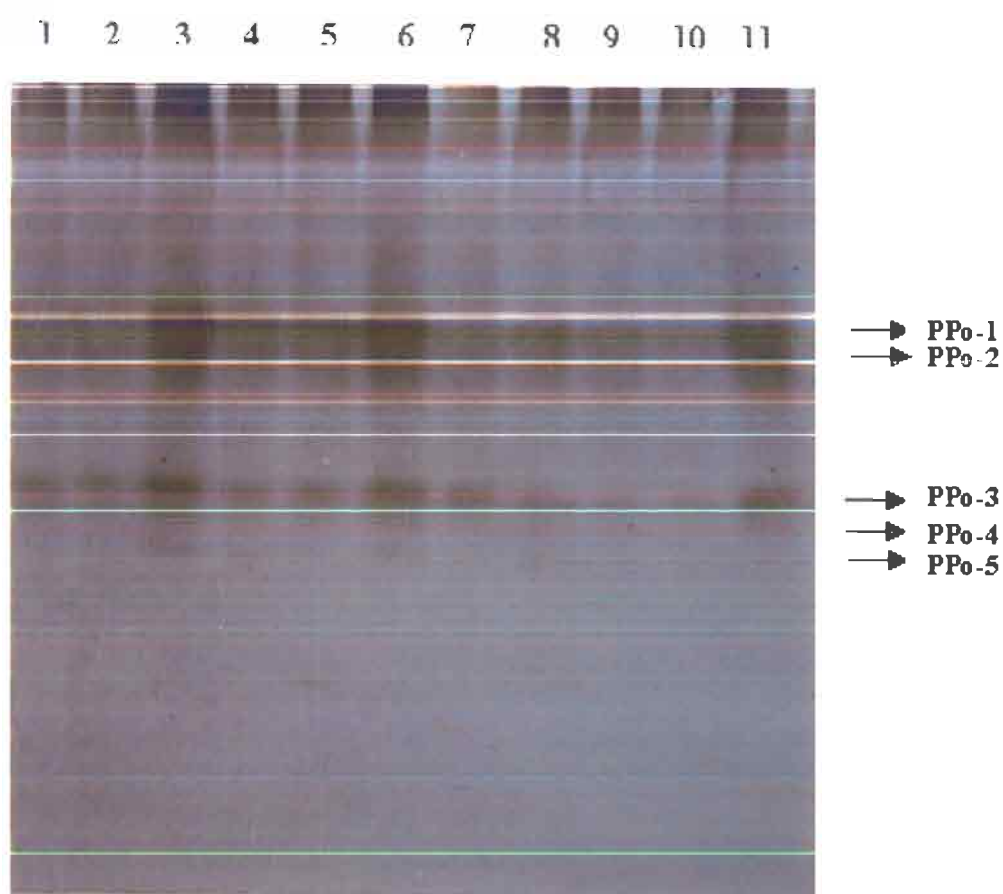
4.9.3.1. Polyphenol oxidase isozymes

The PPO isozyme pattern (Plate-45) on third day revealed that, even though five PPO isoforms viz., PPO1, PPO2, PPO3, PPO4 and PPO5 were present in most of the treatments, the intensity varied between the treatments. For example, the PPO1, PPO2 and PPO3 isoforms were induced more in *P. fluorescens* seed treatment, salicylic acid seed treatment, and salicylic acid and bion foliar applications. (lanes 3, 6, 8 and 11). The above results indicated the triggering of PPO activity upon application of biotic and abiotic inducers and their possible involvement in plant defense mechanism.

4.9.4. Phenylalanine ammonia lyase (PAL)

A similar trend was observed in PAL activity also like total phenols, peroxidase and PPO. On third day, bion and salicylic acid foliar sprays and *P. fluorescens* foliar sprays ranked top in PAL activity and the activity was 46.3, 45.2 and 43.3 respectively (Table-25). The remaining treatments were found to be similar with each other except for the healthy control. On the sixth day, the PAL activity reached the maximum and as in the other assays, bion foliar spray recorded the higher activity with 57.2 followed by *P. fluorescens* seed treatment with the activity of 56.3 and 55.0 in bion seed treatment.

45.
Plate Induction of polyphenol oxidase isozymes due to application of biotic and abiotic agents against SCMV (Third day)



Lane 1 : Healthy

Lane 2 : SCMV Inoculated

Lane 3 : *P. fluorescens* seed treatment

Lane 4 : *P. fluorescens* soil application

Lane 5 : *P. fluorescens* foliar application

Lane 6 : Salicylic acid seed treatment

Lane 6 : Salicylic acid soil application

Lane 8 : Salicylic acid foliar application

Lane 9 : Bion seed treatment

Lane 10 : Bion soil application

Lane 11 : Bion foliar application

Table-25. Effect of application of biotic and abiotic agents on phenylalanine ammonia-lyase (PAL) activity in SCMV inoculated sorghum plants

Treatments	PAL activity (nmol transcinamic acid min ⁻¹ g ⁻¹ fresh tissue) at different intervals (Days after SCMV inoculation)			
	0	3	6	9
<i>P. fluorescens</i> ST	21.2 ^a	43.3 ^{ab}	56.3 ^{ab}	51.0 ^a
<i>P. fluorescens</i> SA	20.3 ^a	40.7 ^b	48.3 ^c	44.2 ^{bc}
<i>P. fluorescens</i> FA	21.6 ^a	40.3 ^b	47.3 ^c	43.1 ^{bc}
Bion ST	19.7 ^a	41.7 ^b	55.0 ^{ab}	50.4 ^a
Bion SA	20.2 ^a	39.7 ^b	46.1 ^c	42.2 ^c
Bion FA	21.1 ^a	46.3 ^a	48.9 ^c	44.4 ^{bc}
Salicylic acid ST	22.7 ^a	41.3 ^b	57.2 ^b	51.0 ^a
Salicylic acid SA	21.4 ^a	40.7 ^b	47.0 ^c	42.4 ^c
Salicylic acid FA	20.6 ^a	45.2 ^a	53.5 ^a	52.9 ^a
SCMV inoculated control	22.2 ^a	40.2 ^b	48.4 ^c	46.4 ^b
Healthy control	19.9 ^a	20.3 ^c	19.0 ^d	20.7 ^d

L.S.D (5%): I X T=1.285

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

ST-Seed treatment; SA- Soil application; FA-Foliar application

and 53.5 in salicylic acid foliar spray. When compared to healthy plants, the PAL Induction was nearly 2.5 to 3 times higher in the above four treatments on 6th day. The remaining treatments were found on par with each other. On 9th day after inoculation, fast decline in PAL activity was observed but with same trend in the PAL activity among the treatments.

4.9.5. Assessment of SCMV infection by ELISA method

By DAC ELISA procedure, the SCMV infection was assessed in the inoculated sorghum plants at different intervals, by using the SCMV antiserum. The assay was conducted from the tissue samples on 0, 3, 9, 12, 24 and 48 days after inoculation. The results revealed that up to six days after SCMV inoculation, there was not significant difference in ELISA readings between, healthy and SCMV inoculated plants (Table-26). However from 9th day onwards, the plants started showing symptoms. The reduction in virus concentration was observed in bion and salicylic acid foliar applied plants and also in *P. fluorescens* seed treated plants when compared to SCMV inoculated control.

After 12 days also, a similar trend was observed. After 24 days a slight increase in virus concentration was observed as indicated by ELISA readings. However the salicylic acid and bion foliar applied plants still maintained their lower rate of SCMV infection when compared to other treatments. In these two treatments the ELISA readings were 0.791 and 0.783 respectively when compared to 0.128 in the healthy control and 1.201 in the inoculated control. In the remaining treatments, the readings ranged from 0.902 to 1.149. However, after 48 days, there was not much difference between the treatments.

The results revealed that there was initial slow down in the SCMV infection in the foliar applied plants with bion and salicylic acid followed by seed treatments with *P. fluorescens*, bion and salicylic acid.

Table-26. Effect of application of biotic and abiotic agents on SCMV titre in sorghum plants

Treatments	ELISA readings at different intervals (Days after SCMV inoculation)						
	0	3	6	9	12	24	48
<i>P. fluorescens</i> ST	0.131	0.137	0.184	0.484	0.559	0.983	1.301
<i>P. fluorescens</i> SA	0.128	0.137	0.151	0.664	0.798	1.102	1.412
<i>P. fluorescens</i> FA	0.168	0.154	0.174	0.684	0.763	1.149	1.481
Bion ST	0.135	0.124	0.188	0.438	0.491	0.902	1.303
Bion SA	0.136	0.154	0.172	0.708	0.800	1.120	1.521
Bion FA	0.132	0.139	0.186	0.248	0.383	0.783	1.261
Salicylic acid ST	0.135	0.153	0.171	0.456	0.488	0.942	1.421
Salicylic acid SA	0.121	0.144	0.144	0.711	0.801	1.140	1.490
Salicylic acid FA	0.121	0.138	0.163	0.239	0.351	0.791	1.264
SCMV inoculated control	0.124	0.139	0.153	0.662	0.828	1.201	1.583
Healthy control	0.108	0.124	0.128	0.117	0.119	0.128	0.120

L.S.D(5%): I X T=0.565

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

ST-Seed treatment; SA- Soil application; FA-Foliar application

4.10. Resistant mechanism against SCMV in sugarcane genotypes and hybrids

Different clones of *Saccharum spontaneum*, *Erianthus bengalense* and two hybrids were inoculated with SCMV and the changes in total phenol and different defense proteins were assessed calorimetrically as well as by isozyme studies, at different intervals. The SCMV infection was also assessed at different stages by ELISA method and the results are presented here under.

4.10.1. Total phenol

The changes in total phenol content were assessed at 0, 3, 6, 9 and 12 days after SCMV inoculation colorimetrically. On 0 day, the inoculated and un inoculated plants, recorded 607 to 669 μg catechol equivalent content. Among the various clones, the content was lesser in the hybrids and 607 to 620 μg was present per gram of tissues. When compared to *S. spontaneum* and *E. bengalense*, the content was significantly low in the interspecific hybrids (Table-27). After 3 days of inoculation, in the uninoculated plants, the phenolic content was between 664 to 788 μg per gram of fresh tissue and the hybrids *i.e* CoC 671 and Co 740 recorded lesser phenolic activity and there was no significant increase from 0 day. Among the various *S. spontaneum* clones, the activity ranged from 670 to 680 μg and in the inoculated plants and the increase was upto 16 per cent (Table-27 and Fig. 20).

The hybrids behaved similarly as a group with comparatively lesser Induction of phenols and the *S. spontaneum* and *E. bengalense* clones behaved similarly as a separate group with higher phenolic content. In the inoculated category, the activity reached maximum on 6th day. Among the different clones, *S. spontaneum* clones *viz.*, IND 81-80 and IND 81-142 were found to be on par and the increased phenolic activity over hybrids was 12 per cent. The remaining two clones *i.e* IND 81-161 (*S. spontaneum*) and IND 81-147 (*E. bengalense*) were also inducing more phenolic activity than the interspecific hybrids.

Table-27. Changes in total phenol content in SCMV inoculated resistant and susceptible sugarcane genotypes

Sugarcane genotype	Phenolic content ($\mu\text{g catechol g}^{-1}$ fresh tissue) at different intervals (Days after SCMV inoculation)									
	0		3		6		9		12	
	Un	Ino	Un	In	Un	In	Un	In	Un	In
<i>S. spontaneum</i> IND 81-80	644 ^{ab}	654 ^{ab}	674 ^a	744 ^b	670 ^a	782 ^b	652 ^{ab}	764 ^b	650 ^{ab}	707 ^b
<i>S. spontaneum</i> IND 81-142	651 ^a	659 ^{ab}	680 ^a	749 ^b	672 ^a	779 ^b	660 ^a	751 ^b	647 ^{ab}	701 ^b
<i>S. spontaneum</i> IND 81-161	643 ^{ab}	638 ^{bc}	670 ^a	788 ^b	664 ^a	841 ^a	649 ^{ab}	827 ^a	652 ^{ab}	753 ^a
<i>E. bengalense</i> IND 81-47	657 ^a	669 ^a	680 ^a	789 ^a	680 ^a	831 ^a	672 ^a	820 ^a	660 ^a	768 ^a
CoC 671	620 ^{bc}	609 ^d	638 ^b	666 ^c	624 ^b	701 ^d	631 ^{bc}	704 ^c	629 ^{bc}	696 ^b
Co 740	607 ^c	614 ^{cd}	627 ^b	664 ^c	631 ^b	694 ^d	614 ^c	699 ^c	610 ^c	690 ^b

L.S.D_{5%}: I X TXV=24.7

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Un: Un inoculated plants

In : Inoculated plants

Fig. 20. Changes in total phenol content in SCMV inoculated resistant and susceptible sugarcane genotypes

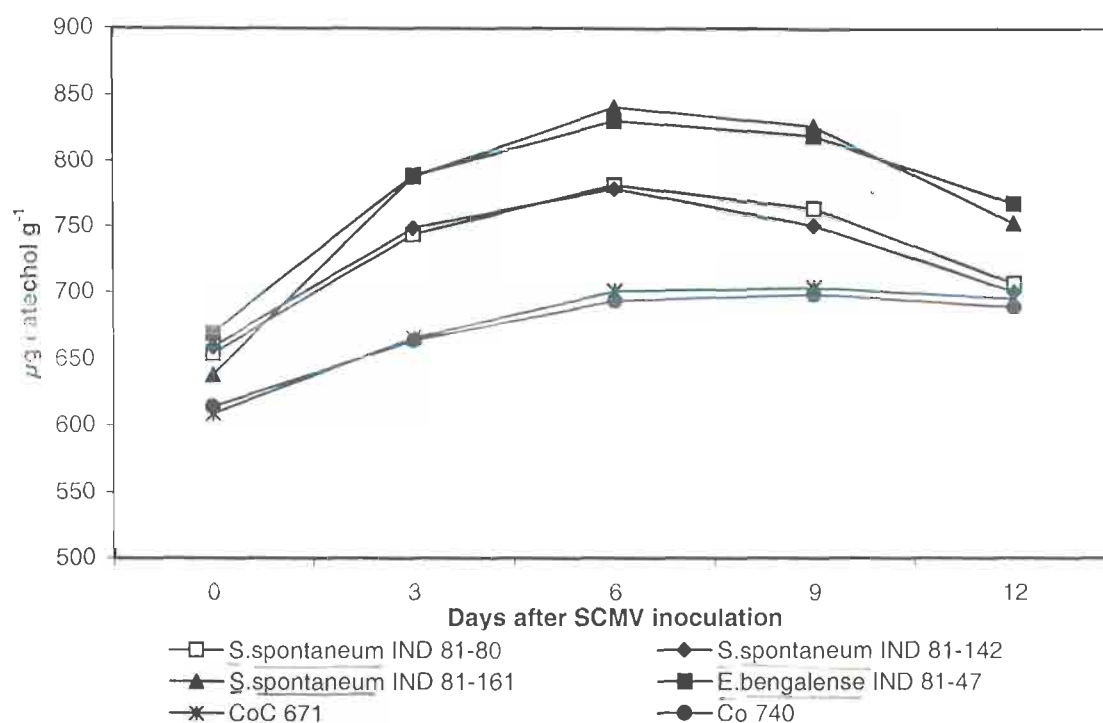
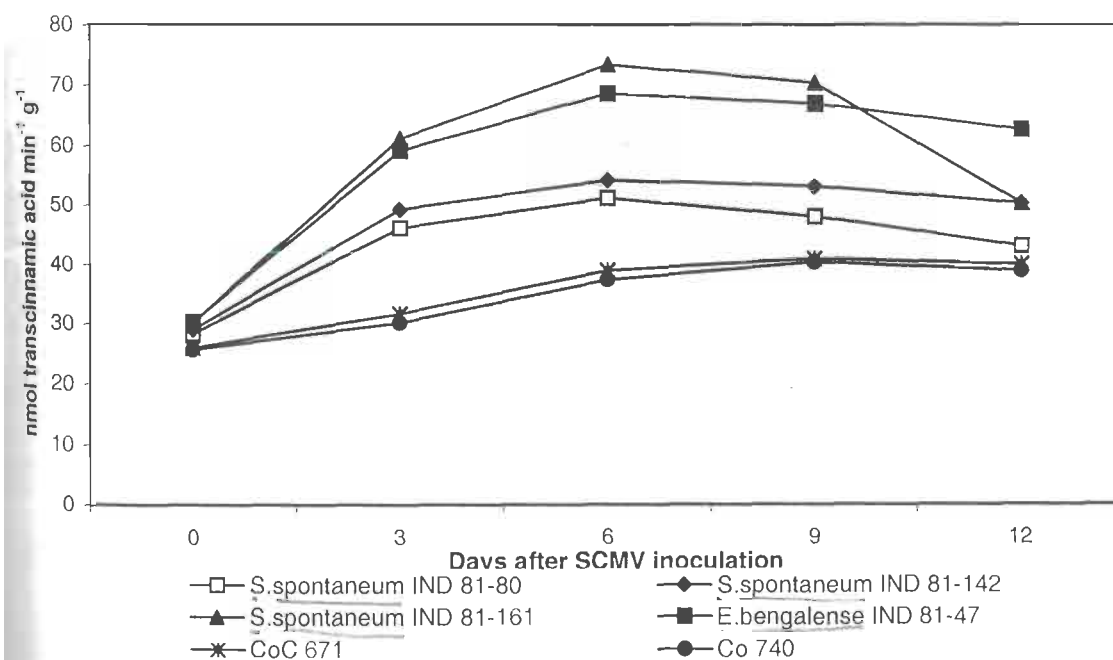


Fig. 21. Changes in phenylalanine ammonia lyase activity (PAL) in SCMV inoculated resistant and susceptible sugarcane genotypes



On 9th day, the phenolic content reached maximum in the hybrids. However the rate of increase was very less. In the remaining clones, the phenolic content started declining gradually. Even then, it was 18 per cent more than the hybrids in IND 81-161 and IND 81-147. In the remaining two clones namely IND 81-80 and IND 81-142, the phenolic content was 9 per cent more than the hybrids. In the uninoculated plants, the phenolic content was almost static and there was not much variation when compared to 6th day. After 12 days of SCMV inoculation, slightly further decrease in the phenolic content was observed. The above results indicated that upon SCMV inoculation, the phenolic content increased and the rate of increase was faster and quantum was also high in the *S. spontaneum* and *E. bengalense* clones when compared to interspecific hybrids.

4.10.2. Phenylalanine ammonia lyase (PAL)

Three days after SCMV inoculation, there was not much increase in PAL activity in the un inoculated plants and significant increase in PAL activity was observed in all the inoculated clones. But the rate of increase was very high in IND 81-161, which recorded 61.2, where the increase was more than 2 times. It was followed by IND 81-47 where the increase was 95 per cent from 0 day. When compared to the hybrids, these two clones recorded two times more phenolic content. In the hybrids, the PAL activity was 31.7 and 30.2 respectively in CoC 671 and Co 740 varieties. After six days of inoculation, the PAL activity was further increased in all the clones (Table-28 and Fig. 21). The activity was maximum in the *S. spontaneum* and *E. bengalense* clones. The maximum activity was recorded in IND 81-161 followed by IND 81-47 and was 90 per cent more than the hybrids. The PAL activity started declining from ninth day onwards in the *S. spontaneum* and *E. bengalense* clones. However, maximum activity of PAL was recorded by the hybrids on 9th day only. Here also the similar trend was followed as in the previous intervals.

Table-28. Changes in phenylalanine ammonia lyase activity (PAL) in SCMV inoculated resistant and susceptible sugarcane genotypes

Sugarcane genotype	PAL activity (nmol transcinamic acid min ⁻¹ g ⁻¹ fresh tissue) at different intervals (Days after SCMV inoculation)									
	0		3		6		9		12	
	Un	Ino	Un	In	Un	In	Un	In	Un	In
<i>S.spontaneum</i> IND 81-80	28.5 ^d	28.3 ^c	29.7 ^c	46.2 ^d	29.0 ^c	51.2 ^d	28.4 ^c	48.1 ^d	30.2 ^c	43.2 ^d
<i>S.spontaneum</i> IND 81-142	28.9 ^c	29.0 ^b	30.4 ^b	49.3 ^c	28.0 ^d	54.2 ^c	31.0 ^d	53.1 ^c	30.4 ^b	50.4 ^c
<i>S.spontaneum</i> IND 81-161	29.8 ^b	30.2 ^a	30.9 ^a	61.2 ^a	29.2 ^b	73.4 ^a	31.1 ^b	70.4 ^a	30.4 ^b	50.4 ^a
<i>E.bengalense</i> IND 81-47	30.1 ^a	30.3 ^a	29.4 ^d	59.2 ^b	29.9 ^a	68.6 ^b	31.2 ^a	67.0 ^b	30.9 ^a	62.7 ^b
CoC 671	26.4 ^e	26.0 ^d	26.8 ^e	31.7 ^e	26.9 ^e	39.0 ^e	26.0 ^e	41.0 ^e	26.9 ^d	40.1 ^e
Co 740	24.7 ^f	25.7 ^e	26.0 ^f	30.2 ^e	25.1 ^f	37.4 ^f	24.3 ^e	40.4 ^f	25.9 ^e	39.0 ^f

L.S.D (5%): I X TXV=0.162

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Un: Un inoculated plants

In : Inoculated plants

On 12th day, further decrease in the PAL activity in the inoculated clones was observed. However, when compared to the hybrids, higher phenolic content was recorded in the *S. spontaneum* and *E. bengalense* clones. Throughout the experiment period, the PAL activity in the uninoculated plants remained without much variation. The results indicated that upon SCMV inoculation, the phenyl propanoid metabolism was activated and enhanced PAL activity was observed in the *spontaneum* and *bengalense* clones when compared to interspecific hybrids.

4.10.3. Peroxidase activity

A similar trend was observed in peroxidase activity as like phenols and PAL. The estimation of peroxidase activity at different intervals indicated that upon SCMV inoculation, the activity was enhanced in *S. spontaneum* and *E. bengalense* clones when compared to interspecific hybrids. After three days of inoculation, the peroxidase activity increased to more than two times in IND 81-161. It increased to 12.7 from 6.3 (Table-29 and Fig. 22). In the case of IND 81-47, the activity increased to nearly 3 times from 5.9 to 14.2. When compared to hybrids, 98 per cent increase in peroxidase activity was recorded in IND 81-161 and 122 per cent increase was observed in IND 81-47. After 6 days of inoculation, the peroxidase activity reached the maximum in all the IND clones, in the range of 11.3 to 20.9. *E. bengalense* clone namely IND 81-47 recorded the maximum peroxidase activity of 20.9 followed by 18.7 in IND 81-161, and the rate of increase from third day was more than 47 per cent in both the cases. When compared to the hybrids more than two times increase in peroxidase activity was observed. In case of IND 81-80 and IND 81-142, the rate of increase from the third day was 33 and 27 per cent respectively from the third day. When compared to the hybrids, more than 25 per cent peroxidase activity was recorded in IND 81-80 and IND 81-142 clones.

After 9 days, the peroxidase activity was maintained as in the sixth day or else had started slightly receding in all the IND clones. Whereas in the hybrids, it reached the maximum of 9.6 and 9.9 respectively in CoC 671 and Co 740 canes. In the IND

Table-29. Changes in peroxidase activity in SCMV inoculated resistant and susceptible sugarcane genotypes

Sugarcane genotype	Peroxidase activity (Changes in absorbance at 420 nm min ⁻¹ g ⁻¹ fresh tissue) at different intervals (Days after SCMV inoculation)									
	0		3		6		9		12	
	Un	Ino	Un	In	Un	In	Un	In	Un	In
<i>S.spontaneum</i> IND 81-80	5.4 ^a	5.3 ^{ab}	5.8 ^{ab}	8.7 ^c	5.2 ^{ab}	11.6 ^c	6.9 ^a	10.8 ^{cd}	6.2 ^a	10.4 ^c
<i>S.spontaneum</i> IND 81-142	5.3 ^a	5.5 ^{ab}	6.7 ^a	8.9 ^c	6.5 ^a	11.3 ^c	5.9 ^{abc}	11.0 ^c	5.3 ^{ab}	10.9 ^c
<i>S.spontaneum</i> IND 81-161	6.1 ^a	6.3 ^a	6.6 ^a	12.7 ^b	5.8 ^{ab}	18.7 ^b	6.4 ^{ab}	17.9 ^b	5.7 ^{ab}	16.2 ^b
<i>E.bengalense</i> IND 81-47	5.5 ^a	5.9 ^{ab}	5.8 ^{ab}	14.2 ^a	6.4 ^a	20.9 ^a	5.3 ^{bc}	20.2 ^a	5.9 ^{ab}	19.4 ^{ab}
CoC 671	4.9 ^a	4.8 ^b	5.4 ^{ab}	6.0 ^e	5.2 ^{ab}	8.9 ^d	6.0 ^{abc}	9.6 ^d	4.8 ^b	9.0 ^c
Co 740	4.8 ^a	4.6 ^b	5.1 ^b	6.4 ^c	4.7 ^b	9.2 ^d	4.9 ^c	9.9 ^{cd}	5.3 ^{ab}	9.0 ^c

L.S.D (5%): I X TXXV=1.118

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Un: Un inoculated plants

In : Inoculated plants

Fig. 22. Changes in peroxidase activity in SCMV inoculated resistant and susceptible sugarcane genotypes

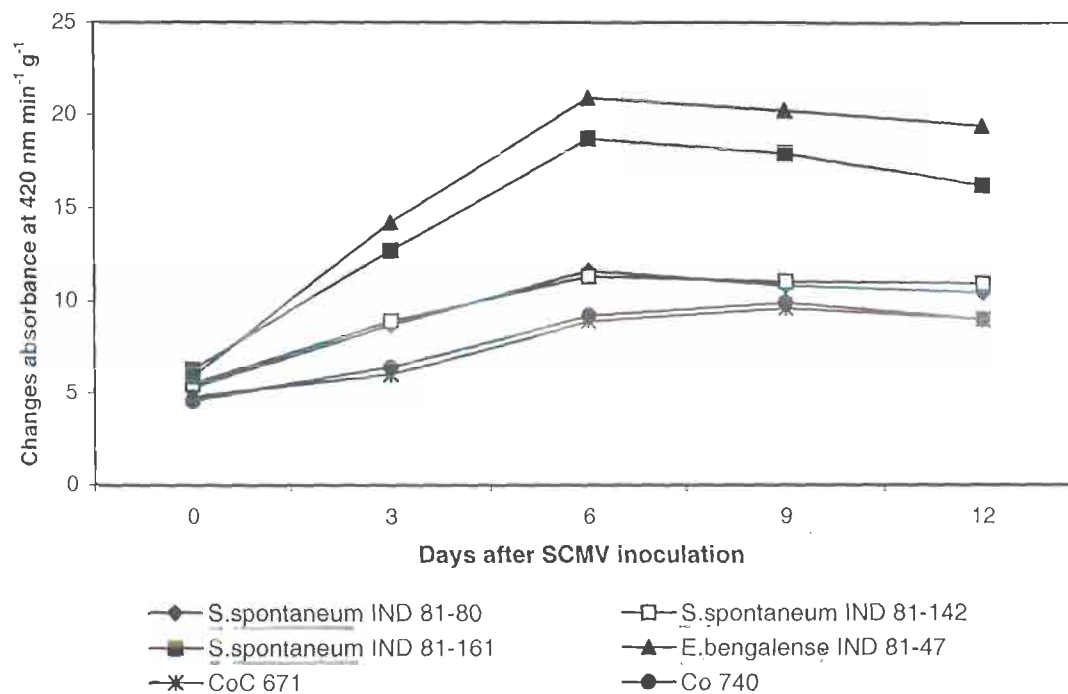
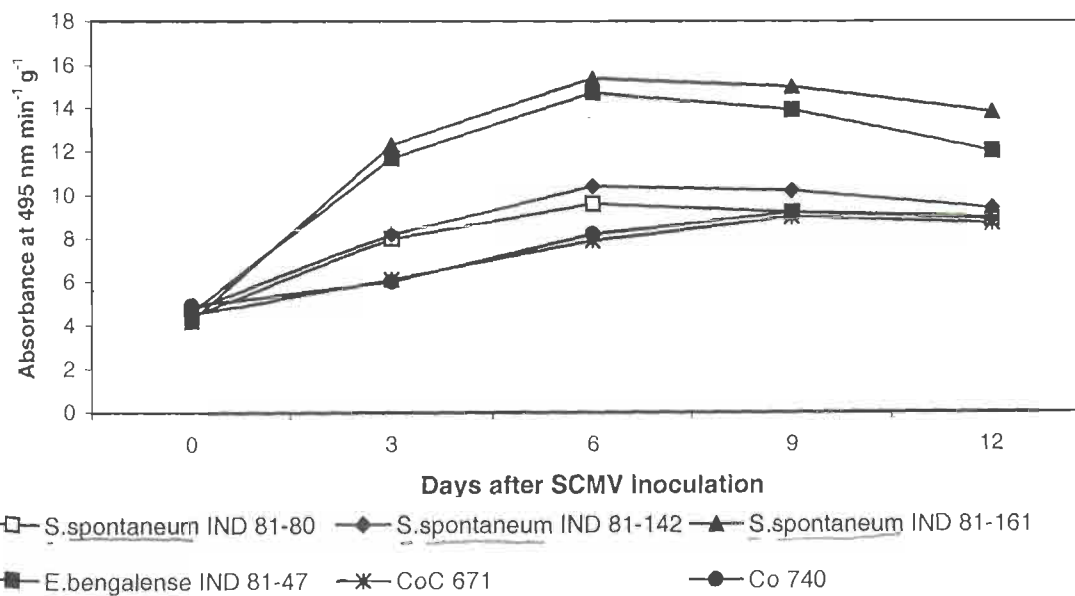


Fig. 23. Changes in polyphenol oxidase activity (PPO) in SCMV inoculated resistant and susceptible sugarcane genotypes



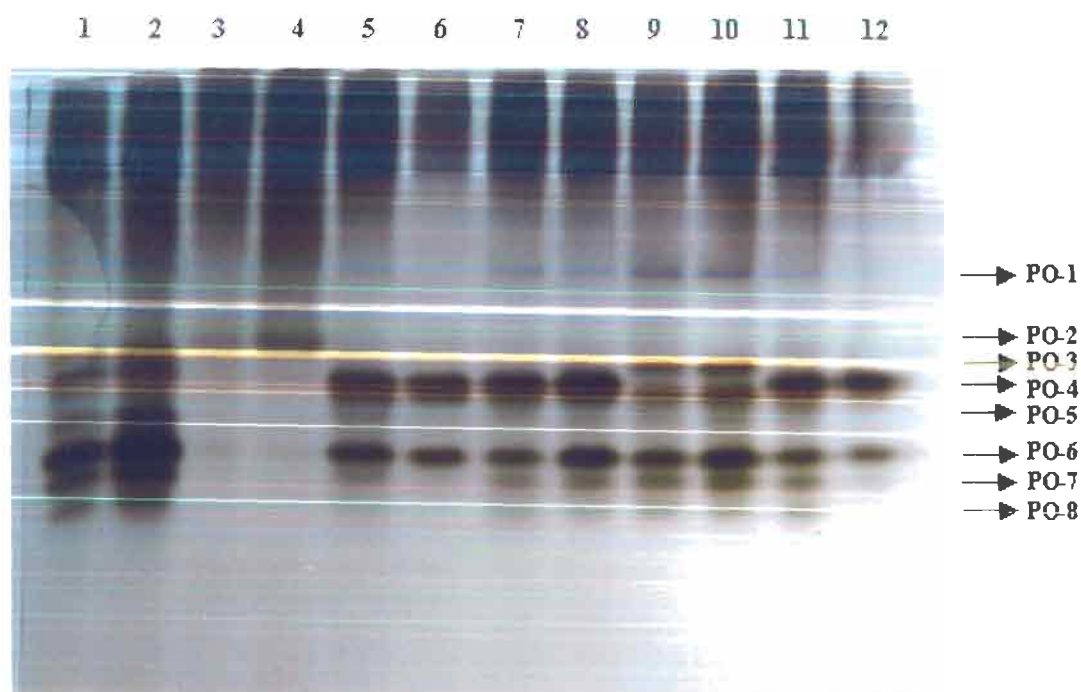
clones the activity ranged from 10.8 to 20.2. As in the previous intervals, the maximum peroxidase activity was recorded in IND 81-47 followed by IND 81-161. After 12 days of observation, the peroxidase activity was slightly declining further.

4.10.3.1. Peroxidase isozymes

The isozyme analysis peroxidase three days after inoculation (Plates - 46 and 47) revealed the significant difference between the uninoculated and inoculated clones. Eight different isoforms *viz.*, PO1, PO2, PO3, PO4, PO5, PO6, PO7 and PO8 were recorded in all the clones put together. The lanes 1,3,5,7 and 9 and 11 were the uninoculated clones of CoC 671, Co 740, IND 81-80, IND 81-161, IND 81-47 and IND 81-142 and lanes 2,4,6,8, 10 and 12 were the inoculated clones in the same order. Clear difference was observed between the uninoculated and inoculated clones. The intensity of peroxidase isoforms was very high in the inoculated plants when compared to the corresponding isoforms in the uninoculated control. PO1 was present in all the IND clones and higher intensity was observed in the inoculated plants. This PO1 isoform was not present in the hybrids. However PO 2 was present only in Co 740 and CoC 671 and in the inoculated control, the intensity was high. The PO 3 was present in IND 81-47 and CoC 671 and in Co 740. In the inoculated clones of IND 81-47, higher intensity of PO3 was observed. The PO4 isoform was very prominent in all the IND clones and intensity was very high in the inoculated plants. The PO5 was present in CoC 671 and in the inoculated plants more induction was observed. The PO6 was also present in all the clones and appreciable increase was observed in the inoculated plants. Especially in CoC 671, IND 81-161 and IND 81-47, remarkable increase was observed in the inoculated plants. Likewise, PO7 and PO8 were present in all the clones and increase in the inoculated plants was also observed.

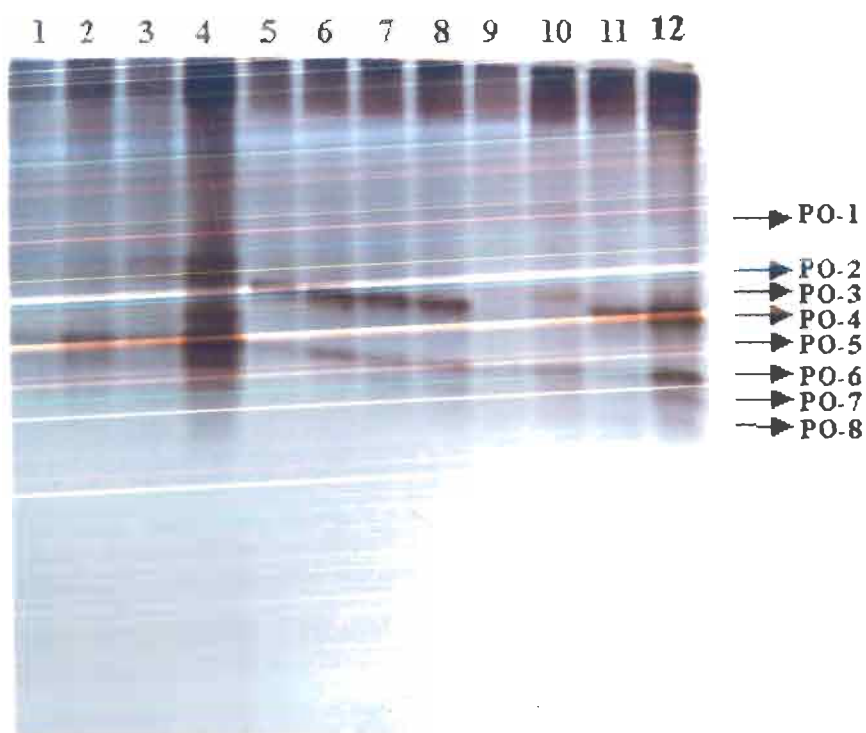
During the sixth day, clear variation in the isoform pattern were observed between different IND clones and hybrids. Eight peroxidase isoforms were observed in all the clones put together. There was characteristic difference between the inoculated and uninoculated clones in the induction of peroxidase isoforms. More induction was

Plate 46. Induction of peroxidase isozymes in resistant and susceptible sugarcane genotypes against SCMV (Third day)



Lane 1: CoC 671 uninoculated;	Lane 2 :CoC 671 inoculated
Lane 3: Co 740 uninoculated;	Lane 4: Co 740 inoculated
Lane 5: IND 81-80 uninoculated;	Lane 6: IND 81-80 inoculated
Lane 7: IND 81-161 uninoculated;	Lane 8: IND 81-161 inoculated
Lane 9: IND 81-47 uninoculated;	Lane 10: IND 81-47 inoculated
Lane 11: IND 81-142 uninoculated;	Lane 12 :IND 81-142 inoculated

Plate 47. Induction of peroxidase isozymes in resistant and susceptible sugarcane genotypes against SCMV (Sixth day)



Lane 1 Co 740 uninoculated;	Lane 2 Co 740 inoculated
Lane 3 CoC 671 uninoculated;	Lane 4 CoC 671 inoculated
Lane 5 IND 81-80 uninoculated;	Lane 6 IND 81-80 inoculated
Lane 7 IND 81-161 uninoculated;	Lane 8 IND 81-161 inoculated
Lane 9 IND 81-47 uninoculated;	Lane 10 IND 81-47 inoculated
Lane 11 IND 81-142 uninoculated;	Lane 12 IND 81-142 inoculated

observed in the SCMV inoculated clones. The PO1 was present in all the IND clones and more induction was observed in the inoculated clones especially in IND 81-161, IND 81-47 and IND 81-142. The PO1 was not present in the hybrids.

The PO2 was present only in the hybrids and more induction was observed in the inoculated clones. The PO3 was present in hybrids and in IND 81-47. Here also more induction was observed in the inoculated clones. The PO4 was predominantly present in all the IND clones and the induction was increased in the inoculated clones particularly in lane 12 representing the inoculated IND 81-142 clone. The PO5 was present only in the hybrids particularly in CoC 671. Here also more induction was observed in the inoculated clones. The PO6 was present in all the IND clones and hybrids. Very clear increase in the peroxidase isoforms observed after SCMV inoculation in all the clones. Likewise PO7 and PO8 were also present in all the clones with more induction in the inoculated clones.

4.10.4. Polyphenol oxidase (PPO)

Regarding the polyphenol oxidase activity, a similar trend like total phenol, PAL and peroxidase was observed. On third day, among the inoculated plants, IND 81-161 recorded the maximum PPO activity of 12.3 followed by IND 81-47 with 11.7. The hybrids recorded comparatively lesser PPO activity. It was 6.1 and 6.0 respectively in CoC 671 and Co 740 (Table-30 and Fig. 23). When compared to the hybrids, IND 81-161 and IND 81-47 recorded more than two times PPO activity. On 6th day after SCMV inoculation, further increase in PPO activity was recorded. In all the IND clones maximum PPO activity was recorded on 6th day. The PPO activity was high in case of IND 81-161 with 15.4 followed by 14.7 in IND 81-47. When compared to the hybrids, the PPO activity in these two clones were more than 90 per cent higher. The other two IND clones namely IND 81-80 and IND 81-142 also recorded higher PPO activity than the hybrids. The PPO activity of 9.6 and 10.4 were recorded respectively in IND 81-80 and IND 81-142.

Table-30. Changes in polyphenol oxidase activity (PPO) in SCMV inoculated resistant and susceptible sugarcane genotypes

Sugarcane genotype	PPO activity (Changes in absorbance at 495 nm min ⁻¹ g ⁻¹ fresh tissue) at different intervals (Days after SCMV inoculation)									
	0		3		6		9		12	
	Un	Ino	Un	In	Un	In	Un	In	Un	In
<i>S.spontaneum</i> IND 81-80	4.3 ^d	4.4 ^d	4.7 ^c	8.0 ^d	5.1 ^b	9.6 ^d	4.4 ^c	9.2 ^d	4.2 ^c	8.9 ^e
<i>S.spontaneum</i> IND 81-142	4.2 ^d	4.7 ^b	4.9 ^b	8.2 ^c	5.4 ^a	10.4 ^c	5.0 ^b	10.2 ^c	4.8 ^b	9.4 ^c
<i>S.spontaneum</i> IND 81-161	4.7 ^b	4.2 ^e	4.5 ^d	12.3 ^a	4.4 ^c	15.4 ^a	5.1 ^b	15.0 ^a	4.8 ^b	13.8 ^a
<i>E.bengalense</i> IND 81-47	4.9 ^a	4.6 ^{bc}	4.7 ^c	11.7 ^b	5.1 ^b	14.7 ^b	5.0 ^b	13.9 ^b	4.8 ^b	12.0 ^b
CoC 671	4.6 ^{bc}	4.5 ^{cd}	5.1 ^a	6.1 ^e	4.4 ^c	7.9 ^f	5.4 ^a	9.0 ^e	4.7 ^b	8.7 ^e
Co 740	4.5 ^c	4.9 ^a	5.0 ^{ab}	6.0 ^e	5.1 ^b	8.2 ^e	4.4 ^c	9.2 ^d	5.3 ^a	9.0 ^d

L.S.D (5%): I X TXV=0.161

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Un: Un inoculated plants

In : Inoculated plants

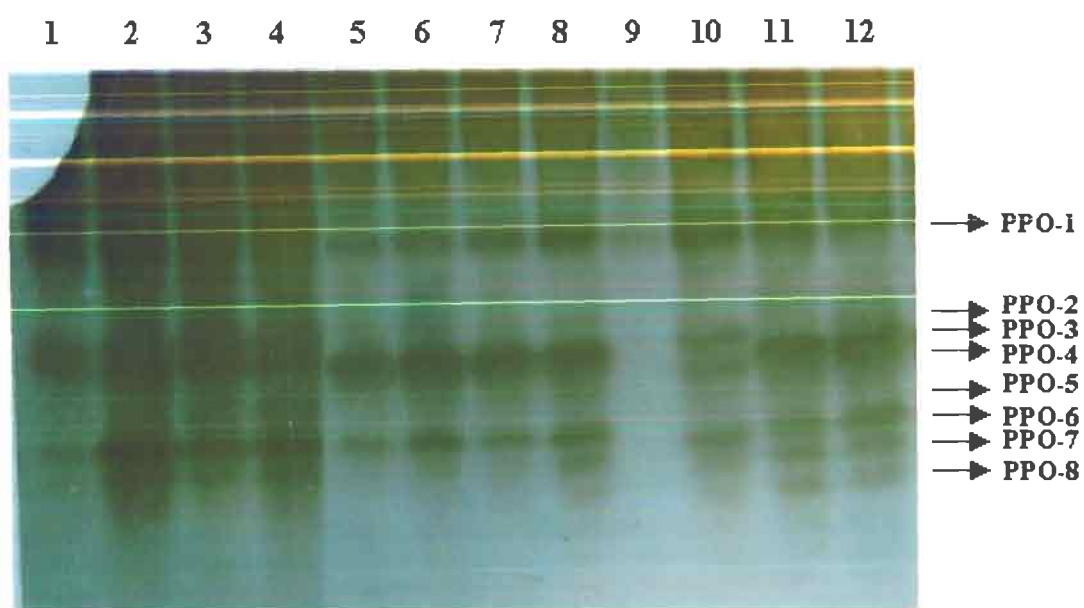
The PPO activity on 9th day revealed that in all the IND clones, no further increase was observed. The activity was static or started slightly declining. On 9th day, the PPO activity in the hybrids reached the maximum. But it was 60 per cent lesser when compared to IND 81-161 and IND 81-47 clones. The hybrids recorded 9.0 and 9.2 PPO activity respectively in CoC 671 and Co 740 varieties. After 12 days of inoculation, the PPO activity was further reduced in all the inoculated clones.

4.10.4.1. PPO isozymes

The PPO isoform pattern on third day revealed eight PPO isoforms from PPO 1 to PPO 8. In all the clones, the PPO activity was increased due to SCMV inoculation (Plates 48 & 49). The PPO1 was present in all the IND clones (lanes 5 to 12) and not in the hybrids. The increase in PPO1 isoform intensity was observed in the inoculated IND clones (lanes 6,8, 10 and 12). The PPO2 isoform was present in the hybrids, and the PPO3 isoform was also present in the hybrids as well as in IND 81-47. The PPO4 isoform was predominantly present in all the IND clones and corresponding increase PPO4 due to SCMV inoculation was observed in the above clones. The PPO5 was present only in CoC 671. The PPO6, PPO7 and PPO8 isoforms were present in all the clones. Even though the increased activities of PPO6 and PPO7 were noticed in all the SCMV inoculated clones, only in the IND clones, the increased activity was very predominant.

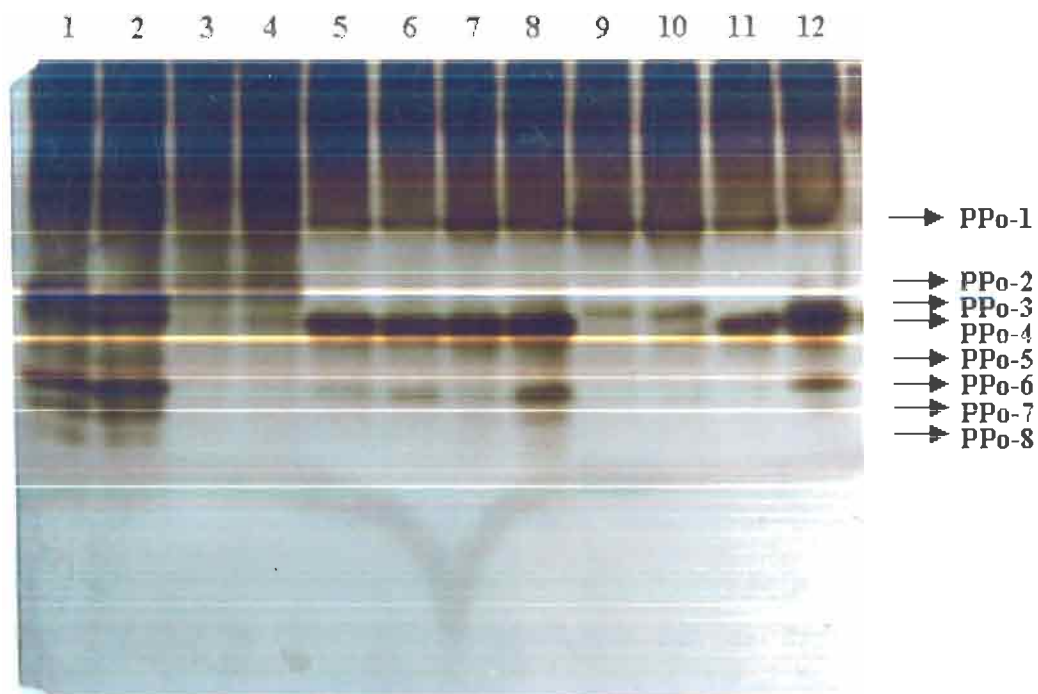
After 6 days, the PPO isoform analysis revealed that, more induction of PPO isoforms were observed in all the SCMV inoculated IND clones. The induction of PPO1 in the SCMV inoculated clones of IND 81-161, IND 81-47 and IND 81-142 was very well observed. Likewise more induction of PPO3 in IND 81-47 was also noticed. The PPO 4 was very well increased in SCMV inoculated clones of IND 81-161, IND 81-47 and IND 81-142 (lanes 8, 10 and 12). Likewise, more induction of PPO6 was observed in CoC 671, IND 81-161 and IND 81-142 (lanes 2,8 and 12) due to SCMV inoculation.

Plate 48. Induction of polyphenol oxidase isozymes in resistant and susceptible sugarcane genotypes against SCMV (Third day)



Lane 1: CoC 671 uninoculated; Lane 2: CoC 671 inoculated
 Lane 3: Co 740 uninoculated; Lane 4 :Co 740 inoculated
 Lane 5: IND 81-80 uninoculated; Lane 6: IND 81-80 inoculated
 Lane 7: IND 81-161 uninoculated; Lane 8: IND 81-161 inoculated
 Lane 9: IND 81-47 uninoculated; Lane 10: IND 81-47 inoculated
 Lane 11: IND 81-142 uninoculated; Lane 12: IND 81-142 inoculated

Plate 49. Induction of polyphenol oxidase isozymes in resistant and susceptible sugarcane genotypes against SCMV (Sixth day)



Lane 1: CoC 671 uninoculated

Lane 2: CoC 671 inoculated

Lane 3: Co 740 uninoculated

Lane 4: Co 740 inoculated

Lane 5: IND 81-80 uninoculated

Lane 6: IND 81-80 inoculated

Lane 7: IND 81-161 uninoculated

Lane 8: IND 81-161 inoculated

Lane 9: IND 81-47 uninoculated

Lane 10: IND 81-47 inoculated

Lane 11: IND 81-142 uninoculated

Lane 12: IND 81-142 inoculated

4.10.5. Assessment of SCMV infection in the resistant and susceptible sugarcane genotypes

By using the DAC ELISA method, tissues from the inoculated and uninoculated clones were assessed for the presence of SCMV at different intervals in all the clones. On 10th day, there was not much variation in ELISA readings among the various clones. However, the readings on 15th day revealed the presence of SCMV in the inoculated clones of CoC 671 and Co 740 varieties (Table-31). After 30 days, the ELISA readings revealed the higher SCMV infection in the interspecific hybrids. Among the different clones, IND 81-161 recorded the lowest absorbance of 0.112 followed by 0.139 in the case of IND 81-47 clone. The other IND clones namely IND 81-80 and IND 81-142 recorded 0.196 and 0.191 respectively. After 45 days of SCMV inoculation, IND 81-47 recorded the lowest absorbance of 0.131, followed by the remaining three IND clones. When compared to the hybrids, the ELISA readings were nearly three times lesser in the IND clones indicating the resistance against SCMV infection (Plate-50).

4.11. Virus elimination from infected sugarcane

Two different experiments were carried out separately to find out their effect on virus elimination from the infected sugarcane. In the first experiment, different chemotherapeutants were amended with the MS medium and meristem tip culture was followed, by using CoC 671 variety. In the second experiment, serial hot water treatment was followed in CoC 671 and Co 740 varieties.

4.11.1. Combined effect of antiviral chemotherapeutants and meristem tip culture

Two chemptherapeutants namely ribavirin and 8- Azaguanine were amended with the MS medium at different concentrations (5, 10, 25 and 50 ppm). Meristem from SCMV infected CoC 671 variety was subjected for virus elimination by meristem tip culture.

Table-31. Assessment of SCMV infection in the virus inoculated resistant and susceptible sugarcane genotypes

Sugarcane genotypes	ELISA readings at different intervals (Days after SCMV inoculation)							
	10 days		15 days		30 days		45 days	
	Un	In	Un	In	Un	In	Un	In
<i>S.spontaneum</i> IND 81-80	0.096 ^a	0.102 ^b	0.105 ^a	0.121 ^d	0.119 ^a	0.196 ^c	0.120 ^a	0.219 ^c
<i>S.spontaneum</i> IND 81-142	0.093 ^a	0.094 ^{bc}	0.105 ^a	0.148 ^c	0.116 ^a	0.191 ^c	0.116 ^{ab}	0.224 ^c
<i>S.spontaneum</i> IND 81-161	0.090 ^a	0.089 ^c	0.109 ^a	0.109 ^e	0.117 ^a	0.112 ^c	0.119 ^b	0.224 ^c
<i>E.bengalense</i> IND 81-47	0.087 ^a	0.094 ^{bc}	0.110 ^a	0.124 ^d	0.114 ^a	0.139 ^d	0.116 ^{ab}	0.131 ^d
CoC 671	0.094 ^a	0.197 ^a	0.109 ^a	0.273 ^b	0.117 ^a	0.484 ^a	0.123 ^a	0.576 ^b
Co 740	0.089 ^a	0.202 ^a	0.103 ^a	0.284 ^a	0.121 ^a	0.451 ^b	0.124 ^a	0.590 ^a

L.S.D (5%) I X V: 0.011

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Un: Un inoculated plants

In : Inoculated plants

Plate 50. SCMV resistant sugarcane genotypes

a) *Saccharum spontaneum* (IND - 81- 161)



b) *Erianthus bengalense* (IND- 81- 47)



Within three to four days, shoot tips started growing from the meristem and within 3 to 4 weeks it attained two to three leaf stage. When it was transferred to the rooting medium, rooting process completed within five to six weeks and subsequently transferred for hardening (Plates-51, 52 and 53).

4.11.1.1.Regeneration

Among, the two chemotherapeutants, from 8- Azaguanine there was reduction in the number of plantlets derived and only 52 to 56 per cent plantlets were derived from 5,10 and 25 ppm of 8-azaguanine amended medium. From 50 ppm, the plantlets derived was reduced further to 44 per cent. However, another chemotherapeutant ribavirin did not affect the regeneration. From all the concentrations, more than 80 per cent of the plantlets were derived and found to be on par with the meristem tip culture where 90 per cent of the plantlets were derived. The data clearly indicated that amending the MS medium with 8-azaguanine affected the regeneration of sugarcane meristem up to 48 per cent.

4.11.1.2.Virus elimination

At different stages, virus elimination was assessed by DAC- ELISA method from the meristem-derived plantlets. In meristem tip culture alone, 62 per cent virus elimination was obtained and found on par with 5,10 and 25 ppm ribavirin, whereas 95 per cent virus elimination was obtained from 50 ppm ribavirin (Fig. 24) and found superior than other treatments (Table-32). Likewise, 8-azaguanine was not superior in SCMV elimination when compared to meristem tip culture. Two to three times difference in ELISA readings were recorded (Table-33) between virus eliminated and infected plantlets derived from the meristem

4.11.2.Effect of serial hot water treatment

The infected setts of Co 740 and CoC 671 varieties were subjected to different serial hot water treatments as given in the chapter 3.10. Different temperature and time schedule were followed for the treatment of setts for 3 to 4 successive days.

51.
Plate Combined effect of antiviral chemotherapeutants and meristem culture on SCMV elimination

a) Meristem in liquid medium



b) Shoot initiation

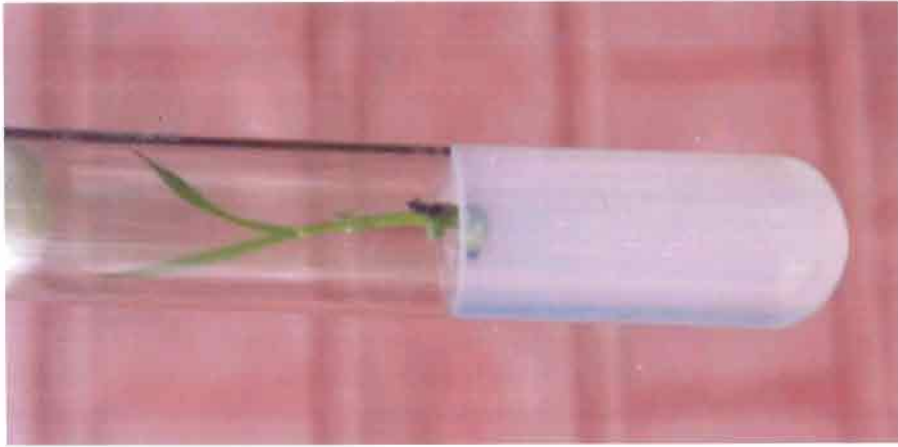


c) Emerged shoots

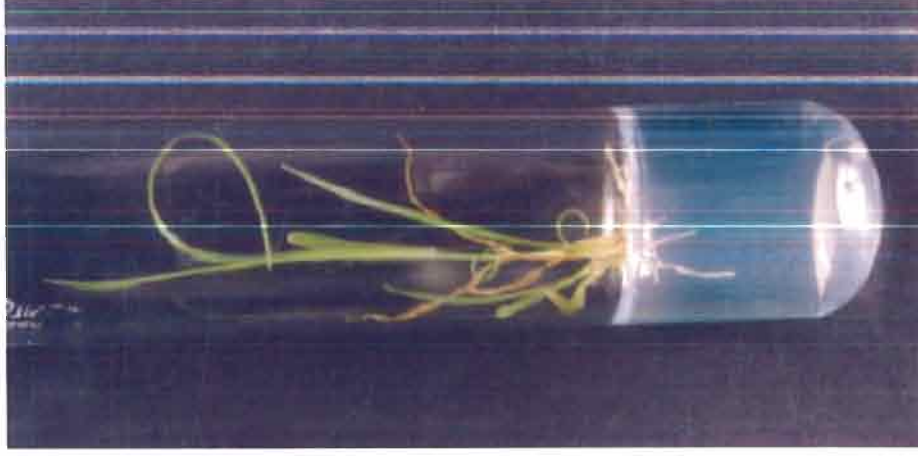


Plate 52. Root induction of emerged sugar cane shoots in half MS solid medium

a) Shoots in solid medium



b) Rooting



c) Rooted plants



Plate Hardening of tissue culture sugar cane plants

A) Rooted plants in plastic cups



b) Hardened plants in poly bags

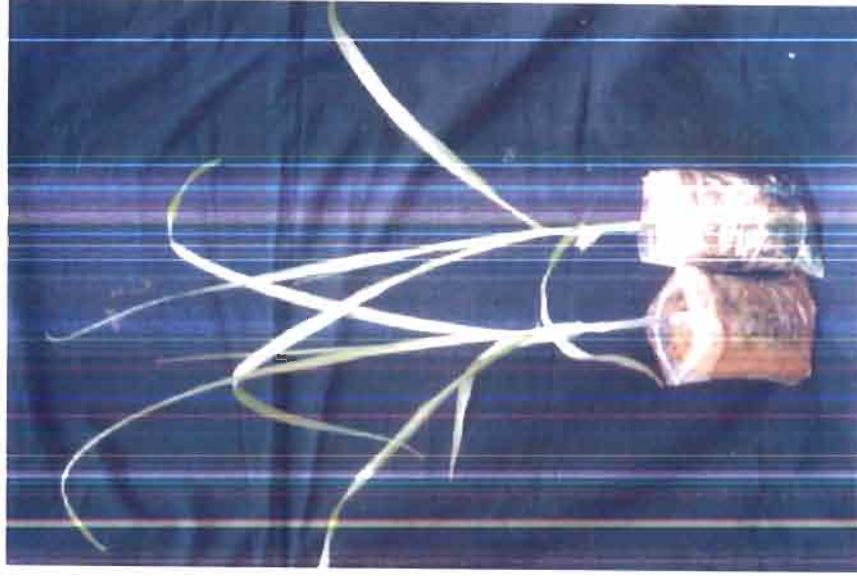


Fig. 24. Combined effect of antiviral chemotherapeutants and meristem tip culture against SCMV in sugarcane (CoC 671)

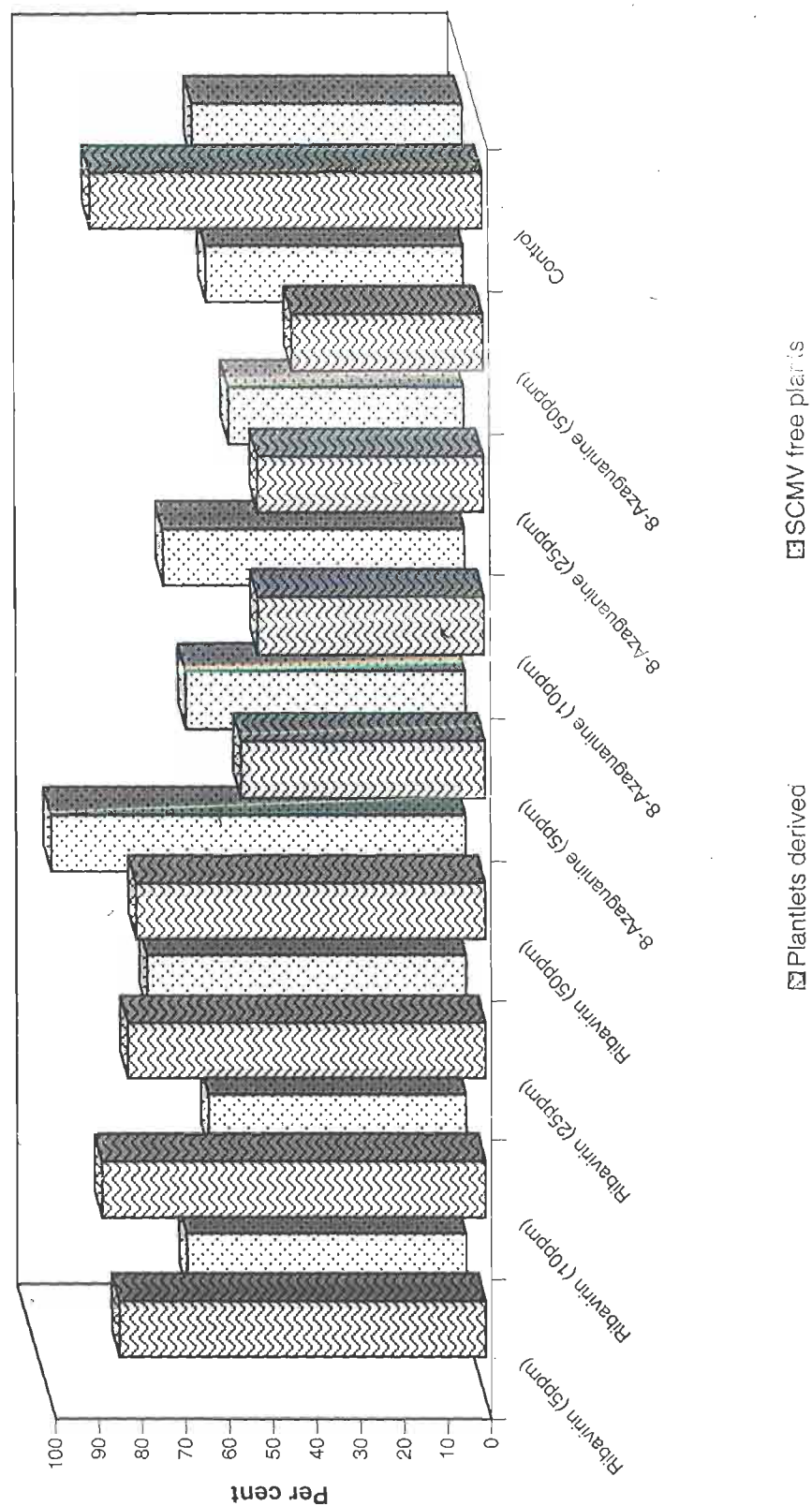


Table-32. Combined effect of antiviral chemotherapeutants and meristem tip culture against SCMV in sugarcane (CoC 671 variety)

Treatments	Concn (ppm)	No.of meristem used	No.of plantlets derived	No.of virus free plants
Ribavirin	5	50	42 ^c	27 ^b
Ribavirin	10	50	44 ^c	26 ^b
Ribavirin	25	50	41 ^c	30 ^b
Ribavirin	50	50	40 ^c	38 ^c
8-Azaguanine	5	50	28 ^b	18 ^a
8-Azaguanine	10	50	26 ^{ab}	18 ^a
8-Azaguanine	25	50	26 ^{ab}	14 ^a
8-Azaguanine	50	50	22 ^a	13 ^a
Control (Meristem culture alone)		50	45 ^c	28 ^a

L.S.D (5%)

5.2

6.1

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table-33. Assessment of SCMV elimination by chemotherapeutants in DAC-ELISA method

Treatments	Concn (ppm)	Mean ELISA readings of derived plants in each treatment (405nm)	
Ribavirin	5	Virus free plants	SCMV infected plants
Ribavirin	10	0.092	0.239
Ribavirin	25	0.081	0.241
Ribavirin	50	0.097	0.241
8-Azaguanine	5	0.107	0.197
8-Azaguanine	10	0.101	0.230
8-Azaguanine	25	0.107	0.219
8-Azaguanine	50	0.099	0.292
Control (Meristem culture alone)		0.112	0.283
		0.110	0.259

The number of plantlets derived were recorded in each treatment. ELISA was followed to determine the virus elimination from the plants emerged.

4.11.2.1. Effect of hot water treatment on Co 740 variety

The data (table-34) revealed that in all the serial hot water series, germination and emergence of plantlets were severely affected. Only in the treatment series A, 40 per cent of the plantlets were derived. In the remaining five treatments, the percentage of plantlets derived was 20 or less than 20 per cent. When the temperature duration was increased, the plantlets derived were also reduced. In the treatment series A, the setts were treated for three successive days for 20, 15 and 5 min at 52, 58 and 60°C respectively and 40 per cent plantlets derived whereas, in treatment series B the setts were treated for more duration *ie* 10 min on third day at 60°C, hence the plantlets derived were reduced to 23 per cent. When the treatment was increased for one more day at 62°C for 5 and 10 min respectively in treatment series C and D, the germination of the setts was further affected. Only 20 and 13 per cent of the setts germinated respectively from the treatment series C and D. In the series E and F when the temperature was increased to 60°C on the second day itself, only 13 and 10 per cent of the setts germinated respectively in both the treatment series. From the healthy control, 83 per cent of the setts germinated while in the diseased untreated control, it was 73 per cent. The untreated diseased control and healthy control were found to be on par whereas treatment series F was superior in affecting the germination and C, D and B were on par with each other. While the treatment series A was comparatively better regarding the germination of setts.

over In none of the treatment series, virus elimination could be achieved completely and showing visible symptoms when the ELISA readings were taken up after eight weeks of planting. All the plants reacted positive for the presence of SCMV. However, reduction in the virus load was recorded in the treatment series A, B and C when compared to untreated diseased plants. The treatment series D, recorded 41 per cent reduction while the treatment series E and F recorded nearly 50 per cent reduction in the virus concentration.

Table-34. Effect of serial hot water treatment on SCMV elimination in CoC 671 sugarcane variety

Treatments	No of setts planted	No of plantlets derived	No of SCMV free plants	Mean ELISA Readings after two months (405 nm)	
				Virus free plants	SCMV positive plants
A	30	15 ^c	0	—	0.408 ^b
B	30	9 ^b	0	—	0.398 ^b
C	30	8 ^{ab}	0	—	0.383 ^b
D	30	6 ^{ab}	0	—	0.361 ^b
E	30	5 ^{ab}	0	—	0.320 ^b
F	30	4 ^a	0	—	0.321 ^b
Untreated control	30	24 ^d	0	—	0.551 ^c
Healthy control	30	26 ^d	26	0.108	0.108 ^a

L.S.D (5%)

4.54

0.090

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

4.11.2.2. Effect of serial hot water treatment on CoC 671 variety

A slightly better trend was observed in CoC 671 variety when compared to Co 740 variety regarding the germination of treated setts and up to 50 per cent of the plantlets were derived in the treatment series. Thirty per cent of the plantlets were derived from the treatment series B. In the treatment series C, D and E up to 27 per cent of the plantlets were derived. However in the treatment series F only 13 per cent of the plantlets were derived. It showed that even though the CoC 671 variety withstands the heat treatment slightly better than Co 740 variety, only very few plantlets were derived when the treatment severity was higher.

Regarding the virus elimination, similar trend was observed in CoC 671 variety as in the previous experiment. All the treatment series were similar in recording the ELISA readings. All the derived plantlets produced visible symptoms. However up to 27 per cent reduction in the SCMV concentration was observed as indicated by lesser ELISA readings (Table-35).

Table-35. Effect of serial hot water treatment on SCMV Elimination in Co 740 sugarcane variety

Treatment	No of setts planted	No of plantlets derived	No of SCMV free plants	Mean ELISA Readings (405 nm)	
				Virus free plants	SCMVpositive plants
A	30	12 ^b	0	—	0.399 ^d
B	30	7 ^a	0	—	0.402 ^d
C	30	6 ^a	0	—	0.401 ^d
D	30	4 ^a	0	—	0.368 ^c
E	30	4 ^a	0	—	0.301 ^b
F	30	3 ^a	0	—	0.298 ^b
Untreated control	30	22 ^c	0	—	0.632 ^c
Healthy control	30	25 ^c	25	0.131	0.131 ^a

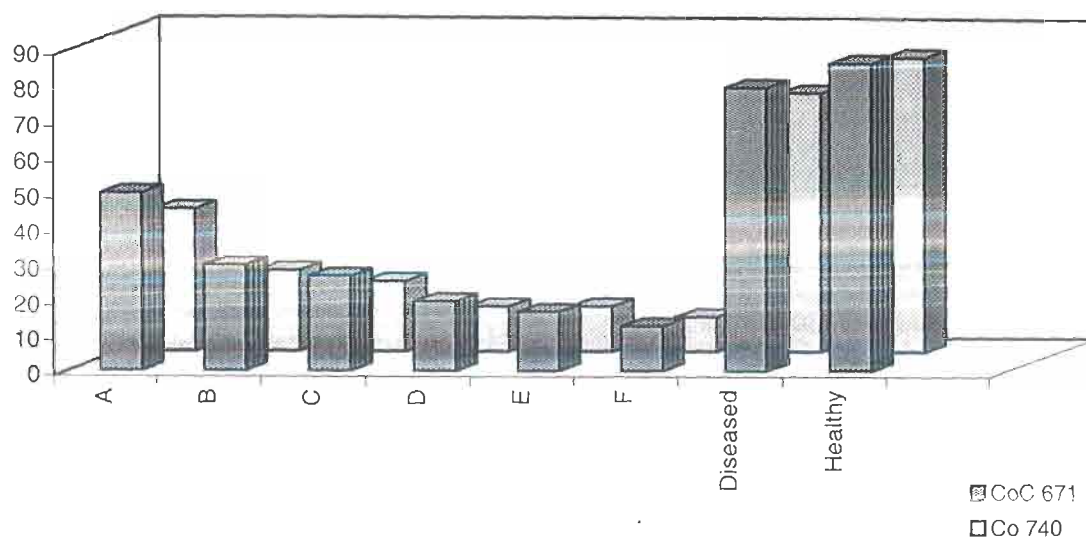
L.S.D(5%)

4.58

0.029

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Fig. 25. Effect of serial hot water treatment on sett germination in sugarcane



Treatment series	Duration (min) and corresponding temperature (°C) at different days			
	First day	Second day	Third day	Fourth day
	52°C	58°C	60°C	62°C
A	20	15	5	—
B	20	15	5	5
C	20	15	10	—
D	20	15	10	5
	52°C	60°C	62°C	—
E	20	15	10	
F	20	15	15	

DISCUSSION

CHAPTER V

DISCUSSION

Mosaic disease caused by sugarcane mosaic virus is an important disease of sugarcane. Its presence is felt worldwide since it causes crop loss to varying intensities in different sugarcane growing countries. In India, practically no variety is free from the virus infection in the field. Detailed studies were undertaken previously on various aspects of the virus such as virus strains, virus-vector relation, detection and management. However, information on prevailing virus strains in Tamil Nadu, molecular diagnosis of the virus, their molecular characterization, impact of the virus on cane growth and yield, resistance mechanism, elimination of the virus from the infected cane materials *etc.* is not available. Hence, detailed studies were undertaken on the above aspects and the results are discussed here under.

Survey of SCMV incidence in Tamil Nadu

SCMV symptoms are characterized by elongated yellow characteristic streaks intermingled with light and dark green patches, as noticed in susceptible cultivars like CoC 671, Co 740, Co 859 and Co 1158. The survey conducted in different sugarcane belts of Tamil Nadu revealed that none of the sugarcane variety was found free from SCMV symptoms. The various symptoms exhibited in different varieties were broadly grouped into three as streak type, chlorotic patches with green islands, and yellow blotches and systemic yellowing. Among the three different groups, symptoms of chlorotic patches and green islands expressed in CoC 671 were widely prevalent. It was found that when different sugarcane cultivars are maintained together as collections as in different sugar factory farms and Sugarcane Breeding Institute, Coimbatore, the severity of SCMV was high. It may be due to the continuous availability of different preferred clones so that various SCMV strains easily move between the sugarcane clones and expressed severe symptoms.

The varietal response plays a major role in the symptoms expression. It was observed that even in the adjacent rows, the CoC 671 and Co 740 varieties expressed different characteristic symptoms. It may be due to the preference of particular variety by a set of SCMV strains and also due to varietal susceptibility. Bhargava (1975) after the extensive survey in Uttar Pradesh reported that SCMV symptoms were more prominent during pre monsoon and monsoon periods but became inconspicuous during, post– monsoon period. During their survey, Rao *et al.* (1998a) identified severe incidence of SCMV in Poaceous crops like sugarcane, maize and sorghum in different regions of the country like, Uttar Pradesh, Gujarat and Andhra pradesh during 1991 to 1996. The present results indicate the importance of SCMV especially in the susceptible sugarcane varieties, which are grown extensively in many sugarcane belts. The other sugarcane diseases such as red rot, smut, wilt and grassy shoot cause significant yield loss to sugarcane production in India (Alexander and Viswanathan, 1996). However, their incidence and severity depends on many factors including epidemic nature of the disease. The present study also indicates that the virus can also cause significant loss to the cultivation since no variety in the field is free from SCMV.

Characterization of SCMV strains

The SCMV from several sugarcane varieties in the present study produced overlapping symptoms in different hosts, which might be due to the presence of strain mixtures. Among the different sugarcane varieties, Co 740 and CoC 671 showed the possibility of maximum number of strains namely SCMV A, B, D, SrMV H and MDMV B, followed by UP 95-30 and CoJn 86141 which recorded all the above strains except SCMV B. The CoLk 8001 variety recorded SCMV B, SCMV D, SCMV E and SrMV H. The remaining varieties showed the presence of SCMV B and SCMV D strains. In the serological studies also, the virus from most of the varieties reacted positively with SCMV A, D, SrMV H and MDMV B. The reaction with polyclonal antiserum was very strong when compared to the antisera of individual strains. It may be due to production of polyclonal antiserum raised against the virus, possibly a mixture of strains. Hence, there was always a very strong reaction with the polyconal

antiserum for a mixture of strains. The serological results also revealed the absence of some of the SCMV strains in Tamil Nadu, namely SCMV-YU, SCMV-Sabi and also the JGMV. In India, presence of SCMV strains A, B, D, E and F was reported by Bhargava (1975) and Rishi and Rishi (1985). Farrag (1978) reported the presence of SCMV strains, A, B, C, D, E, F, H, I and M in Tamil Nadu. The presence of mixture of SCMV strains were reported by Joshi (1989) from the CoC 671 collected from Coimbatore. The strains reported were SCMV A, E, F, I and K strains along with SCMV H (SrMV H) based on differential host reactions. SCMV A, F and SCMV H were reported from the Co 859 variety, which was also collected from Coimbatore. Gujarat isolate from CoC 671 was also a mixture of SCMV strains. Grisham (1994), reported mixture of SrMV strains namely SCMV H, I and M in Louisiana of commercial sugarcane cultivars.

It is in line with the present findings that only severe symptoms are expressed in some differential hosts. For example, CP 31-294 expressed strong SCMV-D strain symptoms *viz.*, severe stunting, chlorosis and death of the growing points. In the other differential hosts, the strong symptoms of some other SCMV strains were expressed. Some ungrouped symptoms were also noticed in the present experiment, like group-A, in Co 281 and CP 31-558, where severe chlorosis was present like bleached appearance. It may be due to combined infection by a mixture of strains, which requires further detailed studies. Gillaspie and Mock (1987), also reported the presence of strainal mixtures from the mosaic-infected samples collected from Pakistan (SCMV A and F), South Africa (SCMV A and D) and Venezuela (SCMV B and J).

Purification and molecular characterization of SCMV

The morphology of the purified SCMV particles under electron microscope appeared as long, flexuous rod shaped particles measuring 690 to 740nm. Several workers have already described the morphology and size of the SCMV. It was reported as flexuous rods of 750 x 13 nm (Chen, 1978); 750 nm (Tosic *et al.*, 1978), Moghal and Francki (1981); 670-770 nm for strain A and F (Rishi and Rishi, 1985) 750 x 13 nm (Teakle *et al.* 1989) and 700 – 755 nm (Rao *et al.*, 1998c). UV absorbance

ratio of 260/280 was 1.2. The value is characteristic for nucleoproteins containing 5 – 6 per cent nucleic acid. The 260/280 ratio reported earlier were 1.2 – 1.4 (Rishi and Rishi, 1985), 1.4 (Rao *et al.*, 1998b), 1.28 (Rao *et al.*, 1998c). The maximum UV absorbance of 1.49 was recorded at 268 nm. The value obtained now is in confirmity with the earlier findings. The purified SCMV upon inoculation in sorghum cultivar 'Rio', the characteristic mosaic mottling symptoms were noticed. The virus yield was 5.25 mg per 100 gram of infected leaves. Varying virus yield were reported earlier. It ranged from 1.1-mg/100 g to 70–110 mg/100 gm infected leaves in different purification procedures (Rao *et al.*, 1998a, b).

The studies on the serological relationship of the purified SCMV, revealed that it reacted with the polyclonal antisera of different SCMV strains *viz.*, SCMV A, D, MDMV B and also with the related potyviruses *viz.*, SrMV H. There was no postive reaction with SCMV strains BC, Sabi and YU and also with JGMV. The reaction was very strong with the SCMV polyclonal antiserum in ELISA and electroblot immuno assay. The above findings revealed that either the purified SCMV might have contained a mixture of strains or the purified SCMV might have closer serological relationship with SCMV-A, SCMV-D and SrMV as revealed by ELISA and electroblot immunoassay. Serogrouping of SCMV was carried out earlier based on the serological relationship among the different SCMV strains and related potyviruses. As per that, SCMV-A, B and D along with MDMV-B comprised one serogroup and SCMV-H, E and M the other group (Jarjees and Uyemoto, 1984). Gillaspie and Harris (1987) placed the SCMV-A, B, D and K as one serogroup and SCMV-H, I and M as another group and SCMV-E as separate serogroup. In another study, SCMV-A, B and D were placed as one serogroup and MDMV-D as another serogroup (Chen *et al.*, 1987). Shukla *et al.* (1989a, b) after comparing 17 strains of SCMV and MDMV based on serological reactions, grouped these strains as four distinct potyviruses namely, SCMV, MDMV, SrMV and JGMV. The present results and the earlier findings revealed that, SCMV strains have close serological relationship with each other. Tosic *et al.* (2001) concluded that MDMV, SCMV, JGMV and SrMV and their strains are serologically

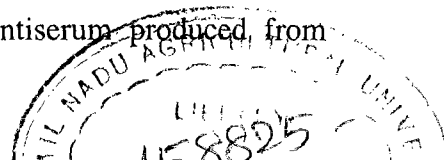
related, overlapping each other and can be differentiated by use of more specific or cross-absorbed antisera.

In the electroblot immunoassay, appearance of three to four bands might be due to degradation of the coat protein from the purified SCMV. Hema *et al.* (1999) observed that storage of purified viruses led to degradation of coat protein and three bands with molecular weight of 40, 34.5 and 28.5 kDa. More number of virus bands due to degradation of coat protein was also observed by Shukla *et al.* (1988).

In the present study, the coat protein of the purified SCMV migrated as a single band in gel electrophoresis and the molecular weight was 40.1 kDa. SCMV appeared to be the only potyvirus that consistently gives a single band on SDS-PAGE but the molecular weight reported has varied: 33kDa (Moghal and Francki, 1976); 33.7 kDa (Baudin, 1977); 36 kDa (Hill *et al.*, 1973); or 33.7 – 40 kDa depending on the strains (Gough and Shukla, 1981) and 34 kDa (Rao *et al.*, 1998b). Joshi (1989) reported that SCMV isolates collected from Coimbatore from CoC 671 and Co 859 yielded 40.5 kDa coat protein. The molecular weight of the purified SCMV in the present experiment is within the earlier reported range. When viral genome was separated from the purified SCMV and analysed on agarose gel, addition of ribonuclease resulted in the absence of any visible band confirming the presence of RNA. Upon amplification with the SCMV specific primer, 870 bp amplified DNA product was observed in the agarose gel, which is in confirmation with the results of Yang and Mirkov (1997).

Serology

The present study on the DAC-ELISA reactions of early and late bleed antisera with single and multiple immunization revealed that only low titre antiserum can be obtained with single immunization but the reaction was very specific with SCMV. From multiple immunizations, high titre antisera can be obtained. Antiserum from early bleed single immunization produced SCMV polyclonal antibodies, which did not react with any of the three potyviruses namely banana bract mosaic virus, sorghum mosaic virus and papaya ring spot virus. Whereas, the antiserum produced from



multiple immunization, reacted with the related potyviruses. Early bleed antisera was reacting with two poty viruses namely sorghum mosaic virus and banana bract mosaic virus when compared to all the three viruses in the late bleed antisera.

Polyclonal antisera against SCMV have been produced against several strains and isolates of SCMV (Pirone, 1972), SCMV-D (Devergne *et al.*, 1982), SCMV-A and F (Rishi and Rishi, 1985) and Indian isolate (Rao *et al.*, 1998b). The above results are in line with the earlier findings. According to Shukla *et al.* (1989c), two early bleed antisera to JGMV obtained after three and five intravenous infections (1 mg virus /injection) contained antibodies only to epitopes located in the surface-exposed amino-terminal regions of the coat protein and reacted only with strains of JGMV but not with five other potyviruses tested. Early bleed antisera to potyviruses predominantly contain antibodies to the amino terminus of the coat proteins. It has been shown that antisera recovered one week after single immunization with the strains of JGMV, MDMV and SCMV reacted only with their homologous viruses whereas those obtained four or more weeks after 1-6 immunizations reacted also with coat proteins of heterologous viruses (Lenardon *et al.*, 1993). The variation in the specificity of potyvirus antisera may be due to partial or complete degradation of N and C – terminus of coat proteins during purification and storage, by enzymes of plant or microbial origin which co-sediment with the virus particles (Shukla *et al.*, 1988). Removal of N-terminus containing the virus specific epitopes, results in virus particles containing only cross-reactive core epitopes. The immunization procedure might have an influence on the antiserum specificity. Wide differences in the reactivities of antisera taken at different stages of same animal were reported earlier (Koenig and Bercks, 1968). Antisera from early bleedings contain virus – specific antibodies whereas cross reactive antibodies begin to appear in later stages of immunization (Van Regenmortel and Von Wechmer, 1970). Recently, Rao *et al.* (1998b) reported that polyconal antibodies, developed against an Indian SCMV isolate from an early and late bleeds with multiple immunization reacted strongly only with homologous antigen and no reaction was obtained with many other potyvirus.

Molecular Diagnostics

The accurate identification of the viral diseases like SCMV in the field, glass house and quarantine is very essential not only for maintaining the productivity and also to contain the spread of SCMV within the country and between the countries. Increase in understanding of the molecular genetics of viruses combined with an acceptance of detection techniques based on nucleic acid has led to the development of faster, more sensitive detection tests for sugarcane virus diseases (Braithwaite and Smith, 2001).

In the present experiment, DAC-ELISA method was followed with 10^{-3} antiserum titre, the SCMV was detected in leaf blade, sugarcane buds, leaf sheath and midrib but not in the root. Among the leaves taken from various positions, the top most emerging leaf recorded the highest SCMV concentration. The ELISA method of SCMV detection has been reported by several workers like sandwich type ELISA to detect up to 1mg/ ml of purified SCMV-D (Devergne *et al.*, 1982), in different sugarcane parts like leaves, rind and buds (Rishi and Rishi, 1985), use of biotin conjugated antisera (Hewigh *et al.*, 1986). Ultra micro DAS-ELISA method (Alfonso and Peralta, 1993) and indirect ELISA (Viswanathan, 1997) were reported. SCMV concentration in sugarcane roots was very minimum and highest concentration was observed in leaves (Rishi and Rishi, 1985) in the ELISA. Rao *et al.* (1998a) identified sugarcane mosaic and maize dwarf poty viruses from different Poaceous crops using DAC-ELISA method.

The EBIA technique otherwise known as Western blot was first applied by O' Donnel *et al.* (1982) for the detection of potato leaf roll virus and subsequently used against many plant viruses. The technique is highly sensitive and viruses can be detected at very low concentrations. Against 11 different poty viruses, this technique was successfully used (Shukla *et al.*, 1989b). In the present experiment, the SCMV from the infected sugarcane leaves was detected with the early bleed antisera obtained both from the single and multiple immunization methods. Along with the DAC-ELISA, this technique can be combined as a further confirmation method for SCMV diagnosis.

This technique was used to find out the taxonomic relationship among 17 different strains of SCMV and MDMV (Shukla *et al.*, 1989d) and taxonomic relationship between MDMV and JGMV (Mckern *et al.*, 1990). In different Poaceous crops, the SCMV and MDMV were detected based on Western blot (Rao *et al.*, 1998a). The present studies also indicated that dot-blot can also be used along with DAC-ELISA for large scale screening of sugarcane setts.

ISEM was successfully used for the detection of numerous isometric and filamentous plant viruses including poty viruses (Milne and Lesemann, 1984). The procedure was used for the differentiation of closely and distantly related strains of SCMV (Shukla and Gough, 1984), and for determining taxonomic relationship of different potyviruses (Shukla *et al.*, 1992). In the present study, the trapped virus particles were flexuous rods with 690 to 740 nm. Rao *et al.* (1998a) used the ISEM method for the detection of SCMV and MDMV from different Poaceous crops.

For the viruses like SCMV, which contains RNA genome, RT-PCR is being used where the RNA genome is converted to cDNA before amplification. In the present experiment, using SCMV specific primer, amplification was achieved from the RNA extracted from infected leaf blade, buds, midrib and leaf sheath but not from the roots and central pith region. Potyvirus specific primer also amplified the RNA from the infected leaf samples. No amplification of RNA extracted from the roots and pith of SCMV infected sugarcane plants may be due to reduced virus concentration in these tissues. Because of its extreme sensitivity, RT-PCR can be an ideal tool for sugarcane quarantine operations to prevent the movement of SCMV. Detection of SCMV and FDV has been successfully achieved in a duplex RT-PCR (Smith and Van de Velde, 1994). Huckett and Botha (1996) developed a RT-PCR assay, to diagnose SCMV isolates and strains found in South Africa, while Yang and Mirkov (1997) developed primers and conditions to amplify all the SCMV/SrMV isolates and also an RFLP refinement for strain discrimination.

Influence of age of sugarcane plant on SCMV infection

The results revealed that the progress of SCMV infection in sugarcane declined with ageing. According to Bhargava *et al.* (1971), four to six weeks old plants of Co 281 were most susceptible and the infection percent decreased with age. Upon increased age, the modification in the host plants occur like thickening of cell walls, lignin formation etc. and which might influence the establishment of the virus inside.

Economic impact of SCMV

In the present field experiments with two sugarcane varieties, CoC 671 and Co 740, different growth parameters and quality parameters were found to be affected by SCMV infection. In the two plant crop experiments at Coimbatore, the impact was high in Co 740 variety. Ten per cent reduction in cane germination was noticed in Co 740 variety. Ahmed *et al.* (1991) in Pakistan observed, five to eleven per cent reduction in sugarcane sett germination due to SCMV infection. The tillering and plant height were also affected due to SCMV infection. An increase of 1.34 tillers/plant was observed in the healthy plants when compared to SCMV infected plants (Ahmed *et al.*, 1991). In the present experiments up to 1.7 tillers per plant was reduced due to SCMV infection in Co 740 variety. Plant height was affected in both the varieties but severity was more in Co 740. Likewise, the cane thickness and number of millable canes were also reduced in Co 740. The reduction in sett germination affected the cane population and number of millable canes. Ahmed *et al.* (1990) observed the reduction in tillers, cane length, number of internodes and cane thickness in Co 975 upon SCMV infection. Reductions of 7.8 and 9.2 per cent in cane height were observed in UP 22 and CoS 87220 varieties respectively. Singh *et al.* (1997) have reported 7.8 and 17.6 per cent reductions in tillering in UP 22 and CoS 87220 varieties, respectively.

SCMV causes chlorotic streaks, pale green patches, yellow blotches and sometimes systemic yellowing in the cane varieties. This led to loss of chlorophyll which reflected as the loss in effective area for photosynthesis. The net photosynthesis results in the present experiments clearly supported this, and up to 27 per cent

reduction was recorded in Co 740 variety where as 10 per cent loss was observed in CoC 671 variety. Cane weight reduction of 30 and 36 per cent were recorded in NCo 310 and CP 52-68 varieties respectively (Villalan, 1980). The reduction in net photosynthesis could have resulted in reduced plant height and cane thickness. A reduction of 30 and 20 per cent in total chlorophyll content was observed during seventh month in the SCMV infected UP 22 and CoS 87220 varieties respectively (Singh *et al.*, 1997). The reduction in net photosynthesis decreased the chlorophyll content of the affected leaf tissue. Decrease in chlorophyll is often associated with stimulation in chlorophyllase activity that can catalyse the dephytylation of chlorophyll led to enhanced breakdown of chlorophyll. The photosynthetic changes are accompanied by related metabolic changes in the utilization of the products. There is often a decrease in the sugar content of leaves and shift towards the production of amino acids and organic acids (Agrios, 2000).

The reduction in net photosynthesis, cane thickness and plant height were reflected in cane weight in Co 740 variety and up to 37 per cent reduction in single cane weight was recorded. Sixteen per cent reduction in the single cane weight was recorded in UP 22 variety and 10 per cent in CoS 87220 variety (Singh *et al.*, 1997). In the larger field trials, the reduction in germination, tillering, plant height, and cane thickness due to SCMV was reflected in the cane yield, and 40 per cent reduction per hectare was observed in Co 740. This clearly indicates that all the growth parameters are interrelated and finally reflected in the cane yield. Many workers reported the yield reduction due to SCMV. The reductions reported were up to 10 per cent in India (Varma, 1989), 24.2 and 30.8 per cent respectively in F 177 and F 178 varieties respectively in Taiwan (Chen *et al.*, 1989), 14.5 and 34.5 per cent in BO 70 and Co 1148 respectively in Bangladesh (Rahman *et al.*, 1990).

In ratoon crop, further decrease in germination, tillering, net photosynthesis, cane thickness and cane weight was observed. The virus build up could have been more over the period of plant crop and affected the subsequent ratoon crop. Increased yield loss in the ratoon crop was already reported (Ahmed *et al.*, 1990). Between the

two varieties, CoC 671 showed recovery from the SCMV infection in the older leaves in plant crop and did not cause much impact on cane yield. However, the same variety could not escape from the adverse impact on juice quality, even in the plant crop. Significant yield loss was observed in CoC 671 variety in ratoon crop whereas yield loss was not observed in plant crop. According to Varma (1989), even 10 per cent yield loss due to SCMV is enormous considering the vast sugarcane area in our country.

The juice quality characters *viz.*, brix, sucrose and purity percent were severely affected due to SCMV infection. In both CoC 671 and Co 740 varieties, the above juice quality parameters were affected. The impact was more in the ratoon crop than plant crop. The reduction in net photosynthesis might have affected the carbohydrate content, which could have reflected on brix and sucrose content further. The reductions in the juice quality ultimately reflected on the sugar yield. The commercial cane sugar yield was reduced up to 7 to 8 per cent in the plant crop and more than 10 per cent in the ratoon crop. The build up of SCMV inoculum in the ratoon crop could have severely affected the host metabolism, which could have more adversely reflected in the juice quality and sugar yield in the ratoon crop. Ahmed *et al.* (1990, 1991) also indicated reduction in juice weight, sucrose content and sugar recovery due to SCMV in sugarcane.

Induced resistance

Hull (1994) emphasized that plant viral disease management strategies typically include the use of genetically resistant varieties, the integration of selected cultural practices, and the application of insecticides to control the insect vectors. Two additional approaches for managing viruses include cross protection and development of genetically engineered plants that express a viral structural or non-structural protein (Lomonossoff, 1995). In the recent years, induced resistance strategies are being explored against the plant viruses also, by application of biotic and abiotic agents. According to Fraser (2000), many plants contain substances, which will inhibit viruses of other plants, especially when applied at the stage of initial infection. Other substances appear to induce a form of resistance in the donor plant, which may act against establishment or against virus multiplication.

In the present studies, there was a significant decrease in the virus concentration as evidenced by ELISA readings at the early growth phase upon application with some of the abiotic and biotic agents. Foliar application of bion and salicylic acid was effective followed by seed treatment with *P. fluorescens*, bion and salicylic acid. From ninth day onwards, the plants started showing the symptoms and the difference in ELISA readings started piling up between the healthy and in the inoculated plants. These results clearly showed that application of these above treatments reduced the initial virus inoculum at a significant level.

Effectiveness of bion, salicylic acid and *P. fluorescens* against different plant viruses was already reported. Salicylic acid application reduced the TMV incidence in tobacco (White, 1979). The foliar application of bion reduced the disease symptoms of tomato spotted wilt virus, tobacco mosaic virus, cucumber mosaic virus and potato virus Y (Oostendorp *et al.*, 2001). The induction of systemic resistance of *P. fluorescens* strains 89B -27 and *Serratia marcescens* strain 90-166 had consistently reduced the number of cucumber mosaic virus infected plants and delayed the development of symptoms in cucumber and tomato (Raupach *et al.*, 1996). *P. fluorescens* strain CHAO naturally produced salicylic acid under conditions of iron limitation and also induced ISR in tobacco against Tobacco necrosis virus (Maurhofer *et al.*, 1998).

Resistance mechanism in sugarcane against SCMV

In the present experiment, three clones of *S. spontaneum* (IND 81-80, IND 81-142 and IND 81-161), one clone from *Erianthus bengalense* (IND 81-47) and two inter specific hybrids (CoC 671 and Co 740) were inoculated with SCMV and the progress of virus infection was assessed by DAC-ELISA up to 45 days after inoculation. The concentration of SCMV was high in both the inter specific hybrids indicating their susceptibility.

There is a good resistance to most sugarcane pathogens within the hybrid gene pool, or within the larger gene pool of the '*Saccharum* complex'. While the complex genetic background makes identification of loci for resistance difficult, these resistance

genes have been very successfully exploited in breeding programmes against Fiji disease virus (Smith and Harding, 2001). Different sugarcane clones resistant to SCMV have been reported within the '*Saccharum*. complex'. According to Zhou *et al.* (1989), *Saccharum spontaneum* clones were immunue to SCMV. Among the different *Saccharum* spp. and relatives tested against SCMV-H, to assess the resistance, it was found that *Erianthus*, *S. spontaneum* and *S. barberi*/*S. sinense* clones were the most resistant and *S. robustum* clones were intermediate (Grisham and Burner, 1992).

The *S. spontaneum* clones as reported by earlier workers were found to be SCMV resistant (Zhou *et al.*, 1989; Grisham and Burner, 1992). The SCMV resistance from *E. bengalense* clones and susceptibility from interspecific hybrids were also reported (Grisham and Burner, 1992).

Mechanism of virus resistance

In general, different defense gene products were activated upon application of biotic and chemical agents against fungal and bacterial diseases. The activated defense gene products are phenylalanine ammonia lyase, peroxidases, polyphenol oxidases and phenols. In the virus resistant plants, different types of resistance mechanisms were reported.

Total Phenols

Enhanced accumulation of total phenols was observed upon inoculation with SCMV in the resistant clones when compared to susceptible ones. In the induced resistance experiment, foliar applications of bion and salicylic acid, seed treatment with *P. fluorescens* resulted in the enhanced accumulation of phenolics up to six days after inoculation and started declining from ninth day. Appreciable amount of increase was also recorded in bion and salicylic acid seed treatments. Rapid incorporation of phenolic materials into plant cell walls either by sample esterification of hydroxycinnamic acid derivaties or by peroxidase catalysed polymerization of phenyl propane alcohols to yield lignin is often associated with resistance (Friend, 1981; Matern and Kneusel, 1988).

Phenolics and their oxidative products have been reported as defense chemicals, against many pathogens (Sathianathan and Vidhyasekaran, 1981). Phenolic compounds are often positively associated with increase in disease resistance (Nicholson and Hammerschmidt, 1992). Higher phenolic contents were detected in the sugarcane varieties resistant to SCMV than in the susceptible ones (Molina *et al.*, 1993). This enhanced phenolic content might have contributed the resistance against SCMV.

Phenylalanine ammonia lyase (PAL)

Enhanced accumulation of PAL activity was observed in the resistant clones upon SCMV inoculation when compared to susceptible interspecific hybrids. The PAL accumulation was very fast in the resistant clones whereas in the susceptible clones it was comparatively slower. This enhanced and faster accumulation of PAL in the resistant clones might have contributed the SCMV resistance. In the present experiment, PAL activity was dramatically increased upon foliar application of bion and salicylic acid followed by seed treatments with *P. fluorescens*, bion and salicylic acid. The maximum activity was recorded on sixth day.

PAL catalyses the conversion of L-phenylalanine into transcinnamic acid in the first step of the phenylpropanoid pathway which supplies the precursors for flavonoid pigments, lignin, UV protectants and furanocoumarin phytoalexins in plants (Grisebachk, 1981; Hahlbrock and Scheel, 1989; Lewis and Yamamoto, 1990). The phenylpropanoid metabolism is activated in response to a wide range of developmental and environmental cues and consequently, phenylpropanoids are synthesized during the normal process of development and under various stress conditions such as UV irradiation, mechanical wounding or pathogens attack (Dixon and Lamb, 1990). The lignin deposition and thickened walls have been proposed as a contributory factor to the restriction of virus translocation. (Favali *et al.*, 1978; Collendavelloo *et al.*, 1982; 1983). They may represent a secondary defense to possible fungal or bacterial infection.

Peroxidase (PO)

Enhanced PO activity was observed in the resistant clones upon inoculation with SCMV. Among the IND clones, IND 81-161 and IND 81-47 recorded the maximum peroxidase activity in all the intervals. The enhanced accumulation of peroxidase was observed after foliar application of bion and salicylic acid, and *P. fluorescens* seed treatment. This could have contributed the initial reduction in the SCMV inoculum by inducing the plant resistance. The peroxides activity increased nearly three times on third day in the above treatments and on sixth day nearly 60 per cent increase was observed and reached maximum.

Peroxidase participates in a variety of plant defense mechanisms (Moerschbacher, 1986) in which H_2O_2 often supplied by an oxidative burst, a common event in defense responses (Dixon and Lamb, 1990). The cell wall of plants appears to be a major site for defense-related peroxidase polymerization relations such as lignification (Hammerschmidt *et al.*, 1982b), suberisation (Espelie *et al.*, 1986) and cross-linking of structural cell wall profiling (Fry, 1986). The virus infection appears to stimulate the peroxidase activity in all hosts in which necrotic or chlorotic symptoms are induced, with a degree of stimulation, which correlates with symptom severity. The stimulation is greatest in hosts reacting hypersensitively and also in systemic infections. The peroxidase activity in the SCMV susceptible sugarcane varieties (PC 21-294 and PR-980) increased with time after SCMV inoculation (Pinon and Sanchez, 1993). Increased PO activities were observed in SCMV infected leaves (Singh *et al.*, 1997).

In the experiment, the intensity and number of PO isozymes were high in the inoculated IND clones when compared to the interspecific hybrids. In the experiment on induced resistance, more PO isoforms were observed on third, sixth and ninth day in foliar applied plants with salicylic acid and bion and also in bion and *P. fluorescens* seed treated plants when compared to inoculated and healthy control. These increased inductions of peroxidase isoforms could have contributed for the increased peroxidase activity, which in turn might have induced the plant resistance against SCMV.

The above findings revealed that increased number of PO isoforms with high intensity in the SCMV inoculated IND clones could have contributed for the resistance mechanism against SCMV, when compared to the interspecific hybrids. Stimulation in the PO isozyme activity arising from virus infection could conceivably reflect a perturbation in the regulation of the biosynthesis of an enzyme already present at a low level in the uninfected plant. Alternatively, it could represent biosynthesis of a new isozyme, the biosynthesis of which does not occur in healthy plants. Most of the higher plants possess a number of different PO isozymes and/or isoforms of peroxidase. Many cDNAs and chromosomal DNAs encoding PO isozymes have been isolated and cloned from dicotyledonous plants (Intahruk *et al.*, 1994). In case of monocotyledonous plants, two genes for peroxidases were reported in barley (Rasmussen *et al.*, 1991) and wheat (Hertig *et al.*, 1991; Rebmann *et al.*, 1991). In hosts reacting hypersensitively, including soybean mosaic virus infected bean and TMV infected tobaccos, new peroxidase isozymes were present (Gaborjanyi *et al.*, 1973). In systemically infected tissues including tobacco (Gaborjanyi *et al.*, 1973) and bean (Bates and Chant, 1970) and cucumber infected with CMV (Wood and Barbara, 1971) quantitative changes in the PO isozymes in the infected tissues were reported.

Polyphenol oxidase (PPO)

Increased PPO activity was observed in all the intervals upon SCMV inoculation in resistant genotypes. The rate of increase was slower in the susceptible interspecific hybrids and reached maximum on ninth day only, whereas in the IND clones rapid increase was observed and reached maximum on sixth day. When compared to the interspecific hybrids, more than two times increase in the PPO activity was observed in the IND clones. This increased polyphenol oxidase activity might have contributed for the resistance mechanism. In the experiment on induced resistance, the plants applied with bion and salicylic acid as foliar spray and seed treated with *P. fluorescens*, bion and salicylic acid enhanced the accumulation of polyphenol oxidase. Two to three times increased PPO activity was observed on third day in the above treatments. The PPO activity reached maximum on sixth day and started

declining on ninth day. The initial enhanced PPO activity could have contributed for the reduction in SCMV inoculum at the early growth phase. PPO seems to be associated with resistance by catalysing the final polymerization step of lignin synthesis (Hammerschmidt *et al.*, 1982b). A significant stimulation in the PPO activity accompanies the expression of hypersensitivity in many virus-host combinations like tobacco mosaic virus infected tobacco (Van Loon and Geelen, 1971) and tobacco necrosis virus infected cowpea (Wagih and Coutts, 1982) were reported. A lesser stimulation in the PPO activity in the systemic infection was also reported.

There was increase in the PPO isoforms in the IND clones upon inoculation with SCMV when compared to interspecific hybrids. The intensity was also high in the SCMV inoculated IND clones especially in IND 81-47 and IND 81-161. These increased PPO isoforms in the IND clones could have contributed the increased polyphenol oxidase activity, which in turn might have contributed the SCMV resistance. Likewise in the sorghum plants treated with bion and salicylic acid as foliar spray and seed treated plants with bion, salicylic acid and *P. fluorescens* accumulation of additional PPO isoforms were observed. This increased PPO isoforms with increased intensity could have contributed for the increased polyphenol oxidase activity, which in turn contributed for the initial reduction in the SCMV infection in sorghum plants.

In sugarcane, new varieties/genotypes are being evolved every year through hybridization and selection. The varieties are screened for major diseases such as red rot, smut and wilt before their release. However, severity of the mosaic is also taken into consideration while selecting a variety for promotion. Many agronomically superior clones were rejected due to their mosaic susceptibility. Present studies on resistance mechanism in sugarcane genotypes revealed that commercial hybrids are susceptible to SCMV while certain *S. spontaneum* clones are resistant to the virus. Resistant mechanism to SCMV in sugarcane has not been studied in detail. The current studies revealed that there is a definite resistant mechanism to SCMV operates in *S. spontaneum* and *E. bengalense* while the same is lacking in commercial cultivars.

Hence further work on utilising the *S. spontaneum* clones in the breeding programme would lead to developing genetic stocks with SCMV resistance. Such genotypes could be very useful donors of SCMV resistance. Since the virus infection has drastically affected the cane yield and juice quality in commercial hybrids and such work may reward in selecting agronomically elite cultivars with mosaic resistance.

Likewise, the induction of resistance mechanism against SCMV in sorghum during the early growth phase by application of biotic and abiotic agents might be exploited against susceptible cultivars of sugarcane also. Induction of defense related enzymes such as chitinase, PO, PPO, PAL due to *P. fluorescens* treatment followed by challenge inoculation of red rot pathogen *Colletotrichum falcatum* in sugarcane was recorded in susceptible cv CoC 671. Such enhanced induction of these enzymes reduced the red rot development in sugarcane stalks (Viswanathan, 1999; Viswanathan and Samiyappan, 1999). Studies conducted by Ramesh Sundar *et al.* (2001) revealed that bion an abiotic signal inducer, induced systemic resistance against red rot disease of sugarcane and induced several defense enzymes. The studies conducted in sugarcane and other crops revealed that the inducers trigger various defense enzymes in susceptible cultivars and increased the resistant level. The enzymes singly or combinely restrict the pathogen infection and further development.

SCMV elimination from infected setts

The antiviral compounds could augment the effectiveness of virus eradication by meristem tip culture particularly for those viruses that are difficult to eradicate by the standard heat treatment and meristem culture combination. Ribavirin (virazole), a chemical that has been extensively tested against human viruses and which appears to be effective against both RNA and DNA viruses. The purine base analogues like 8 – azaguanine is also reported against plant viruses (Hansen, 1989).

In the present study on SCMV elimination by combined method of chemotherapy and meristem tip culture, addition of ribavirin at 50 ppm along with the MS medium has greatly enhanced the SCMV elimination. About 95 per cent virus

elimination was attained with the incorporation of ribavirin at 50 ppm in MS medium when compared to 62 per cent in the meristem tip culture alone. The increased SCMV elimination without any phytotoxicity is a major advantage because nearly 80 per cent of the plantlets were derived in this treatment. The ELISA results also revealed that nearly 3 times decrease was attained in ribavirin treated virus free plants when compared to infected plants. In sugarcane, meristem tip culture is being widely followed, for SCMV elimination. Hendre *et al.*, (1975) obtained more than 90 per cent of SCMV free plants from the virus infected Co 740 variety after growing it aseptically on modified MS liquid medium and subsequently on modified white's medium. Explants taken from SCMV infected apical meristem and regenerated on MS medium were free from the virus.

Virus free shoots can be produced on meristems by incorporation of 20 to 50 ppm of the ribavirin compound in the medium, and 10 ppm was sufficient to reduce the infection of protoplast to a minimum (Hansen, 1989). According to Cassells and Long (1982), incorporation of ribavirin into potato explant and meristem culture media increased the percentage of virus free progeny. The viruses eliminated singly or as complexes included potato viruses X, Y, S and M. Hansen and Lane (1985) successfully eliminated apple chlorotic leaf spot virus from apple shoot cultures by growing them at rates of 10, 20, 40 and 80 μ m for a 4week period.

The mode of action studies of ribavirin against tobacco mosaic virus revealed that ribavirin inhibited replication during an early step lasting 8 hr. after inoculation (Dawson and Lozoya-Saldana, 1984). According to Lerch (1987), 5' triphosphate formed from ribavirin could inhibit RNA cap formation in analogy to the inhibition of mammalian viruses described by Goswami *et al.* (1982). Regarding the 8-azaguanine, nearly 3 per cent of the guanine in the nucleic acid was replaced and this incorporation greatly reduced the infectivity of the virus. However, 8-azaguanine was generally too phytotoxic eventhough it inhibited several plant viruses (Hansen, 1989).

Sugarcane being a vegetatively propagated crop, yield reduction is more due to major virus diseases. After infection, mosaic spreads systemically throughout the plant and the problem was very severe under favourable conditions like ratooning and presence of high population of virus vectors. Being one of the staple crop the demand for the sugarcane sett material is very high and move very fastly within the country and also outside the country. This leads to faster spread of SCMV through infected material. Accumulation of viruses has been a common problem in vegetative propagated crops like sugarcane and as a result many useful clonal materials often became so crippled by viruses that its cultivation had to be abandoned. This phenomenon is referred to as varietal deterioration or varietal decline. This may also be due to infection of SCMV and other pathogens such as sugarcane bacilliform virus, ratoon stunting bacterium and grassy shoot phytoplasmas etc. The need for virus free stock therefore became essential. Especially the crops like sugarcane are being maintained year after year permanently for the hybridization programme. Hence, the above combined method of chemotherapy and meristem culture can be effectively utilized to enhance the SCMV elimination.

Serial hot water treatment

In sugarcane, SCMV elimination with thermotherapy and later in combination with tissue culture were reported earlier. Apical meristem tissues after treatment at 52°C for 10 or 20 min and cultured on Vacin and Went medium supplemented with 25 per cent coconut milk were used to obtain healthy plants from SCMV, ratoon stunting disease and white leaf or chlorotic streak virus (Lev, 1978). Likewise, El-Nasr *et al.* (1989) observed 40 per cent of SCMV elimination with hot water treatment at 55 and 57°C starting two days after harvesting followed by meristem tip culture in Van Hoof medium.

According to Steib and Cifuentes (1977), treatment of sugarcane setts with aerated steam at 56- 57°C for 2-5 hr. effectively controlled the sugarcane mosaic virus and ratoon stunting. Likewise, Benda *et al.* (1989) reported that serial hot water

treatment was effective in the elimination of virus from infected setts. The setts were serially submerged in hot water (51.5, 59, 61 and 63 °C) for 0-20 min at 20-24 hr intervals.

In the present experiment involving six different treatment series and two sugarcane cultivars namely CoC 671 and Co 740, germination failure was observed in both the varieties and between the two varieties, higher germination failure was observed in Co 740 variety. The temperature of 60°C and 62°C as well as increase in treatment duration severely affected the sett germination. The susceptibility of the canes to higher temperatures varied between two varieties and was more in Co 740. In both the varieties eventhough the concentration of the virus was reduced as evidenced by ELISA readings, none of the treatment series yielded complete elimination of the SCMV from the infected setts. The treatment series E and F reduced the virus concentration up to 50 per cent. This itself was sufficient for further multiplication and to attain severe form at a later stage. Moreover, the germination was severely affected due to the alteration in plant metabolism. According to Stace-Smith (2000), the virus is not completely eradicated but the concentration is drastically reduced in thermotherapy and the virus replication resumed when the plant is returned to normal growing conditions. Heat treatment can be frequently combined with meristem tip culture to eradicate those viruses that are not readily eliminated by either treatment alone.

Benda *et al.* (1989) also reported that survival of sugarcane setts tends to decrease as the severity of the treatment series increased *i.e* when the duration lengthened, the temperature rose or when the treatment number increased. But they attained symptom free sugarcane plants from SCMV by subjecting to maximum temperature of 60 to 65°C. But germination was drastically reduced even up to less than 10 per cent. The present experiment on heat treatment could be utilized for some other purpose. Since the treatment reduced the SCMV concentration sufficiently, it can be further subjected for meristem tip culture so that virus elimination will be improved.

Present studies revealed the extensive spread of mosaic in different sugarcane growing regions in Tamil Nadu state, a major sugarcane growing region and records highest cane yield in the country. Major SCMV strains prevailing in different sugarcane varieties were identified. The virus was purified and polyclonal antisera were developed. Sensitive serological techniques such as ELISA, dot- blot, EBIA and ISEM and RT-PCR were standardized for the precise detection of the virus. Impact of the virus infection on cane and sugar yield was established. Some of the enzymes involved in host defense against SCMV were identified and resistance in wild sugarcane clones was identified. Further, meristem culture combined with ribavirin has been standardized for virus elimination. The sensitive detection techniques can be exploited while quarantining sugarcane germplasm materials. The impact of the virus on cane yield necessitates the need for assessing virus resistance in the clones and monitoring the SCMV incidence in the field. Since combination of chemotherapy and meristem tip culture yielded successful virus elimination, many elite commercial varieties infected with SCMV can be freed of the virus in future to obtain full yield potential of that variety.

SUMMARY

CHAPTER VI

SUMMARY

All the major sugarcane belts were infected with SCMV, either in mild or severe form. The SCMV symptoms expressed were broadly classified into three types as a) streak type (eg: Co 740) b) chlorotic patches with green islands (eg: CoC 671) and iii) yellow blotches and systemic yellowing (eg: CP 44-101). When different clones were maintained together, the severe symptom expression of SCMV was noticed. In majority of the survey areas, chlorotic patches with green islands were predominant.

Differential host reactions in sorghum and sugarcane revealed the presence of mixture of SCMV strains viz., A, B, D, E, MDMV-B and SrMV-H in many sugarcane varieties.

The two sugarcane varieties Co 740 and CoC 671 recorded the maximum number of SCMV strains followed by UP 95-30 and CoJn 86141. The SCMV-B and SCMV-D strains were widely present in most of the sugarcane varieties. Serological reactions also revealed that each sugarcane variety reacted with more number of SCMV antisera.

Most of the SCMV infected varieties tested were reacting positively with the antisera of SCMV- A, D, SrMV-H and MDMV-B. The reaction with the polyclonal antiserum was very strong than with the antisera of individual strains. None of the SCMV infected sugarcane variety reacted positively with SCMV-YU, SCMV-Sabi and also with JGMV. Along with the antiserum of SCMV-BC strain, only Co 740 and CoM 9217 varieties reacted positively.

From the infected 'Rio' cultivar, the SCMV was purified after the sucrose gradient centrifugation. Yield of the virus was 5.25 mg from 100gm SCMV infected sorghum (Rio) leaves. The molecular weight of the coat protein for the purified SCMV was 40.1 kDa and 260/280 ratio of UV absorbance was 1.2. The purified SCMV appeared as flexuous rods with 690 to 740 nm. The purified SCMV produced characteristic symptoms in sorghum cultivar 'Rio' and 'Atlas'.

The purified SCMV strongly reacted with different strain specific antisera such as SCMV-A, D, polyclonal antiserum, SrMV-H and MDMV-B in DAC-ELISA. However, the reaction was very strong with the polyclonal antiserum. The electro blot immunoassay also revealed that, the purified SCMV reacted with SCMV-A, D, polyclonal antiserum and SrMV-H and not react with SCMV-BC, Sabi, YU and JGMV.

The viral genome was separated from the purified nucleoproteins and after the ribonuclease treatment, did not produce any visible band in agarose gel. The viral genome after separation from the purified SCMV was amplified in RT-PCR method with SCMV specific primer. The amplified viral genome was 870 bp in length.

Polyclonal antisera against the purified SCMV were produced by administering single and four intramuscular injections in rabbits. Early bleed and late bleed antisera were obtained for two times at weekly intervals after the last injection. The titre was low from the early and late bleed antisera with single immunization when compared to multiple immunization. However, in single immunization, the reaction was specific with SCMV and there was no positive reaction with the related poty viruses, like papaya ring spot virus, banana bract mosaic virus and sorghum mosaic virus. However, the late bleed antisera from single immunization reacted with the sorghum mosaic virus.

The titre of the antiserum was high from multiple immunizations. The reaction was positive up to 1:5000 antiserum dilution. The antigen was detected up to 1:500 dilution. In both the immunization methods, the titre of the late bleed antisera was higher than the early bleed antisera.

The early and late bleed antiserum (10^{-3} dilution) in both the immunization methods reacted with SCMV from infected tissues in dot blot immunoassay and there was no reaction with the healthy tissues. However, the reaction was stronger with the

late bleed antisera. The late bleed antiserum (10^{-3}) reacted well in ISEM and flexuous rod shaped virus particles of 690-740nm were visible from the infected sap. In electro blot immuno assay, both the antisera (10^{-3}) reacted with the infected sugarcane tissues.

Maximum concentration of SCMV was recorded in leaf blade followed by midrib, leaf sheaths and buds in DAC-ELISA. The tissues taken from the top most leaf blade recorded the highest SCMV concentration. The root tissues did not react positively indicating the reduced SCMV concentration.

The SCMV genome was amplified from the infected leaf blade, bud region, midrib and leaf sheath by RT-PCR method with SCMV specific primer and the amplified DNA was 870bp in size. The viral genome from the SCMV infected sugarcane and sorghum tissues were also amplified by potyvirus specific primers and the amplified DNA was 750bp in size and the molecular weight matched with the cloned cDNA of SCMV genome.

Sugarcane, up to two months old were found to be highly susceptible for SCMV infection in both Co 740 and CoC 671 varieties. Whereas, five and six months aged plants of same varieties were not showing much infection upon mechanical inoculation with SCMV.

Between the two popular sugarcane varieties, the SCMV infection caused heavy yield loss in Co 740 than CoC 671 variety. The net photosynthesis, cane yield, juice quality and sugar yield were affected both in plant crop and ratoon crop of Co 740 variety. Among the various growth parameters, the effect of SCMV was felt very much on germination, tillering, plant height, cane thickness and number of millable canes in Co 740 variety both in plant crop and ratoon crop. In CoC 671 variety, the impact on net photosynthesis and juice quality was observed in plant crop and in ratoon crop.

SCMV infection caused loss in single cane weight. The loss due to SCMV infection was ranging from 27.5 to 36.1 per cent in the plant crop and 35.6 to 41.0 per cent in ratoon crop of Co 740 variety. In CoC 671 variety, 11.5 to 13.0 per cent loss

in cane weight was observed only in the ratoon crop. Regarding the yield loss, in Co 740, about 40.1 per cent loss per hectare was observed in the plant crop. The SCMV infection reduced the brix and sucrose content that led to the reduction in the juice purity as a result, sugar yield was also reduced in both the varieties. In Co 740 variety, loss in sugar recovery ranged from 3.2 to 10 per cent in main crop and in the ratoon crop it ranged from 8.3 to 22.3 per cent. In CoC 671 variety, in the plant crop, the reduction in commercial cane sugar ranged from 7.9 to 11.3 per cent and in the ratoon crop it ranged from 8.3 to 22.3 per cent.

Foliar applications of bion and salicylic acid as well as seed treatment with *P. fluorescens*, bion and salicylic acid induced resistance mechanism against SCMV in sorghum. The above treatments significantly slowed down the SCMV infection in sorghum during the earlier growth phase (up to four weeks).

Among the different sugarcane genotypes and interspecific hybrids tested for SCMV resistance, the interspecific hybrids namely Co 740 and CoC 671 were highly susceptible. The *S. spontaneum* clone IND 81-161 and *E. bengalense* clone IND 81-47 were highly resistant followed by *S. spontaneum* clones namely IND 81-80 and IND 81-142.

Clones exhibiting resistance to SCMV recorded higher levels of phenols, PO, PPO and PAL. The increase was also recorded upon treatment with biotic and abiotic inducers viz. bion, salicylic acid and *P. fluorescens*.

The combined method of antiviral chemotherapy and meristem tip culture was found to be more effective in SCMV elimination than the meristem tip culture alone. Amending the MS medium with 50ppm of ribavirin increased the SCMV elimination from the infected meristem up to 95 per cent and it did not affect the emergence of sugarcane shoots. The other antiviral chemotherapeutant 8-Azaguanine was phytotoxic and affected the emergence of sugarcane plants even at 5ppm lower concentration. The serial hot water treatment involving different combinations of temperature from 52 to 62°C with 5 to 20 min duration for three to four days had partially reduced the SCMV. However these treatments affected the sett germination drastically.

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*originals not seen

