BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF YELLOW MOSAIC VIRUS INFECTING RIDGE GOURD (*Luffa acutangula)* AND CUCUMBER (*Cucumis sativus*)

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IN

PLANT PATHOLOGY

BENGALURU **JULY, 2014**

Affectionately

Dedicated to

My Beloved Parents

For

Their hope, dream and

Endless prayer

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BENGALURU – 560 065

CERTIFICATE

This is to certify that the thesis entitled **"BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF YELLOW MOSAIC VIRUS INFECTING RIDGE GOURD (*Luffa accutangula)* AND CUCUMBER (*Cucumis sativus*)"** submitted by **Mr. MANJUNATH S. HURAKADLI, ID No. PALB 2264,** in partial fulfillment of the requirements for the award of degree of **MASTER OF SCIENCE (Agriculture) in PLANT PATHOLOGY** to the University of Agricultural Sciences, GKVK, Bengaluru, is a record of research work carried out by him during the period of his study in this university under my guidance and supervision and the thesis has not previously formed the basis of the award of any other degree, diploma, associate ship, fellowship or any other similar titles.

Bengaluru
13th June, 2014

(K. T. RANGASWAMY)
CHAIRPERSON

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4. _____
(K. KARUNA)

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June, 2014 ***(Manjunath S. Hurakadli)***

BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF YELLOW MOSAIC VIRUS INFECTING RIDGE GOURD (*Luffa acutangula)* AND CUCUMBER (*Cucumis sativus)*

Manjunath S. Hurakadli.

THESIS ABSTRACT

Yellow mosaic virus disease of Ridge gourd and Cucumber caused by begomovirus is a major constraint in cucurbits cultivation in Karnataka. Survey conducted during 2013-14 to assess the incidence of yellow mosaic virus in ridge gourd and cucumber in four districts of southern Karnataka, revealed the occurrence of the disease incidence ranging from 37.5 to 86.0 per cent in ridge gourd and 31.0 to 78.5 per cent in cucumber. Among the districts, the highest disease incidence of Yellow mosaic virus (YMV) on ridge gourd was recorded in Chikkaballapur (67.09%) and least in Ramanagar (49.88%). Whereas the incidence of YMV on cucumber was highest in Kolar (63.64%) and lowest in Ramanagar (49.69%). YMV disease was successfully transmitted through *B. tabaci* whitefly which were given 12 h of Acquisition access period (AAP) and incubation period. Ten adult *B. tabaci* whiteflies were sufficient to cause 100 % transmission and the whitefly retained the infectivity up to be 6-9 days. YMV of both Ridge gourd and cucumber was successfully transmitted to bottle gourd, pumpkin, ash gourd and squash through *B. tabaci* whitefly vector. The virus causing yellow mosaic disease was identified by PCR amplification of the Coat protein (CP) gene of the virus using CP specific primers. The CP sequences showed maximum nucleotide identity of 92% with *Tomato Leaf Curl New Delhi Virus* (ToLCNDV)-*Luffa cylindrica* and ToLCNDV-severe strains. Based on the high level of nucleotide sequence similarity of the viral CP gene, the virus causing yellow mosaic disease in Ridge gourd and Cucumber is identified as strain of ToLCNDV.

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Major Advisor

ಹೀರೆಕಾಯಿ ಮತ್ತು ಸೌತೆಕಾಯಿ ಹಳದಿ ವರ್ಣವಿನ್ಯಾಸ ನಂಜಾಣುವಿನ ಜೈವಿಕ ಮತ್ತು ಅಣ್ವಿಕ ಗುಣಲಕ್ಷಣಗಳ ಧೃಡೀಕರಣ

ಮಂಜುನಾಥ ಎಸ್ ಹುರಕಡ್ಲಿ

ಪ್ರಬಂಧ ಸಾರಾಂಶ

ಹೀರೆಕಾಯಿ ಮತ್ತು ಸೌತೆಕಾಯಿಯ ಹಳದಿ ವರ್ಣವಿನ್ಯಾಸ ನಂಜಾಣು ರೋಗವು ಬೆಗೊಮೊನಂಜಾಣು ಗುಂಪಿಗೆ ಸೇರಿದ್ದು, ಕರ್ನಾಟಕದಲ್ಲಿ ಸೌತೆಕಾಯಿ ಗುಂಪಿನ ಬೇಸಾಯಕ್ಕೆ ಪ್ರಮುಖ ಹಾನಿಕಾರಕವಾಗಿದೆ. ಹೀರೆಕಾಯಿ ಮತ್ತು ಸೌತೆಕಾಯಿಯಲ್ಲಿ ಬರುವ ಹಳದಿ ವರ್ಣವಿನ್ಯಾಸ ನಂಜಾಣು ರೋಗದ ಹಾವಳಿಯನ್ನು ನಿರ್ಣಯಿಸಲು ೨೦೧೩–೧೪ ಅವಧಿಯಲ್ಲಿ ದಕ್ಷಿಣ ಕರ್ನಾಟಕದ ನಾಲ್ಕು ಜಿಲ್ಲೆಗಳಲ್ಲಿ ಸಮೀಕ್ಷೆ ಕೈಗೊಂಡಾಗ, ಹೀರೆಕಾಯಿಯಲ್ಲಿ ಶೇಕಡಾ ೩೭.೫ ರಿಂದ ೮೬ ರ ವರಗೆ ಹಾಗೂ ಸೌತೆಕಾಯಿಯಲ್ಲಿ ಶೇಕಡಾ ೩೧ ರಿಂದ ೭೮.೫ ರಷ್ಟು ರೋಗದ ಹಾವಳಿ ಇರುವುದು ಕಂಡುಬಂದಿದೆ. ಸಮೀಕ್ಷೆ ನಡೆಸಿದ ಜಿಲ್ಲೆಗಳಲ್ಲಿ ಹೀರೆಕಾಯಿಯ ಹಳದಿ ವರ್ಣವಿನ್ಯಾಸ ರೋಗದ ಅತೀ ಹೆಚ್ಚು ಹಾವಳಿಯು ಚಿಕ್ಕಬಳ್ಳಾಪುರ ಜಿಲ್ಲೆಯಲ್ಲಿ (೬೭.೦೯%) ಮತ್ತು ಅತೀ ಕಡಿಮೆ ರೋಗದ ಹಾವಳಿಯು ರಾಮನಗರ ಜಿಲ್ಲೆಯಲ್ಲಿ (೪೯.೪೮%) ದಾಖಲಿಸಲಾಗಿದೆ. ಸೌತೆಕಾಯಿ ಬೆಳೆಯಲ್ಲಿ ರೋಗದ ಹಾವಳಿಯು ಅತೀ ಹೆಚ್ಚು ಕೋಲಾರ ಜಿಲ್ಲೆಯಲ್ಲಿ (೬೩.೬೪%) ಮತ್ತು ಅತೀ ಕಡಿಮೆ ರಾಮನಗರ ಜಿಲ್ಲೆಯಲ್ಲಿ (೪೯.೬೯%) ದಾಖಲಿಸಲಾಗಿದೆ. ಹಳದಿ ವರ್ಣವಿನ್ಯಾಸ ನಂಜಾಣುವು ಯಶಶ್ವಿಯಾಗಿ ಪ್ರಸರಣಗೊಳ್ಳಲು ೧೨ ಗಂಟೆಯ ಅಕ್ವಿಸಿಷನ್ ಅಕ್ವೆಸ್ ಪೀರಿಯಡ್ (ಎಎಪಿ) ಮತ್ತು ಇನ್‌ಕ್ಯೂಬೇಷನ್ ಪೀರಿಯಡ್‌ಗಳು ಬೇಕಾಯಿತು. ಹತ್ತು ಬಿಳಿನೊಣಗಳಿಂದ ಶೇ. ೧೦೦ ರಷ್ಟು ರೋಗದ ಪ್ರಸರಣವಾಗಿರುವುದು ಕಂಡುಬಂದಿದೆ ಮತ್ತು ಬಿಳಿನೊಣದಲ್ಲಿ ಹಳದಿ ವರ್ಣವಿನ್ಯಾಸ ನಂಜಾಣುವು ೯–೧೦ ದಿನಗಳ ಧೃಡತೆಯನ್ನು ಹೊಂದಿದೆ ಎಂದು ತಿಳಿದು ಬಂದಿದೆ. ಹೀರೆಕಾಯಿ ಮತ್ತು ಸೌತೆಕಾಯಿ ಹಳದಿ ವರ್ಣವಿನ್ಯಾಸ ನಂಜಾಣುವು ಸೋರೆಕಾಯಿ, ಬೂದು ಕುಂಬಳಕಾಯಿ, ಕುಂಬಳಕಾಯಿ, ಸಿಹಿ ಕುಂಬಳಕಾಯಿ ಬೆಳೆಗಳಿಗೂ ಬಿಳಿನೊಣಗಳ ಮೂಲಕ ಯಶಸ್ವಿಯಾಗಿ ಹರಡಿ ರೋಗವನ್ನು ಉಂಟುಮಾಡುವುದೆಂದು ಕಂಡುಬಂದಿದೆ. ನಂಜಾಣುವಿನ ಕೋಟ್ ಪ್ರೋಟೀನ್ (ಸಿಪಿ) ಜೀನ್ ಅನ್ನು ಸಿಪಿ ನಿರ್ದಿಷ್ಟ ಫ್ರೈಮರ್‌ಗಳನ್ನು ಬಳಸಿ ವರ್ಧನೆಗೊಳಿಸಿ ಅನುಕ್ರಮಗೊಳಿಸಲಾಯಿತು. ಸಿಪಿ ಅನುಕ್ರಮವು ಅತೀ ಹೆಚ್ಚು ನ್ಯೂಕ್ಲಿಯೋಟೈಡ್ ಗುರುತು ಶೇಕಡ ೯೨% ರಷ್ಟು ಸಮರೂಪತೆಯನ್ನು ಟೊಮಾಟೊ ಎಲೆ ಮುದುಡು ನವ ದೆಹಲಿ ನಂಜಾಣು – ಲೂಫ್ಫಾ ಸಿಲಿಂಡ್ರಿಕಾ ಮತ್ತು ಟೊಮಾಟೊ ಎಲೆ ಮುದುಡು ನವ ದೆಹಲಿ ನಂಜಾಣು –ತೀವ್ರ ತಳಿ ಜೊತೆ ಹೊಂದಿದೆ. ಗರಿಷ್ಠ ಸಿಪಿ ನ್ಯೂಕ್ಲಿಯೋಟೈಡ್ ಅನುಕ್ರಮದ ಆಧಾರದ ಮೇಲೆ ಹೀರೆಕಾಯಿ ಮತ್ತು ಸೌತೆಕಾಯಿಯ ಹಳದಿ ವರ್ಣವಿನ್ಯಾಸ ನಂಜಾಣುವು ಟೊಮಾಟೊ ಎಲೆ ಮುದುಡು ನವ ದೆಹಲಿ ನಂಜಾಣು ಗುಂಪಿಗೆ ಸೇರಿದ ವಿಭೇದ ಎಂದು ಗುರುತಿಸಲಾಗಿದೆ.

ಸಸ್ಯ ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ,
ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ,
ಜಿ.ಕೆ.ವಿ.ಕೆ., ಬೆಂಗಳೂರು–೬೫

(ಕೆ. ಟಿ. ರಂಗಸ್ವಾಮಿ)
(ಮಾರ್ಗದರ್ಶಕರು)

Biological and Molecular characterization of Yellow Mosaic Virus infecting Ridge gourd (*Luffa accutangula* L.) and Cucumber (*Cucumis sativus* L.).

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INTRODUCTION

Cucurbitaceae is an extensive and heterogeneous family that includes 118 genera and approximately 825species (Robinson and Decker-Walters,1997). Among cucurbits, Ridge gourd (*Luffa acutangula* L.) and Cucumber (*Cucumis sativus*) are important vegetable crops for Indian agriculture and are good sources of carbohydrates, vitamin A, vitamin C and minerals (Nath, 1994). Cucurbits suffers from few viral diseases. Among viral diseases, yellow mosaic has been considered as an important limiting factor in cucurbits productivity. The disease was caused by Begomovirus and transmitted by whitefly (*Bemisia tabaci* Genn.) (Sutic et al., 1999). The present work focuses at characterization of yellow mosaic virus.

OBJECTIVES

- To assess the incidence of Yellow mosaic virus disease on ridgegourd and cucumber in southern dry zone of Karnataka.
- Biological characterization by determining the host range and virus-vector relationship.
- Molecular characterization of yellow mosaic virus by cloning and sequencing partial genome of the virus.

MATERIAL & METHODS

- Survey for disease incidence was carried out in Southern districts of Karnataka. The Per Cent Disease incidence (PDI) was assessed by counting the infected plants out of total number of plants examined in the location.
- Host range of Yellow mosaic virus (YMV) was determined in selected crops of cucurbitaceae family by raising healthy seedlings in an insect proof cages through inoculation of viruliferous whiteflies.
- To determine the virus-vector relationships viz.,, Acquisition acces period(AAP), Inoculation access period(IAP) and number of whiteflies required for transmission was determined.
- Total DNA was extracted from leaf of ridge gourd and cucumber infected with YMV disease, using a cetyl trimethyl ammonium bromide (CTAB)method (Namba *et al.*,1993).Further, PCR was carried out with begomovirus specific primers.
- The PCR amplified products were separated on a 1.5% agarose gel electrophoresed in 1xTAE buffer and gel eluted products were cloned and sequeced.
- The obtained nucleotide sequence of Coat protein of YMV was subject to blast in NCBI to know the percent homology with other begomoviruses. Selected sequences from NCBI database were used for phylogenetic analysis.

RESULTS

- Survey revealed the occurrence of yellow mosaic disease on cucumber and ridge gourd was in the range of 49 to 67%

Table 1 :Mean Per cent Disease Incidence of yellow mosaic virus disease on ridge gourd and cucumber

Districts	Mean Per cent Disease Incidence (PDI)	
	Ridge gourd	Cucumber
Bangalore rural	57.09	55.77
Ramanagar	49.88	49.69
Kolar	60.278	63.64
Chikkaballapur	67.098	56.702

- Out of 15 plant species belonging to Cucurbitaceae, yellow mosaic virus was successfully transmitted to ridge gourd, cucumber, bottle gourd, ash gourd and pumpkin under glass house conditions. Symptoms were observed 10-15 days after inoculation.

Table 2: Transmission characteristics of YMV

Acquisition Access Period (AAP)			Inoculation Access Period (IAP)			No. of viruliferous whiteflies		
Period of acquisition	Per cent transmission*	No. of days taken for symptom expression	Period of Inoculation	Per cent transmission*	No. of days taken for symptom expression	No. of viruliferous whiteflies used for transmission	Per cent transmission*	No. of days taken for symptom expression
30 min	20	8- 20	30 min	20	8-25	1	10	10 – 30
1 hour	30	8- 15	1 hour	40	8 – 20	3	40	8 – 20
6 hour	60	8 –15	6 hour	60	8 – 15	5	80	8 – 15
12 hour	100	8- 15	12 hour	100	8 – 15	10	100	8 – 15
24 hour	100	8- 15	24 hour	100	8 – 15	20	100	8 - 15

- The PCR product of 750 bp was amplified from YMV infected plants by using begomovirus specific primers which was absent in healthy plants. The amplified product was eluted and sequenced. The sequences obtained were subjected to Blast of NCBI. The nucleotide sequence of YMV showed 92% homology with *Tomato Leaf Curl New Delhi Virus* (ToLCNDV)-severe strain, ToLCNDV-mild strain and bottle gourd isolate of ToLCNDV.
- Phylogenetic analysis of coat protein gene sequences revealed that YMV clustered with ToLCNDV-severe strain and ToLCNDV-mild strain.

DISCUSSION

- Mean PDI of YMV in ridgegourd was highest in Chikkaballapur (67.09%), and least in Ramanagar (49.88%). The PDI of YMV in cucumber was highest in Kolar (63.64%) and least in Ramanagar district (49.69%). There are already reports of ToLCNDV incidence in cucurbits varied from 4.7 to 36% from northern india (Sohrab, *et al*., 2003).
- The virus was transmitted to ridgegourd, cucumber, bottle gourd, ash gourd and pumpkin. Similar results were obtained by Sohrab, *et al*., (2003), ToLCNDV was transmitted to *Cucumis sativus*, *Luffa acutangula* and *L. cylndrica*.
- Studies on virus-vector relationship revealed that an acquisition and inoculation period of 12 h and minimum 10 number of viruliferous whiteflies were sufficient for 100% transmission (Nair and nene, 1973).
- The PCR amplified products of coat protein gene (750 bp) from Luffa and pumpkin isolates were separately cloned and sequenced. The phylogenetic analysis revealed RYMV clustered with ToLCNDV isolates which was in confirmation with Sohrab *et al.*, (2013).

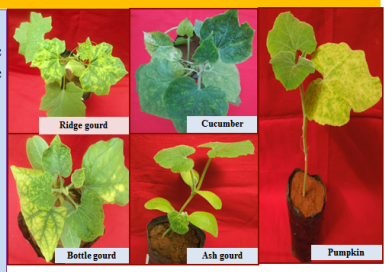
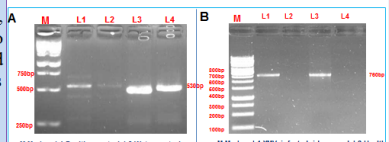


Fig 1. Host range studies of yellow Mosaic Virus(YMV)



M-Marker, L1-Positive control, L2-Water control , L3-YMV infected ridge gourd, L4-YMV infected cucumber

M-Marker, L1-YMV infected ridgegourd, L2-Healthy ridgegourd,L3-YMV infected cucumber, L4-healthy cucumber

Fig 2. Molecular detection of YMV in Ridge gourd and Cucumber using Universal Deng Primers(A) and Complete coat protein primers(B)

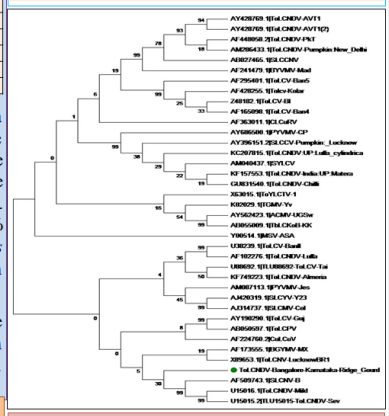


Fig 3. Phylogenetic tree of nucleotide sequence of coat protein of YMV from Bangalore with other Begomovirus sequences

SUMMARY

• The percent disease incidence of YMV in ridge gourd and cucumber in Southern Karnataka varied from 49.88 to 67.09% and 49.69 to 63.64% respectively.

• The virus was transmitted to ridge gourd, cucumber, bottlegourd, ashgourd and pumpkin

•Virus was efficiently transmitted after 12 h of AAP and IAP and 10 number of whiteflies were sufficient to cause 100 % transmission

•The PCR product 750 bp was amplified from YMV infected plants by using specific primers.

• Phylogenetic analysis of coat protein sequence of YMV with other bipartite begomoviruses revealed that YMV clustered with ToLCNDV-severe strain and ToLCNDV-mild strain

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I INTRODUCTION

Cucurbitaceae is an extensive and heterogeneous family that includes 118 genera and approximately 825 species (Robinson and Decker-Walters, 1997). It includes Cucumber (*Cucumis sativus* L.), Ridge gourd (*Luffa acutangula*), Melon (*Cucumis melo* L.), West Indian Gherkin (*Cucumis anguria* L.), Squash (*Cucurbita* spp) and Pumpkin (*Cucumis pepo* L.). In India the Cucurbitaceae family is represented by 36 genera with 100 species (Chakravarthy, 1982). Nearly twenty different kinds of cucurbits are cultivated as vegetables in India.

Ridge gourd (*Luffa acutangula* L. Roxb) popularly known as Kalitori or angled gourd and belongs to *Cucurbitaceae* family. The name "Luffa" is of Arabic origin and refers to the spongy characteristics of the mature fruit (Bose and Som, 1986). It contains a gelatinous compound called luffein which has medicinal importance. Fruits contains protein (0.5%), carbohydrate (3%), carotene (37 mg) and vitamin C (18 mg) per 100 g of edible portion.

Ridge gourd originated in India and it is cultivated in the tropics for its tender edible fruits both on commercial scale and in kitchen gardens throughout India. It is a popular vegetable both as spring summer and rainy season crop (Yawalkar, 1985).

It is cultivated in India, Indonesia, Malaysia, Myanmar, Philippines, SriLanka and Taiwan. The total area under cucurbits in India is about 0.5 million ha, which is about 12 percent of the total vegetable growing area excluding potato (Seshadri, 1996)**.** In India, it is largely grown in Karnataka, Andhra Pradesh, Kerala, Tamil Nadu, Uttar Pradesh, Madhya Pradesh and Maharashtra states. Among the cucurbitaceous vegetables grown in India, gourd vegetables occupy an area of 73273 ha with an annual production of 685224 tonnes. In Karnataka, it occupies an area of 2,753 ha with an annual production of 18,706 tonnes of fleshy fruits (Anon., 2004).

Cucumber (*Cucumis sativus* L.) is native to the tropics and is one of the oldest cultivated vegetable crops grown commonly throughout India (Yawalkar, 1985). Cucumbers are a rich source of triterpene phytonutrients called cucurbitacins. It contains 0.28 gm protein, 0.42 gm carbohydrate, 10 mg calcium, 17 mg Phosphorus per 100 mg of edible portion. It also ranks fourth among vegetables in world next to tomatoes, cabbage and onion. The tender fruits are eaten raw or with salt in salad and are also used as cooked vegetable.

Cucumber is believed to be indigenous to an area in India between the Himalayas and the Bay of Bengal and was introduced to West Africa by the Europeans in 1940 (Sinnadurai, 1992). The total area under cucumber cultivation in India is reported to be around 0.02 million ha with production 0.12 million tonnes (Anon., 2004).

Cucurbits suffers from many fungal, bacterial and viral diseases. The major constraint to cucurbits production is downy mildew caused by *Pseudoperonospora cubensis*), powdery mildew (*Erysiphe cichoracearum*), Anthracnose, Fusarium wilt,

Gummy stem blight, Target leaf spot, Bacterial leaf spot, Bacterial wilt, Root-knot nematode ,Cucumber Mosaic Virus (CMV), Watermelon Mosaic Virus (WMV), Zucchini Yellow Mosaic Virus (ZYMV) etc. Among viral diseases, yellow mosaic has been considered as an important limiting factor in cucurbits productivity.

Characteristic symptoms of cucurbits yellow mosaic disease include extensive chlorosis and mosaic mottling on newly emerged leaves, vein banding and severe mosaic mottling. The most characteristic symptoms of the disease on *Luffa cylindrica* are leaf curling, yellow spot on the newly emerged leaves, chlorosis and mosaic (Tiwari *et al*., 2012).

Geminiviruses have been reported to be the cause of yellow mosaic disease. Geminiviruses transmitted by the whitefly *Bemisia tabaci* are wide spread in tropical and subtropical regions of the world, where they cause numerous diseases in dicotyledonous plants including cassava, pulses, vegetables, tobacco and cotton (Muniyappa and Veeresh, 1984; Harrison, 1985; Brown and Bird, 1992). More than 50 geminiviruses have been reported to be transmitted by whiteflies (Bedford *et al*., 1994 ; Markham *et al*., 1994).

Geminiviruses (Family *Geminiviridae*) have circular single stranded DNA genomes that are encapsidated in twinned quasi-isometric particles and are classified into four genera: *Begomovirus, Mastrevirus, Curtovirus* and *Topocuvirus.* The largest genus, *Begomovirus* comprises of one/two circular components viz., DNA-A and DNA-B. The DNA-A component encodes all viral functions required for replication, control of gene expression, encapsidation and vector transmission. The DNA-B, code for two proteins which are involved in movement of the virus between and within plant cells. Both components of bipartite begomoviruses are required for systemic infection and symptom induction (Singh *et al*., 2012).

Phaneendra *et al*. (2012), observed yellow vein mosaic and leaf curl symptoms in pumpkin plants under field conditions at IARI in successive years. Two different begomovirus species Squash leaf curl China virus (SLCCNV) and ToLCNDV along with betasatellite were reported to be the cause of the disease in pumpkin.

Natural infection of begomoviruses also recorded on cucumber, bottle gourd and muskmelon with yellow leaf symptoms in Thailand. The complete nucleotide sequences of DNA-A and DNA-B were amplified, cloned and sequenced. Sequence analyses showed that the DNA-A component of cucumber isolate shares 97% similarity with isolates of tomato leaf curl New Delhi virus (ToLCNDV) while bottle gourd and muskmelon isolates share more than 99% sequence identity with ToLCNDV-[Cuc:Tha] (Phaneendra *et al*., 2012).

Several virus diseases affecting Luffa crop in India have been reported (Varma and Giri, 1998). A begomovirus was detected by PCR from *L. cylindrica* (Sohrab et al., 2003) and later the association of Tomato leaf curl New Delhi virus and Yellow mosaic virus (Sohrab et al., 2006). Varma *et al*. (2006) were the first to report the association of

Tomato leaf curl New Delhi virus (ToLCNDV) with yellow mosaic and leaf curl disease of *L. cylindrica.*

In the recent years ridge gourd and cucumber have been severely affected by yellow mosaic disease. The incidence some time reach hundred percent. Review of literature revealed the lack of information on causal virus, mode of spread and source of reservoirs. Hence attempts were made to study various aspects of the disease with the following objectives.

Objectives of the research work:

1) Survey of yellow mosaic virus disease in ridge gourd and cucumber in southern dry zone of Karnataka.
2) Biological characterization of the causal virus by determining the host range and virus-vector relationship.
3) Molecular detection and characterization of the causal virus by sequencing Coat protein (CP) gene of the virus.

II REVIEW OF LITERATURE

The virus causing yellow mosaic disease of cucurbits are Geminiviruses which are transmitted efficiently by whiteflies. The literature pertaining to yellow mosaic disease, spread, host range, virus-vector relationship and molecular characterization has been reviewed and presented in this chapter.

The availability of literature related to yellow mosaic disease of ridge gourd and cucumber is meager, hence whitefly transmitted Geminiviruses on other cucurbits are reviewed for the support of present investigation.

2.1 Economic importance of cucurbits:

Cucurbits form an important and a big group of vegetable crops cultivated extensively in India. This group consists of a wide range of vegetables either used as salads, cooking, pickling, desert fruits or preserved foods. They are good sources of carbohydrates, vitamin A, vitamin C and minerals. Being warm season crops, these are of tropical origin, grown mostly in Africa, tropical America and Asia, chiefly in the southeast. Cucurbits belong to the family *cucubitaceae* with a number of different genera in the family including *Cucumis* (Cucumber and muskmelon), *Citrullus* (watermelon), *Cucurbita* (pumpkin, squash and marrow), *Lagenaria* (Bottle gourd), *Momordica* (Sponge gourd and ridge gourd) and *Tricosanthes* (Snake gourd and Pointed gourd) (Seshadri, 1996). The genus *cucurbita* comprises about 27 species, both wild and cultivated. There are only five cultivated species in the genus *cucurbita; C. moschata*, *C. maxima*, *C. pepo*, *C.ficifolia* and *C.mixta*.

A large number of diseases and pests affect cucurbits at different stages of growth in India. These results in losses through reduction in growth and yield and are responsible for distortion and mottling of fruit, making the product unmarketable. The most important diseases of cucurbits in India include powdery mildew, downy mildew, anthracnose, fusarium wilt and root-knot nematode diseases (Seshadri, 1996).

2.2. Major viral diseases of cucurbits

Viruses are the most common causes of diseases affecting cucurbits. These diseases result in losses through reduction in growth and yield. A complex of viruses is able to infect cucurbits. A plant group that includes cucumber, melon, squash, pumpkins, and watermelon. The most important viruses are cucumber mosaic virus (CMV), squash mosaic virus (SqMV), watermelon mosaic virus-I (WMV-1), watermelon mosaic virus-2 (WMV-2), and zucchini yellow mosaic virus (ZYMV). With the exception of SqMV, which is seed borne in melon and transmitted by beetles, the other major viruses are transmitted by several aphid species in a non-persistent manner (Varma and Giri, 1998).

Pumpkin has been found to be affected by PYVMV, which is transmitted by the whitefly, *B. tabaci* (Varma, 1955; Capoor and Ahmad, 1975, Babitha, 1996; Jayashree *et al*., 1999; Muniyappa *et al*., 2003).

In India, cucurbits found to be infected by two important begomoviruses viz., Squash leaf curl china virus (SLCCNV) (Singh *et al*., 2008 and Maruthi *et al*., 2007) and *Tomato leaf curl New Delhi virus (ToLCNDV)* on pumpkin (Phaneendra *et al*., 2012), on bottle gourd (Sorhab *et al*., 2010), on sponge gourd (Sohrab *et al*., 2003) and on bitter gourd (Tiwari *et al*., 2010).

2.3 Morphology and genome organization of Begomoviruses.

Begomoviruses have circular single stranded DNA bipartite genome components, DNA-A and DNA-B, each of the size of 2500-2800 nt. Only a few begomoviruses have monopartite genome, which lack DNA-B. Six protein-coding genes are universally present in the bipartite genome of DNA-A and DNA-B on the virus strand and complementary strand (Brown, *et al*., 2001).

DNA-A contains four genes, AV1 in the virus strand and AC1, AC2 and AC3 in the complementary strand. AV1 gene codes for virus coat protein (CP), AC1 codes for Replication initiation protein (Rep) which is essential for DNA replication, AC2 is a transcriptional activator for the virion-sense genes in both DNA-A and DNA-B components and AC3 has no role in infectivity but enhances the viral replication. The DNA-B has BV1 gene in the virus strand and BC1 in the complementary strand. These genes together code for virus movement. BV1 coded protein controls viral DNA movement from nucleus to cytoplasm and BC1 coded protein controls its cell-to-cell movement. In addition AV2 gene that overlaps AV1 in the virus strand of DNA-A component occurs in begomoviruses of the Old World but not in those of New World (Brown, et al., 2001). The CP is hypervariable at N-terminal ~55-60 amino acids and the central portion contains both variable and conserved regions while the C-terminal regions are nearly identical between begomoviruses. AC4 is found in many begomoviruses in the complementary strand of DNA-A within but in a different ORF from AC1.

An intergenic region (IR) exists between the initiation codons of AV2 and AC1 in DNA-A and in DNA-B this IR lies between BV1 and BC1. The nucleotide sequences in the IR regions of DNA-A and DNA-B of a begomovirus are very identical. The known exceptions are Cabbage leaf curl virus and Yellow mosaic viruses infecting legumes, where the IR sequences of DNA-A and DNA-B are only 80% identical (Hill *et al*., 1998; Usharani *et al*., 2004). The IR contains regulatory elements. The nonanucleotide sequences where Rep protein leaves (Ori) is present in IR. The two TATA motifs seen in IR, one near the 3' end is probably involved in the transcription of AV1 and/or AV2 and another one in the 5' end in transcription of AC1 and/or AC4

Phylogenetic studies show that begomoviruses can be broadly divided into two groups, the Old World begomoviruses (eastern hemisphere, Europe, Africa, Asia) and the New World begomoviruses (western hemisphere, the Americas) (Padidam *et al.*, 1999; Paximadis *et al*., 1999; Rybicki, 1994). Begomovirus genomes have a number of characteristics that distinguish Old World and New World viruses. All New World begomoviruses are bipartite, whereas both bipartite and monopartite begomoviruses are present in the Old World. In addition, all Old World begomoviruses have an extra AV2 ORF in DNA A that is not present in New World begomoviruses (Rybicki, 1994; Stanley

et al., 2005). New World begomoviruses also have an N-terminal PWRsMaGT motif in the coat protein (CP) encoded by AV1, which is absent from Old World begomoviruses (Harrison *et al*., 2002). In most Old World begomoviruses, there are two iterons upstream of the AC1 TATA box, with a complementary iteron downstream. This downstream iteron is lacking in most New World begomoviruses (Arguello-Astorga *et al*., 1994).

2.4 Symptoms of begomoviruses on cucurbits.

The PYVMV produces a typical yellow vein type of symptoms in infected plants of pumpkin. The initial symptoms of the disease appeared on the young developing leaves as faint vein clearing symptoms developed first in the tertiary veins of pumpkin plants. The symptoms extended gradually to secondary and primary veins as a prominent vein yellowing and later, coalesced to a yellow mosaic. As disease symptoms progresses, leaves of older plants displayed a mixed pattern of vein yellowing in smaller areas and chlorotic patches over larger areas of the leaf laminae (Capoor and Ahmad, 1975; Muniyappa *et al*., 2003) causing early senescence. Infected plants stunted and flowers drop prematurely, greately reducing yields (Muniyappa *et al*., 2003). In contrast, PYVMV infected summer squash (*C. pepo*,) and winter squash (*C. maxima*) showed leaf distortion and mosaic symptoms. Infection of bottle gourd (*L. siceraria*) was associated with only a mild mosaic but no vein yellowing (Muniyappa *et al.,* 2003).

Yellow mosaic disease on sponge gourd showed yellow spots on newly emerged leaves, mosaic, mild leaf curling and distortion, small leaves and misshapen fruits (Sohrab *et al*., 2003).

Begomovirus disease symptoms such as yellow mosaic, leaf curling, puckering and vein bending were observed on bitter gourd, pointed gourd, sponge gourd and pumpkin (Tiwari *et al*., 2012). The typical symptoms of leaf curling, blistering, yellowing, mottling of leaves and small deformed fruits in squash, cucumber, and melons were reported (Ali *et al*., 2010).

Infection of begomoviruses cause chlorotic mottling, mild curling and excessive reuction in leaf size and stunting of bottle gourd plants (Sohrab *et al*., 2010).The yellow mosaic disease infected Chayote (*Sechium edule*) plant shown to be associated with symptoms such as yellow spots, mosaic and upward curling with occasional enations (Mandal *et al*., 2004).

Symptoms caused by yellow mosaic begomoviruses are known to be largely dependent on host species and susceptibility. Initially symptoms appear as small yellow specks along the veins and then spread over the leaf. In severe infection the entire leaves of legumes may become chloratic (Javaria *et al.,* 2007).

The association of ToLCNDV causing yellow mosaic disease in pumpkin characterized by marginal rolling and curling of leaves and yellow patches on lamina. The affected plants were stunted and produced less flowers and fruits (Phaneendra *et al*., 2012).

2.5 Occurrence of Begomoviruses on cucurbits.

Begomoviruses infect a broad range of dicotyledonous plants and constitute the largest group of geminiviruses with at least 196 virus species recognized (ICTV, 2011). The production of economically important crops such as cassava, cotton, cucurbits, legumes, papaya, pepper, potato, sweet potato and tomato, have been severely affected by diseases caused by begomoviruses in different parts of the world (Varma and Malathi 2003; Wang *et al*. 1996; Brown et al. 2005).

In 1990, a severe epidemic of yellow vein mosaic disease in pumpkin caused by begomovirus was reported in northern India (Varma, 1990). This epidemic was caused by the increase in whitefly populations early in the cucurbit-growing season. In 2003, yellow vein mosaic disease was reported to cause severe yield losses in pumpkin (Muniyappa et al., 2003).

The Pumpkin yellow vein mosaic virus disease (PYVMV) was first reported in and around Delhi by Vasudeva and Lal (1943) and causal virus was designated as pumpkin yellow vein mosaic virus (PYVMV). Subsequently the disease was reported from different parts of India (Varma, 1955; Capoor and Ahmad, 1975; Bhargava and Bhargava, 1977; Ghosh and Mukhopadhyay, 1979; Latha and GopalKrishnan, 1993; Jayashree *et al*., 1999) and the disease was mainly confined to central western India until the 1980s. In 1990, a severe epidemic of yellow vein mosaic in pumpkin caused by begomovirus, appeared in northern india (Varma, 1990).

Yellow leaf disease caused by whitefly-transmitted begomoviruses was first observed on wax gourd, cantaloupe and muskmelon in Thailand. Partial sequences of the begomoviruses indicated that they were the species of Tomato leaf curl New Delhi virus (ToLCNDV) (Samretwanich *et al.* 2000a, b, and c). Subsequently, the complete genome sequences of ToLCNDV isolates were determined from diseased bottle gourd, cucumber and muskmelon (Ito *et al*. 2008b) as well as luffa in Thailand. Isolates of squash leaf curl China virus (SLCCNV) infecting pumpkin and wax gourd were also detected (Ito *et al*. 2008a).

The begomovirus diseases have been detected in cucurbitaceous crops, such as bottle gourd, bitter gourd, cucumber, ivy gourd, musk melon sponge gourd, pumpkin and water melon through PCR methods using primers of putative CP gene of Tomato leaf curl New Delhi Virus (Raj and Singh, 1996; Varma and Giri, 1998; Singh *et al*., 2001; Mandal *et al*., 2004; Sohrab *et al.*, 2006).

Epidemics of Whitefly transmitted geminiviruses (WTGs) have increased in number, prevalence and distribution during the past two decades (Harrison, 1985; Duffus, 1987; Otim-Nape *et al*., 2000; Varma and Malathi, 2003) and the impact has been devastating. WTGs occur predominantly in the tropics where their vectors are most abundant (Muniyappa, 1980; Muniyappa and Veeresh, 1984; Brown, 1994) and they usually have a devasating impact on agricultural and horticultural crops.

Table 1. Occurrence begomoviruses on different cucurbits.

Crop	Name of the disease	Name of the virus	Nature of the virus particle	Symptoms	References
Ash gourd	Ash gourd yellow stunt disease	*Tomato leaf curl New Delhi virus*(ToLCNDV)	Bipartite	Yellowing of leaves and stunted growth	Anirban *et al*.(2013)
Bitter gourd	Bittergourd yellow mosaic disease	Bitter gourd yellow mosaic virus	Bipartite	Leaf yellowing and mosaic	Raj *et al*. (2005)
	Bitter gourd leaf crumpling virus	Tomato leaf curl palampur virus (ToLCPV)	Bipartite	Chlorosis, leaf crumpling, and stunting of plant	Ali *et al*.(2010)
	Bitter gourd yellow mosaic disease	ToLCNDV	Bipartite	Severe yellow mosaic with slightly leaf curling	Tiwari et al. (2010)
Bottle gourd	Bottle gourd chlorotic stunt disease	ToLCNDV	Bipartite	Small chlorotic and curled leaves and stunted growth	Sohrab *et al*.(2003)
	Yellow leaf curl disease	ToLCNDV	Bipartite	Yellow leaf curling	Ito *et al*. (2008b)
Cucumber	Cucumis yellow mosaic disease	Cucumis yellow mosaic disease associated virus	Bipartite	Leaf yellowing and mosaic	Raj and Singh, 1996
	Cucumber yellow mosaic disease	ToLCNDV	Bipartite	Yellow mosaic and upward leaf curling	Mizutani *et al*.(2011)
Chayote	yellow mosaic disease of Chayote	ToLCNDV	Bipartite	Yellow spots, mosaic and upward curling with occasional enations	Mandal *et al*. (2004)
Oriental melon plants	Yellow mosaic disease	ToLCNDV	Bipartite	Mosaic, leaf curl and puckering	Chang *et al*., 2007
Pumpkin	Pumpkin leaf curl disease	ToLCNDV	Bipartite	Upward leaf curl with Chlorotic patches and stunting of plant.	Phaneendra *et al*.(2012)
	Pumpkin yellow vein mosaic disease	PYVMV	Bipartite	Yellowing leaf veins, chlorotic patches on leaves, stunting of plant	Muniyappa *et al*.(2003)
	Pumpkin yallow leaf curl disease	Squash leaf curl chinavirus (SLCCNV)	Bipartite	Leaf curling and yellowing	Ito *et al*. (2008)
Sponge gourd	Sponge gourd yellow mosaic disease	ToLCNDV	Bipartite	Yellow mosaic symptoms	Sohrab *et al*. (2014)
Water melon	Watermelon chlorotic stunt disease	Watermelon chlorotic stunt virus (WmCSV)	Bipartite	Mottling, yellowing, leaf curling and stunting of plant	Al-Musa *et al*. (2011)
Muskmelon	Yellow leaf curl disease	ToLCNDV	Bipartite	Yellow leaf curling	Ito *et al*. (2008a)

Sohrab *et al*. (2003) conducted surveys of begomovirus disease in cucurbits in Delhi and Haryana and reported that Bottle gourd (*Lagenaria siceraria*) was affected by begomovirus like disease. Incidence of the disease varied from 4.7 to 36%.

Nagaraja (2005) conducted a survey to identify yellow mosaic disease in bhendi, transmitted by *B. tabaci*, in different districts of Southern Karnataka. BYVMV incidence ranged from 19.1 to 95.4 per cent, the highest disease incidence was recorded in Mandya district (36.80 to 95.4 per cent), followed by Mysore (43.3 to 85.5 per cent), Bangalore (21.62 to 37.5 per cent) and Kolar (19.1 to 24.3 per cent).

Tomato leaf curl New Delhi virus-{Luffa} (ToLCNDV-Luffa) in sponge gourd (Sohrab *et al*., 2003) and chayote (Mandal *et al*., 2004) have been identified as serious viral pathogens of cucurbits in India. ToLCNDV- luffa was detected in bottle gourd, ivy gourd, pumpkin, ridge gourd and watermelon in northern India (Sohrab *et al*., 2006). Sponge gourd was affected by yellow mosaic distortion disease, which was recorded to occur in epidemic proportion in the vegetable growing tract of Delhi and Haryana. Occurrence of 4 begomoviruses such as ToLCNDV in sponge gourd, ridge gourd and bitter gourd: ToLCPV in pumpkin; SLCCNV in pumpkin reported in Northern India (Tiwari *et al.*, 2012).

2.6 Host range

PYVMV causes vein clearing symptoms in *C. moschata*, prominent yellow veins in *Citrullus vulgaris*, faint chlorotic mottle in *Luffa acutangula*, vein clearing in *Callistephus chinensis* (L.) and mild leaf curling in *Nicotiana tabacum*, (Capoor and Ahmad, 1975). Pumpkin, winter squash, summer squash, bottle gourd and non-cucurbitaceous crops such as *N. tabacum*, 'White Burley' and 'Xanthi' were considered as symptomatic hosts for PYVMV (Muniyappa *et al.*, 2003).

Sohrab *et al.* (2003) studied the host range of Tomato leaf curl New Delhi virus infecting *Luffa cylindrica*. The virus was transmitted to *Cucumis sativus, Luffa acutangula, L. cylndrica, Lycopersicon esculentum, Nicotiana tabacum* and *Praecitrullus fistulosus* but not to *Citrullus lunatus*, *Cucumis melo, Cucurbita moschata* and *Vigna unguiculata*.

The ToLCNDV associated with yellow mosaic disease of chayote (*Sechium edule*) was experimentally transmitted to *Luffa acutangula* and *Nicotiana benthamiana* by sap inoculation and to *L. acutangula* by whitefly transmission using *Bemisia tabaci* (Mandal *et al*., 2004).

Tomato yellow leaf curl virus (TYLCV) was transmitted to different species belonging to the families *Amarnthaceae, Chenopodiaceae, Cruciferae, Cucurbitaceae, Leguminaceae (Fabaceae), Malvaceae and Solanaceae* through whiteflies, *B. tabaci* (Ajlan *et al*., 2006).

Host range studies of kenaf leaf curl virus revealed that the East Indian isolate produced leaf crumpling symptom in *V. unguiculata* with a transmission efficiency of

10%. The south Indian isolate could produce leaf crumpling in *Solanum lycopersicum* and leaf spot in *Nicotiana tabacum* with transmission efficiencies of 50% and 30%, respectively (Paul *et al* ., 2009).

To study the host range of WmCSV (Watermelon chlorotic stunt virus) has been found to infect plants belonging to *Cucurbitacea* and *Solanaceae* families, including watermelon, pumpkin, melon, cucumber, squash, *Nicotiana benthamiana*, and *N. glutinosa* were found susceptible to WmCSV infection (Al-Musa *et al.*, 2011).

ToLCNDV has a wide host range, including pepper, potato, tomato and cucurbit plants (Hussain *et al*., 2005). Watermelon chlorotic stunt virus and Tomato leaf curl Palampur virus have been reported to infect cucurbit plants in Iran (Kheyr-Pour *et al*., 2000).

2.7 Virus vector relationship

2.7.1 Number of *B. tabaci* required for transmission.

Varma (1955) was the first to report successful transmission of PYVMV by *B. tabaci*, which was later confirmed by Capoor and Ahmad (1975) and Jayashree *et al*. (1999) suggested that PYVMV may be a begomovirus. Single whitefly was able to transmit PYVMV with 30 per cent efficiency, which increased to 60 per cent when three whiteflies were caged on healthy pumpkin seedlings. This contrasts with 1.6 per cent transmission rate reported by Capoor and Ahmad (1975). A 100 per cent efficiency of transmission was achieved with five (Muniyappa *et al*., 2003) and fifteen (Jayashree *et al*., 1999) whiteflies per test plant but Capoor and Ahmad (1975) noticed a maximum infection of only 77.3 per cent with 20 whiteflies. It was also observed that the days required for the symptom expression became progressively less as the number of whiteflies used for inoculating the test plants was gradually increased from one to 20. A single adult whitefly could transmit *Croton yellow vein mosaic virus* (CYVMV) with 40.4 per cent active transmission. A minimum of 15 viruliferous *B. tabaci* per plant causes 100 per cent infection of CYVMV (Mandal, 1989) and Cotton leaf curl virus (CoLCV) in cotton (Nateshan *et al*., 1996).

Capoor and Varma (1950) had used 20 to 30 viruliferous whiteflies to transmit the dolichos yellow mosaic virus, while Raj *et al*. (1988) had used 20-25 whiteflies per test plant.

2.7.2 Aquisitiion access period (AAP)

A minimum of 30 sec was required by *B. tabaci* to transmitt PYVMV (Capoor and Ahmad, 1975). A minimum AAP period of 5 min (Jayashree *et al*., 1999) and 30 min (Muniyappa *et al*., 2003) was necessary for whiteflies to acquire PYVMV which resulted in 8.3 and 20.0 per cent transmission, respectively. An AAP of 6 hour or more resulted in 100 per cent transmission (Jayashree *et al*., 1999; Muniyappa *et al*., 2003). With increase in AAP, the percentage of insect becoming viruliferous also increased, as observed higher percentage of infected plants and the days required for symptom expression became less.

B. tabaci acquired SLCV after 30 min of AAP but 100 per cent transmission was achieved only with 6 h AAP (Cohen *et al*., 1983). A minimum of 1 h AAP required to transmit yellow mosaic virus of *Phaseolus lunatus (*L.) was reported by Capoor and Varma (1948) and the similar findings were recorded for Bhendi yellow vein mosaic virus (BYVMV) (Varma, 1952), Beet pseudo yellow virus (Daffus, 1965), Tobacco leaf curl virus (TbLCV) (Retuerma *et al*., 1971) and Cotton leaf crumple virus (CLCmV) (Brown and Nelson, 1987).

A minimum of 30 min AAP was required for whiteflies to transmit Euphorbia mosaic virus (EMV) (Costa and Bennet, 1950). Similar period was also reported for TYLCV (Cohen and Nitzany, 1966), Horsegram yellow mosaic virus (HYMV) (Muniyappa and Reddy, 1976), ToLCV (Reddy and Yaraguntaiah, 1981), SLCV (Cohen *et al.*, 1983) and CYVMV (Mandal and Muniyappa, 1991). For successful transmission of ICMV and CoLCuV by *B. tabaci*, AAP of 10 min (Mathew and Muniyappa, 1991) and one hour (Nateshan *et al*., 1996) were required, respectively.

Costa and Benett (1950) reported that whiteflies require minimum of 30 min. AAP to transmit Euphorbia mosaic virus. Similar time period was also reported for tomato yellow leaf curl virus transmission (Cohen and Nitzany, 1966), Horse gram yellow mosaic virus (Muniyappa and Reddy, 1976), tomato leaf curl virus (Reddy and Yaraguntaiah, 1981; Ramappa, 1993), squash leaf curl virus (Cohen *et al*., 1983) and croton yellow vein mosaic virus (Mandal, 1989).

ToLCNDV-[Luffa:Del] and SqLCV-[Pum:Del] were transmitted efficiently by even single whitefly, which resulted in infection of 90% by Luffa:Del isolate and 80% by Pum:Del isolate. The symptoms developed however, took slightly longer time when single whiteflies were used for inoculation as compared to 20 whiteflies used per test plant. The efficiency of transmission varied with the variation in AAP. For a single whitefly, the minimum of 60 min AAP required to transmit the both Luffa:Del and Pum:Del isolates, whereas, for a group of 20 whiteflies, the minimum of 15min AAP required. With the increase in AAP, percent transmission was also increased (Sohrab *et al*., 2013).

2.7.3 Inoculation access period (IAP)

An IAP of 30 min by the viruliferous vectors caused 50 per cent transmission. With increase in IAP, there was a gradual increase in the percentage of infected plants. An IAP of 3 hour or more resulted in 100 per cent disease transmission (Jayashree *et al*., 1999). Viruliferous whiteflies required a minimum of 10 min IAP to cause one per cent transmission, where as a 6 hour IAP resulted in 100 per cent transmission (Muniyappa *et al.*, 2003). Minimum IAP was found to be 2 h to transmit PYVMV by *B. tabaci* (Capoor and Ahmad, 1975).

For transmission of CoLCuV, a minimum of 30 min IAP was required (Kirkpatrick, 1931; Ripper and George, 1965). Similar IAP was also observed by Varma (1952), Cohen and Nitzany (1966), Reddy and Yaraguntaiah (1981) and Cohen *et al*., (1983) transmitting BYVMV, TYLCV and SLCV, respectively. *B. tabaci* required 3 min

IAP to transmit TbLCV (Retuerma *et al*., 1971) and 10 min minimum IAP for HYMV (Muniyappa and Reddy, 1976). Similar results were observed by Brown and Nelson (1987), Mathew and Muniyappa (1991) and Mandal and Muniyappa (1991) for CLCmV, ICMV and CYVMV, respectively. IAP of 5 min was sufficient for successful transmission of CoLCuV (Nateshan *et al*., 1996).

ToLCNDV-[Luffa:Del] and SqLCV-[Pum:Del] were transmitted efficiently by even single whitefly, which resulted in infection of 90% plants by Luffa:Del isolate and 80% by Pum:Del isolate. The efficiency of transmission varied with the variation in AAP and IAP. Minimum IAP required was 15 min, but the efficiency of transmission increased with the increase in IAP (Sohrab et *al.*, 2013)

2.7.4 Persistance of virus in *B. tabaci*

The PYVMV transmission was sporadic after initial acquiring and persisted in B. tabaci for atleast eight days (Muniyappa *et al*., 2003). SLCV was found to be retained by individual viruliferous *B. tabaci* for 26 days in serial transfers (Cohen *et al*., 1983). The whiteflies that acquired the CoLCuV remained infective for 12 days (Kirkpatrick, 1931) and infective for 7-9 days (Ripper and George, 1965). Virus inducing yellow mosaic on *P. lunatus* was retained in *B.tabaci* for 15-20 days (Capoor and Varma, 1948) and whitefly could retain HYMV up to 12 days and not throughout the life span of the insect (Muniyappa and Reddy, 1976).

Once the whiteflies acquired ToLCV, they could transmit the virus throughout their life period (Reddy and Yaraguntaiah, 1981). SLCV was retained for 26 days in single female *B. tabaci* (Cohen *et al*., 1983). Maximum of 6-8 days persistence of CLCmV in *B. tabaci* was observed by Brown and Nelson (1987) and ICMV was retained for a maximum of eight days (Mathew, 1988). CYVMV persisted for 11 days (Mandal, 1989) and CoLCuV for 8 days (Nateshan *et al*., 1996). The association of SLCV with *B. tabaci* was noticed (Cohen *et al*., 1983) and they found that viral nucleic acid could be detected readily in adults of *B. tabaci* for up to 120 h after AAP.

Bird (1957) found that viruses causing yellow mosaic on *Jatropha gossypifolia* and infectious chlorosis on *Sida cardifolia*, were retained in *B. tabaci* for four and seven days respectively.

Reddy and Yaraguntaiah (1981) found that once the whiteflies acquired TLCV could transmit the disease throughout their life period. Mathew (1988) observed that ICMV was retained in *B. tabaci* for a maximum of seven to eight days.

2.7.5 Incubation period in vector

PYVMV required a minimum latent period of 6 h, after which time five per cent of *B. tabaci* were capable of transmitting the virus (Muniyappa *et al*., 2003). Two h of incubation period was required by *B. tabaci* to transmitt PYVMV (Capoor and Ahmad, 1975).

A minimum of 3 h incubation period was required to transmit CoLCuV (Ripper and George, 1965). 4 h incubation period was required by B. tabaci to transmit euphorbia mosaic virus (Costa and benett, 1950), TobLCV (Varma, 1963) and ICMV (Mathew and Muniyappa, 1991). Incubation period of 6 h was sufficient for successful transmission of beet pseudo yellows by greenhouse whitefly. *T. vaporarium* (Duffus, 1965) and similar reports were also found by Muniyappa and Reddy (1976) with HYMV and ToLCV (Reddy and Yaraguntaiah, 1981). A latent period of 24-28 h was found enough for CLCmV transmission (Brown and Nelson, 1987).

2.8 Sap transmission

In sap transmission, the Luffa:Del isolate of ToLCNDV was trans-mitted with greater efficiency by sap inoculation to both ridge gourd and sponge gourd. Sap transmission from ridge gourd to pumpkin, ridge gourd and sponge gourd resulted in 80 to 90% transmission, whereas no sap transmission was obtained in tomato which was infected up to 50 to 60% by whitefly inoculation by both isolates. Back inoculation from the sap and whitefly inoculated ridge gourd and tomato plants to ridge gourd seedlings was positive for ridge gourd but not of tomato (Sohrab, *et al*., 2014).

2.9 Molecular characterization of yellow mosaic begomoviruses.

2.9.1 PCR amplification of begomoviruses and Phylogenetic analysis.

Padidam *et al*. (1995a) collected Chilli plants exhibiting symptoms similar to tomato leaf curl disease from Punjab province of Pakistan and subjected to molecular analysis. The PCR product of 2.7kb corresponding to DNA-A component was cloned and sequenced. Sequence analysis showed 95% sequence identity with DNA-A of Tomato leaf curl New Delhi virus (ToLCNDV).

Sohrab *et al.* (2003) first reported association of Tomato leaf curl New Delhi virus with yellow mosaic disease of *Luffa cylindrica* in India, The causal virus was identified on the basis of whitefly transmission and sequence identity of putative coat protein (CP) and replication initiator protein (Rep) genes. Sequence analysis revealed that the CP and Rep genes of *Luffa* isolates had 100% similarity with ToLCNDV-Severe strain.

The complete nucleotide sequence of the PYVMV CP gene was determined and Sequence alignments showed the CP gene of PYVMV to be most closely related to that of ToLCNDV-[Svr] with 95% nucleotide sequence identity. Amongst the other begomoviruses from the Indian sub-continent, CP gene sequences of PYVMV showed 82% similarity with ToLCKV, 81% with ToLCBDV and less than 80% identical to the remaining begomoviruses (Muniyappa *et al.*,2003).

Sequence analysis of putative CP gene showed that the virus associated with the yellow mosaic disease of chayote occurring in India showed maximum sequence identity (95%) was found with ToLCNDV Pakistan isolate (AF448058), but shared only 70·7% identity with the uncharacterized Chayote yellow mosaic virus (ChYMV; AJ223191),

reported from Nigeria. This was thought to be first report of occurrence of a new yellow mosaic disease of chayote in India, associated with a begomovirus (Mandal *et al.*, 2004).

Ito *et al*. (2008b) collected the leaves from cucumber, bottle gourd and muskmelon plants showing typical yellow mosaic symptoms and subjected to molecular analysis. The amplified product of 2.7kb corresponding to DNA-A and DNA-B were cloned and sequenced. The obtained sequences were subjected to BLAST. The BLAST analysis showed that DNA-A shares 97% similarity with isolates of tomato leaf curl New Delhi virus (ToLCNDV) and thus named tomato leaf curl New Delhi virus-[Cucumber: Thailand] (ToLCNDV-[Cuc:Tha]). The sequences obtained from bottle gourd and muskmelon isolates shared more than 99% sequence identity with ToLCNDV-[Cuc:Tha].

Geminivirus, which causes yellow leaf curl disease of wax gourd plants, was detected using polymerase chain reaction with geminivirus-specific primers. The PCR product of 2.7kb corresponding to DNA-A component was cloned and sequened. The sequence analysis showed maximum sequence identity (94%) with that of DNA-A of *Squash leaf curl China virus*-Thailand (SLCCNV-TH). Based on sequence comparisons and phylogenetic tree analyses, the virus isolate from wax gourd plants were identified as a new strain of SLCCNV and named as *Squash leaf curl China virus* (Sopid *et al*., 2009).

Tiwari *et al*. (2010) reported a severe yellow mosaic disease on bitter gourd (*M. charantia*) in Eastern Uttar Pradesh. The total DNA isolated from infected leaf samples and PCR was carried out using begomovirus specific primers. The obtained amplified product of 800bp corresponding to coat protein gene of DNA-A cloned and sequenced. The sequence data analysis revealed highest 98-93% similarities with several isolates of Tomato leaf curl New Delhi virus (ToLCNDV) at both nucleotide and amino acid levels. The phylogenetic analysis also showed closest relationships of the isolate with various variant of ToLCNDV. Based on highest sequence similarities and closest relationships with ToLCNDV the virus isolated from bitter gourd was considered as an isolate of Tomato leaf curl New Delhi virus (ToLCNDV).

Pratap *et al*. (2011) reported the incidence of yellow mosaic disease on eggplant in central India. The total DNA was isolated from infected leaf sample and PCR carried out by using full length primers. The expected size of 2.7kb amplified products corresponding to DNA-A and DNA-B genomic components were cloned and sequenced. Nucleotide sequence analysis of DNA-A showed 97.6% identity with Tomato leaf curl New Delhi virus-India [India:Udaipur:Okra:2007] and lowest of 87.9% identity with Tomato leaf curl New Delhi virus- India[India:NewDelhi:Papaya:2005], while DNA-B showed highest 94.1% identity with ToLCNDV-IN[IN:UD:Ok:07] and lowest 76.2% identity with ToLCNDV-India [India:Lucknow].

Begomovirus associated with PYVMD was amplified by the PCR using the primer pairs P1F/ P1R. A product of 750 bp obtained from pumpkin samples were cloned into pDrive vector and sequenced. BLAST analysis showed high nucleotide identity to Squash leaf curl China virus (SLCCNV) (Accession No: DQ026296), Tomato leaf curl

New Delhi Virus (ToLCNDV) (Accession No: GQ225732) and Tomato leaf curl Palampur Virus (ToLCPMV) (Accession No: GQ225738) (Namrata *et al*., 2012).

Based on the highest sequence similarity of 97-93% at nucleotide level and 100-97% at amino acid level, the closest phylogenetic relationships was obtained with various isolates of Tomato leaf curl New Delhi virus. The isolate from Luffa (under study, EU439261) was considered as a variant of Tomato leaf curl New Delhi virus. However, isolate also shared 92% at nucleotide and 100% at amino acid similarity with Squash leaf curl China virus isolate, which indicate that isolate under study may be the recombinant of two begomovirus (ToLCNDV and SqLCCV) (Tiwari *et al*., 2012).

Tiwari et al. (2012), subjected *Luffa* plants exhibiting yellow mosaic and leaf curling disease symptoms to molecular analysis. A 800bp amplicons amplified by nested PCR was cloned, sequenced and sequence data was submitted in GenBank database (Accession no. EU439261). Based on highest 97% and 100% identities at nucleotide and amino acid levels, respectively and closest phylogenetic relationships with strains of Tomato leaf curl New Delhi virus (ToLCNDV), *L. cylindrica* begomovirus was considered as a variant of ToLCNDV.

The sequence comparison and phylogenetic analysis of the complete DNA genome revealed the association of Tomato leaf curl New Delhi virus (ToLCNDV) with the leaf curl of pumpkin (*Cucurbita moschata*) in northern India (Phaneendra *et al*., 2012).

The PCR amplified products of coat protein gene (750 bp) from Luffa and pumpkin isolates were separately cloned and sequenced. The BLAST result showed highest of 97.5% nucleotide identity with ToLCNDV (AY939926) and 96.1% with ToLCNDV-Luffa (AF102276) and 95.1% identity with ToLCNDV-[Svr] (U15015) and the lowest nucleotide identity of 55% with *Squash mild leaf curl virus* (Sohrab *et al*., 2014).

III. MATERIAL AND METHODS

In the present investigation, the following objectives were carried out *viz*., survey of yellow mosaic disease in ridge gourd and cucumber, host range, virus-vector relationship, partial characterization of virus. The details regarding the materials used and methodologies followed during the present study are presented in this chapter.

3.1 Survey

Surveys were carried out during the 2013-2014 to know the per cent disease incidence of yellow mosaic disease of ridge gourd and cucumber in Southern districts of Karnataka; Bengaluru rural, Chikkaballapur, Ramanagara and Kolar were surveyed during summer. The percentage of disease incidence was assessed by recording the number of plants showing disease symptoms out of total number of plants examined by using the formula mentioned below. The diseased samples collected during the survey were brought to used for transmission and virus culture was maintained on ridge gourd plants.

$$\text{Per cent disease incidence} = \left| \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \right| \text{X } 100$$

During the survey the additional informations such as, stage of the crop, area, varieties grown, symptoms and presence of whitefly were recorded.

3.1.1 Estimation of Per cent Disease Severity

During the survey, infected ridge gourd and cucumber leaf samples from each field were collected and the disease severity was estimated based on the per cent leaf area affected.

3.2 Transmission studies

3.2.1 Raising of healthy seedlings

Healthy seedlings of Ridge gourd and Cucumber required for various transmission studies were raised from seeds collected from healthy plants. The seedlings were raised in 4”x 6” polyethylene bags filled with soil and compost mixture in 2:1 proportion. These plants were kept in insect proof cages and used throughout the period of investigations.

3.2.2 Maintenance of YMV Culture

Ridge gourd and Cucumber plant samples with characteristic symptoms of yellow mosaic disease was collected from naturally infected plants in the fields during survey. Using whiteflies (*B. tabaci*) the virus cultures were transmitted to the healthy Ridge gourd plants. Whiteflies were released for virus acquisition for 24 h. They were later transferred to 8-10 days old healthy Ridgegourd and Cucumber seedlings in a glasshouse. The inoculation access period of 24 h was given on healthy seedlings. The virus cultures

were maintained in insect proof cages by periodically inoculation to healthy seedlings by *B. tabaci* and used for various studies.

3.2.3 Maintenance of whitefly culture and handling of whiteflies

The type culture of *B. tabaci* used for inoculation was maintained on cotton, *Gossypium hirsutum* cv. varalakshmi plants kept in insect proof wooden cages (45 x 45 x 30 cm) and muslin cloth was pasted on the sides and the top with fevicol. The front side was covered with glass, which could be moved easily in the grooves made in the wooden framework. Healthy cotton plants grown in polythene bags (4 x 6 cm) were introduced into the cages. The cages were maintained at temperature of 28^{o}C to 30°C in an insect proof polyhouse. The whiteflies colonized on the lower surface of young leaflets of cotton (Plate 1).

An aspirator made of a glass tube (30 x 0.5 x 40 cm) and a rubber tube of 40 cm length was used for collection of whiteflies. The leaves colonized with healthy whiteflies were turned slightly upwards and then were sucked in to the glass tube. Later, they were gently blown in to the plastic tubes. Such collected disease free whiteflies were used in this investigation.

3.2.4 Preparation of cages and tubes/bottles for acquisition access feeding by *B. tabaci*

Plastic or Polyvinyl chloride (PVC) bottles (20 x 7.5 cm) tapering towards the narrow mouth were taken, the bottom portion of the bottles was removed with the help of a soldering rod and they were covered with muslin cloth. The narrow mouth of the bottle was cut up to few centimeters above the screw cap and plugged with cotton to prevent flies escaping from the bottle during usage.

3.2.5 Preparation of cages/tubes for inoculation

Plastic tubes (7.5 x 2.5 cm) were taken and the bottom of the tube was removed with the help of a soldering rod. The bottom ends were sealed with a black muslin cloth to avoid accumulation of excess moisture inside the cage and also to provide aeration. A small hole (0.5 cm) was made in the middle portion of the tube to facilitate release of whiteflies. The open end of the tube was plugged with cotton after inserting young leaflets into the tube.

3.2.6 Collection of whiteflies

An aspirator made of a glass tube (30 x 0.5 x 40 cm) and a rubber tube of 40 cm length was used for the collection of whiteflies. The leaves colonized with healthy whiteflies were turned slightly upwards and the flies were sucked in to the glass tube. Later, they were gently blown in to the plastic tubes. Such collected virus free whiteflies were used in this investigation.

Plate 1: Maintenance of indigenous Whitefly colony on Cotton (1) and Bean (2)

Plate 2. Nylon net cage for raising healthy seedlings

Plate 3. Nylon net cages used for maintenance of inoculated plants for symptoms expression

Plate 4. Inoculation of seedlings using whiteflies

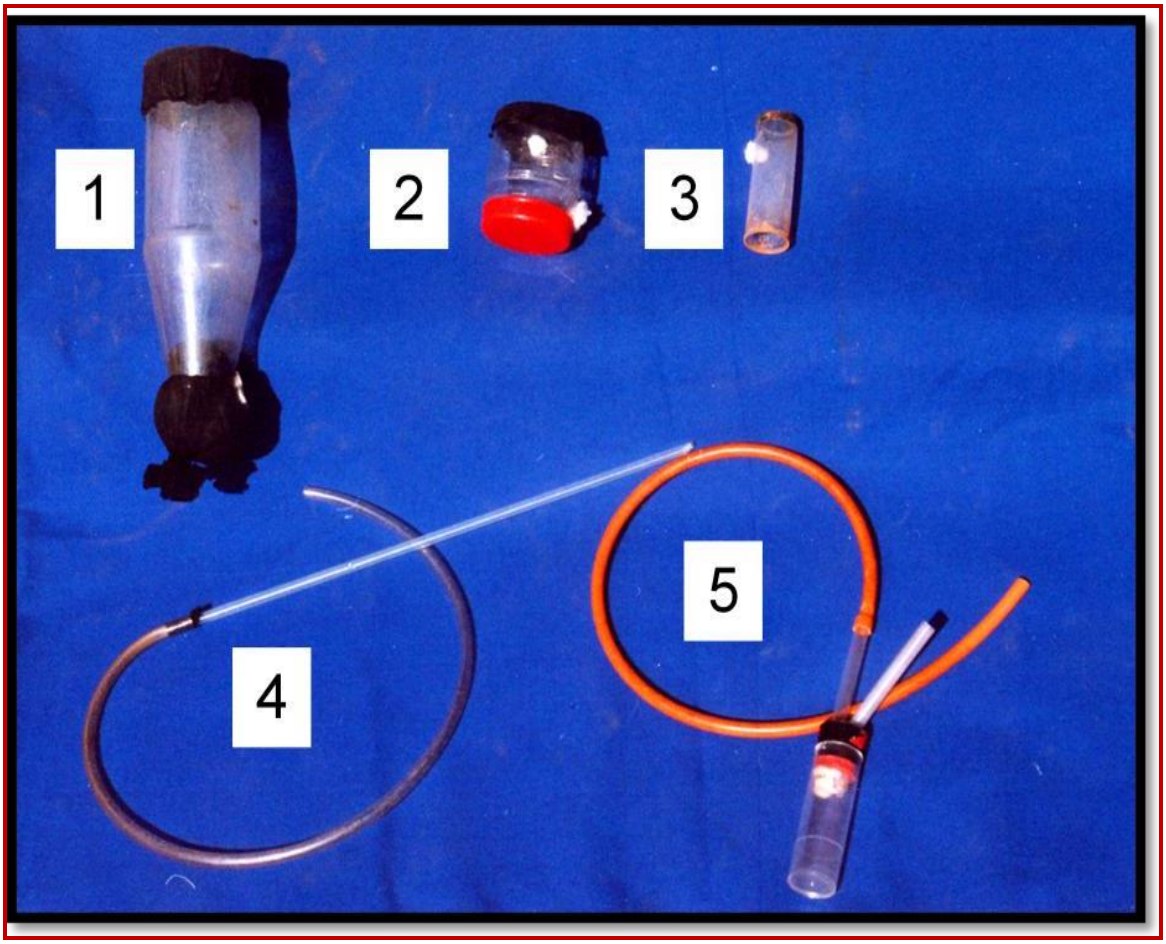


Plate 5. Materials used for transmission

1. Cage for acquisition
2. Cage for inoculation
3. Microtube used for inoculation
4. Lab aspirator
5. Field aspirator

3.2.7 Whitefly transmission

Relative transmission efficiencies of *B. tabaci* on transmission of YMV were determined. Adult whiteflies were collected from their respective cages in PVC bottle with the help of an aspirator and the YMV infected Ridge gourd or Cucumber twig was inserted inside the bottle. Whiteflies were allowed to feed for 24 hr and were then released at the rate of 10 whiteflies per seedling on the healthy Ridge gourd and Cucumber seedlings covered with cylindrical plastic cages (18 x 6 cm). After 48 hr IAP, whiteflies were removed from individual plants. The plants were sprayed with 0.03 per cent Imidachloprid and kept in insect-proof cages for symptom development. The experiment was repeated four times using 10 plants.

3.3. Virus vector relationship

Virus-vector relationship was determined only for ridge gourd isolate of YMV using *B. tabaci* maintained on cotton.

3.3.1 Minimum number of *B. tabaci* required for transmission

To determine the number of *B. tabaci* required for the successful transmission of YMV, non-viruliferous *B. tabaci* were given an AAP of 24 hr on YMV -infected plant separately. Viruliferous whiteflies were then transferred to 8-10 days-old young healthy ridge gourd seedlings at the rate of 1, 3, 5, 10, and 20 per seedling separately, and 10 plants were inoculated in each treatment. After an IAP of 24 hour, whiteflies were killed by spraying 0.03 per cent Imidachloprid. The plants were kept in an insect-proof glasshouse for symptom expression and per cent transmission was recorded.

3.3.2 Acquisition access period (AAP)

The effect of different AAP on the rate of transmission of YMV was tested by allowing *B. tabaci* to feed for 30 min, 1,6,12 and 24h on YMV infected plants separately. After the prescribed AAP, the whiteflies were transferred on to 8-10 days-old healthy ridge gourd seedlings at the rate of 10 whiteflies per plant. For each treatment 10 ridge gourd plants were inoculated. After 24 hr of IAP, insects were killed by spraying 0.03 per cent Imidachloprid. Plants were kept in the glasshouse for symptom development.

3.3.3 Inoculation access period (IAP)

To determine the influence of different IAP on transmission of YMV, *B. tabaci* were allowed for a 24 hr AAP on YMV-infected plants separately. Viruliferous whiteflies were then transferred to 8-10 days old ridge gourd seedlings for IAP of 30 min, 1,6,12 and 24 hr at the rate of 10 per seedling. Ten plants were inoculated for each treatment. Whiteflies were then killed by spraying 0.03 per cent Imidachloprid and plants were kept in an insect-proof glasshouse for symptom development.

3.3.4 Persistence of virus in vector

To determine the persistence of YMV in adult *B. tabaci*, the whiteflies were allowed for 24 h AAP on YMV infected ridge gourd plant. Then single whitefly was released on ridge gourd seedlings. The whiteflies were serially transferred to the healthy

ridge gourd seedlings 24 h intervals until the insects were alive in each case. After each IAP the plants were sprayed with 0.03 per cent Imidachloprid and kept in insect proof cages.

3.3.5 Incubation period in vector

To estimate the incubation period of YMV, the whiteflies were given a minimum acquisition access period of 1 h on infected Ridge gourd plant. Groups of 10 whiteflies were released on healthy ridge gourd seedlings after 30min, 1, 6, 12 and 24 hr inoculation access periods separately. Inoculated plants were kept in an insect proof glasshouse for symptom production.

3.4 Host range studies

Healthy seedlings of ridge gourd, cucumber plants and other plant species of family *Cucurbitaceae, solanaceae* and *leguminoceae* were raised in an insect proof glasshouse. Seedlings at the two leaf stage were inoculated with viruliferous indigenous whiteflies, *B. tabaci* after 24 h acquisition access feeding on yellow mosaic virus infected ridge gourd and cucumber plant. The viruliferous whiteflies were fed on healthy test seedlings for 24 h in insect proof tubes. The inoculated seedlings were kept in insect proof glasshouse for development of symptoms.

3.5 Molecular characterization of Yellow mosaic begomovirus

3.5.1 Collection of virus isolates

Diseased leaf samples of Ridge gourd and Cucumber plants showing symptoms of begomovirus infection were collected during survey. The leaves from the top of the plants that showing viral symptoms were selected for sampling. The samples were put in plastic bags, labelled and stored in -80^0C at virology lab, UAS, Bangalore. These samples were used for total DNA extraction and screened for the presence of begomovirus using begomovirus specific primers (Deng *et al*., 1994). The leaf samples were stored in $-80^{\mathbf{0}}$c before PCR analysis.

3.5.2 PCR Detection of yellow mosaic begomoviruses

Total DNA was extracted from the symptomatic leaf samples of ridge gourd and cucumber collected during survey. DNA was subjected to PCR using begomovirus specific primers (Deng *et al.,* 1994) to identify the presence of begomoviruses. These primers amplify from the origin of replication in the IR to part of the AV1 (CP) gene on DNA-A of most of the begomoviruses.

3.5.2.1 DNA extraction from host plants

Total genomic DNA of YMV virus was extracted by following CTAB (Cetyl Trimethyl Ammonium Bromide) method of Lodhi *et al*. (1994) modified by Maruthi *et al*. (2002a) as follows.

Table 2. Chemicals and their sources

S. No.	Materials	Source(s)
1	General chemicals and reagents	HiMedia Laboratories Pvt Ltd, India; Sigma Aldrich Chemicals Pvt Ltd, India
		Sisco Research Laboratories Pvt Ltd (SRL), Mumbai-400099, India
		NICE Chemicals, Kochi-682024, India
		Merck Limited, Mumbai-400018, India
		Qualigens Fine Chemicals, Mumbai-400030, India
2	PCR ingredients, Restriction enzymes, DNA markers	Fermentas Life Sciences, Germany
		Bioline Ltd, UK
		Sigma-Aldrich Inc, USA
		Chromous Biotech Pvt Ltd, India
		HiMedia Laboratories Pvt Ltd, India
3	Oligonucleotides	Bioserve Biotechnologies (India) Pvt Ltd, Hyderabad-500076, India
		Sigma Aldrich Chemicals Pvt Ltd, No. 12, Bommasandra-Jigani Link Road, Bangalore-560100, India

Table 3. Instruments used in the present study

Instrument	Manufacturer /Model
Autoclave	PSM vertical autoclave (PSM/VA-01), PSM Scientific Instruments (P) Ltd, Peenya III Phase, Bangalore-560058, India
Cooling Centrifuge	REMI® zcentrifuge, Remi Instrument Ltd, Mumbai, 400053, India (Cat. No. C-24 BL/1/2006)
Deep freezer (-200C)	Vestfrost®, Denmark
Deep freezer (-800C)	-800C ultra-low freezer (U41085), New Brunswick Scientific, Germany
Digital camera	Sony, DSC-W320, Sony Crop, Japan
Digital scanner	HP Scanner, China
Electrophoresis power pack	Biometra® P25 power pack Nr, Biomedizinische Analytik, GmbH, Germany
Electrophoresis unit	SCOTLAB (SL-H2-SET and SL-H3-SET), Anachem Ltd, Anachem house, Charles Street, Luton, Bedfordshire LU2 OBE, UK
Gel documentation unit	IMAGO Compact Imaging System, B & L Systems, Isogen Lifescience, The Netherlands
Gradient PCR thermal cycler	Eppendorf Mastercycler gradient, 22331, Hamburg, Germany
High speed centrifuge	Sigma® philip harris (1-15), Sigma, D-37520, Osterode am, Harz, Germany
Hot air oven	LBB mechanical convection hot air oven-Digital, Culture Instruments, Bangalore, India
Ice maker	SCOTSMAN® Faimont automatic ice machine (AF80AS-E 230/50/1)
Laminar air flow	SONAR® laminar air flow bench
Magnetic stirrer	REMI® (1MLH), Magnetic stirrer, Remi Instrument Ltd, Mumbai, 400053, India
Micropipettes	Eppendorf® Research, 22331, Hamburg, Germany
Microwave oven	LG (MC-7649 DW), LG Electronics India Pvt Ltd, India
Millipore water plant	MILLIPORE (TANK PE 030), MILLIPORE Elix 3 Century (BM7MN3074 E) and DIAPHRAGM PUMP, Millipore India, Bangalore-560058, India
pH meter	ELICO® (LI 610), India
Refregerator	LG (GL-244GP), LG Electronics India Pvt Ltd, India
Rocker	NEOLAB (2985 05), Neolab Instruments, Mumbai, India
Scanner	HP Scanjet 3500C, HP, China
Shaker incubator	LABLINE shaking Incubator, India
Spectrophoto meter	Cintra 5, UV visible spectrometer, GBC Scientific Equipment, Braeside VIC 3195, Australia
Top loading electronic balance	ANAMED® (MX-7301A), Anamed Instruments Pvt Ltd, D-165, T.T.C. Area, MIDC, Mumbai 400706, India
UV transilluminator	FOTO/UV21(30-3027), FOTODYNE Incorporated, USA
Vacuum dryer	Savant DNA Speed Vac® (Model- DNA 120)
Vertexer	CYCLO MIXER (CM 101), Remi Instrument Ltd, Mumbai, 400053, India
Water bath	Magni Whirl® constant temperature bath, Blue M Electric Company, Illinois, USA

DNA extraction buffer

Reagents

2 % (w/v) CTAB
1.4 M NaCl
20 mM EDTA
100 mM Tris- HCl (pH 8.0)
1% Sodium sulphite*
2% PVP-40*
0.2 % (v/v) ß - mercapto ethanol (add after autoclaving)

*****PVP and Sodium sulphite added fresh to aliquot of stock buffer (containing first four reagents immediately prior to extraction)

Procedure

1. Approximately 100 mg of diseased leaf tissue was placed into a thick-gauge plastic bag. The tissue was ground using a roller and mixed with 10 volumes (1 ml) of CTAB extraction buffer.
2. About 750 µl of the sample was poured into a 1.5 ml eppendorf tube and the samples were heated at 60°C for 30 min.
3. The samples were mixed with an equal volume (750 µl) of chloroform: isoamylalcohol (24:1) and centrifuged at 13000 rpm for 10 min.
4. The top aqueous phase was transferred into a new 1.5 ml eppendorf tube and DNA was precipitated by adding 0.6 volumes (300 µl) of cold (-20°C) isopropanol and incubated at –20°C for at least 1 h.
5. The samples were centrifuged at 13000 rpm at 4°C for 10 min and the supernatant was discarded.
6. The pellet was washed in 0.5 ml 70 per cent ethanol by vertexing and then centrifuged for 5 min at 13000 rpm.
7. The ethanol was removed and the pellet was vacuum dried for 5 min and the dried pellet was suspended in 100 µl 1x TE buffer and stored at –20°C.
8. All the DNA extracts were further diluted 10-fold in single distilled water (SDW) before using for PCR amplifications.

3.5.2.2 Quantification of DNA

Quantification of DNA by using the NanoDrop instrument

3.5.3 Amplification of viral DNA by PCR

Reagents

a. PCR buffer

200 mM Tris (pH 8.3)	-1.0 ml
500 mM KCI	-2.5 ml
0.01% Gelatin	-0.5 mg
H_20 (SDW)	-1.0 ml

b. dNTP mixture

Each 25µl of dATP, dCTP, dGTP and dTTP from a 100mM stock was mixed. The concentration of each dNTP in this mixture was 25 mM. Further the final concentration of each dNTP was made to 2.5 mM by diluting it by ten times.

c. Primers:

Two sets of oligonucleotide primers used for amplification of the coat protein of DNA-A fragment are as follows

Primer	Oligonucleotides	Reference
P1F	5'- ATGGCGAAGCGACCAGC - 3' (17 nt)	Namrata *et al*., 2012
P1R	5'- TTAATTTGTTACGCAA TCATA -3' (21nt)	
Deng A	5' TAATATTACCKGWKGVCCSC -3' (20 nt)	Deng *et al.,* 1994
Deng B	5'- TGGACYTTRCAWGGBCCTTCACA -3' (23 nt)	

d. Procedure

1. 0.5 ml eppendorf tubes were selected, labeled and kept on ice crystals.
2. Samples were taken for PCR along with positive control (CTAB extracted YMV of ridgegourd and cucumber DNA) and negative control (distilled water).
3. 25 µl PCR mixture was prepared by adding the following ingredients into the eppendorf tube.

Sterile distilled water	13.4 µl
10 x PCR buffer (Supplied with the enzyme)	2.5 µl
25 mM $MgCl_2$	1.0 µl
2.5 mM dNTP mixture	2.0 µl
Primer CP-F (20 mM)	2.0 µl
Primer CP-R (20 mM)	2.0 µl
Taq polymerase	0.1 µl
Viral DNA	2.0 µl
Total	25.0 µl

4. After preparing the cocktail, the DNA template was added and tubes were spun briefly. The PCR was performed in a thermal cycler (Techne Genius/ Eppendorf) using the following parameters:

Initial denaturation	94º C for 2 min	35 cycles
Denaturation	94º C for 1 min	
Annealing	55º C for 2 min	
Extension	72º C for 3 min	
Final extension	72º C for 10 min	

After the completion of the reaction the products were kept at 4^{o}C prior to gel analysis (Wyatt and Brown, 1996).

3.5.4 Analysis of PCR products by agarose gel electrophoresis

Reagents

- 10X TBE (Tris borate EDTA buffer, 1L): 108 g Tris base, 55 g Boric acid, 0.5 M EDTA (pH 8.0); volume was made up to 1 litre with distilled water and stored at RT.
- 6X Loading dye: 10 mM Tris HCl (pH 7.6), 0.03 per cent bromophenol blue, 0.03 per cent xylene cyanol, 60 per cent glycerol, 60 mM EDTA
- 1 per cent ethidium bromide stock solution (10 ml): 100 mg ethidium bromide was dissolved in 10 ml distilled water; Stored in dark bottle at 40C
- 1kb DNA ladder (Cat. No. SM0311, Fermentas Life Sciences, Germany, 60 µl): 10µl 1 kb ladder (0.5 µg/µl), 10µl 6X DNA loading dye, 40 µl deionized water.
- Six microlitres of loading mixture is required for an agarose gel lane.

Procedure

The gel casting tray was cleaned by washing and subsequent wiping with 70 per cent ethanol. Required volume of 1 per cent agarose was prepared by melting agarose in 1X TBE buffer (300 mg in 30 ml 1X TBE buffer) in a microwave oven. Once agarose solution was cooled up to 45-500C, ethidium bromide was added at the rate of 0.5 µg/ml of agarose. Gel casting tray was prepared with the desired number of wells and taping the ends. After comb was placed in the boat, molten agarose was poured in to the boat without forming air bubbles and allowed 30 min for proper solidification (polymerization). Five microlitres of each PCR product was separately mixed with 2 µl of loading dye on a clean polythene strip. After removing the tapes from the ends of the casting tray, tray was placed in the electrophoresis tank filled with 1X TBE buffer maintaining the buffer level 2 mm above the gel slab. The comb was removed carefully. Samples of PCR amplified products were loaded into wells of the gel along with 1 kb DNA ladder (Fermentas Life Sciences, Germany) and run at 90 V for 1hr until the dye front reached the other end of the gel. Gel was removed from the casting tray and visualized under UV and photographed in a gel documentation unit.

3.5.5 Elution of DNA from agarose gel using QIAquick gel extraction kit

DNA from agarose gel in TBE buffer was extracted and purified using QIAquick gel extraction kit (Cat. No. 28704; Qiagen, Germany) according to the instructions given by the manufacturer. PCR amplified product (about 50-100µl) containing desired fragment of PCR product was loaded into 0.8 per cent low melting agarose fresh gel in fresh buffer. The desired DNA fragment was excised from the agarose gel with a clean sharp scalpel while observing through UV illuminator at 360 nm. The gel slice was weighed in a 1.5-ml eppendrof tube. Three volumes of Buffer QG (300 µl buffer for each 100 mg gel weight) were added. The sample was then incubated at 500C in a water bath for 10 min until the gel slice completely dissolved with occasional mixing the tube 2-3 times during the incubation. One volume of isopropanol was added into the yellow colored mixture which was subsequently mixed properly by inverting the tube several times. The sample was loaded into a QIAquick spin column placed in a 2-ml collection tube and kept for 3-5 min. The spin column with mixture was centrifuged for 1 min at 13,000 rpm. The flow-through was discarded. The spin column was placed back in the same collection tube and centrifuged at 13,000 rpm for 1 min after adding 500 µl of Buffer QG into the column. 750µl of Buffer PE (or 70 per cent ethanol) was added into the column and kept for 5 min. The column was centrifuged for 1 min at 13,000 rpm. After discarding flow-through, column was centrifuged for an additional 1 min at 13000 rpm to remove traces of Buffer PE. The column was placed in a fresh 1.5-ml effendrof tube and dried for 5 min to remove any ethanol smell. 30µl of elution buffer (Buffer EB) was added carefully onto the center of the QIAquick membrane and incubated for 5 min at room temperature. The DNA was eluted by centrifuging the column at 13,000 rpm for 1 min. To increase the concentration of DNA, eluted DNA was again added onto the center of the QIAquick membrane of the same column and allowed to stand for another 3-5 min. The column was then centrifuged for 1 min at 13,000 rpm. The eluted DNA was stored at -200C until further use.

3.5.6 Sequence of eluted product

Eluted product sent to the National Centre for Biological Sciences, Sahakaranagar Bangalore, for the sequencing by Sanger's primer walking method.

3.5.7 Coat protein gene sequencing of YMV

Total DNA was extracted from the leaves of YMV infected ridge gourd and cucumber plants as described above. PCR reactions were set up using the primers of (P1F and P1R as described above) Namrata *et al*., (2012) that were designed to amplify ~760 bp fragment respectively from the complete CP gene of begomoviruses. PCR was performed in a 25-µl reaction mix containing 6.0 mM Tris-HCl, pH 8.8, 1.5 mM $MgCl_2$, 2.5 mM dNTPs, 20 µM primers, 2.5 U of *Taq* DNA polymerase and 10-15 ng DNA. Reactions were run in a Eppendorff thermal cycler programmed with the following amplification cycles:. 94^{o}C for 1 min denaturation, 60^{o}C for 20 sec. primer annealing, 72^{o}C for 30 sec. primer extension for one cycle followed by another 35 cycles of denaturation at 92^{o}C for 1 min denaturation, 60^{o}C for 20 sec. primer annealing, 72^{o}C for 30 sec. primer extension and finally at 72^{o}C for 10 min primer extension. The PCR product (approx. 760 bp) generated with primers P1F and P1R within complete CP gene

cloned and sequenced bi directionally to ensure sequence identity and reliability. Sequences obtained were highly reproducible in total 7 consensus complete CP gene sequence of YMV were obtained. The complete CP gene sequence length of 760 bp was used in the analyses, which were aligned using software package GENEDOC.

PCR products (approx. 750 bp) were separated on 1% agarose gels, purified using the gel extraction kit (QIAGEN, GmbH, Hilden, Germany) and cloned into the Plasmid TA^{TM} or pTZ57R/T cloning kit (MBI, Fermentas) following the manufacturer's instructions. The clones were sequenced using the ABI dye terminator kit in an automated sequencer (ABI Prism 377 DNA sequencer) following the dideoxynuleotide chain termination procedure (Sanger *et al*., 1977) Chromus Biotech Pvt. Ltd., Bengaluru, Karnataka, India**.** Clones were sequenced in both directions using the universal M13 forward and reverse primes to minimise sequencing errors.

3.5.8 Sequence analysis of coat protein gene of YMV

The sequences received from NCBS were aligned and joined together to get full length sequence using 'nucleotide blast' at basic blast programmes and 'align two (or more) sequences' at specialized blast programmes freely assessing in 'Basic Local Alignment Search Tool (BLAST)' at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). BLAST search was carried out at each step for confirmation of the correct orientation and position of sequences. The complete coat protein sequence was subjected to BLAST search. Sequences were compared with other respective viral sequences of the NCBI database using BLAST.

3.5.9 Phylogenetic analysis

Clones were sequenced in both directions using the universal forward and reverse primes to minimize sequencing errors. Sequences were assembled and analysed using Bioedit sequence alignment editor (version 5.0.9), multiple alignments were performed and a phylogenetic tree was constructed and bootstrapped using Neighbour Joining Algorithm of CLUSTAL W (version 1.7) (Higgins *et al*., 1996; Sujay Paul *et al.*, 2012), Mega6. The sequences were analyzed by parsimony methods (Swofford, 2002) using the software PAUP version 4.0 b10 for Mac, and finally displayed, manipulated and printed using Treeview software (version 1.6.6). Sequences of other begomoviruses and associated betasatellites used for comparison were taken from the NCBI database (http://www.ncbi.nlm.nih.gov/).

The heuristic method of search with 60% confidence levels was employed for reconstructing phylogenetic trees with 100 bootstrap replicates. The identity of the sequences was confirmed by BLAST search analysis.

Table 4. List of begomoviruses used in the phylogenetic analysis for comparision with yellow mosaic begomovirus coat protein gene sequences

Geminivirus species used	Abbreviation	Gene bank accession No
Afrcan cassava mosaic virus-Uganda severe	ACMV-UGSvr	AF126802.1
Cooton leaf curl Rajasthan virus	CLCuRV	AF363011.1
Cucurbit leaf curl virus	CuLCuV	AF224760.1
Maize streak virus-A[South Africa]	MSV-A[SA]	Y00514.2
Pumpkin yellow vein mosaic virus-[Jessore] AV2 gene, isolate 2	PYVMV-Jes	
Solanum yellow leaf curl virus partial cp gene for coat protein	SYLCV	AM040437.1
Sri Lankan cassava mosaic virus-[Colombo] DNA-A, complete genome, isolate SLCMV-Col	SLCMV-Col	AJ314737.1
Tobacco leaf curl Kochi virus-[KK]	TbLCK-[KK]	AB055009.1
Tomato golden mosaic virus-yellow vein	TGMV-Yv	K02029.1.1
Tomato leaf curl Bangalore virus	ToLCBV	Z481182.1.1
Tomato leaf curl Bangalore virus-[Ban4]	ToLCBV-[Ban4]	AF165098.1
Tomato leaf curl Bangalore virus-[Ban5]	ToLCBV-[Ban5]	AF295401.1
Tomato leaf curl Bangalore virus-[Kolar]	ToLCBV-[Kol]	AF428255.1
Tomato leaf curl Gujarat virus	ToLCGV	AY190291.1
Tomato leaf curl New Delhi virus isolate Almeria 661	ToLCNDV-Almeria 661	KF749223.1
Tomato leaf curl New Delhi virus isolate Murcia 11.1	ToLCNDV-Murcia 11.1	KF749225.1
Tomato leaf curl New Delhi virus isolate -Spain-Almeria	ToLCNDV-Spain-Almeria	KF891468.1
Tomato leaf curl New Delhi virus-[Pumpkin:New Delhi]	ToLCNDV-Pumpkin:New Delhi	AM286433.1
Pumpkin yellow vein mosaic virus coat protein gene	PYVMV-CP	AY686500.1
Tomato leaf curl New Delhi virus-Mild coat protein	ToLCNDV-Mild	U15016.1
Tomato leaf curl New Delhi virus from potato	ToLCNDV-Potato	DQ272541.1
Tomato leaf curl New Delhi virus-Severe	ToLCNDV-Severe	U15015.2
Tomato leaf curl New Delhi virus isolate India:UP:Bahraich:Luffa cylindrica:	ToLCNDV-UP:Luffa cylindrica	KC207815.1
Tomato leaf curl New Delhi virus isolate-AVT1	ToLCNDV-AVT1	AY428769.1
Tomato leaf curl virus AV1 gene-isolate 26	ToLCNDV-AV1-Isolate 26	AJ810365.1
Tomato leaf curl Philippines virus	ToLCPV	AB050597.1

IV. EXPERIMENTAL RESULTS

The results of the various experiments carried out on survey, incidence, host range, transmission efficiency, and molecular characterization are presented below.

4.1 Survey for the incidence of Yellow mosaic virus disease on ridge gourd and cucumber

Surveys were carried out in major cucurbits growing areas of southern districts of Karnataka viz., Bengaluru rural, Ramanagara, Kolar and Chikkaballapur during June, 2013 to March, 2014. The details of places surveyed, age of the crop, area, variety grown and per cent disease incidence in the field based on symptoms are presented in Table 5 and 7.

The symptoms noticed on yellow mosaic virus infected ridge gourd and cucumber plants were; mild to severe yellow mosaic, chlorosis, leaf curling, mottling, networking of yellow veins, followed by thickening of veins and vein lets, puckering, leaf distortions, stunting of whole plant, misshapen fruits and reduced fruit yield (Plate 6,7,8 ,9 and 10).

4.1.1 Incidence of Yellow mosaic virus disease on ridge gourd

The incidence of yellow mosaic virus (YMV) disease on ridge gourd was ranged from 37.5 to 86.0 per cent (Table 5). Among the districts surveyed, highest disease incidence recorded in Chikkaballapur district (67.098%), followed by Kolar (60.278%), Bangalore (57.09%) and Ramanagar district (49.88%) (Table 6 and fig. 1).

In Bangalore district the incidence of yellow mosaic disease on ridge gourd varied from 47 to 68.2% (Table 5). Incidence of disease on ridge gourd was highest in Nelamangala taluk (59.50%) and lowest in Hoskote taluk (53.3%) (Table 6).

In Ramanagar district the incidence of disease on ridge gourd varied from 37.5 to 72% (Table 5). Among the 4 different taluks surveyed, the incidence of disease recorded highest in Bidadi taluk (61%) and lowest in Channapattana taluk (43.5%) (Table 6).

The incidence of disease in Kolar district on varied from 55.2 to 68.5% (Table 5). Among the 5 different taluks of Kolar surveyed, the incidence of yellow mosaic on ridge gourd was highest in Kolar taluk (63%) and lowest in Bangarpet taluk (58.86%) (Table 6).

In Chikkaballapur district, the incidence of disease on ridge gourd varied from 55.3 to 86% (Table 5). Chikkaballapur taluk recorded highest disease incidence of 69.2% and Chintamani taluk recorded lowest incidence of 62.2% (Table 6).

4.1.2 Incidence of Yellow mosaic virus disease on cucumber.

The incidence of yellow mosaic virus (YMV) disease on cucumber in southern Karnataka ranged from 31.0 to 78.5 per cent (Table 7). Among the districts surveyed, the highest disease incidence of YMV on cucumber was recorded in Kolar (63.64%)

Table 5. Incidence of yellow mosaic virus disease of ridge gourd in Bengaluru, Ramanagara, Kolar and Chikkaballapur districts

District	Taluk	Village	Age of the crop (days)	Area (acre)	Variety grown	Average disease incidence (%)
Benguluru	Devanahalli	Yaluvahalli	50	1.5	Arka sumeet	50.0
		Mandibele	40	1.0	Naga	58.0
		Boodihal	55	1.0	MHRG7	63.2
	Doddaballapur	Konaghatta	45	0.5	Naga	53.6
		Dodda Belavangala	30	2.0	MHRG7	68.2
		Halekote	40	0.5	Arka sumeet	52.0
	Hoskote	Magabala	50	0.5	Jindian	53.0
		Manchappanahalli	45	1.0	Naga	60.0
		Thavekare	25	1.0	Naga	47.0
	Nelamangala	Tavarekere	55	0.5	Local	55.2
		Byranhalli	40	1.5	Akshay	65.0
		Thymagandlu	30	1.0	Naga	58.5
Ramanagara	Ramanagara	Abburu	35	1.0	Naga	37.5
		Dasawara	45	1.0	Arka sumeet	55.9
	Magadi	Bilagumba	40	1.0	Naga	54.6
		Sathnur	35	0.5	MHRG7	42.0
	Channapattana	Kali Hosure	50	1.0	Arka sumeet	42.0
		Doddamallur	60	1.5	Arka sumeet	45.0
	Bidadi	Hejala	35	1.0	Naga	72.0
		M.Karenahalli	40	1.0	Arka summet	53.0
		K.Karenahalli	50	1.0	Naga	58.0
Kolar	Bangarpet	Chattakamadenahalli	40	1.5	Akshay	65.0
		Bethamangala	50	0.5	Akshay	55.2
		Kavaraganahalli	40	1.0	Arka sumeet	56.4
	Malur	Huralagere	50	1.5	MHRG7	59.4
	Kolar	Vakleri	55	0.4	Arka sumeet	56.4
		Baterhalli	50	1.5	MHRG7	68.5
		Hudukulla	60	0.8	Local	64.3
	Srinivaspura	Laxmipura	35	1.0	Arka sumeet	62.9
		Kolature	45	0.8	Akshay	55.2
		Neeltur	35	1.5	Naga	62.2
	Mulbagal	Tayalure	40	1.5	Naga	68.0
		Vaddahalli	50	1.5	Arka sumeet	52.1
Chikkaballapur	Chintamani	Tupahalli	45	1.2	Naga	53.6
		Kamatanapalli	50	2.0	Naga	70.9
	Chikkaballapur	Manchenahalli	50	2.0	MHRG7	68.0
		Upparahalli	55	1.5	MHRG7	86.0
		Kanaganakoppa	50	2.0	Arka sumeet	55.3
	Sidlaghatta	Chikadasahalli	35	1.0	Naga	68.0
		Marappanahalli	55	1.5	Arka sumeet	72.7
		Handighalli	45	2.0	Jindian	66.0
	Bagepalli	Kothakote	50	1.0	MHRG7	66.9
	Gauribidanur	Karekallahalli	55	0.5	Arka sumeet	72.3
		kalludi	60	1.5	MHRG7	64.9
		Kaderpalli	50	1.5	Arka sumeet	67.5

Table 6. Average incidence of yellow mosaic virus disease on ridge gourd in 4 districts of southern dry zone of Karnataka

District	Taluk	Average disease incidence (%)	Average disease incidence of a district (%)
Bengaluru rural	Devanahalli	57.60	57.09
	Doddaballapur	57.93	
	Hoskote	53.33	
	Nelamangala	59.50	
Ramanagar	Ramanagar	46.70	49.88
	Magadi	48.33	
	Channapattana	43.50	
	Bidadi	61.00	
Kolar	Bangarpet	58.86	60.28
	Malur	59.40	
	Kolar	63.00	
	Srinivaspura	60.10	
	Mulbagal	60.03	
Chikkaballapur	Chintamani	62.26	67.09
	Chikkaballapur	69.20	
	Sidlaghatta	68.90	
	Bagepalli	66.90	
	Gowribidanur	68.23	

followed by Chikkaballapur (56.702%), Bangalore (55.77%) and Ramanagar district (49.69%) (Table 8 and Fig 2).

The incidence of yellow mosaic virus (YMV) disease on cucumber in Bangalore district varied from 31 to 68% (table 7). Incidence of yellow mosaic disease on cucumber was highest in Doddaballapur taluk (63.46%) and lowest in Hoskote taluk (49.06%) (Table 8).

In Ramanagar district the incidence of disease on cucumber ranged from 35.6 to 70% (Table 7). Highest Incidence of the disease was recorded in Bidadi taluk (54.33%) and lowest in Ramanagara taluk (44.53%) (Table 8).

In Kolar district the incidence of disease on varied from 45.8 to 78.5% (table 7). The survey was carried in 5 different locations and incidence of yellow mosaic disease on cucumber was highest in Kolar taluk (68.7%) and lowest in Srinivaspura taluk (60.5%) (Table 8).

In Chikkaballapur district the incidence of disease on varied from 39.4 to 68.8% (table 7). Incidence of the disease was recorded highest in Bagepalli taluk (63.3%) and lowest in Chintamani taluk (51.7%) (Table 8).

4.2 Biological characterization by determining the host range and virus-vector relationship

4.2.1 Transmission of yellow mosaic virus:

4.2.1.1 Mechanical transmission

The yellow mosaic virus infected ridge gourd and cucumber leaves were mechanically transmitted experimentally by sap inoculation technique to different cucurbit seedlings in glass house. None of the isolates of cucurbits were sap transmissible to other cucurbits.

4.2.1.2. Vector transmission

The indigenous whitefly, *B. tabaci* was used to test the potentiality in transmitting the yellow mosaic virus isolates from ridge gourd and cucumber. The vector was allowed to acquire the virus for 24 hours and inoculation period given was 24 hours. The rate of transmission of yellow mosaic virus disease was 100 per cent when 24 h AAP and IAP were given. The time taken for symptom expression was between 7 to 15 days after inoculation.

4.2.1.2.1 Symptomatology

The leaves from infected ridge gourd and cucumber plants showing typical yellow mosaic symptoms were collected during field survey and were inoculated to healthy ridge gourd and cucumber plants, respectively through whitefly, *B.tabaci*. One-week old seedlings, when inoculated by viruliferous whiteflies, developed symptoms in 8-12 days after inoculation.

Table 7. Incidence of yellow mosaic virus disease of cucumber in Bengaluru, Ramanagara, Kolar and Chikkaballapur districts

District	Taluk	Village	Age of the crop (days)	Area (acre)	Variety grown	Average disease incidence (%)
Benguluru	Devanahalli	Mandibele	80	1.0	IIHR177-1	51.0
		Yaluvahalli	75	1.0	Sedona	58.3
	Doddaballapur	Haniyur	100	1.5	Mullu southe	72.2
		Kakolu	90	0.5	Local white	68.0
		Sonneppanahalli	85	0.5	Dharwad green	56.2
	Hoskote	Magabala	75	1.0	Mullu southe	60.0
		Manchappanahalli	70	0.5	Local white	56.2
		Thavekere	60	1.0	IIHR177-1	31.0
	Nelamangala	Byranhalli	78	0.5	Local white	48.2
		Tavarkere	65	1.0	Dharwad green	55.0
		Thymagandlu	70	1.0	Tasty	64.5
Ramanagara	Ramanagar	Dasawara	95	1.0	Sedona	53.4
		Abburu	90	0.5	Sedona	35.6
	Magadi	Hullenhalli	75	0.5	Tasty	52.0
		Sathnur	55	1.0	Dharwad green	49.0
	Channapattana	Kali Hosure	70	0.5	Mullu southe	49.4
	Bidadi	Hejala	100	1.0	Mullu southe	70.0
		M.Karenahalli	85	1.0	IIHR177-1	40.0
		K. Karenahalli	70	0.5	Sedona	53.0
Kolar	Bangarpet	Kamasandra	75	1.5	Mullu southe	72.0
		Bethamangala	80	1.5	IIHR177-1	45.8
		Kavaraganahalli	85	1.0	Local green	68.2
	Malur	Hanumantapura	70	0.5	Dharwad green	64.1
	Kolar	Vakleri	95	1.2	IIHR177-1	78.5
		Baterhalli	75	1.0	IIHR177-1	66.8
		Hudukulla	85	1.4	Mullu southe	70.8
	Srinivaspura	Kolature	100	1.2	IIHR177-1	69.2
		Laxmipura	95	0.8	IIHR177-1	63.3
		Neeltur	60	1.0	Local green	49.2
	Mulbagal	Tayalure	85	1.0	Dharwad green	64.8
		Hemmenatha	70	0.5	Local	61.0
Chikkaballapura	Chintamani	Ramakunte	85	1.0	Mullu southe	55.5
		Gadidasarahalli	80	08	Sedona	39.4
		Kamatanapalli	95	1.0	Dharwad green	60.2
	Chikkaballapur	Manchenahalli	95	1.0	IIHR177-1	63.0
		Upparahalli	80	0.6	Sedona	43.0
		Kanaganakoppa	90	1.0	Sedona	52.3
	Sidlaghatta	Marappanahalli	65	1.0	Dharwad green	59.7
	Bagepalli	Kothakote	80	1.2	Sedona	58.2
		TB Cross	90	2.0	Mullu sothe	68.8
	Gauribidanur	Karekallahalli	85	0.8	Local white	59.3
		Kalludi	75	2.0	IIHR177-1	45.9
		Kaderpalli	50	1.5	Sedona	62.8

Table 8. Average incidence of yellow mosaic virus disease on Cucumber in four districts of southern dry zone of Karnataka

District	Taluk	Average disease incidence (%)	Average disease incidence of a district (%)
Bengaluru rural	Devanahalli	54.66	55.77
	Doddaballapur	63.46	
	Hoskote	49.06	
	Nelamangala	55.90	
Ramanagar	Ramanagar	44.53	49.69
	Magadi	50.5	
	Channapatana	49.4	
	Bidadi	54.33	
Kolar	Bangarpet	62.0	63.64
	Malur	64.1	
	Kolar	68.7	
	Srinivaspura	60.50	
	Mulbagal	62.9	
Chikkaballapura	Chintamani	51.7	56.702
	Chikkaballapur	52.76	
	Sidlaghatta	59.75	
	Bagepalli	63.3	
	Gowribidanur	56.0	

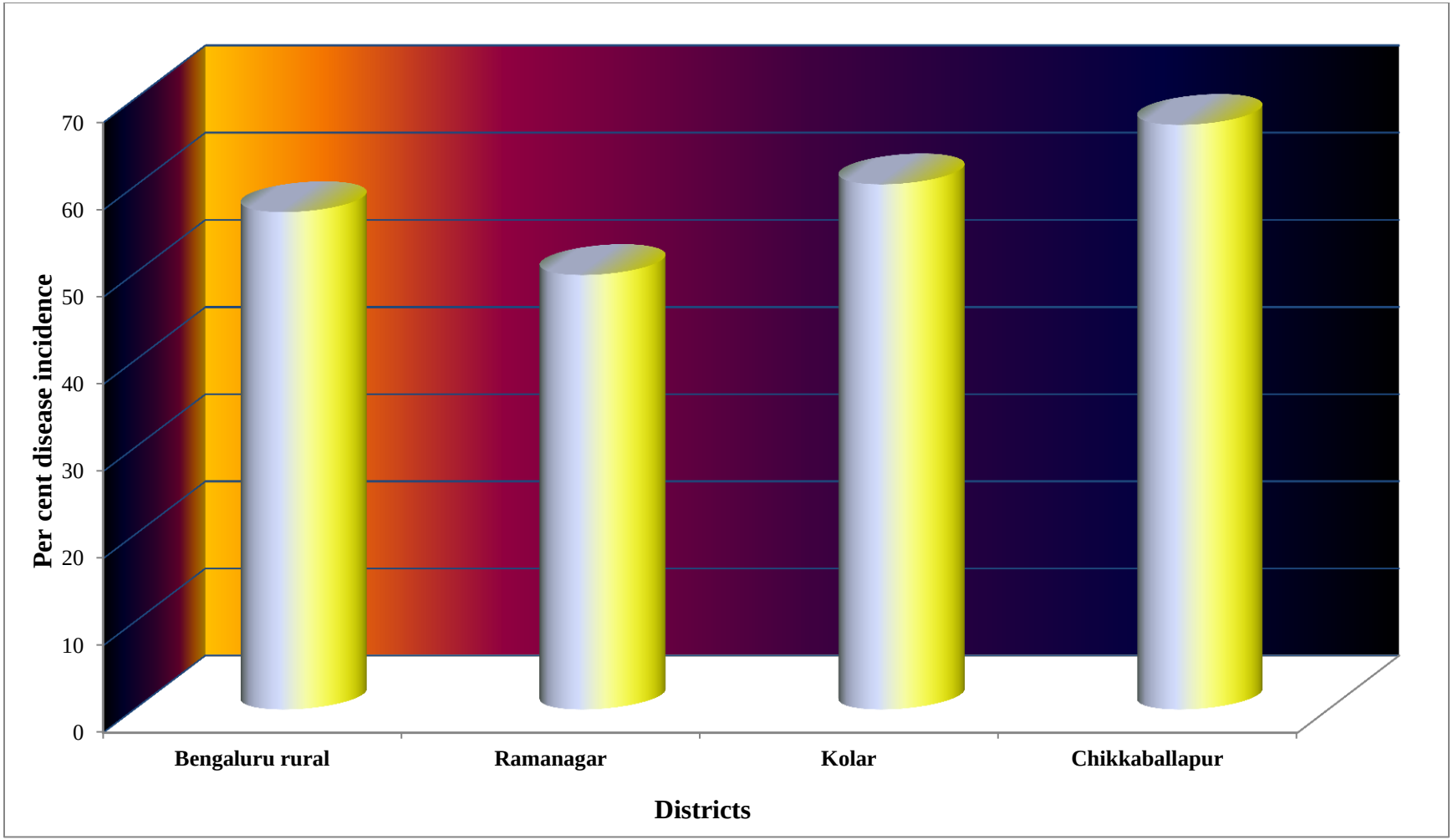


Fig. 1. Average incidence of yellow mosaic virus disease on ridge gourd in Bengaluru, Ramanagara, Kolar and Chikkaballapur districts

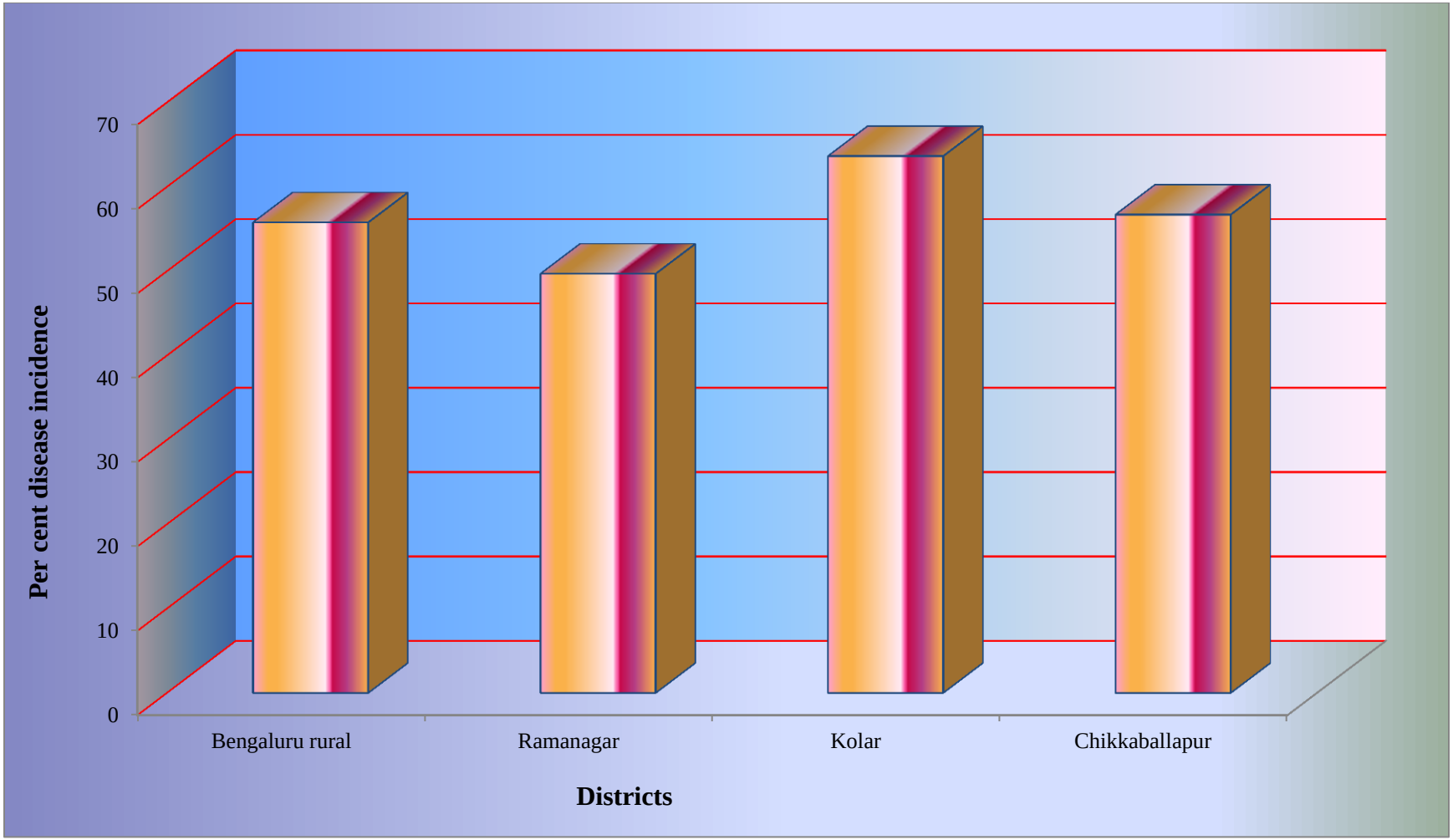


Fig. 2. Average incidence of yellow mosaic virus disease on cucumber in Bengaluru, Ramanagara, Kolar and Chikkaballapur districts.

Plate 6. Field view of ridge gourd crop infected with yellow mosaic disease in Chikkaballapur district

Plate 7. Field view of ridge gourd crop infected with yellow mosaic disease in Kolar district

Plate 8. Different types of symptoms of YMV on ridge gourd

Development of yellow spots on leaves (A),
Yellow mosaic with green and yellow patches(B),
Yellow mottling (C),
Downward leaf curling (D),

Complete chlorosis of leaves with severe yellow mosaic symptoms(E),

Mishapen fruits(F).

Plate 9. Field view of cucumber crop infected with yellow mosaic disease in Kolar district

Plate 10. Field view of cucumber crop infected with yellow mosaic disease in Chikkaballapur district

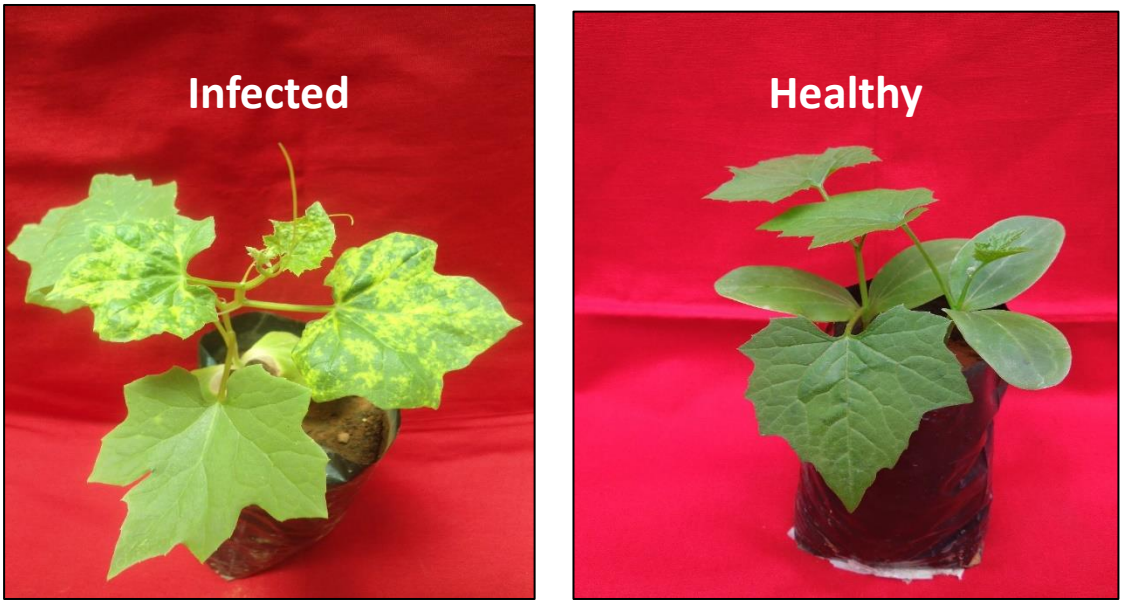


Plate 11. **Ridge gourd seedling inoculated with YMV by whitefly showing chlorotic yellow patches**

Plate 12. **Cucumber plants inoculated with YMV using whiteflies showing yellow mosaic and upward leaf curling symptoms.**

Yellow mosaic virus inoculated ridge gourd developed initial symptom of yellow mosaic disease as small chlorotic specks after eight days of inoculation with mild mosaic sympyoms (Plate 11). As the disease progresses, yellow mosaic with puckering was observed 12 days after inoculation. After 20 days, the diseased plant was stunted with complete chlorosis of leaves. Cucumber plant showed upward leaf curling, mottling and yellow mosaic symptoms on inoculation of yellow mosaic virus-cucumber isolate by *B. tabaci* (Plate 12).

4.2.2 Virus-vector relationships

The virus transmission characteristics were determined only for the yellow mosaic virus-ridge gourd isolate using indigenous whitefly *B. tabaci* vector.

4.2.2.1 Determination of number of whiteflies required for virus transmission.

To ascertain the minimum number of *B. tabaci* required for efficient transmission, different groups of whiteflies (eg. 1, 3,5,10 and 20) per plant were used for virus inoculation. Plants were enclosed on test plants with AAP and IAP of 24h each. Single adult whitefly could able to transmit the yellow mosaic virus with 20 per cent efficiency. The transmission efficiency increased to 40 and 80 per cent when three and five whiteflies were inoculated to healthy ridge gourd plants, respectively. Transmission efficiency was 100 per cent with ten or more whiteflies per plant (Table 9 and fig. 3).

4.2.2.2 Acquisition access period

A group of 10 non-viruliferous adult whiteflies were allowed to feed on YMV infected leaves for 30 min to 24h. The whiteflies were then enclosed on healthy plants for 24h IAP to estimate the efficiency of AAP of *B. tabaci*. A minimum AAP of 30 min was necessary for whiteflies to aquire the yellow mosaic virus, which resulted in 20 per cent transmission. An AAP of 1h and 6h resulted in 30 and 60 per cent transmission, respectively. An AAP of at least 12h and 24h was required for 100 per cent transmission. The number of days taken for symptoms expression varied from 7 to 23 days depending upon period of acquisition. Results of this experiment also revealed that the percentage of transmission increased with the increase in AAP (Table 10 and fig. 4).

4.2.2.3 Inoculation access period

A group of 10 viruliferous adult whiteflies were allowed for inoculation of yellow mosaic virus. The inoculation period ranged from 30 min to 24h. Viruliferous whiteflies required a minimum IAP of 30 min to achieve 20 per cent transmission efficiency. An IAP of 1h and 6h resulted in an increased transmission efficiency of 40 and 60 per cent, respectively. An IAP of 12h or more resulted in 100 per cent transmission. The days taken for symptoms expression varied from 8 to 23 days when 10 viruliferous whiteflies per plant were used depending upon the IAP. The results also indicated that percentage transmission increased with the increase of IAP (Table 11 and fig. 5)

The hundred per cent transmission of yellow mosaic virus in was obtained with an optimum AAP, IAP and number of whiteflies in 12 h, 12 h and 10 whiteflies respectively (Table 12).

Table 9. Determination of minimum number of viruliferous indigenous whiteflies, *B. tabaci* required for transmission of yellow mosaic virus.

No. of viruliferous whiteflies used for transmission	No of plants infected out of 10 inoculated plants	Per Cent transmission	No of Days taken for Symptom development
1	2	20	10-30
3	4	40	8 -20
5	8	80	8-15
10	10	100	8-15
20	10	100	8-15

AAP: 24 hrs
IAP: 24 hrs

Date of sowing: 02/02/14
Date of inoculation: 11/02/14

Table 10. Effect of different Acquisition access periods (AAP) on transmission of ridge gourd yellow mosaic virus disease through indigenous whitefly, *Bemisia tabaci*.

Period of aquisition	No of plants infected out of 10 inoculated plants	Per Cent transmission	No of Days taken for Symptom development
30min	2	20	8-20
1 hour	3	30	8-15
6 hour	6	60	8-15
12 hour	10	100	8-15
24 hour	10	100	8-15

Average no. of viruliferous whiteflies used per plant: 10
IAP: 24 hrs

Date of sowing: 10/01/2014
Date of inoculation: 18/01/2014

Table 11. Effect of different Inoculation access periods (IAP) on transmission of ridge gourd yellow mosaic virus disease through indigenous whitefly, *Bemisia tabaci*

Period of Inoculation	No of plants infected out of 10 inoculated plants	Per Cent transmission	No of Days taken for Symptom development
30min	2	20	8-23
1 hour	4	40	8-20
6 hour	6	60	8-15
12 hour	10	100	8-15
24 hour	10	100	8-15

Average no. of viruliferous whiteflies used per plant: 10
AAP: 24 hrs

Date of sowing: 19/01/2014
Date of inoculation: 27/01/2014

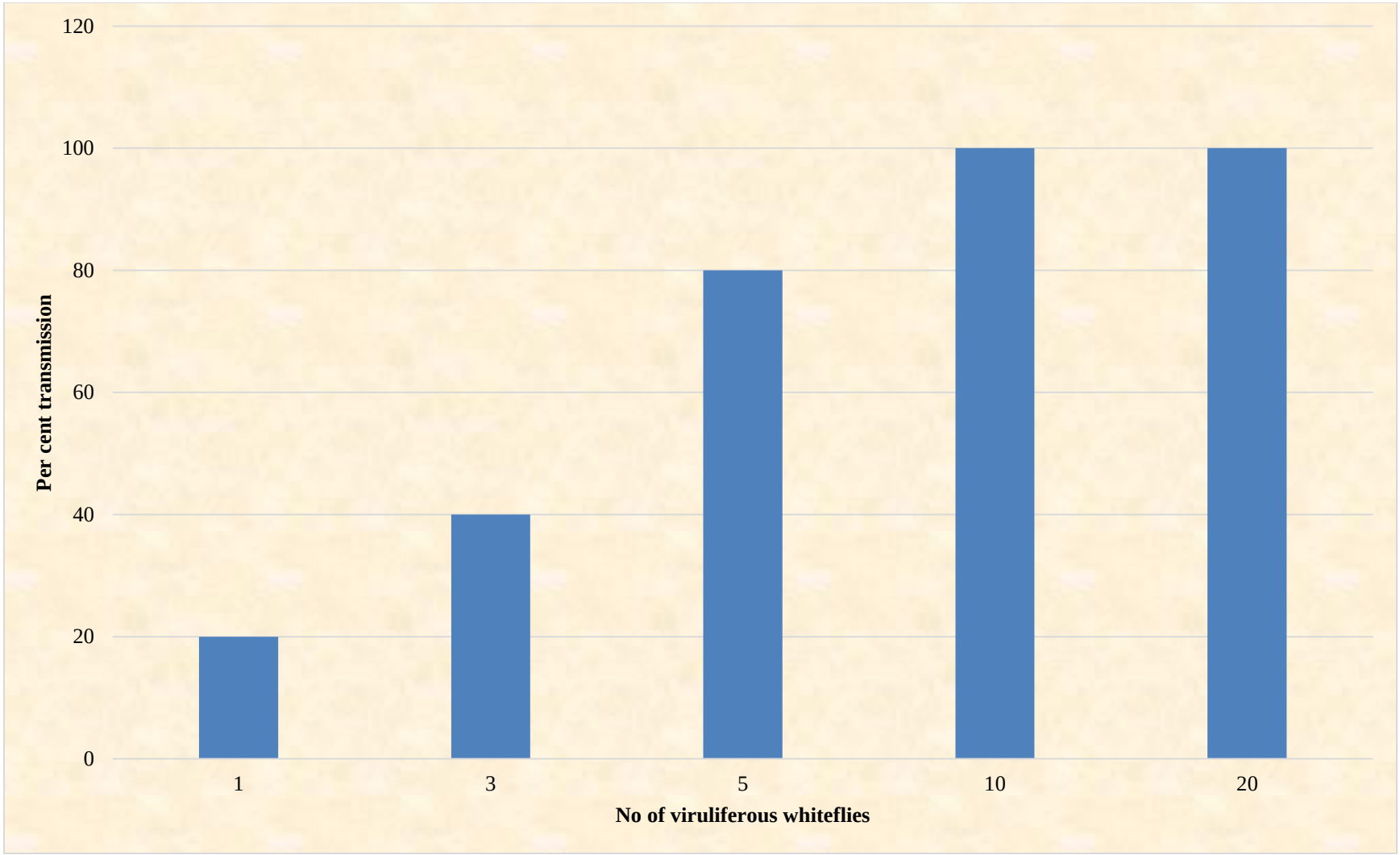


Fig 3. Effect of number of *Bemisia tabaci* on transmission of yellow mosaic virus disease of ridge gourd

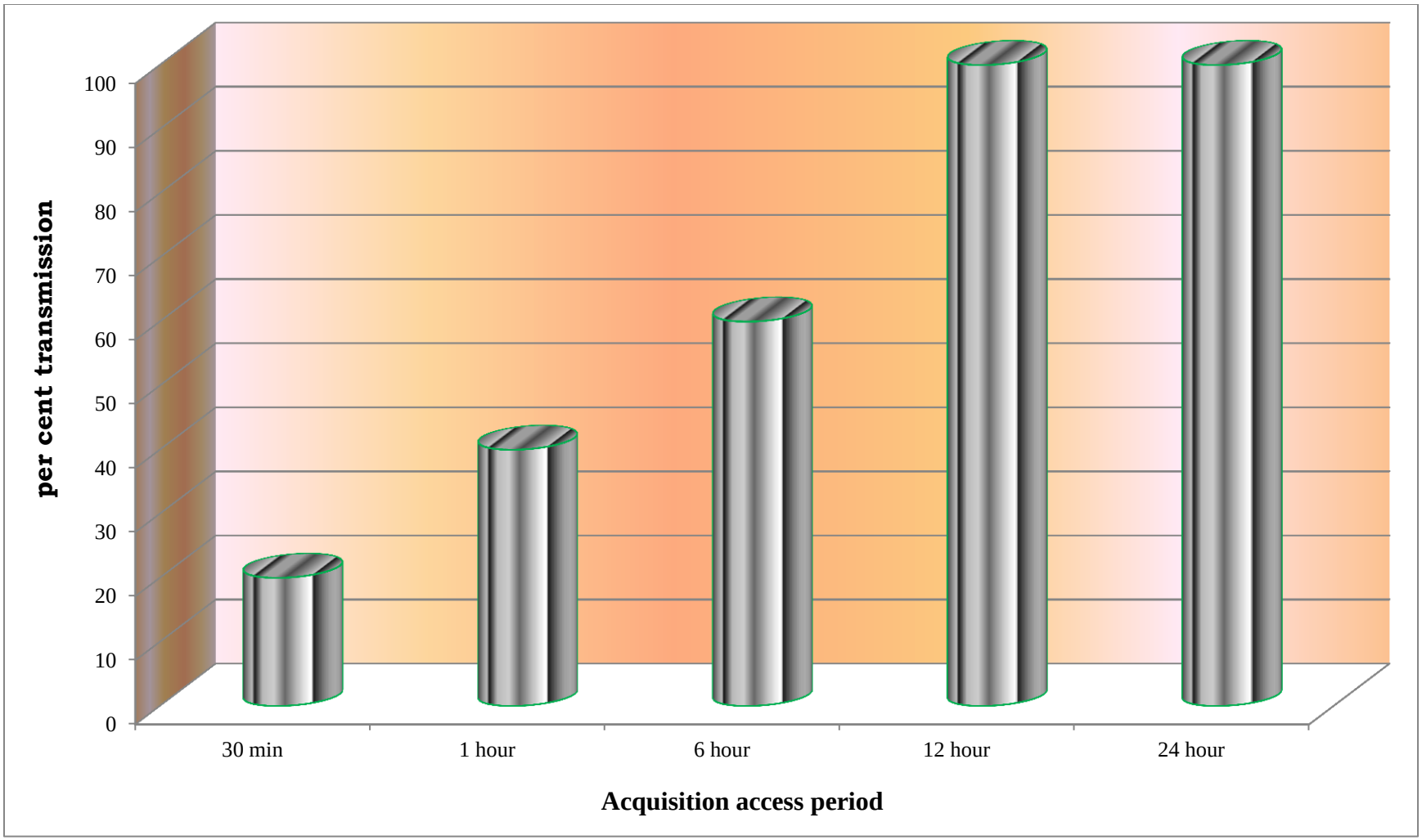


Fig 4. Effect of different Acquisition Access Period (AAP) on transmission of yellow mosaic virus disease of ridge gourd through *Bemisia tabaci*

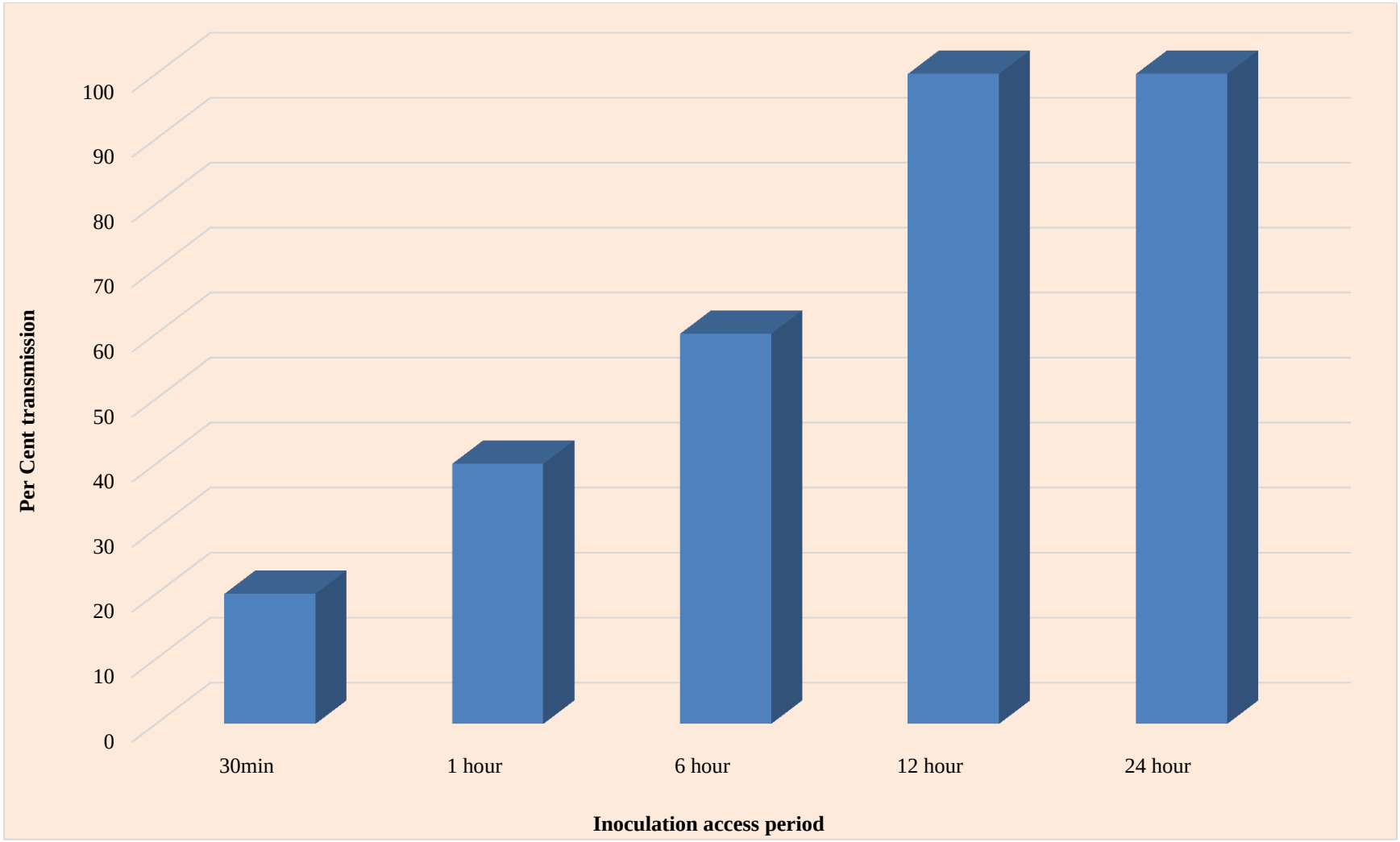


Fig 5. Effect of different Inoculation Access Period (IAP) on transmission of yellow mosaic virus disease of ridge gourd through *Bemisia tabaci*

Table 12. Ridge gourd yellow mosaic virus transmission characters

Acquisition Access Period (AAP)			Inoculation Access Period (IAP)			No. of viruliferous whiteflies		
Period of acquisition	Per cent transmission*	No. of days taken for symptom expression	Period of Inoculation	Per cent transmission*	No. of days taken for symptom expression	No. of viruliferous whiteflies used for transmission	Per cent transmission*	No. of days taken for symptom expression
30 min	20	8- 20	30 min	20	8 - 23	1	10	10 – 30
1 hour	30	8– 15	1 hour	40	8 – 20	3	40	8 – 20
6 hour	60	8 –15	6 hour	60	8 – 15	5	80	8 – 15
12 hour	100	8- 15	12 hour	100	8 – 15	10	100	8 - 15
24 hour	100	8- 15	24 hour	100	8 - 15	20	100	8- 15

*: No. of plants inoculated = 10

4.2.2.4 Incubation period of yellow mosaic begomovirus in vector

The whiteflies were allowed for a minimum AAP of 24h on YMV infected plant to determine the incubation period. Groups of 10 viruliferous whiteflies were released on 10 healthy ridge gourd plants after 30min, 1h, 6h, 12h and 24h of incubation period. After each incubation period, whiteflies were given 20h IAP. The results revealed that a minimum of 30 min incubation period, which resulted in 10 per cent transmission. An incubation period of 12h and 24h resulted in 100 per cent transmission. The results also indicated that the transmission efficiency increased with increase in incubation period (Table 13 and fig. 6).

4.2.2.5 Persistence of yellow mosaic begomovirus in vector

Experiments were conducted in two sets with groups of five and ten viruliferous whiteflies. Groups of five viruliferous whiteflies were serially transmitted to healthy ridge gourd plants at 24h interval (Table 14). The whiteflies retained and transmitted YMV successfully to all ridge gourd test plants on the first day after virus acquisition. The transmission was sporadic, thereafter, for one week and YMV persisted in whitefly for at least 7 days, after which all of the whiteflies had died. A similar sporadic transmission pattern was obtained with groups of 10 whiteflies per plant.

4.3 Host range

The host range studies of the viruses were conducted to know the host plants susceptible to the virus. Twenty one crop plants and three weed species belonging to six different families (Table 15) grown in an insect proof glasshouse were inoculated with indigenous whitefly, *B. tabaci*.

Upon inoculation of yellow mosaic virus-ridge gourd isolate to different crop plants and weeds. Hundred per cent infection was observed on cucumber, ridge gourd, bottle gourd, pumpkin, ash gourd and squash after 8-12 days of inoculation (Plate 13). The virus induced yellow mosaic symptoms on ridge gourd (Plate 13A), yellow mosaic and upward leaf curling in cucumber (Plate 13B), yellow vein mosaic, chlorosis and mottling on bottle gourd (Plate 13C), yellow vein mosaic on pumpkin (Plate 13D), yellow mosaic and reduced leaf size on ash gourd (Plate 13E) and yellow vein mosaic and stunted growth on squash (Plate 13F). The virus could not infect the plants of Solanaceae, Fabaceae, Asteraceae and weed plants (Table 15).

Host range of cucumber isolate of yellow mosaic virus was also determined. On inoculation of yellow mosaic virus-cucumber isolate to different hosts by whiteflies, hundred per cent infection with similar symptoms (Plate 13) were observed after 8-15 days of inoculation such as cucumber, ridge gourd, bottle gourd, pumpkin and squash. The virus could not transmitted to the plant species of other families and weeds tested (Table 16).

Table 13. Determination of incubation period of yellow mosaic virus in indigenous Whitefly, *Bemicia tabaci*.

Incubation period	No of plants infected out of 10 inoculated plants	Per Cent transmission	No of Days taken for Symptom development
30min	1	10	8-20
1 hour	3	20	8-15
6 hour	5	60	8-15
12 hour	10	100	8-15
24 hour	10	100	8-15

Average no. of viruliferous whiteflies used per plant: 10

AAP: 24 hrs
IAP : 24 hrs

Date of sowing: 05/03/2014
Date of inoculation: 13/05/2014

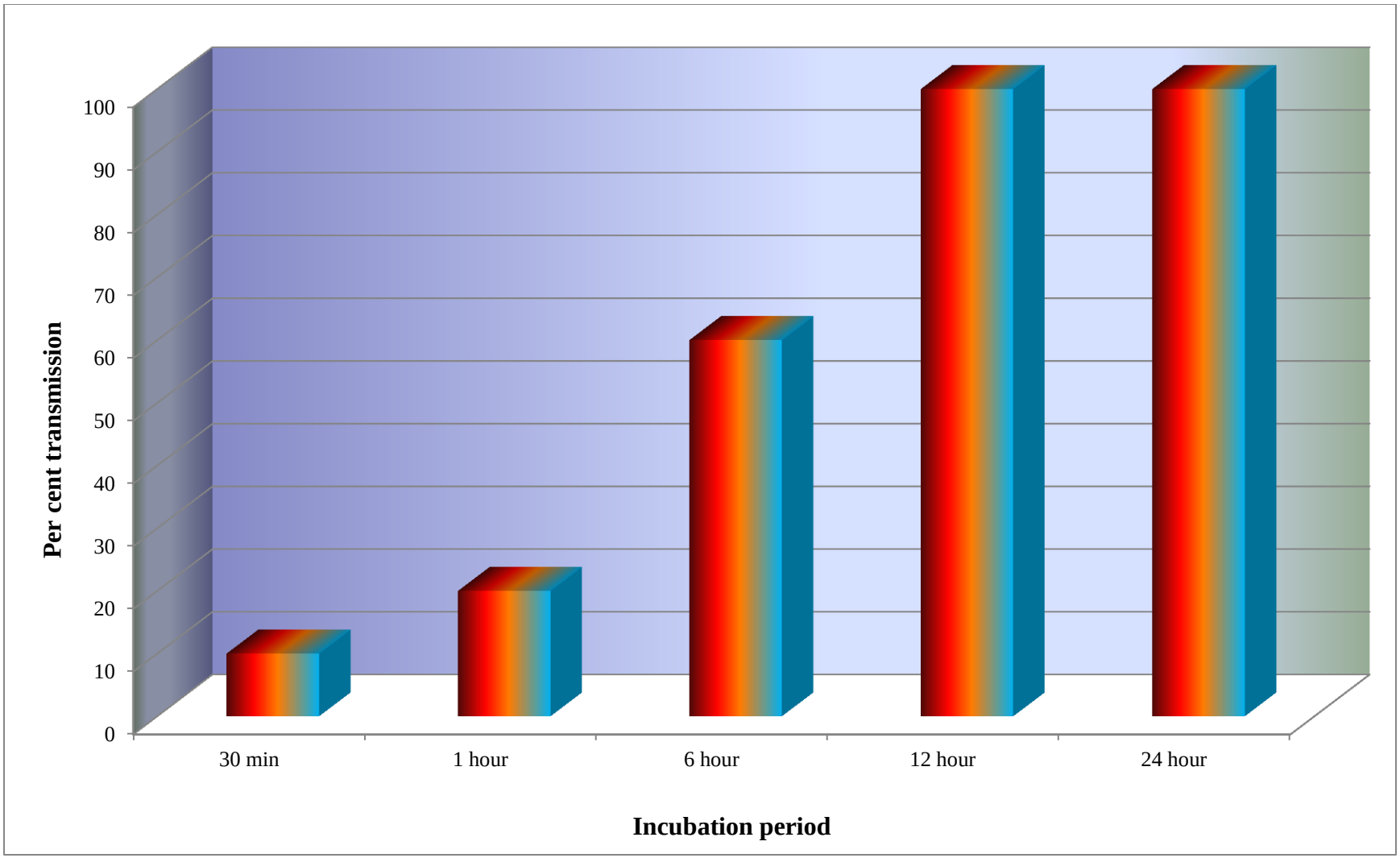


Fig 6. Effect of Incubation period on transmission of yellow mosaic virus disease of ridge gourd through *Bemisia tabaci*

Table 14. Persistence of yellow mosaic virus in viruliferous indigenous *Bemisia tabaci* [1]

No. of whiteflies/ seedlings	Plant number	Serial transfer in days									
		1	2	3	4	5	6	7	8	9	10
1	1	+	+	+	+	+	+	D			
	2	+	+	+	+	+	+	+	-	D	
	3	+	+	+	+	+	+	+	_	D	
	4	+	+	+	+	+	+	+	-	D	
	5	+	+	+	+	+	+	D			
5	1	+	+	+	+	+	+	+	-	D	
	2	+	+	+	+	+	+	+	+	-	D
	3	+	+	+	+	+	+	+	-	D	
	4	+	+	+	+	+	+	-	+	-	D
	5	+	+	+	+	+	+	+	-	-	D

Bemisia tabaci [1] were given 24 h acquisition and inoculation access period each,
+ = Positive transmission, - =Negative transmission, D=Death of whiteflies

Table 15. Host range of yellow mosaic virus -ridge gourd assessed by symptom expression after virus transmission by *B. tabaci.*

Plant species inoculated[a]	Common name	family	% infection	Sympotoms[b]	No of Days taken for Symptom development
Luffa cylindrica	Sponge gourd	Cucurbitaceae	0 (0/10)	-	-
***Cucumis sativus* L.**	**Cucumber**	Cucurbitaceae	**100 (10/10)**	**Yellow mosaic, mottling and leaf curling**	**12-15**
Cucurbita moschata	**Pumpkin**	**Cucurbitaceae**	**100 (10/10)**	**Yellow vein mosaic**	**12-15**
Cucurbita pepo L.	**Squash**	**Cucurbitaceae**	**100 (10/10)**	**Yellow mosaic**	**10-15**
Cucumis melo L.	Musk melon	Cucurbitaceae	0 (0/10)	-	-
Citrullus vulgaris	Watermelon	Cucurbitaceae	0 (0/10)	-	-
Lagenaria siceraria	**Bottle gourd**	**Cucurbitaceae**	**100 (10/10)**	**Yellow mosaic, chlorosis and reduced leaf size**	**10-12**
***Luffa acutangula* (L.)**	**Ridge gourd**	Cucurbitaceae	0 (0/10)	**Yellow mosaic**	**8-10**
Momordica charantia L.	Bitter gourd	Cucurbitaceae	0 (0/10)	-	-
Trichosanthes charantia	Snake gourd	Cucurbitaceae	0 (0/10)	-	-
Benincasa hispida	**Ash gourd**	**Cucurbitaceae**	**100 (10/10)**	**Yellow mosaic and reduced leaf size**	**10-15**
Lycopersicon esculentum Mill.	Tomato	Solanaceae	0 (0/10)	-	-
Nicotiana tabacum L.	Tobacco	Solanaceae	0 (0/10)	-	-
Solanum melongena L.	Eggplant	Solanaceae	0 (0/10)	-	-
Capsicum annuum L.	Bell pepper	Solanaceae	0 (0/10)	-	-
Nicotiana glutinosa L.	Tobacco	Solanaceae	0 (0/10)	-	-
Phaseolus lunatus L	Lima bean	Fabaceae	0 (0/10)	-	-
Macrotyloma uniflorum	Horse gram	Fabaceae	0 (0/10)	-	-
Vigna radiata (L.)	Greengram	Fabaceae	0 (0/10)	-	-
Vigna ungiculata (L.)	Cowpea	Fabaceae	0 (0/10)	-	-
Vigna mungo (L.)	Blackgram	Fabaceae	0 (0/10)	-	-
Weeds					
Parthenium hysterophorus L		Asteraceae	0 (0/10)	-	-
Chenopodium amaranticolor		Chenopodiaceae	0 (0/10)	-	-
Euphorbia geniculata Orteg.		Euphorbiaceae	0 (0/10)	-	-

a Two . Three-week-old plants were inoculated using 15-20 *B. tabaci* per plant provided with 24 h AAP and 24 h IAP.
b Expression of symptoms was recorded weekly for 3 months after virus inoculation.

Ridge gourd

Cucumber

Bottle gourd

Pumpkin

Ash gourd

Squash

Plate 13. Cucurbits showing symptoms of mild mosaic to yellow mosaic upon inoculation with YMV using White fly, B. *tabaci*

Table 16. Host range studies of yellow mosaic virus -Cucumber assessed by symptom expression after virus transmission by *B. tabaci.*

Plant species inoculated[a]	Common name	Family	% infection	Symptoms[b]	No of Days taken for Symptom development
***Luffa acutangula* (L.)**	**Ridge gourd**	**Cucurbitaceae**	**100 (10/10)**	**Yellow mosaic**	**10-12**
***Cucumis sativus* L.**	**Cucumber**	**Cucurbitaceae**	**100 (10/10)**	**Yellow mosaic and mottling**	**12-15**
Momordica charantia L.	Bitter gourd	Cucurbitaceae	0 (0/10)	-	-
***Cucurbita pepo* L.**	**Squash**	**Cucurbitaceae**	**100 (10/10)**	**Yellow mosaic**	**10-15**
Cucumis melo L.	Musk melon	Cucurbitaceae	0 (0/10)	-	-
Citrullus vulgaris Schrad.	Watermelon	Cucurbitaceae	0 (0/10)	-	-
Lagenaria siceraria.	**Bottle gourd**	**Cucurbitaceae**	**100 (10/10)**	**Yellow mosaic and reduced leaf size**	**10-12**
Luffa cylindrica	Sponge gourd	Cucurbitaceae	0 (0/10)	-	-
Cucurbita moschata	**Pumpkin**	**Cucurbitaceae**	**100 (10/10)**	**Yellow vein mosaic**	**12-15**
Trichosanthes charantia L.	Snake gourd	Cucurbitaceae	0 (0/10)	-	-
Benincasa hispida	**Ash gourd**	**Cucurbitaceae**	**100 (10/10)**	**Yellow mosaic and reduced leaf size**	**12-15**
Solanum melongena L.	Eggplant	Solanaceae	0 (0/10)	-	-
Nicotiana tabacum L.	Tobacco	Solanaceae	0 (0/10)	-	-
Lycopersicon esculentum Mill.	Tomato	Solanaceae	0 (0/10)	-	-
Capsicum annuum L	Bell pepper	Solanaceae	0 (0/10)	-	-
Phaseolus lunatus L	Lima bean	Fabaceae	0 (0/10)	-	-
Macrotyloma uniflorum (Lam.)	Horse gram	Fabaceae	0 (0/10)	-	-
Vigna radiata (L.) *Wilczek*	Greengram	Fabaceae	0 (0/10)	-	-
Vigna ungiculata (L.) *Walp.*	Cowpea	Fabaceae	0 (0/10)	-	-
Vigna mungo (L.) *Hepper*	Blackgram	Fabaceae	0 (0/10)	-	-
Weeds					
Euphorbia geniculata Orteg.		Chenopodiaceae	0 (0/10)	-	-
Chenopodium amaranticolor		Asteraceae	0 (0/10)	-	-
Parthenium hysterophorus L		Euphorbiaceae	0 (0/10)	-	-

a Two . three-week-old plants were inoculated using 15-20 *B. tabaci* per plant provided with 24 h AAP and 24 h IAP.
b Expression of symptoms was recorded weekly for 3 months after virus inoculation.

4.4 Molecular detection and characterization of the yellow mosaic begomovirus

4.4.1 PCR detection of yellow mosaic begomovirus using degenerate primers

Polymerase chain reaction technique was employed to confirm the association of *Begomovirus* with yellow mosaic disease through amplification of PCR product approximately 570 bp CP gene fragments using Begomovirus group specific universal primers (Deng *et al*., 1994). A band of approximately 570 bp corresponding to viral coat proein was consistently amplified from total DNA template extracted from infected ridge gourd and cucumber plants (Plate 14). No such virus specific products were obtained with DNA template extracted from healthy leaf material.

4.4.2 PCR detection of begomovirus using virus coat protein specific primers.

In order to identify the begomovirus responsible for causing the disease, PCR was carried out with specific complete coat protein primers (Namrata *et al*., 2012). The PCR product of 750 bp corresponding to coat protein (CP) gene was amplified from YMV infected ridge gourd and cucumber plants which was absent in healthy plants (Plate 15). The amplified product was eluted and sequenced.

4.4.3 Coat protein (CP) gene sequence homologies of yellow mosaic begomovirus with other begomoviruses.

The sequences obtained were subjected to BLAST of NCBI. BLAST search analysis of the sequence from ridge gourd virus isolate showed 93% nucleotide identity with *Tomato Leaf Curl New Delhi Virus* (ToLCNDV)-isolate TC-309 (KF551576.1) and 92% nucleotide identity with ToLCNDV-severe strain (U15015.2), ToLCNDV-mild strain (U15016.1), ToLCNDV-Lucknow (KC513822.1), ToLCNDV-*Luffa cylindrica* (KC207815.1) and bottle gourd isolate of ToLCNDV(DQ 272540.2) (Table 17).

The sequence of yellow mosaic begomovirus-cucumber isolate showed 95% nucleotide identity with ToLCNDV-Almeria 661 (KF749223.1) and ToLCNDV-murica (KF74925.1) and 92% homology with ToLCNDV-severe strain (U15015.2), ToLCNDV-mild strain (U15016.1), ToLCNDV-Lucknow (KC513822.1), ToLCNDV-*Luffa cylindrical* (KC207815.1) and bottle gourd isolate of ToLCNDV (DQ 272540.2) (Table 18).

4.4.2 Phylogenetic relationship of yellow mosaic begomovirus based on coat protein (CP) gene sequences

The phylogenetic tree was constructed by MEGA6 programme based on nucleotide sequence data of YMV of ridge gourd and cucumber with selected isolates of ToLCNDV and other begomoviruses. The phylogenetic dendrogram of coat protein gene sequence of ridge gourd and cucumber isolate are as shown in Plate 17. The two begomoviruses under study and other selected begomoviruses formed three clusters. The major Cluster-I grouped all the ToLCNDV isolates and cluster II grouped all isolates of tomato leaf curl virus. Phylogenetic analysis of coat protein gene sequences of YMV revealed that both YMV-ridge gourd and cucumber isolates clustered with ToLCNDV-strains supported by 92% bootstrap score (Plate 16).

Table 17. Nucleotide sequence identity of yellow mosaic virus- ridge gourd isolate with other begomoviruses by BLAST analysis.

Sl. No	Homology with the nucleotide sequence in GenBank	% Homology	Accession Number
1	Tomato leaf curl New Delhi virus isolate TC309	93	KF551576.1
2	Tomato leaf curl New Delhi virus from bottle gourd	92	DQ272540.2
3	Tomato leaf curl New Delhi virus-Severe	92	U15015.2
4	Tomato leaf curl New Delhi virus : India: UP: Bahraich: Luffa cylindrica	92	KC207815.1
5	Tomato leaf curl New Delhi virus-Mild	92	U15016.1
6	Tomato leaf curl New Delhi virus from potato	91	DQ272541.
7	Tomato leaf curl New Delhi virus isolate ToLCNDV-AVT1	92	AY428769.1
8	Tomato leaf curl New Delhi virus - chili pepper	92	DQ141676.1
9	Tomato leaf curl New Delhi virus-[Pumpkin: New Delhi	92	AM286434.1
10	Tomato leaf curl New Delhi virus - Bitter Gourd	92	AM747291.1
11	Tomato leaf curl New Delhi virus: Pakistan: Solanum	92	FN435310.1
12	Tomato leaf curl New Delhi virus-India isolate Lucknow	92	KC513822.1
13	Pumpkin yellow vein mosaic virus coat protein	91	AY686500.1
14	Squash leaf curl China virus coat protein (AV1)	91	FJ232922.1
15	Squash leaf curl China virus - [Cucurbita pepo: Lahore] AV2	91	AM286794.1

Table 18. Nucleotide sequence identity of yellow mosaic virus- cucumber isolate with other begomoviruses by BLAST analysis.

Sl. No	Homology with the nucleotide sequence in GenBank	% Homology	Accession Number
1	Tomato leaf curl New Delhi virus isolate Almeria 661	95	KF749223.1
2	Tomato leaf curl New Delhi virus isolate Murcia 11.1	95	KF749225.1
3	Tomato leaf curl New Delhi virus isolate ToLCNDV-Spain-Almeria	95	KF891468.1
4	Tomato leaf curl New Delhi virus isolate TC309	93	KF551576.1
5	Tomato leaf curl New Delhi virus from bottle gourd	92	DQ272540.2
6	Tomato leaf curl New Delhi virus-Severe strain	92	U15015.2
7	Tomato leaf curl New Delhi virus isolate India: UP: Luffa cylindrica	92	KC207815.1
8	Tomato leaf curl New Delhi virus-Mild	92	U15016.1
9	Tomato leaf curl New Delhi virus from potato	91	DQ272541.
10	Tomato leaf curl New Delhi virus isolate ToLCNDV-AVT1	92	AY428769.1
11	Tomato leaf curl New Delhi virus – chili pepper	92	DQ141676.1
12	Tomato leaf curl New Delhi virus-[Pumpkin:New Delhi	92	AM286434.1
13	Tomato leaf curl New Delhi virus – Bitter Gourd	92	AM747291.1
14	Tomato leaf curl virus AV1 gene, isolate 26	92	AJ620187.1
15	Tomato leaf curl New Delhi virus isolate India: Ash gourd	92	JN208136.1

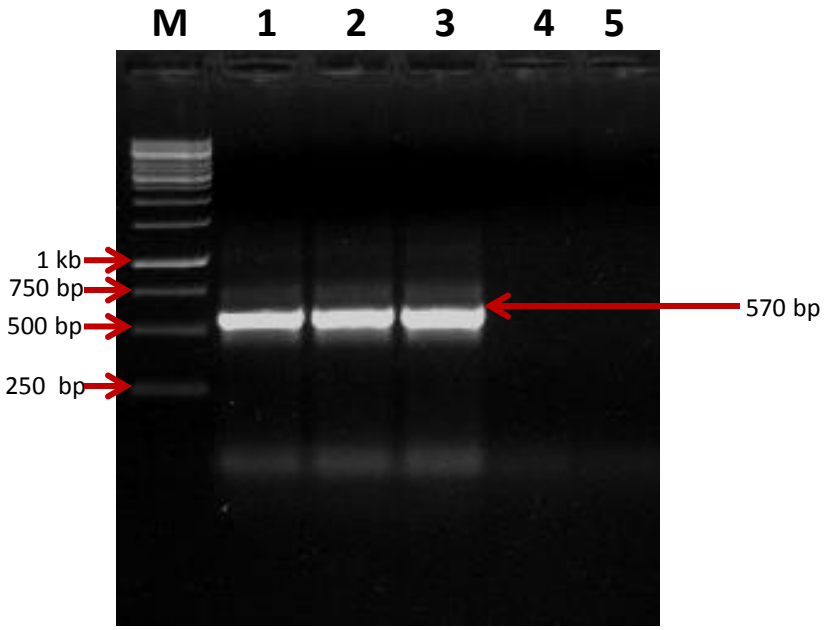


Plate 14: PCR detection of YMV using begomovirus group specific primers

Lane M=1kb Marker

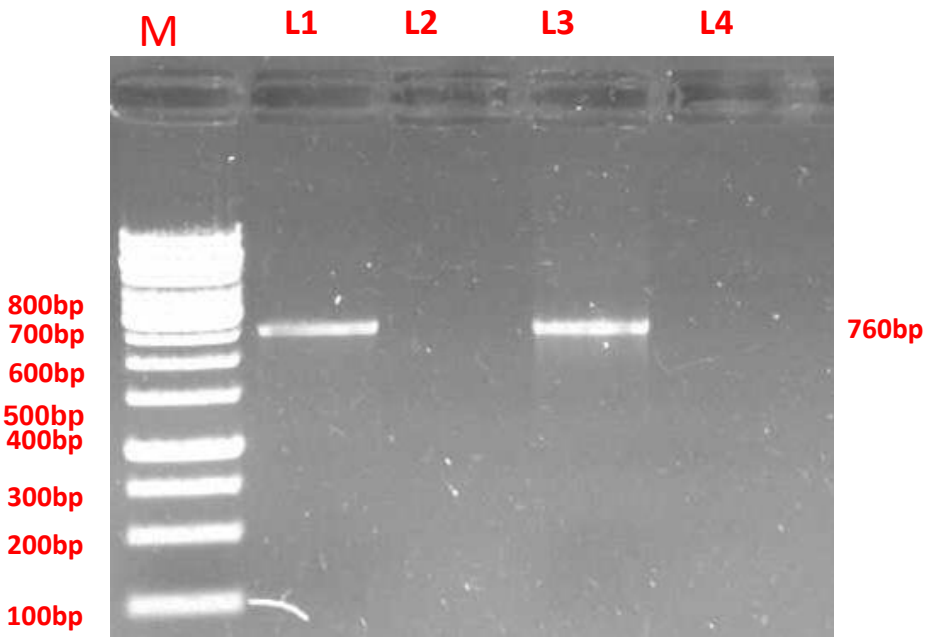
Lane 1= positive control

Lane 2 = YMV infected Ridge gourd

Lane 3 = YMV infected cucumber

Lane 4= healthy Ridge gourd

Lane 5= healthy cucumber



M-Marker, L1-YMV infected ridgegourd, L2-Healthy ridgegourd,L3-YMV infected cucumber, L4-healthy cucumber

Plate 15. PCR Amplification of coat protein gene of yellow mosaic begomo virus infecting Ridge gourd and Cucumber

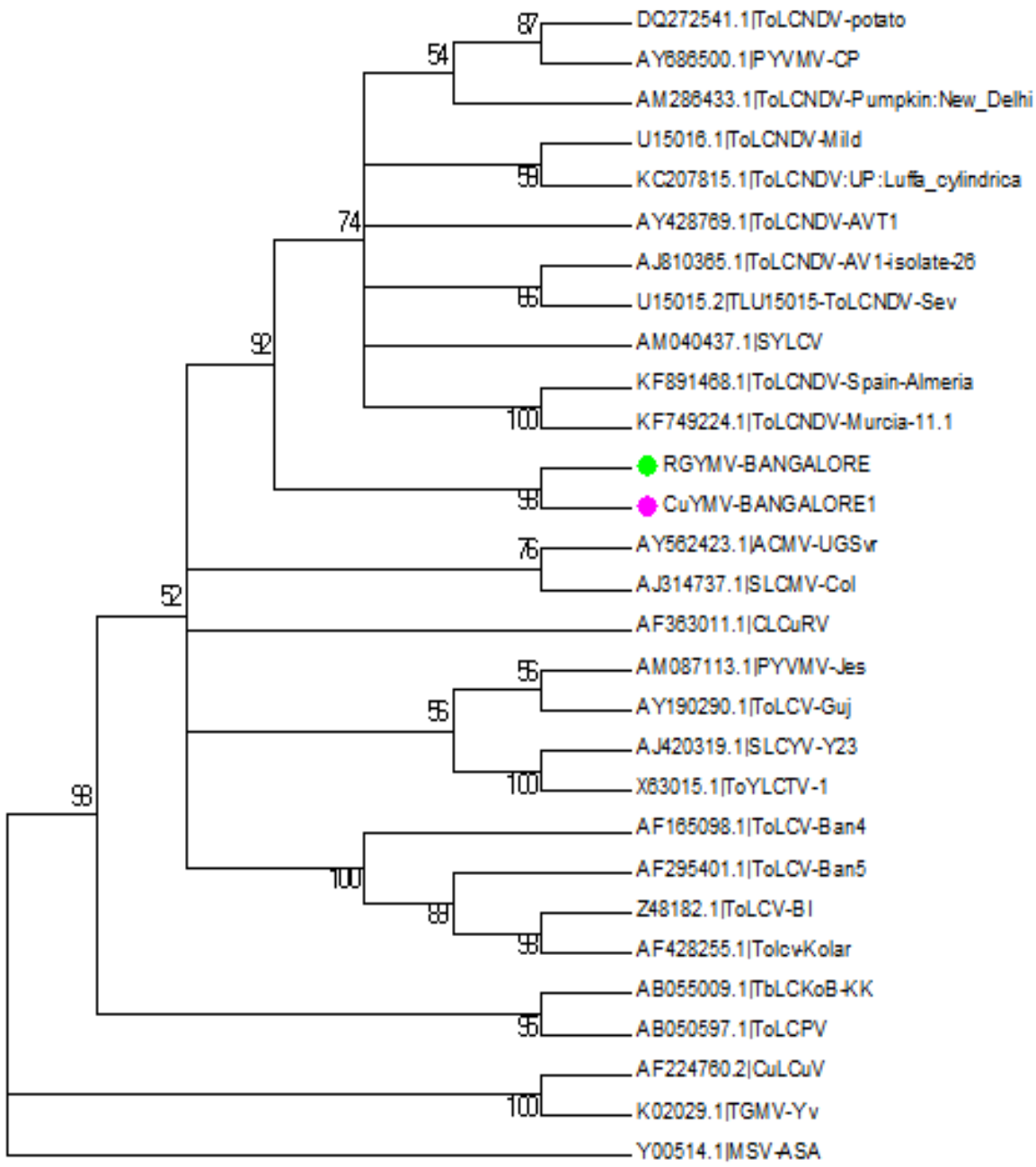


Plate 16. Phylogenetic tree showing the relationship of yellow mosaic begomovirus-ridge gourd and cucumber isolates to other begomoviruses based on coat protein gene sequences. The dendrograms are calculated using neighbor-joining algorithim of MEGA 6.06 version. Numbers at nodes indicate percentage bootstrap confidence scores (1,000 replications).

V DISCUSSION

Studies were undertaken on biological and molecular characterization of the virus causing yellow mosaic diseases. The results obtained there on are discussed briefly here under.

Survey carried out from 2012-2013 in southern dry tract of Karnataka comprising of 4 districts for the assessment of disease status. Survey revealed that occurrence of the disease on ridge gourd and cucumber range between 37.5 to 86.0 per cent and 31.0 to 78.5 per cent, respectively. Chikkaballapur and Kolar districts have the larger area under vegetable crops recorded highest incidence of the disease on both ridge gourd and cucumber. There are many reports on begomviruses infecting wide range of cucurbits from different parts of the country. They are likely to be a major threat to the cucurbits production in the future. The present study confirmed with the occurrence of begomovirus on bottle gourd in Delhi and Haryana varied from 4.7 to 36% (Sohrab *et al*., 2003), on bitter gourd in Gorakhpur up to 20% (Tiwari *et al*., 2010).

The symptoms of naturally infected ridge gourd and cucumber plants by yellow mosaic virus observed during field survey were; mild to severe yellow mosaic, chlorosis, leaf curling, mottling, networking of yellow veins, followed by thickening of veins and vein lets, puckering, leaf distortions, stunting of whole plant, misshapen fruits and reduced fruit yield. Similar observations were obtained by Tiwari, et al., (2012), typical yellow mosaic and leaf curling symptoms on sponge gourd and bitter gourd observed during survey at Gorakhpur, Uttar Pradesh. Namrata *et al.*, (2013) reported various symptoms of PYVMV on pumpkin viz., yellowing of young leaves, curling, thickening of tender stems and severe to mild mosaic of youngest leaves were predominantly observed during survey at Varanasi, UP. Similar symptoms were observed on bottle gourd in New Delhi and Haryana (Sohrab *et al*., 2010), on sponge gourd in New Delhi (Sohrab et al., 2010), on tomato, potato and cucurbits (Jyothsna *et al*., 2013), on pumpkin in Karnataka (Muniyappa *et al*., 2003; Maruthi *et al*., 2007).

Studies carried on transmission of the virus clearly revealed the successful transmission of the virus isolates by whitefly, *B. tabaci* vector. Yellow mosaic begomoviruses from cucurbits such as ridge gourd and cucumber were transmitted successfully up to 100 per cent with 10 viruliferous whiteflies, *B. tabaci* which were given 24h AAP and IAP. Successful transmission of begomoviruses infecting cucurbits by *B. tabaci*, (Jayashree *et al*., 1999; Muniayappa *et al*., 2003). Tiwari *et al*. (2012) reported successful transmission of begomovirus causing yellow mosaic disease on sponge gourd, bitter gourd and pumpkin by whitefly, *B. tabaci*. The ToLCNDV infecting cucurbits has been reported to be transmitted efficiently by even single whitefly, *B. tabaci* (Sohrab *et al*., 2013).

Single adult whitefly has been found capable of transmitting begomovirus, but with low transmission efficiency. Number of whitefly per test plant required to be vary with the nature of the plant species. For plants with fleshy tender tissues required minimum of 5 adult whitefly per plant to cause 100 per cent infection. For woody nature

plant species, 5 or more whiteflies required to get 100% virus transmission. The differences could be due to the vector feeding preference or the rate of virus multiplication in inoculated plants. These results indicates that the number of insects and the transmission efficiency are positively correlated. Jayashree *et al.,* (1999) and Muniyappa *et al* .,(2003), reported that single whitefly was able to transmitt PYVMV with 30 per cent efficiency, which increased to 60 per cent when three whiteflies were used and 100 per cent transmission with five or more viruliferous whiteflies per test plant. A minimum of 15 viruliferous B. tabaci per plant were required to achieve 100 per cent infection of CYVMV (Mandal, 1989) and cotton leaf curl virus (CoLCV) in cotton (Nateshan *et al*., 1996).

Studies on determination of effect of different acquisition access periods revealed that a minimum AAP of 30 min was necessary for whiteflies to aquire the YMV of ridge gourd, which resulted in 20 per cent transmission. An AAP of 1h and 6h resulted in 30 and 60 per cent transmission, respectively. An AAP of at least 12h and 24h was required for 100 per cent transmission. Results revealed that the percentage of transmission increased with the increase in AAP. Similar results were found that, a minimum of 30 sec was required by *B. tabaci* to transmitt begomovirus on pumpkin, PYVMV (Capoor and Ahmad, 1975). A minimum AAP of 5 min (Jayashree *et al*., 1999) and 30 min (Muniyappa *et al*., 2003) was necessary for whiteflies to aquire PYVMV which resulted in 8.3 and 20.0 per cent transmission, respectively. An AAP of 6 hour or more resulted in 100 per cent (Jayashree *et al*., 1999; Muniyappa *et al*., 2003). With increase in AAP, the percentage of insect becoming viruliferous increased, as a result the percentage of disease transmission also increased. However. Sohrab *et al*. (2013) reported that a minimum AAP required to transmit the virus was 60 min for both Luffa: Del and Pum:Del isolates.

Viruliferous whiteflies required a minimum IAP of 30 min to achieve 20 per cent transmission efficiency. An IAP of 1h and 6h resulted in an increased transmission efficiency of 40 and 60 per cent, respectively. An IAP of 12h or more resulted in 100 per cent transmission. The results also indicated that percentage transmission increased with the increase of IAP. Similar virus transmission characters reported for begomoviruses like CoLCuV (Kirkpatrick, 1931; Ripper and George, 1965), BYVMV (Varma, 1952), TYLCV (Cohen and Nitzany, 1966) and SLCV (Cohen et al., 1983).

A minimum of 30 min incubation period, which resulted in 10 per cent transmission of YMV of ridge gourd. An incubation period of 12h and 24h resulted in 100 per cent transmission. The results also indicated that the transmission efficiency increased with increase in incubation period. Incubation period of 6 h was sufficient for successful transmission of beet pseudo yellows by greenhouse whitefly. *T. vaporarium* (Duffus, 1965) and similar reports were also found by Muniyappa and Reddy (1976) with HYMV and ToLCV (Reddy and Yaraguntaiah, 1981) and a latent period of 19 h was observed for SLCV in *B. tabaci* (Cohen *et al*., 1983). Three h minimum incubation period was required to transmit CoLCuV (Ripper and George, 1965).

The whiteflies retained and transmitted YMV successfully to all ridge gourd test plants on the first day after virus acquisition. The transmission was sporadic, thereafter,

for one week and YMV persisted in whitefly for at least 6 days, after which all of the whiteflies had died. A similar sporadic transmission pattern was obtained with groups of 10 whiteflies per plant. These results are in agreement with the report of Babitha (1996) and Muniyappa *et al.,* (2003) who stated that the maximum retension period of PYVMV was eight days in indigenous whitefly using 10 and 15 viruliferous insects. Similar persistence period was found with ICMV (Mathew and Muniyappa, 1991) compared to ToLCV that was retained in *B. tabaci* throughout its life period (Reddy and Yaraguntaiah, 1981).

The host range studies of the viruses were conducted to know the host plants susceptible to the virus. Twenty one crop plants and three weed species belonging to six different families grown in insect proof glasshouse were inoculated with whitefly, *B. tabaci*.

The results of studies on identification of susceptible hosts to the virus indicated that YMV successfully transmitted to ridge gourd, bottle gourd, cucumber, pumpkin, ash gourd and squash. Plant species such as Bitter gourd, snake gourd, musk melon, watermelon, tomato, tobacco, legume crops and weed hosts did not take infection upon inoculation experimentally. The ToLCNDV associated with yellow mosaic disease of Chayote was experimentally transmitted to *Luffa acutangula* by using *B. tabaci* (Mandal *et al*., 2004). But it was interesting note that the virus could not be transmitted to tomato though it is a distinct species of Tomato leaf curl virus in India.

Studies carried out to identify the causal virus responsible for causing yellow mosaic disease. The PCR product of 750 bp was amplified from YMV infected both ridge gourd and cucumber plants. The PCR detection implicated that associated causal agent was a begomovirus. The sequence of coat protein gene of the ridge gourd virus isolate showed 93% nucleotide identity with *Tomato Leaf Curl New Delhi Virus* (ToLCNDV)-isolate TC-309 (KF551576.1) and 92% nucleotide identity with ToLCNDV-severe strain (U15015.2), ToLCNDV-mild strain (U15016.1), ToLCNDV-Lucknow (KC513822.1), ToLCNDV-*Luffa cylindrica* (KC207815.1) and bottle gourd isolate of ToLCNDV(DQ 272540.2).

The sequence of cucumber virus isolate showed 95% nucleotide identity with ToLCNDV-Almeria661 (KF749223.1) and ToLCNDV-murica (KF74925.1) and 92% homology with ToLCNDV-severe strain (U15015.2), ToLCNDV-mild strain (U15016.1), ToLCNDV-Lucknow (KC513822.1), ToLCNDV-*Luffa cylindrical* (KC207815.1) and bottle gourd isolate of ToLCNDV (DQ 272540.2). The present investigation compared with Sohrab *et al*., (2013) who reported the ToLCNDV which is associated with yellow mosaic disease of luffa and pumpkin. The PCR amplified products of coat protein gene (750 bp) from Luffa and pumpkin isolates were separately cloned and sequenced. The BLAST result showed highest homology with *Tomato leaf curl New Delhi virus* and designated as ToLCNDV-Luffa. Comparison of the nucleotides sequence of Luffa:Del isolate (AY309957) with selected begomoviruses showed 96.1% nucleotide identity with ToLCNDV-Luffa (AF102276) followed by 95.1% identity with ToLCNDV-[Svr] (U15015) and 87.7% with SqLCV-[Pum:Del] (AY686500) was observed.

The phylogenetic tree was constructed by MEGA6 programme based on nucleotide sequence data of YMV of ridge gourd and cucumber with selected isolates of ToLCNDV and other begomoviruses. Phylogenetic analysis of coat protein gene sequences revealed that YMV clustered with ToLCNDV-spain Almeria, ToLCNDV-Murcia 11.1, ToLCNDV-severe strain and ToLCNDV-mild strain and ToLCNDV-*luffa cylindrica*. Tiwari *et al*., (2012) reported that phylogenetic analysis of yellow mosaic virus infecting *Luffa cylindrica* clustered with the strains of Tomato leaf curl New Delhi virus (ToLCNDV).

CP genes represent the most conserved gene in the family Geminiviridae and CP sequences can be used as preliminary virus identification, or to infer geographic and vector relationship. On the basis of the various studies, it can be concluded that the yellow mosaic virus disease of ridge gourd and cucumber prevailing in southern karnataka are caused by *Tomato Leaf Curl New Delhi Virus (ToLCNDV)*.

Future Line of Work

- Use of coat protein gene sequence information for coat protein mediated resistance studies in the development of transgenic plants against begomoviruses.
- Use of CP primers as a molecular epidemiological tool for begomovirus detection and preliminary identification and to study closely related viruses.
- The host range of yellow mosaic begomovirus has to be tested further especially using molecular detection techniques in order to understand its epidemiology properly.
- The management of yellow mosaic begomovirus is dependent on control of whiteflies in addition to using resistant varieties. Future studies should concentrate on the development of resistance varieties/hybrids for both yellow mosaic virus and *Bemisia tabaci* biotypes.

VI SUMMARY

The present investigation on biological and molecular characterization of yellow mosaic virus infecting ridge gourd and cucumber was carried out from 2013 to 2014 at GKVK, Bengaluru. The salient findings of the present investigation are summarized here under.

The virus causing yellow mosaic disease is major constraint in cucurbits productivity in Karnataka. The survey under taken to assess the incidence of yellow mosaic virus in ridge gourd and cucumber in four districts of southern Karnataka, revealed that the occurrence of the disease in the range of 37.5 to 86.0 per cent in ridge gourd and 31.0 to 78.5 per cent on cucumber. Among the districts, the highest disease incidence of YMV on ridge gourd was recorded in Chikkaballapur (67.098%), followed by Kolar (60.278%), Bangalore (57.09%) and least in Ramanagar (49.88%). Whereas highest incidence of YMV on Cucumber was recorded in Kolar (63.64%) followed by chikkaballapur (56.702%), Bangalore (55.77%) and lowest in Ramanagar (49.69%).

The ridge gourd and cucumber plants infected with yellow mosaic begomovirus were associated with the symptoms such as mild to severe yellow mosaic, chlorosis, leaf curling, mottling, networking of yellow veins, followed by thickening of veins and vein lets, puckering, leaf distortions, stunting of whole plant, misshapen fruits and reduced fruit yield.

YMV was successfully transmitted through the whiteflies (*Bemicia tabaci*). Single adult whitefly could able to transmit the yellow mosaic virus with 20 per cent efficiency. Transmission efficiency was 100 per cent with ten or more whiteflies per plant. The per cent transmission increased with the increase in number of whiteflies.

The minimum AAP of 30 min was required for the vector (B. tabaci) to transmit the YMV with 20 per cent efficiency. The per cent transmission increased with the increase in AAP of vector.

The minimum IAP required for the vector (*B. tabaci*) to transmit YMV was found to be 30 min. the per cent transmission increased with increase in IAP of the vector. Virus was efficiently transmitted after 12 h of AAP, IAP and 10 number of whiteflies were sufficient to cause 100 % transmission.

A minimum of 30 min incubation period, which resulted in 10 per cent transmission. An incubation period of 12h and 24h resulted in 100 per cent transmission. The results also indicated that the transmission efficiency increased with increase in incubation period.

Persistence of YMV in *B. tabaci* was found to be 6-9 days but failed to transmit continuously.

Upon inoculation of YMV-ridge gourd and cucumber isolates to different crop plants and weeds, the virus able to infect cucumber, ridge gourd, bottle gourd, pumpkin, ash gourd and squash.

Studies on identification of causal agent through detection of yellow mosaic mosaic virus by PCR tests using begomovirus specific primers. The PCR product of 750 bp corresponding to coat protein gene was amplified from YMV infected both ridge gourd and cucumber plants indicating the association of a begomovirus.

The sequence of the 750bp CP gene of virus from ridge gourd and cucumber virus isolates showed 92% nucleotide identity with *Tomato Leaf Curl New Delhi Virus* (ToLCNDV) -severe strain (U15015.2), ToLCNDV-mild strain (U15016.1), ToLCNDV-*Luffa cylindrica* (KC207815.1) and bottle gourd isolate of ToLCNDV (DQ 272540.2).

Phylogenetic analysis of coat protein gene sequences revealed that YMV from ridge gourd and cucumber clustered with ToLCNDV-spain Almeria, ToLCNDV-murcia 11.1, ToLCNDV-severe strain, ToLCNDV-mild strain, ToLCNDV-Luffa isolate.

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