IDENTIFICATION AND CHARACTERIZATION OF VIRUSES CAUSING MOSAIC OF BOTTLE GOURD IN JAMMU SUB-TROPICS

by

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Thesis submitted to
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(PLANT PATHOLOGY)

DIVISION OF PLANT PATHOLOGY
Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu
Main Campus, Chatha, Jammu 180009
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CERTIFICATE - I

This is to certify that the thesis entitled "**Identification and Characterization of Viruses Causing Mosaic of Bottle Gourd in Jammu Sub-Tropics**" submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Agriculture (Plant Pathology) to the Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, is original work and has similarities with published work not more than minor similarities as per UGC norms of 2018 adopted by the University. Further, the level of minor similarities has been declared after checking the manuscript with Urkund software provided by the University.

The work has been carried out by **Ms. Sneha Choudhary,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.

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(Major Advisor)

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We, the members of Advisory Committee of **Ms. Sneha Choudhary**, Registration No. **J-18-D-350-A,** a candidate for the degree of **Doctor of Philosophy in Agriculture (Plant Pathology),** have gone through the manuscript of thesis entitled **"Identification and Characterization of viruses causing Mosaic of bottle gourd in Jammu sub-tropics''** and recommend that it may be submitted by the student in partial fulfillment of the requirements for the degree.

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Dedicated To

my Beloved Parents

Shri. Harshvardhan and
Smt. Shipra Choudhary,
brother Mr. Himanshu

and

my late grandmother

Smt. Krishna Dutta

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Sneha

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ABSTRACT

Bottle gourd (*Lagenaria siceraria*) holds an important place in the *Cucurbitaceae* family and is cultivated in all tropical and sub- tropical areas of world. This multipurpose crop faces many stresses (biotic and abiotic) wherein, viruses are a major group of pathogens causing mosaic disease. Bottle gourd mosaic disease is not only responsible for yield losses but also for inciting epidemics. Extensive survey of Jammu division to assess the status of bottle gourd mosaic disease was conducted during 2020 and 2021 and it was found that the average maximum disease incidence (53.33 %) was recorded from Salaher (R.S. Pura) while the lowest incidence (7.00 %) was recorded from Ratwal (Bishnah) of Jammu district. However, Jammu district recorded higher incidence of mosaic than other districts (Kathua, Samba and Udhampur). About 274 samples were collected from different locations and 55 symptomatic isolates were detected for causal virus/ viruses. Molecular identification of the causal viruses through RT-PCR revealed the presence of *Cucumber green mottle mosaic virus* (CGMMV) and *Papaya ringspot virus* (PRSV-W) by using coat protein specific primers. CGMMV CP primers amplified a 604 bp product while PRSV-W CP primer produced 398 bp amplicon which was further custom sequenced. CGMMV isolate shared maximum sequence identity (99.80 %) at nucleotide level with CGMMV isolate CG003 (MH271409) reported from USA while, PRSV-W isolate shared the highest per cent identity (96.23 %) with PRSV isolate SHK- PM1 from India (KY448325). The host range of the virus was restricted to *Cucurbitaceae* and *Chenopodiaceae* families only. During transmission assays, CGMMV isolate was not transmitted by insect-vector while, PRSV-W was transferred successfully by aphids (*Aphis gossypii* and *A. craccivora*) from infected to healthy plants. However, *A. gossypii* was found an efficient vector for the transmission of the virus. Seventy eight different germplasm were screened against bottle gourd mosaic disease under both field and controlled conditions and it was found that 'MGH-4' was moderately resistant under field conditions but resistant under controlled conditions. However, four germplasm (IC- 40890, IC- 337077, IC-550725 and Pusa Komal) were found moderately resistant against the disease under both the conditions.

Keywords: *Bottle gourd, mosaic disease, Cucumber green mottle mosaic virus, Papaya ringspot virus, molecular characterization, insect vector, host resistance*

Sneha

Signature of Major Advisor **Signature of the Student**

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LIST OF ABBREVIATIONS

%	per cent
@	at the rate of
°C	degree celsius
µg	microgram
µl	microlitre
dpi	Days post inoculation
RBD	Randomized Block Design
RT-PCR	Reverse Transcription polymerase chain reaction
RNA	Ribonucleic acid
cDNA	Complementary Deoxyribonucleic Acid
EDTA	Ethylenediamine tetra acetic acid
Kb	Kilo base (pair)
M	Molar (conc.)
mM	Millimolar
TAE	Tris, Acetic EDTA
EtBr	Ethidium bromide
dNTP's	Deoxynucleotide triphosphate
Taq	*Thermus aquaticus* DNA polymerase
in vitro	Under aseptic conditions
ml	milliliter
cv	cultivar
etc	*etcetera*
Fig.	figure
BLAST	Basic local alignment search tool
MEGA	Molecular evolutionary genetics analysis
CP	Coat Protein
ORF	Open reading frame
et al	*et alia* (and others)
viz.,	*videlicet* (namely)
i.e.	Id est (that is)

INTRODUCTION

CHAPTER-I

INTRODUCTION

Cucurbits are an important group of vegetables under the family *Cucurbitaceae*. The family *Cucurbitaceae* comprises of cucumber, squash, melons and gourds (118 genera and 825 species) grown tropically and sub-tropically worldwide (Jeffrey, 1990). India is one of the centers of origin for many gourds and melons rendered to its rich diversity (Choudhury, 1996) wherein, several major and minor cucurbits are cultivated in commercial cropping systems as well as kitchen gardens throughout the year (Robinson and Decker-Walter, 1999; Wang *et al.,* 2006, 2007; Rai *et al.,* 2008).

Bottle gourd (2n=2x=22), is a white flowered gourd belonging to genus *Lagenaria*, family *Cucurbitaceae*. The genus *Lagenaria* includes six species that are distributed across Africa, Madagascar, Indo-Malaysia and the Neo-tropics. Out of these, only one species i.e. *L. siceraria* is cultivated. The genus consists of two subspecies *viz.*, the African *L. siceraria* spp. *siceraria* and the Asian *L. siceraria* spp. *asiatica* (Schlumbaum and Vandorpe, 2012). De Candolle (1961) mentioned its wild relatives from South Africa and India. It is a monoecious, annual vine which is considered native to Africa, and spread across the globe through trans-oceanic drift or human transport. Bottle gourd is one of the first cultivated plants in the world. It is warm season crop widely cultivated in tropical regions of the world like India, Japan, Sri Lanka, China and Thailand.

The genus *Lagenaria*, represents the meaning ‘bottle’, which is derived from word ‘*lagena’*. In India, the crop is known by various local names like ‘lauki’ ‘Lau’ or ‘ghiya’ (Parle and Kaur, 2011). Though the crop originated in Indian sub-continent and Africa, but its diversity has not been adequately exploited. The major bottle gourd producing states in India are Bihar, Chhattisgarh, Madhya Pradesh, Orissa and Uttar Pradesh.

Bottle gourd occupies an area of 191.88 thousand ha with production of 3142.73 thousand MT at national scenario, while in Jammu and Kashmir it is cultivated over an area of 1.01 thousand ha with the production of 25.29 thousand MT

(Anonymous, 2021). In India, it is cultivated both as a rainy season and summer season vegetable and, grown almost throughout the year in different parts of the country.

Bottle gourd is a popular, multipurpose crop which contains all the essential constituents that are required for normal and good human health (Parle and Kaur, 2011). Some mention worthy uses of bottle gourd are medicinal (cardio-tonic, general and liver tonic, anti-inflammatory etc.), nutritional (rich vitamin B and C source), musical instrument or decorative artefact (Kirtikar and Basu, 2001; Deshpande *et al.*, 2008). Recently bottle gourd has also been utilized as a rootstock for grafting to improve disease resistance especially for watermelon and cold tolerance (Wu *et al.*, 2017).

Like any other cucurbits, bottle gourd too suffers from many biotic factors namely fungi, bacteria and viruses. The fungal key players are *Colletotrichum lagenarium, Erysiphe cichoracearum* and *Pythium aphanidermatum, Alternaria alternata, Fusarium oxysporum* f.sp. *lagenariae* and *Cercospora lagenariae.* In case of bacteria, *Pseudomonas syringae* pv. l*achrymans* and *Erwinia tracheiphila* are major pathogens. However, in case of plant viruses about fifty-nine different viruses are known to cause various diseases in cucurbits (Nagendran *et al.*, 2017a) and their outbreaks have resulted in severe losses (Lecoq and Desbiez, 2012). The prevalent viruses reported and characterized in bottle gourd belongs to Potyviruses, Begomoviruses and Tobamo or Tospoviruses *viz., Cucumber Green Mottle Mosaic Virus* (CGMMV), *Cucumber Mosaic Virus* (CMV), *Watermelon Mosaic Virus* (WMV), *Cucumis Virus-3, Papaya Ringspot Virus-W* (PRSV-W), *Pumpkin Yellow Vein Mosaic Virus* (PYVMV), *Zucchini Yellow Mosaic Virus* (ZYMV), *Squash Mosaic Virus* (SqMV) etc. (Nagendran *et al.*, 2017b).

Among the different viral diseases mosaic disease of bottle gourd is a serious problem in the sub-tropical areas of Jammu. The disease is caused by CGMMV and PRSV- W. Apart from causing wide varieties of mosaic, these viruses cause a wide range of symptoms like mottling, puckering, blisters on leaves and fruits, mosaic with ringspots etc. (Nagendran, 2014). In India, mosaic disease in bottle gourd was first reported by Vasudeva and Lal (1943), which was later identified as CGMMV (Capoor and Varma, 1948; Vasudeva *et al.*, 1949). CGMMV is rod shaped, positive sense, single stranded *Tobamovirus* and is responsible for 100 per cent incidence in Northern parts of India and causes epidemic throughout the world. The virus is responsible for 64 per cent reduction of yield in bottle gourd and 15 per cent in cucumber. In severe cases, the fruit formed are blistered which renders it unmarketable (Fletcher *et al.*, 1969; Singh and Dey, 1976).

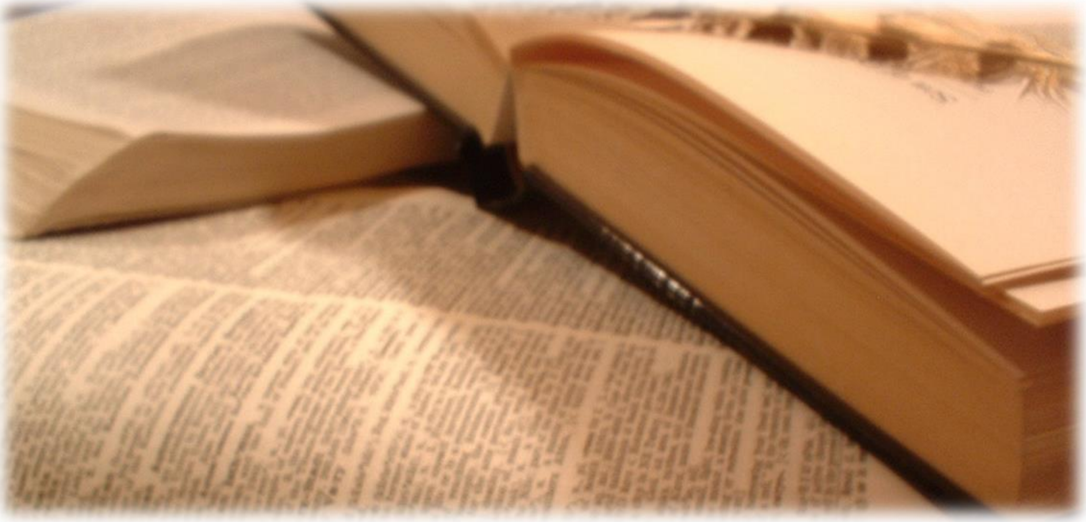
Another virus having a high occurrence in bottle gourd is PRSV-W (earlier known as WMV-1). This virus was first reported in bottle gourd from Maharashtra, India by Mantri *et al*. (2004) resulting an incidence of 10 to 100 per cent. PRSV-W is flexuous, single stranded positive sense RNA virus and a member of *Potyvirus* group. In India, PRSV is responsible for causing 85-90 per cent yield loss in all the geographical areas (Hussain and Varma, 1994).

The viruses causing mosaic in bottle gourd are transmitted in more than one way (infected seeds, farm tools, soil, plant debris etc.) but generally by insect- vectors under field conditions. This increases the chances of losses incurred by them. The members of order Hemipetra like aphids (*Aphididae: Myzus persicae* and *Aphis gossypii*) and whiteflies (*Aleyrodidae: Bemisia tabaci)* are majorly transmitting bottle gourd viruses under field conditions. Therefore, it is important to understand their mode of transmission in order to manage them effectively.

In Jammu, the bottle gourd is grown by small scale or by migrant farmers, predominantly on the banks of Tawi River. However, a very scanty information is available regarding the occurrence and prevalence of the mosaic causing viruses, let alone their characterization. Therefore, keeping in view, the economic and social importance of the crop and the level of losses caused by these viruses either through single or mixed infections, the present study was formulated to investigate and characterize the causal virus with the following objectives:

1. To ascertain the status of mosaic of bottle gourd in different subtropical areas of Jammu division
2. Identification of viruses causing bottle gourd mosaic disease
3. To study host range and insect-vector transmission of causal virus
4. Evaluation of bottle gourd germplasm for host resistance against bottle gourd mosaic disease

REVIEW OF LITERATURE



CHAPTER-II

REVIEW OF LITERATURE

Bottle gourd (*Lagenaria siceraria*) acts as a host to various viruses both *in vivo* and *in vitro* conditions. Although, more than fifty viruses are known to infect various cucurbits like cucumber, squash, pumpkin, watermelon, gherkins and various other gourds (Lecoq and Desbiez, 2012; Nagendran *et al.*, 2017a), the literature relevant to bottle gourd mosaic disease is comparatively sparse. However, the naturally occurring bottle gourd viruses affecting various cucurbits/ bottle gourd in India are enlisted as under:

Family	**Genus**	**Virus**	**References**
Potyviridae	*Potyvirus*	*Zucchini Yellow Mosaic Virus (ZYMV)*	Verma *et al.* (2004)
		Water melon Mosaic Virus (WMV)	Singh (1989)
		Papaya Ringspot virus (-W strain)	Mantri *et al.* (2004)
Bromoviridae	*Cucumovirus*	*Cucumber Mosaic Virus (CMV)*	Ullman *et al.* (1991); Horvath *et al.* (1994)
Virgaviridae	*Tobamovirus*	*Cucumber Mosaic Mottle Virus (CGMMV)*	Capoor and Varma, (1948); Vasudeva *et al.* (1949)
Alphaflexiviridae	Potexvirus	*Lagenaria Mild Mosaic Virus* (LaMMoV)	Chakraborty *et al.* (2019)

Although the disease is caused by different viruses but only two viruses were identified during the study. The literature pertaining to the causal viruses of Bottle gourd mosaic disease has been reviewed here under:

2. 1 *Cucumber green mottle mosaic virus* (CGMMV): A *Tobamovirus*

2.1.1 Taxonomy

Domain: Virus
Family: *Virgaviridae*
Genus: *Tobamovirus*
Species: *Cucumber green mottle mosaic virus*

Bewley (1926) was first to report this virus and used the name Cucumis virus 2. However, CGMMV was first described by Ainsworth (1935) while Adams *et al.* (2009) reported that the virus is a member *Tobamovirus* genus belonging to family *Virgaviridae.*

2.1.2 Occurrence

Cucumber green mottle mosaic virus (CGMMV) a species under the genus *Tobamovirus,* family *Virgaviridae* is a major mosaic causing pathogen. CGMMV has a narrow host range.

Earlier CGMMV was known as Cucumis virus 2 (strains 2A, 2S and 2 C), *Cucumis virus* 3 and *Cucumber virus* 4. Ainsworth (1935) first reported CGMMV (*Cucumis virus* 3 and *Cucumis virus* 4) from England. Even though, first reported from Europe this virus is presumed to be Asiatic in origin due to number of isolates/ strains available and the variability amongst them. CGMMV has been reported from various cucurbits *viz.*, cucumber, bottle gourd, muskmelon, pumpkin, snake gourd, ridge gourd, water melon, gherkins (Lovisolo, 1980).

Inouye *et al.* (1967) first reported CGMMV in cucumber from Western parts of Japan. Four different strains have been described in Japan: cucumber (CGMMV-C; Inoue- Nagata *et al*., 2007), Yodo (CGMMV-Y; Kitani *et al*., 1970), muskmelon (CGMMV-SH; Ugaki *et al*., 1991; Tan *et al*., 2000) and watermelon (CGMMV-W; Komuro *et al*., 1968). Later, Komuro *et al.* (1971) isolated and identified another watermelon strain of CGMMV from watermelon in Eastern Japan.

CGMMV is reported to be widespread in West and East Georgia (USSR) in cucumber and on watermelon, cantaloupe melon, cucumber, *Luffa acutangula* from

Iran (Komuro *et al.*, 1971; Rahimian and Izedpanah, 1977) and melon (Yamashita *et al.*, 1979). In central and northern Europe and Japan, CGMMV was reported to cause heavy yield losses mainly in cucumber grown in the protected cultivation (Conti and Lovisolo, 1982).

The virus had been spread from Europe to Asia, and more recently to North America and Africa (Dombrovsky *et al.*, 2017) causing epidemics across United Kingdom, Germany, Finland, Israel, Saudi Arabia, India, Pakistan, Korea, Japan and Greece (Rao *et al.*, 2017).

i) Occurrence in India

CGMMV occurs commonly and naturally in India infecting several cucurbits in open fields. In India, CGMMV is reported to cause diseases in bottle gourd (Rao and Varma, 1984), muskmelon and watermelon (Capoor and Varma, 1948; Vasudeva *et al.*, 1949; Vasudeva and Nariani, 1952; Janardhan *et al.*, 1969; Jaganathan *et al.*, 1970; Shankar *et al.*, 1971; Shankar and Nariani, 1974; Raychaudhuri and Varma, 1975, 1978; Nariani *et al.*, 1977 and Rao and Varma, 1984) and snake gourd (Verma *et al.*, 1970).

In India, Vasudev and Lal (1943) were the first to report the occurrence of the virus disease of bottle gourd from Delhi, India and named the virus as Cucumis virus-3. Capoor and Varma (1948) reported mosaic disease of bottle gourd from Bombay and later a new strain of Cucumis virus 2 from bottle gourd was also reported by Vasudeva *et al.* (1949). The virus was proposed as Cucumis virus 2C (according to Smith Classification, 1935).

Lal and Chauhan (1961) reported Cucumis virus 2C (CGMMV) in the pollen and embryo of the systemically infected *L. siceraria* plants. But such plants failed to produce seed borne infection. Mitra and Nariani (1965) identified Cucumis virus 3(CGMMV) from *Luffa acutangula*, while Raychaudhuri and Varma (1978) isolated the virus from diseased muskmelon.

Indian strains of CGMMV were often called as bottle gourd mosaic virus, Cucumis Virus 2 B and Cucumis Virus 2 C but later Lovisolo (1980) suggested to use CGMMV to avoid confusion.

Nagendran *et al.* (2017a) reported occurrence of CGMMV from different cucurbits growing regions in all agroclimatic zones of Tamil Nadu. They detected

CGMMV predominantly from pumpkin, bottle gourd, snake gourd, cucumber and sparsely from bitter gourd, ridge gourd and watermelon.

Kumari *et al.* (2021) reported occurrence of CGMMV in twelve different cucurbit species from nine agroclimatic zones of Uttar Pradesh.

2.1.3 Incidence

The incidence of CGMMV in cucumber has been reported by various workers (Yakovleva, 1965; Hollings *et al.,* 1975). Raychoudhuri and Varma (1978) reported the mosaic disease of muskmelon in Delhi with nearly 70 to 80 per cent incidence and identified the virus as CGMMV-M (muskmelon strain). However, Faris-Mukhayyish and Makkouk (1983) determined 3 to17 per cent incidence of CGMMV in cucumber seeds.

Ali *et al.* (2004) surveyed five major cucurbit growing areas and found that CGMMV was the major virus in cucurbits with an incidence of 46.9 per cent. They also reported mixed infection was common in these areas accounting 42 per cent of all the samples collected.

Thonmo and Thummabenjapone (2008) found CGMMV in 25 per cent of cucurbits collected from cucurbit seed production fields of Northeastern Thailand.

Liu *et al.* (2009) surveyed twelve different provinces of China and found that the disease caused 28.31 per cent incidence in watermelon.

Sharma *et al.* (2012) reported CGMMV in bottle gourd with an incidence of 15 per cent from the Trans- Gangetic plains of India.

Ali *et al.* (2014) surveyed twenty-two fields across seven districts of Khyber Pakhtunkhwa Province (KPK) of Pakistan and reported 29.4 per cent incidence of CGMMV from district Dir and 58.1 per cent from Nowshera. CGMMV was reported in bottle gourd with an incidence of 59.3 per cent from the plains and 49.6 per cent from the hilly areas of KPK.

Rao *et al.* (2017) detected CGMMV from bottle gourd growing areas of Dongyang, Quzhou, Weiling and Zhejiang, China during 2013-14. The detection rate for CGMMV from the bottle gourd fields ranged from 0 to 100 per cent.

Nagendran *et al.* (2017a) conducted field surveys from 2012-2014 in Tamil Nadu and reported the occurrence of various viruses from different cucurbits. They reported 22.2 per cent incidence of CGMMV from different cucurbit species (bottle gourd, pumpkin, cucumber, snake gourd, water melon and ridge gourd).

Kumari *et al.* (2021) surveyed 14 different types of cucurbit crops grown across nine agroclimatic zones of Uttar Pradesh and found 39 per cent incidence of CGMMV.

Kehoe *et al.* (2022) conducted an extensive survey of 95 locations covering commercial farms, roadside verges and seedling nurseries and the incidence ranged from 0.2 to 97 per cent.

2.1.4 Symptomatology

Vasudev and Lal (1943) elucidated the symptoms of bottle gourd mosaic as chlorotic spots, dark green blisters and mottling on leaf blade.

Capoor and Varma (1948) observed intermingled pale and dark green patches on leaves. They also reported occasional and random yellow or whitish chlorotic areas on leaf blade. The fruit set was small, light weighed with patchy discoloration.

Valentin (1958) reported *Cucumis Virus strain* 2C (CGMMV) in cucumber seedlings as green mottle mosaic, stunting, vein clearing, vein banding and a pale-yellow mosaic.

Mitra and Nariani (1965) observed light and dark green mottling, downward leaf curling, and stunting from the *L. acutangula* plants infected by Cucumis Virus strain 3 (CGMMV).

In muskmelon, CGMMV caused mosaic mottling, vein banding and blistering. The diseased plants remained stunted and only a few flowers and fruits were borne (Shankar and Nariani, 1974; Nariani *et al.,* 1977; Raychaudhuri and Varma, 1978).

Thomas (1984) found that the plants affected by CGMMV were seriously wilted and delayed fruit production. Leaves had occasional localized necrosis and fruits showed extensive pale green or yellow necrosis together with small necrotic lesions encircled by dark green blistering watery edges.

Ali *et al.* (2014) observed various symptoms *viz.,* mosaic, mottling, chlorosis and leaf deformation in Pakistan during field surveys. Mosaic symptoms were predominantly associated with bottle gourd.

Ling *et al.* (2014) observed green mottle mosaic and necrotic spots on *Cucumis sativus.* In case of early infection light green mottle and blisters were recorded which later turned into darker green blisters and green mottle mosaic symptoms while stunting in severely infected plants.

2.1.5 Host Range

The host range of CGMMV is reported to be narrow and limited to *Cucurbitaceae* and *Chenopodiaceae*. Vasudev and Nariani (1952) studied the host range of Cucumis Virus strain 2C (CGMMV) from *L. leucantha*. The virus caused localized infection on tobacco, *Solanum nigrum* and *S. nudiflorum* while no symptoms were observed on tomato or chilli.

Naqvi and Mahmood (1975) studied the host range of bottle gourd mosaic virus (*Cucumis virus* 2) and found mosaic symptoms from *Commelina nudiflora*, *Mukia maderaspatana* and *Salvia* spp.

Raychaudhuri and Varma (1978) conducted the host range studies of CGMMV- M (watermelon strain) and found this strain restricted to *Cucurbitaceae* only. They observed systemic symptoms like mosaic on *Cucumis sativus, Cucurbita pepo* and *Tridoosanthes anguina,* a mild mosaic on *L. siceraria* cv. long and *L. siceraria* cv. round while a severe mosaic was observed on *Cucumis melo*.

Subbaiah and Gopal (1997) while studying the host range of CGMMV observed local chlorotic and necrotic spots on *C. amaranticolor*, mosaic on squash, *Cucumis* spp., vein banding in pumpkin and severe mosaic on *L. siceraria*

Varveri *et al*. (2002) determined the host range of CGMMV Greek isolates and reported that the virus retains its infectivity for 10 months when stored at 4 °C. They observed systemic mosaic in *Cucumis melo*, *C. sativus* and *Nicotiana benthamiana* while, symptomless but systemic infection in *Citrullus vulgaris* and *L. siceraria.*

In case of *C. amaranticolor* like previous workers local lesions were reported whereas, no infection was observed on *Datura stramonium*. They found *Cucurbita pepo, Chenopodium quinoa, Vicia faba, Nicotiana tabacum L.* cv. *xanthi, N. tabacum, L.* cv. *samsun* and *N. glutinosa* x *N. tabacum* to be immune.

Zhou *et al.* (2008) found the host range of CGMMV in *C. vulgaris, L. siceraria, C. sativus, C. melo, L. siceraria* var. *makinoi, C. moschata* and *C. pepo* with systemic mosaic symptoms.

Crespo *et al.* (2017) conducted a host range study for the CGMMV isolates collected from Spain and found that cucurbits, all the cucurbits (*C. sativus, C. melo, C. lanatus, C. pepo* and *C. maxima*) were susceptible especially cucumber which exhibited severe blisters. *Gompherena globosa* developed localised red spots, *C. amaranticolor* expressed local lesions for few isolates.

2.1.6 Insect Transmission

Insect transmission has time and again been studied by various workers, but no vector specificity by insects has been successfully established till date.

Inouye *et al.* (1967) studied the transmission of CGMMV and found that it was sap transmissible but failed to transmit virus via aphids or cucumber beetle. Similarly, Shankar *et al.* (1971) identified *Cucumis virus* 2 from bottle gourd plants and found that the virus was not transmitted by aphids. Likewise, Hollings *et al.* (1975) reported that the virus was not transmitted by the aphids, (*Myzus persicae, Aphis gossypii*) or by cucumber leaf beetle (*Aulacophora femoralis*)

Raychaudhuri and Varma (1978) studied transmission of *M. persicae, A. gossypii, A. craccivora, A. nerii* and *Rhopalosiphum maidis* from infected muskmelon to healthy muskmelon plants in non-persistent manner and found that the virus was not transmitted in non-persistent manner by any of the aphids. However, Rao and Varma (1984) reported the transmission of CGMMV by *Radidopalpa faeveicollis* (red pumpkin beetle) as a non-specific vector.

Subbaiah and Gopal (1997) identified CGMMV from snake gourd and reported that it was transmitted by seeds but not by *A. craccivora* and *A. gossypii.* While, Darzi *et al.* (2018) reported that CGMMV was transmitted from infected to healthy plants non-specifically by *Apis mellifera*.

2.1.7 Virion morphology and Genome Organization

CGMMV is a rod shaped (300 X 15 nm), single stranded, positive sense RNA virus of 6.4 kb size (Ugaki *et al*., 1991). The CGMMV genome encodes two replication protein (129 KDa and 186 KDa), one movement protein (29 KDa MP) and one coat protein (17.4 KDa CP) using four open reading frames (ORFs). The two replication proteins are translated genomic RNA, while the MP and CP are translated from two sub genomic RNAs (Ugaki *et al.,* 1991; Mandal *et al.,* 2008; Liu *et al.,* 2020).

2.1.8 Molecular Characterization

Ali *et al.* (2004) identified and characterized CGMMV isolates from Pakistan using coat protein specific primers. The CGMMV-Pak isolate (AB127937) shared lower diversity among them. The amino acid identity ranged from 98.1 per cent to 99.4 per cent with GR-7 and Korean and Japanese isolates respectively

Zhang *et al.* (2009) surveyed major cucurbit growing areas of China and detected CGMMV in *C. lanatus, L. siceraria, C. melo* and *C. moschata.* An isolate (GenBank Accession No. DQ997778) from *L. siceraria* obtained from Liaoning province shared 100 per cent and 91 per cent sequence identity with Japan-W (watermelon strain) and Greece GR5 strain respectively.

Liu *et al.* (2009) characterized the complete sequence of CGMMV-LN isolated from watermelon. The phylogenetic analysis revealed that this Chinese isolate (CGMMV-LN) shared a highest sequence identity (99.4%) with Korean strains/ isolates (CGMMV-KOM and CGMMV-KW), and sequence identity of 97.3–97.7 per cent with other CGMMV watermelon isolates indicating evolutionary relationship between the Chinese and Korean isolates.

Ali *et al.* (2014) detected CGMMV from Pakistan using RT-PCR and generated an amplicon of approximately 1 kb. The sequence analysis of the CGMMV isolates when compared with other reported isolates showed low diversity. All the isolates showed 100 per cent amino acid sequence identity among themselves and shared per cent identities in range of 98.052 per cent (Korea, India and Greece) to 99.351 per cent (Japan, France, Korea, China, Indonesia and India) with the other isolates obtained from GenBank.

Sharma *et al.* (2014) reported CGMMV from *L. acutangula* (ridge gourd) by per forming single tube RT-PCR using oligo dT primer and a Tobamovirus coat protein specific primer. The CGMMV isolate RKG (acc. No. KF702319) shared sequence nucleotide similarity ranging from 85 to 93 per cent with other isolates.

Rao *et al.* (2017) characterized seven isolates of CGMMV and through phylogenetic analysis found that, these seven isolates with other 24 isolates formed two sub groups (Asian subtype I and European subtype II) according to their geographical distribution. All the test isolates formed a group in subtype I.

Crespo *et al.* (2017) explored CGMMV isolate Alm08 (CGMMV-SP) from Almeria, Spain. RT-PCR was used to detect CGMMV, and sequence analysis was done. The phylogentic analysis revealed the test isolate closest to Russian isolates (FJ848666, GQ495275 and GQ495274) sharing an identity of 99 per cent and the lowest with the isolates from China and South Korea.

Cho *et al.* (2021) isolated the total RNA from the infected perilla leaves and a cDNA library was prepared and sequenced. De Novo assembly and BLAST analysis revealed this virus to share 96.83 to 99.32 per cent nucleotide identity to the CGMMV reference genome.

Kehoe *et al.* (2022) reported seven new sequences of CGMMV from five regions of Australia. These sequences shared a pairwise identity of 99.1 – 99.9 per cent with each other and 89.4 – 100 per cent with 121 other genome sequences obtained from NCBI. They were most closely related to the USA and the Netherlands strains.

2.1.9 Host resistance

Rajamony *et al.* (1990) screened one hundred eighty-seven accessions of *C. melo* and eight wild *Cucumis* species against CGMMV under both field and controlled conditions. Out of the eight wild species. *C. africanus, C. ficifolius, C. figarei, C. meeussii, C. myriocarpus* and *C. zeyheri* were found resistant.

Pan and More (1996) determined resistance against CGMMV in seventy-two melon germplasm under artificial conditions and found *C. figarei*, Phoot (*C. melo* var. *momordica*) and advance lines *viz.*, VRM 31-1-2, VRM5-10A, VRM 5-10B, VRM 1-3A and VRM 1-3B resistant/ tolerant to CGMMV.

Sugiyama *et al.* (2006) screened 152 melon accessions for resistance against CGMMV using the method of mechanical inoculation. Only ‘Chang Bougi’ (var. *makuwa* Makino), a Korean accession was resistant to CGMMV.

Crespo *et al.* (2018) evaluated fifty-six *C. sativus* accessions against CGMMV and found only *C. anguria* was resistant, whereas all fifty-five *C. sativus* accessions were susceptible showing varied symptoms from soft mottling, mosaic-mottle on leaves, interveinal chlorosis, leaf blisters and mosaic on fruits.

Ruiz *et al.* (2021) screened forty-seven accessions of *C. melo* against European and the Asian strain of CGMMV and found thirty accessions susceptible to CGMMV.

2. 2 ***Papaya Ringspot Virus- Watermelon strain* (PRSV-W): A *Potyvirus***

2.2.1 Taxonomy

Domain: Virus

Family: *Potyviridae*

Genus: *Potyvirus*

Species: *Papaya Ringspot Virus*

Jensen (1949) coined the term papaya ringspot (PRS) while describing a papaya disease reported from Hawaii. Diseases such as papaya mosaic (caused by *Papaya mosaic virus*) and water melon mosaic (caused by *Watermelon mosaic virus*-1) were shown recently to be caused by Papaya ring spot virus (PRSV). PRSV (W and P type) is a major threat to papaya and cucurbit growing areas worldwide. It is believed that PRSV-P emerged from PRSV-W in Australia.

2.2.2 Occurrence

Mantri *et al.* (2005) first reported the occurrence of PRSV-W from infected bottle gourd leaves collected from Nasik, Maharashtra. The isolate did not infect papaya upon inoculation therefore indicated the test isolate to be W strain instead of P strain of PRSV.

In Brazil, PRSV-W has been reported from several states and as one of the economically threatening viruses in squash, zucchini squash, watermelon, melon and other cucurbit species (Yuki *et al.*, 2000; Moura *et al.*, 2001; Lima *et al.*, 2001; Nagata *et al.*, 2007).

The emergence of PRSV from *Coccinia indica*, *Benicasa hispida*, *L. acutangula* and *C. sativus*) has been reported from various workers (Lecoq *et al*., 2001; Yakoubi *et al*., 2008).

Ali *et al.* (2012) reported the occurrence of multiple viruses (CMV, PRSV-W, ZYMV, WMV) infecting cantaloupe, cucumber, pumpkin, squash and watermelon in Oklahoma, USA. PRSV-W was the most prevalent virus associated either as single or as mixed infections in all the cucurbits. Kone *et al.* (2017) reported the widespread occurrence of PRSV-W along with no. of other cucurbit viruses from Cote d'Ivoire.

2.2.3 Incidence

Mantri *et al.* (2005) reported an incidence of 10 to 100 per cent of PRSV-W from Maharashtra. Meanwhile, Papayiannis *et al.* (2005) conducted an intensive of cucurbit growing areas of Cyprus from 200-2002 and determined the prevalence of PRSV-W along with other viruses like *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), *Cucurbit aphid-borne yellows virus* (CABYV), *Cucumber mosaic virus* (CMV), *Squash mosaic virus* (SqMV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Beet pseudo-yellows virus* (BPYV) and *Cucumber vein yellowing virus* (CVYV). They reported that PRSV-W was detected from 20.8 per cent of the samples tested.

Ali *et al.* (2012) reported a high PRSV-W incidence ranging from 48 to 53 per cent from various cucurbits grown at Oklahoma, USA. However, Abdalla and Ali (2012) reported PRSV-W from watermelon crops with an incidence of fifty per cent.

Nagendran *et al.* (2017a) reported an overall incidence of 32.1 per cent for PRSV-W infecting various cucurbit species. This observation was made during 2012-2014 field surveys from different agroclimatic zones of Tamil Nadu.

Ashfaq *et al.* (2021) surveyed and collected samples from major cucurbit growing areas of Gujranwala, Punjab, Pakistan and found that PRSV-W caused an average incidence of 7.2 per cent in cucurbits.

Khanal *et al.* (2021) collected different samples of cucumber, squash, watermelon, pumpkin, cantaloupe and gourds from cucurbit fields and found PRSV-W was the most prevalent virus with an average incidence of 59.1 per cent.

2.2.4 Symptomatology

Mantri *et al.* (2005) recorded symptoms like mosaic, mottling, distortion of leaves, interveinal chlorosis and malformed fruits from PRSV-W isolate infecting bottle gourd.

Omar *et al.* (2011) observed symptoms of mosaic, malformation such as blisters and narrow leaf blades and malformed fruits in squash (*C. pepo*) from Egypt.

Kumar *et al.* (2014) recorded mosaic, blisters on leaf, leaf distortion, mosaic mottling from PRSV-W infected snake gourd plants from Coimbatore, Tamil Nadu.

Udavant (2018) observed various symptoms *viz.,* severe mottling, mosaic, green blistering, puckering, leaf distortion and overall stunting from infected bottle gourd plants under field and controlled conditions.

2.2.5 Host Range

PRSV-W is known to infect about 38 cucurbit species having two strains (-W and -P) which differs in their ability to infect different hosts (Purcifull *et al.*, 1984). PRSV strain P infects both Papaya and cucurbits while strain W doesn’t infect papaya (Yeh and Gonsalves, 1984).

Mantri *et al.* (2005) studied the host range of isolate BgM-NW-3 (PRSV-W) collected from bottle gourd and found that eleven cucurbits, three solanaceous crops expressed mosaic mottling symptoms.

Biswas and Varma (2006) characterized a PRSV-W isolate from pumpkin, and named this WMV-A. They used twenty-four plant species (seven families) for determining the host range and reported its infection to members of *Cucurbitaceae* including *L. acutangula* and *L. cylinderica.* Mosaic and vein clearing were the most common symptom that was observed.

Kumar *et al.* (2014) during the study reported that PRSV-W produced varied symptoms from mosaic to chlorosis in bottle gourd, ridge gourd, cucumber, pumpkin and melon at 15-17 days after inoculation. Chlorotic lesions were noted from *C. amaranticolor*. However, no symptoms were observed from papaya therefore confirming it to an isolate of PRSV-W.

Begum *et al.* (2016) conducted a thorough study of the host range of PRSV-W. They observed that the virus was sap transmissible to all plants of

Cucurbitaceae, *Chenopodiaceae*, *Amaranthaceae* and *Leguminosae* except that of *Solanaceae.* A range of symptoms were observed i.e. chlorotic lesions, necrotic lesions, systemic mosaic and leaf distortion.

Udavant (2018) screened plants of different families (*Cucurbitaceae, Leguminaceae, Solanaceae, Chenopodiaceae, Cruciferae, Malvaceae* and *Poaceae*) against bottle gourd mosaic disease and found only plants from *Cucurbitaceae* family were susceptible and exhibited symptoms like mosaic, mottling, blisters, puckering, vein clearing etc.

2.2.6 Insect Transmission

Quiot-Douine *et al.* (1990) reported that PRSV-W was transmitted by *A. gossypii* with per cent transmission 3 to 86 per cent.

PRSV-W isolates are transmitted by 24 aphid species in 15 genera with *Myzus persicae, Acyrthosiphum solani, Aphis craccivora* and *Macrosiphum euphorbiae* as natural vectors (Purcifull *et al.,* 1984; Shukla *et al.,* 1994).

Mantri *et al.* (2005) studied the aphid transmission of BgM-NW-3 (PRSV-W) isolate from Nasik, Maharashtra from bottle gourd and reported *A. gossypii* to transmit the virus non-persistently.

Biswas and Varma (2006) found *A. craccivora,* an efficient vector for WMV-A (PRSV-W) than *M. persicae.*

Kalleshwaraswamy and Kumar (2008) studied the transmission efficiency of three aphids *viz., M. persicae, A. gossypii* and *A. craccivora* and found *M. persicae* most efficient with 56 per cent transmission followed by *A. gossypii* and *A. craccivora* at 53 per cent and 38 per cent transmission respectively.

Pinto *et al*. (2008) reported that *A. gossypii* and *M. persicae* transmitted PRSV-W with an average transmission efficiency of 92 per cent.

Angelella *et al.* (2015) studied the vector transmission of both colonizing and non- colonizing aphids on pumpkin and identified about 53 aphid species, out of which the *A. gossypii* (pumpkin colonizing, melon aphid) was more prevalent.

Udavant (2018) studied the vector transmission of the test isolate using *M. persicae* and *A. gossypii*. *M. persicae* was reported as more efficient vector than *A. gossypii*. She determined the optimum acquisition feeding period (15 min), infection feeding period (30 min), pre-acquisition starvation period (120 min) and post-acquisition interval period (0 min) for successful transmission of the virus.

2.2.7 Virion morphology and Genome Organization

PRSV is a filamentous, positive sense, single stranded RNA (750 X 12 nm in size). The RNA is made up of approximately 10 kb nucleotides. PRSV has a single ORF which translates into a single polypeptide. This polypeptide is later cleaved into ten functional proteins. The CP (36 KDa) is highly conserved and important for aphid transmission (DAG motif) (Shukla and Ward, 1989; Riechmann *et al.,* 1992)

2.2.8 Molecular Characterization

Bateson and Dale (1992) determined the CP gene sequence of an Australian PRSV-W isolate and the sequence analysis found it to cluster with U.S. isolates of PRSV (P and W) and shared a sequence identity of 96 and 98 per cent for the nucleotide and amino acid sequence, respectively.

Nagata *et al.* (2007) collected two isolates of PRSV-W *viz.,* PRSV-W-1 and PRSV-W-C from zucchini squash. The genome analysis of the two strains revealed that PRSV-W-1 was 10,332 nucleotides long while PRSV-W-C was 10,327 nucleotides long. Both the strains shared 94.63 per cent of nucleotide identity with each other and none of the two strains were recombinant.

Ali *et al.* (2012) used RT-PCR to detect the presence of PRSV-W in cucurbit samples using coat specific primers of PRSV-W.

Abdalla and Ali (2012) explored the 3’ terminal region of sixty-four PRSV-W isolates from watermelon. The PRSV-W Oklahoma isolates shared a per cent nucleotide sequence identity of 95.2 – 100 per cent and an amino acid identity ranging from 97.1 – 100 per cent.

Kumar *et al.* (2014) identified PRSV-W from *T. cucumerina* (snake gourd) from Coimbatore, Tamil Nadu using RT-PCR. They used potyvirus specific primers (HRP 1-4) generating an amplicon of approximately 650 bp.

Ali (2017) determined the whole genome sequence of PRSV-W TUL15 isolate from gourd in Tulsa, Oklahoma, USA. The genome was ~10.3 kb in size and had a poly (A) tail at 3’ end. The isolate shared a per cent identity ranging from 83-92 per cent at nucleotide level and 90-95 per cent at amino acid. The isolate shared maximum similarity with a French isolate E2 (KC345609) i.e. 92 per cent and 95 per cent with nucleotide and amino acid sequence.

Maina *et al.* (2017) characterized the complete genome sequence of seventeen PRSV-W isolates infecting watermelon from East Timor and North Australia. Phylogeny revealed that the East Timor isolates and Taiwanese isolate shared 89.6 per cent of sequence identity while, the Australian isolates were closest to the Mexican and Brazilian with a shared per cent identity of 92.3 per cent. Based on coat protein region the East Timor isolate shared 93.1 per cent nucleotide identity with Thai isolate.

Rajbanshi and Ali (2019) detected PRSV-W, ZYMV and WMV from infected cucurbit samples collected from Oklahoma in 2018 using multiplex PCR. HC-PRO gene specific primer was used for PRSV-W which generated an amplicon of 496 bp.

2.2.9 Host resistance

Baker *et al.* (1991) screened 17 isolates of PRSV-W on eight melon lines (differential) and found the strains to have an incompatible reaction with PI 414753, 7205 and WMR-29.

Hsiao *et al.* (1993) screened 54 cucumber varieties and 60 muskmelon varieties/lines for resistance against PRSV-W. They reported 2 cucumber varieties (Hei- Pei- Ta- Kua and Chang- Ching) and 3 muskmelon lines (B-63-3, PI-180280 and PI- 414723) highly resistant to PRSV-W.

Provvidenti (1993) reported resistance in various cucurbits (*C. sativus*, *C. melo, Cucurbita* spp, *Lagenaria* spp. and *Luffa* spp.) against PRSV-W.

Gibb *et al.* (1994) screened few commercial muskmelon cultivars (cv. Planters Jumbo, Cinbo, Cinco; hybrids Caravel, Meteor, mission and NC-3) and few wild cucurbits against PRSV-W for resistance. Cinco cultivar showed resistance with occasional mild symptoms when plants were grown at 25°C.

Strange *et al.* (2002) screened 1275 PI accessions (from 68 countries) of watermelon. ‘Charleston Gray’ was used as susceptible check. Sixty accessions were resistant to PRSV-W.

Brown *et al.* (2003) studied the inheritance of resistance in *C. moschata* against ZYMV, WMV, PRSV-W and CMV. They elucidated that PRSV resistance was monogenic recessive in nature while others were monogenic dominant in nature.

Dhillon *et al.* (2007) collected and studied the diversity of 36 snap melon germplasm from nine different agroclimatic sub-regions of Punjab, Rajasthan and Haryana, India. For PRSV, except for nine accessions *viz.*, IC 267360, IC 267363, IC 267374, IC 267384, IC 274006, IC 274007, IC 274010, IC 274011 and IC 274013 all remaining were susceptible.

Ling *et al.* (2013) reported three bottle gourd lines (USVL no. 1-8, 6-3 and 7-1) resistant to PRSV-W under field conditions whereas, under greenhouse conditions three USVL lines *viz.*, 3, 4 and 5-5 and two F1 hybrids (USVL no. 6-3 and 7-1) were resistant against PRSV-W. It was observed that USVL no. 6-3 and 7-1 gave complete resistance under both field and greenhouse conditions.

MATERIALS AND METHODS

CHAPTER-III

MATERIALS AND METHODS

The present investigation on **"Identification and Characterization of viruses causing Mosaic of bottle gourd in Jammu sub-tropics"** was carried out at the experimental farm and PG Laboratory at Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (SKUAST- J) during 2020 and 2021. The following materials and methods were followed for conducting various experiments regarding status of disease, biological and molecular assays and screening for host resistance:

3.1 Survey and collection of virus isolates

Periodic survey of bottle gourd growing areas of Jammu sub-tropics, were conducted during the growing seasons of 2020 and 2021. The four districts surveyed for assessing the prevalence of mosaic disease in bottle gourd were Jammu, Samba, Kathua and Udhampur (Table 3.1). During the surveys, infected plants exhibiting symptoms like mosaic, mosaic mottling, blisters, puckering, vein clearing and vein banding, mosaic with ringspots, mosaic with chlorosis etc. were collected randomly from each location.

About four to five young leaves with typical symptoms were collected per location and were kept in well labelled air tight zip lock bags. Each sample was then transported to the laboratory in an ice box for further analysis. In total 274 samples were collected, 149 from district Jammu, 47 from Samba, 45 and 33 from district Kathua and Udhampur, respectively. The observations were recorded on disease incidence, plant symptoms associated, presence or absence of vectors. The disease incidence was calculated as:

$$\text{Disease Incidence (D.I.)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \text{ X } 100$$

Each leaf sample was carefully packed in aluminum foil, placed in an air tight zip lock bag with proper labeling (sample ID, date of collection, location, symptom observed). These samples were stored at -20^{o}C for future use.

Table 3.1: Bottle gourd growing areas surveyed in Jammu sub tropics during 2020 and 2021

District	Block	Village	GPS coordinates	
			Latitude	Longitude
Jammu	R. S. Pura	Badyal Brahmana	32.66537	74.72887
		Kotli Shah Dhula	32.62439	74.76046
		Salaher	32.51794	74.76973
	Marh	PouniChak	32.736347	74.790009
		Brahmana Dhamwal	32.725891	74.757805
		Trilok Pur	32.715019	74.736137
		Bhad-Rode	32.707375	74.726738
		Ghou Manhasa	32.72337	74.73270
		Rathua	32.709925	74.677771
	Bishnah	Deoli	32.54666	74.80861
		Laswara	32.58146	74.83878
		Ratwal	32.590	74.895
	Miran Sahib	Rakh Chatha	32.66488	74.81889
Kathua	Nagri	Kakhyal	32.350	75.462
		Narroha	32.34805	75.44166
		Dadoli	32.34666	75.46000
	Hiranagar	Patwal	32.467	75.297
		Feruchak	32.471	75.273
Samba	Rajpura	Rajpura	32.456	75.168
		Sanoora	32.489	75.179
	Vijaypur	Koulpur	32.557	75.034
		Supwal	32.55325	75.05799
		Raiyan	32.60749	74.87503
		Yakh	32.59002	74.96749
	Ramgarh	Jenda	32.500	74.892
		Abtal	32.490	74.926
Udhampur	Chenani	Chenani	33.03630	75.28450
		Mantalai	32.99914	75.36511
		Kohsar	33.02603	75.36155
		Sudh Mahadev	33.02184	75.35546
		Kamali	32.92491	75.13921
		Chohan	32.877281	75.112865

Plate 1: A view of bottle gourd growing areas of Jammu during the surveys in 2020 & 2021

3.2 Maintenance of virus culture

The virus culture (isolates) collected from various locations were maintained at the greenhouse situated at experimental farm, SKUAST-J, Chatha. The isolates were multiplied on healthy seedlings of susceptible cv. ‘MAHY-1’ by sap inoculation from infected seedlings to healthy ones.

3.2.1 Raising of test plants

Healthy seedlings of the susceptible cv. ‘MAHY-1’, were raised in 9 and 12 inch plastic pots containing sterilized soil and vermicompost (2:1). The test plants were maintained in a greenhouse. To avoid vector infestation and mixing of isolates the greenhouse was sprayed/ drenched regularly with imidachloprid (@0.3ml/ l water) and neem baan (@3ml/l water).

3.2.2 Virus inoculum

Virus culture of test viruses was obtained during surveys from infected plants exhibiting typical viral symptoms and was used as primary source of inoculum for further studies. This primary source was then further maintained on a susceptible cultivar sown in plastic pots containing the sterilized potting mixture through artificial inoculation. The virus culture was then multiplied and maintained on healthy seedling of the same cultivar further on.

3.3 Mechanical transmission of the virus causing bottle gourd mosaic disease

The inoculum of the test viruses was prepared by collecting young leaves infected severely and showing prominent symptoms. The mechanical transmission of bottle gourd mosaic virus was done in the following ways:

1. The infected leaves were first washed once under running tap water and later rinsed with distilled water, dried using blotting sheets and macerated in sterilized pestle and mortar using chilled 0.1M phosphate buffer (sodium phosphate buffer, pH 7.0) (Appendix-I) as an extracting medium in equal amount (w/v, 1:1).
2. The sap obtained was sieved through double layer of muslin cloth and the filtrate was used as standard inoculum.
3. Plants were inoculated at cotyledonary or two leaf stage by usual leaf rub method.

4. An abrasive i.e. carborundum powder (400-600 mesh) was dusted on the leaf surface prior to inoculation.
5. The sap was rubbed gently on the leaves to avoid tissue injury.
6. After inoculation, leaves were washed with distilled water using wash bottle to remove excessive inoculum and specifically, the abrasive to avoid tissue injury.
7. Plants inoculated with plain water were kept as control in each experiment.
8. The plants were then kept under greenhouse with insect proof conditions.

3.4 Symptomatology

The type of symptoms produced by the viral isolates of bottle gourd mosaic disease were observed and recorded both during survey of the bottle gourd growing areas of Jammu as well as during the maintenance of the viral culture under controlled conditions. Under controlled conditions the symptoms were observed at regular intervals both in case of mechanically inoculated or vector inoculated plants. The virus samples showing characteristic symptoms were further utilized for molecular and biological assays.

3.5 Virus identification

3.5.1 RNA isolation

Total RNA of the isolates maintained on healthy seedlings of cv. ‘MAHY 1’ showing prominent symptoms, was extracted from younger leaves using RNA isolation Kit (Plant Total RNA kit, Sigma Aldrich). The standard protocol followed during the isolation was:

1. Infected leaves (100 mg) were crushed in liquid nitrogen using sterilized chilled pestle and mortar.
2. The crushed powder was transferred into 2 ml eppendorf tubes and 500 µl of lysis solution was added and vortexed vigorously for 30 secs. The tubes were incubated at 56°C for 5 minutes.
3. The samples were centrifuged at 12000 g for 10 minutes at 4°C and the lysate supernatant was transferred into a filtration column (blue ring) seated in a 2ml eppendorf tubes.

4. The samples were again centrifuged at maximum speed for a minute and the flow through was retained.
5. Binding solution (500 µl) was added into the clarified lysate and mixed by pipetting
6. About 700 µl of the mixture from each sample was transferred into binding column (red ring) placed on a fresh 2 ml collection tube.
7. The tubes were centrifuged at maximum speed for a minute for binding the RNA to the column. The flow through was discarded and the collection tube was placed on absorbent tissue to remove residual liquid.
8. Remaining sample from step 5 was added back to the same binding column and step 7 was repeated.
9. Wash solution- I (500 µl) was added to the column and centrifuged at maximum speed for a minute. The flow through was discarded and column was reseated on the collection tube.
10. About 500 µl of Wash solution- II was added to the same column and centrifuged at maximum speed for 30 seconds. The flow through was discarded, the collection tube was placed on absorbent issue and column was reseated on the collection tube.
11. The column was again centrifuged at max. speed for a minute to remove any remaining solution.
12. The column was then transferred to a fresh 2ml microfuge tube and 50 µl of elution buffer was directly added to the center of the binding matrix and centrifuged at maximum speed for 2 minutes to elute the RNA.
13. RNA obtained was stored in deep freeze at -20°C.

3.5.2 RNA check

Three µl of the total RNA along with 2 µl of 6X gel loading dye was loaded on 1 per cent agarose gel containing ethidium bromide (0.5 µg/ml). The gel was electrophoresed at 90 volts for 1 hour in 0.5 X TAE buffer (Appendix-II). Gel was viewed using UV transilluminator of the Gel documentation system (Genetix) and image was captured and saved.

3.5.3 cDNA synthesis

The cDNA was synthesized by using Verso cDNA synthesis kit (Thermo scientific) according to the manufacturer’s instructions. For a final reaction, volume of 20 µl reaction, 4µl of 5x cDNA synthesis buffer, 2 µl of dNTP mix (5mM), 1µl of Oligo-dT (500 ng/µl),1µl of verso enzyme mix and 5 µl of total RNA was used in the reaction mix, the remaining volume was made to 20 µl by adding PCR grade water. The contents were mixed gently and incubated at 42°C for 30minutes, 1 cycle in thermalcycler (Mastercycler Gradient, Eppendorf, USA). Later the cDNA was stored at -20°C for future use in RT-PCR.

3.5.4 Primer designing and synthesis

Six pairs of coat protein specific primer pre-designed and synthesized were used for the amplification of the coat protein gene of a *Tobamovirus* (CGMMV), a *Cucumovirus* (CMV), a *Tospovirus* (ZLCV) and three *Potyviruses* (ZYMV, PRSV-W and WMV) (Table 3.2).

3.5.5 PCR amplification

RT-PCR (reverse transcription-polymerase chain reaction) was carried out using CP gene specific primers (Table 3.2) RT-PCR amplification was carried out in 25 µl reaction volume using 12.5 µl PCR master mix (Promega), 2.5 µl of 10 mM forward and reverse primer, 5.0 µl of cDNA and final volume was adjusted with nuclease free water. Amplification was performed in Gradient Mastercycler (Eppendorf) with the detail of the PCR cycle mentioned here under:

VIRUS	INITIAL DENATURATION	DENATURATION	ANNEALING	EXTENSION	FINAL EXTENSION
CGMMV	94º C	94º C	48º C	72º C	72º C
	2:00	0: 30	0:30	1:00	10:00
ZYMV	94º C	94º C	52º C	72º C	72º C
	2:00	0: 30	0:30	1:00	10:00
PRSV-W	94º C	95º C	52º C	72º C	72º C
	5:00	0:30	1:30	4:00	8:00
CMV	94º C	94º C	54º C	72º C	72º C
	2:00	1: 00	1:00	1:30	10:00
WMV	94º C	95º C	55º C	72º C	72º C
	5:00	1:00	1:00	1:00	10:00
ZLCV	94º C	95º C	52º C	72º C	72º C
	5:00	0:30	1:30	4:00	8:00

Table 3.2: Coat protein specific primers used for the detection of various viruses causing mosaic disease in bottle gourd

S. No.	Virus	Primer pair used	Sequence	Product Size (bp)	Reference
1.	**CGMMV**	CGMMV F	TAAGCGGCATTCTAAACCTCCA	604	Nagendran *et al.* (2017a)
		CGMMV R	CACTATGCACTTTGGTGTGC		
2.	**ZYMV**	ZYMV F	AAATGCAGAGGCACCATAC	1004	Nagendran *et al.* (2017a)
		ZYMV R	AGGCTTGCAAACGGAGTC		
3.	**PRSV-W**	PRSV-W F	TGGGTTATGATGGATGGGG	~398	Aguiar *et al.* (2018)
		PRSV-W R	ATACCCAGGAGAGAGTGCAT		
4.	**CMV**	CMV RNA3-F	GAGTTCTTCCGCGTCCCGCT	1216	Nagendran *et al.* (2017a)
		CMV RNA3-R	AAACCTAGGAGATGGTTTCA		
5.	**WMV**	WMV F	GCCGAGGTATGGTTTGCTTCG	~283	Aguiar *et al.* (2018)
		WMV R	GCTTCCGACGGACTACGGCA		
6.	**ZLCV**	ZLCV F	GAGTTTCACTGTAATGTTCCATAGC	~244	Aguiar *et al.* (2018)
		ZLCV R	AGYTTTGAGATGATCAGTGTTGT		

3.5.6 Agarose gel electrophoresis

The amplicon (3.0 µl) mixed with 2.0 µl of gel loading dye was loaded and electrophoresed in 1.2% agarose gel in 0.5X TAE buffer containing 0.5 µg/ml of ethidium bromide using horizontal agarose slab gel (genetix) apparatus at 90 volts for 1.5 hours. One kb DNA ladder (GeneRuler, Thermo Scientific™) for CGMMV and 100 bp plus DNA Ladder for PRSV-W, WMV and ZLCV (GeneRuler, Thermo Scientific™) were used as standard marker and product size was viewed under Gel documentation system using UV light and image was captured.

3.6 Coat Protein sequencing

3.6.1 Purification of PCR products

Desired RT-PCR amplified products of test virus (CGMMV and PRSV-W) were eluted from gel using DNA Gel Extraction Kit (HiYield^{TM} Gel/ PCR DNA Mini kit, Real Biotech Corporation, Taiwan) as follows:

1. Using scalpel blade the amplicon (about 300 mg) was excised and put in a 2 ml sterile microfuge tube having 500 µl DF buffer.
2. The tube was vortexed and incubated at 55°C - 60°C for 5-10 minutes until the gel dissolves completely.
3. The gel mixture was cooled down at room temperature and DF column was placed onto a collection tube. About 800 µl of dissolved gel mixture was loaded into the column. Centrifugation was done at maximum speed for 30-60 seconds and the flow-through was discarded.
4. Column was washed once with 400 µl of W1 buffer by centrifuging for 30-60 seconds at maximum speed. Flow through was discarded.
5. Column was washed with 600 µl of W2 containing 100 per cent ethanol, the column was kept at stand for 1 min and later centrifuged for 30-60 seconds at full speed. Again flow-through was discarded and the column matrix was dried by centrifugation for 3 minutes at full speed.
6. Further, the column was placed onto a new 2 ml centrifuge tube and 30-50 µl of sterile water was poured in the center of the membrane.

Column was incubated at RT for 2 minutes and centrifuged for 2 minutes to elute DNA. Eluted DNA was checked on 1 per cent agarose gel and stored at -20°C for further use `

3.6.2 Nucleotide sequencing and Phylogenetic analysis

Nucleotide sequences obtained after custom sequencing were manually assembled using Bioedit software. The assembled sequence was subjected to nucleotide blast at NCBI BLASTn (www.ncbi.nlm.nih.gov./blast) (Altschul *et al.,* 1997) to confirm the identity of CGMMV and PRSV-W at molecular level. Later the assembled sequences were submitted to NCBI GenBank for accession number.

Complete genome and coat protein sequences of different strains/ isolates of PRSV-W and CGMMV were selected from NCBI GenBank for sequence comparison with the test strain of PRSV-W and CGMMV (Table 3.3 and 3.4). The phylogenetic analysis was done by using MEGAX software (Kumar *et al.,* 2018) and tree was constructed by neighbour joining method using bootstrap test (500 replicates). The pair wise similarity indices and their multiple alignment was obtained using ClustalW (www.ebi.ac.uk/Tools/ msa / clustalw2/) (Higgins *et al.,* 1994).

Further the nucleotide sequences of the test strains (CGMMV and PRSV-W) along with the other strains were translated using ExPASy Translator Tool (www.expasy.org/tools/dna.html). The phylogenetic analysis, pair similarity indices and multiple alignments were done as in case of the nucleotide sequences as well as the amino acid sequences.

3.6.3 Recombination analysis

For the estimation of the recombination that occurred in the test virus, identification of possible recombination break points using maximum χ^2 (Maynard, 1992) and to find its contributing parent strains, the nucleotide sequence of PRSV-W and CGMMV was analyzed in Recombination Detection Program (RDP4) v.4.80 beta software (Martin and Rybicki, 2000). Seven different methods (RDP, GENECONV, MaxChi, Bootscan, Chimaera, SiScan and 3Seq) with a very high degree of confidence were used to detect the recombination event of both the viruses under study. All methods implemented in RDP4 were executed with default parameters.

Table 3.3: Nucleotide sequences of CGMMV isolates used for phylogenetic analysis

S. No.	Accession No.	Virus	Country
1	MH271435.1	CGMMV CG030	Greece
2	MH427279.1	CGMMV-NT	Australia
3	DQ767631.2	CGMMV isolate	India
4	MT184944.1	CGMMV CG048	USA
5	DQ217778.1	CGMMV isolate	China
6	KR232571.1	CGMMV JN	China
7	KY040049.1	CGMMV Inxg	China
8	KY115174.1	CGMMV WA-1	Australia
9	KP868653.1	CGMMV-TG	China
10	KM873788.1	CGMMV-TZ4	China
11	MW430125.1	CGMMV WA-8	Australia
12	AF225984.1	CGMMV – W (K)	Korea
13	KY753929.1	CGMMV pXT1	China
14	KP868654.1	CGMMV-XG	China
15	KM873789.1	CGMMV DY13	China
16	AJ429090.1	CGMMV	France
17	V01551.1	CGMMV-W	Japan
18	KJ754196.1	CGMMV SD LUFFA	China
19	KJ729039.1	CGMMV TN THON SG1	India
20	JF432067.1	CGMMV Wh1	China
21	KU175639.1	CGMMV AH-FT197	China
22	KU175632.1	CGMMV AH-FX3	China
23	MH271409	CGMMV CG003	USA

Table 3.4: Nucleotide sequences of PRSV isolates used for phylogenetic analysis

S. No.	Accession No.	Virus	country	S. No.	Accession No.	Virus	Country
1	LC457969.1	PRSV CM2-2015	India	18	MG564601.1	PRSV SD51	China
2	AB583208.1	PRSV MP18	Myanmar	19	MG564618.1	PRSV SD56	China
3	AB583202.1	PRSV MP19	Myanmar	20	AF506900.1	PRSV THP-12	Thailand
4	AB583205.1	PRSV MP5	Myanmar	21	KT884449.1	PRSV Andong	South Korea
5	AB583223.1	PRSV Mbo 12	Myanmar	22	KF649243.1	PRSV 1116_19	USA
6	AB583212.1	PRSV Mp14	Myanmar	23	KF649240.1	PRSV 1112_15	USA
7	AB583214.1	PRSV Mp 4	Myanmar	24	KY448324.1	PRSV SHK-BG	India
8	AB583222.1	PRSV Mbi11	Myanmar	25	KY448325.1	PRSV SHK-PM1	India
9	KY448332.1	PRSV YMN-BG	India	26	AB583221.1	PRSV Mbo 3	Myanmar
10	AB127935.1	PRSV PAK	Pakistan	27	AB583211.1	PRSV Mbo2	Myanmar
11	KC345567.1	ZTMV RE01-19	France	28	AB583207.1	PRSV Mbo4	Myanmar
12	KC345566.1	ZTMV RE01-31A	France	29	MK531548.1	PRSV 01MBZ	India
13	KC345568.1	ZTMV Fre08-04	France	30	LC457968.1	PRSV CM1-2015	India
14	KC345569.1	ZTMV Mu98-68	Mauritius	31	MW002438.1	PRSV SC	China
15	MG564629.1	PRSV S50	China	32	AF305545.1	MWMV	USA
16	DQ449533.1	PRSV BN	China	33	EF063361.1	PRSV- W CONGO	Democratic Republic Of The Congo
17	MG564626.1	PRSV H10	China	34	KF649241.1	PRSV 1113_16	USA

3.7 Host Range Studies

About twenty plant species (*Cucumis sativus, Cucurbita pepo, C. melo, Citrullus lanatus, C. maxima, Luffa acutangula, Praecitrullus fistulosus, Momordica charantia, Solanum lycopersicum, S. melongena, Capsicum annum, Abelmoschus aeculentus, Phaseolus vulgaris, Vigna unguiculate, Brassica oleracea* var *capitata, B. oleracea* var *botrytis, Amaranthus* spp.*, Chenopodium* spp.*)*, belonging to six different families (*Cucurbitaceae, Solanaceae, Malvaceae, Fabaceae, Brassicaceae* and *Amaranthaceae)* (Table 3.5) were grown under greenhouse conditions and inoculated with PRSV-W isolate (J-SA-BG3) by leaf rub method as mentioned in section 3.3. The plants belonging to families *Cucurbitaceae, Leguminaceae* and *Malvaceae* were inoculated at the cotyledonary leaf stage while plants of remaining three families (*Solanaceae, Chenopodiaceae* and *Cruciferae*) were inoculated at 4-6 leaf stage. The inoculated plants were then kept for observation up to 30-40 days and symptoms developed were recorded. The plants which showed no symptoms were back inoculated on susceptible cultivar ‘MAHY-1’.

Table 3.5 List of plant species belonging to different families used in the host range study

Family	Scientific name	Common name	Family	Scientific name	Common name
Cucurbitaceae	*Cucumis sativus*	Cucumber	*Solanaceae*	*Solanum lycopersicum*	Tomato
	Cucurbita pepo	Pumpkin		*Solanum melongena*	Brinjal
	Cucumis melo	Muskmelon		*Capsicum annuum*	Capsicum
	Citrullus lanatus	Watermelon		*Capsicum annuum*	Chilli
	Luffa acutangula	Ridge gourd	*Fabaceae*	*Phaseolus vulgaris*	Rajmash
	Praecitrullus fistulosus	Round gourd		*Vigna unguiculata*	Cow pea
	Momordica charantia	Bitter gourd	*Brassicaceae*	*Brassica oleracea var. capitata*	Cauliflower
Malvaceae	*Abelmoschus esculentus (L.)*	Okra		*Brassica oleracea var. botrytis*	Broccoli
			Amaranthaceae	*Amaranthus* spp.	Amaranthus
				Chenopodium spp.	Chenopodium

3.8 Vector transmission Studies

3.8.1 Aphid collection and preservation

The aphids associated with the infected plant samples were collected during the field survey. The aphids collected were subjected to a fasting period of 1 hour and then maintained as aviruliferous colonies on healthy bottle gourd plants for further biological assays. The aphids were gently collected with the help of camel hair brush, before picking the aphids, they were gently tapped or the antennae were slightly touched, so that the aphid could retract the proboscis (while feeding on the leaf), and injury to the stylet could be avoided. The aphids used for identification were preserved in 70 per cent ethyl alcohol in 2 ml microfuge tubes. These aphids were used further for preparation of permanent mounts.

3.8.2 Identification of aphid species

Aphids were duly processed and viewed with the aid of compound microscope. The procedure used for aphid processing was followed as under:

1. Five aphids were put into 10 percent KOH solution (overnight) at room temperature.
2. Next day the aphids were given a washing using acetic acid followed by washing with water thrice and they were dried for approximately 5 minutes.
3. Ethyl alcohol was diluted in different concentrations (30,60,70,95 and 100%).
4. Dehydration in 30, 60, 70, 95 and 100 percent alcohol was done for 5 minutes respectively.
5. Samples were transferred for 5 minutes to Petriplates containing Ethanol, xylene in 1:1 ratio (5ml:5ml) and then transferred into a normal xylene for 5 minutes.
6. Sample was mounted in DPX mount for next 24 hours for drying.

Slide was viewed under compound microscope at 5/10/45 X. Images of the aphids were captured with help of compound microscope. Aphids were identified based on morphological characters (Blackmann and Eastop, 1984) (Table 3.6).

Plate 2: Various plant species screened for determining the host range of mosaic virus of bottle gourd

Table 3.6: Aphid identification key

Features		*Aphis gossypii*	*Aphis craccivora*
Cauda	Tongue shaped pale dusky or dark	+	-
	Tongue shaped, bearing 4-7 hairs	-	+ (dome shaped)
Colour	Dark blackish green to pale whitish yellow	+	-
	Shining black	-	+
	Whitish green, pale yellow green, gray-green, mid green, pink	-	-
Cornicles	Much longer than Cauda	-	-
	Twice the length of cauda	+	+
Antennal Tubercles	Broadly rounded, without forwardly directed	+	- (or reduced)
	Directed anterior	-	-

3.8.3 Maintenance and Rearing of aphids

A) Aphid Transmission:

Transmission study was carried to establish the relation between aphids and bottle gourd mosaic virus. The aviruliferous aphid colonies were maintained in an insect cage covered using muslin cloth to avoid mixing. The aphids were maintained on the cv. ‘MAHY-1’ and identified from Division of Entomology, SKUAST-Jammu. The inoculation was done on 15 days old bottle gourd plants at 2-4 leaf stage. Wingless adult females (apterous) were used in all insect transmission experiments. Aphids were gently disturbed on their feeding leaf by slightly toughing their antennae which made them to withdraw their stylets.

They were carefully picked up and transferred with the moistened tip of a camel hair brush. During starvation i.e. pre-acquisition fasting period for 1-hour period, insects were placed in the test tubes after collecting from their colonies by means of a camel hair brush. Prior to each test, the aphids were starved by keeping them in a Petri dish covered with carbon paper. The aphids were placed on virus infected detached young leaves, separately in a small clean Petri plates for acquisition feeding period for 15 minutes. After acquisition feeding, 10 aphids per plant were liberated for inoculation feeding for 30 minutes on healthy young bottle gourd plants and the test plants were completely covered in cages to protect them from other sources of infection.

B) Virus- vector relationship

i) Minimum number of aphids required for transmission

Per cent virus transmission of bottle gourd mosaic virus by varying number *viz.,* 3, 5, 10, 15 and 20 aviruliferous aphids (*A. gossypii* and *A. craccivora)* were transferred to infected samples. After 1 hour of pre-acquisition fasting, they were allowed for acquisition feeding for 15 minutes on four viral isolates (J-SA-BG3, J-NA-BG-1, J-RJ-BG3 and J-MN-BG1) source separately and then transferred to healthy young bottle gourd plants (cv. MAHY-1) for transmission feeding for 30 minutes. They were killed with imidachlorpid (@ 0.3ml/ l water). Twenty plants seedlings were used per assay.

ii) Pre -acquisition fasting

The effect of pre-acquisition fasting on transmission efficiency of the vector, was studied for *A. gossypii* and *A. craccivora*. Aphids were starved for varying periods *viz.,* 5, 15, 30, 45, 60 and 240 minutes and were released on each test plant.

iii) Acquisition Access period

Prior to each test the aphids *viz., A. gossypii* and *A. craccivora* were starved for 1 hr before acquisition feeding and then provided with different feeding period *viz.,*5, 15, 30, 45, 60 and 240 minutes. A minimum number of 10 aphids were used on each test plant and allowed to feed on test plants for 30 min. For each test 20 seedlings of bottle gourd were used.

iv) Inoculation Access Period

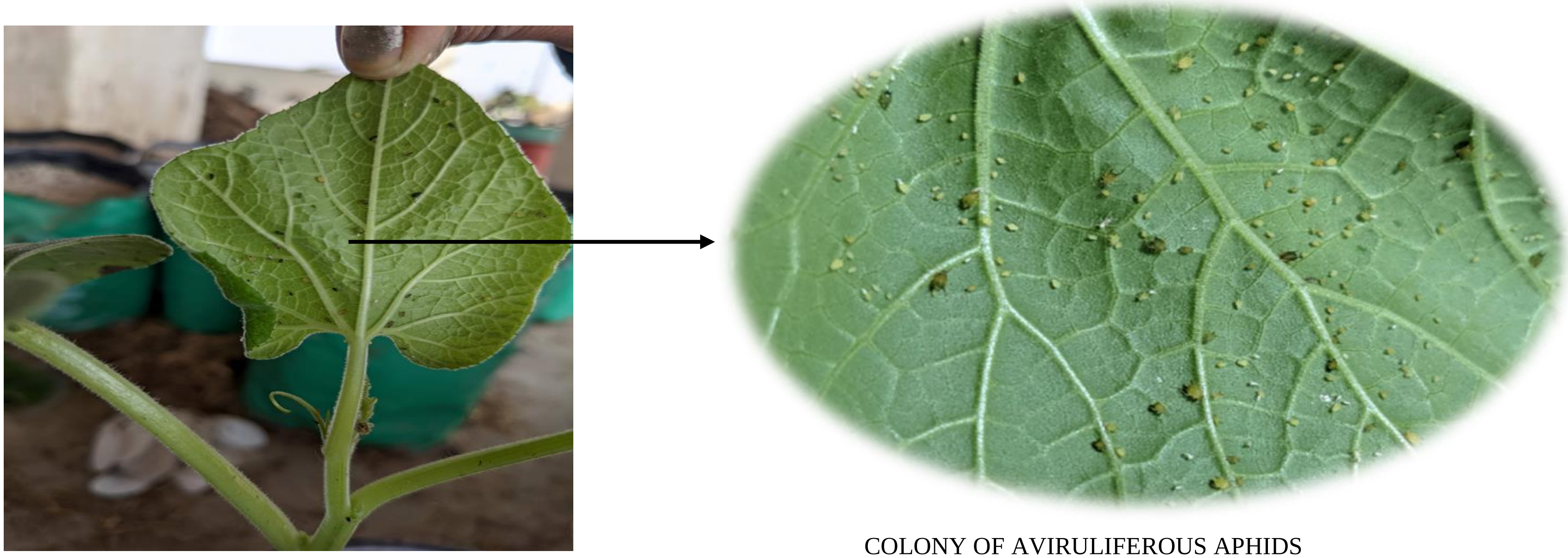
Aphids *(A. gossypii* and *A. craccivora)* were starved for 1 hr and allowed acquisition feeding for 15 min on virus source plant and then released on 20 test

Aphid colony observed during survey

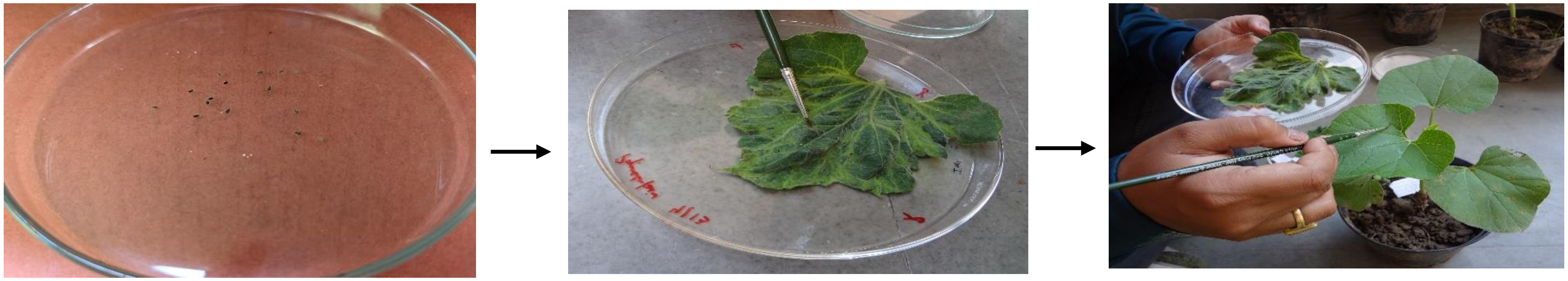
Rearing of aviruliferous aphid colonies

Plate 3: Collection and maintenance of Aphid colonies

Plate 4: Preparation of permanent mounts of *A. gossypii* and *A. craccivora*



COLONY OF AVIRULIFEROUS APHIDS

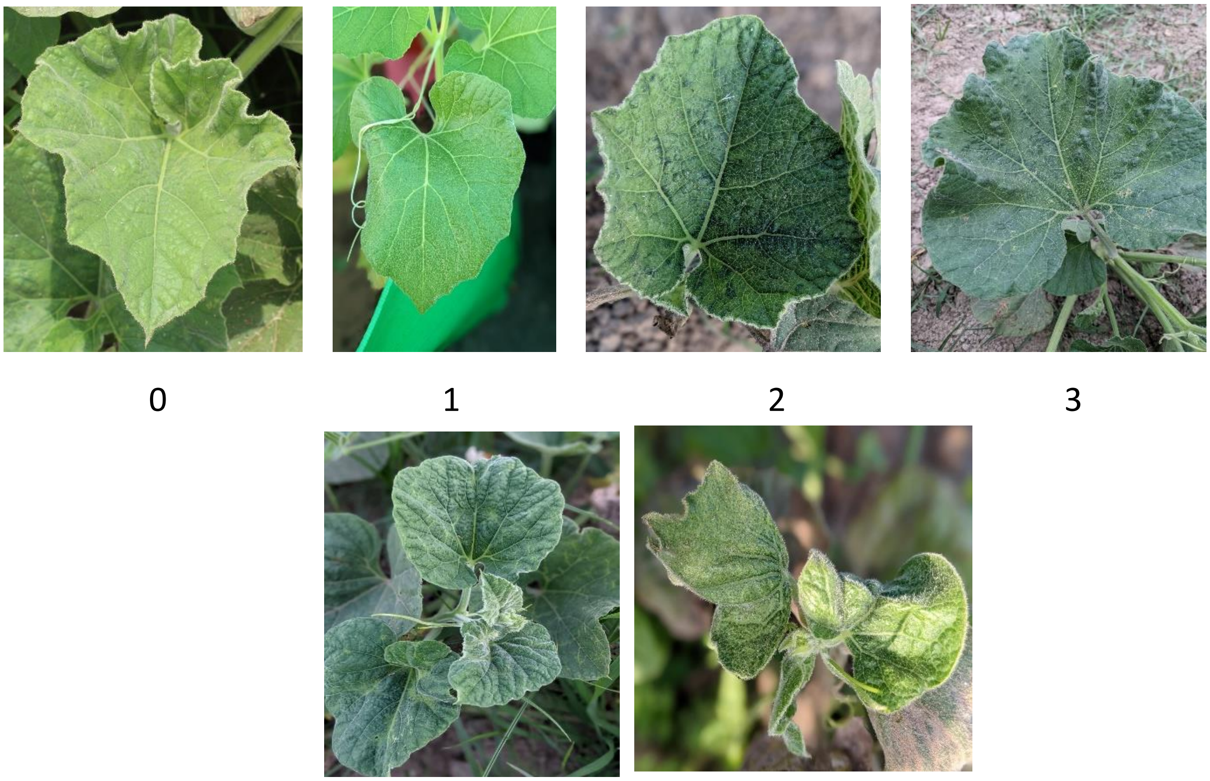


PRE AQUISITION FASTING

AQUISITION ACCESS PERIOD

INOCULATION ACCESS PERIOD

Plate 5: Procedure of virus inoculation using aphid



0 1 2 3

4 5

Plate 6: Disease rating scale for bottle gourd mosaic

0- Immune 1- Resistant 2- Moderately resistant 3- Moderately Susceptible 4- Susceptible 5- Highly Susceptible

seedlings in groups of 10 aphids for infection feeding of varying periods *viz.*, 5, 15, 30, 45, 60 and 240 minutes.

3.9 Host resistance

3.9.1 Evaluation of germplasm

Twenty germplasm lines comprising indigenous accessions procured from NBPGR, New Delhi and fifty-four cultivars (*viz.*, hybrids, open pollinated and locals) were screened for resistance against bottle gourd mosaic under field and controlled conditions (Table 3. 8). For scoring for resistance to susceptibility ranging from 0-5, a scale by Rajamony *et al.* (1990) was followed:

i) Field evaluation

The germplasm was evaluated under field conditions using RBD design. After approximately ten days or when the seedlings were at their true leaf stage, the seedlings of different genotype/cultivar were inoculated using standard leaf rub method. The symptoms were observed up to one-month post inoculation.

Table 3. 7: Scale for grading of different germplasm against bottle gourd mosaic

Scale	Symptoms	Reaction
0	Immune (I)	No symptoms
1	Resistance (R)	Slight vein clearing, very light mottling of light and dark green colour in younger leaves
2	Moderately Resistance (MR)	Mottling of leaves with light and dark green colour
3	Moderately Susceptible (MS)	Blisters and raised surfaces on the leaves
4	Susceptible (S)	Distortion of leaves
5	Highly Susceptible (HS)	Stunting of the plants with negligible or no flowering and fruiting

ii) Under greenhouse conditions

Different seeds of each genotype were raised under insect proof greenhouse following proper agronomic practices as per the method described in section 3.2.1. Each plant was sap inoculated at cotyledonary leaf stage or at first true leaf stage using standard leaf rub method (section 3.3). One plant of each accession and cultivar was kept as control by inoculating with distilled water. The plants were observed for appearance of symptoms up to one month after inoculation. The sap collected from plants showing no symptoms were inoculated on healthy plants of susceptible cv. ‘MAHY 1’ for detecting any symptom less carriers. The data was recorded, and plants were graded either as resistant or susceptible using a disease rating scale (Table 3.7). Cultivars exhibiting resistant reaction were tested twice and the resistance/ susceptibility was further confirmed by RT-PCR using CGMMV and PRSV-W coat protein specific primers respectively (sections 3.5.5).

Table 3.8: List of germplasm/ varieties used against bottle gourd mosaic disease

S. No	Germplasm/ varieties	S. No	Germplasm/ varieties	S. No	Germplasm/ varieties	S. No	Germplasm/ varieties	S. No	Germplasm/ varieties
1	IC- 371695	18	IC- 546147	35	Gaurav	52	Lata	69	Akash
2	IC- 342081	19	IC- 550725	36	Earth Co. lauki	53	Makhmal	70	HP local-1
3	IC- 40890	20	IC- 342077	37	Nirmal lauki	54	Rani	71	HP local-2
4	IC- 371675	21	MGH-1/ MAHY-1	38	Neo lauki	55	Pooja-9	72	HP local-3
5	IC- 276524	22	Vardan	39	PSPL- 101	56	Vardan (GI)	73	HP local-4
6	IC- 284965	23	Sharada	40	Manvik lattu	57	Ratan	74	JK local-1
7	IC- 322274	24	Bhushan	41	Urvashi F1	58	Megha Star	75	JK local-1
8	IC- 331101	25	MHBG-8	42	Pooja	59	Lata	76	JK local-1
9	IC- 337077	26	MGH-4/ Warad	43	NE lauki	60	Makhmal	77	JK local-1
10	IC- 382192	27	JS-651	44	NE lattu	61	Research-10	78	Bihar local
11	IC- 339215	28	GC-S27	45	VC- 038	62	RS-11		
12	IC- 382240	29	GC-S28	46	Komal Kiran	63	RS-101		
13	IC- 385816	30	NO.17	47	Kanchan	64	M-11		
14	IC- 392192	31	HY- 401	48	Imperial G2	65	Kundal BSS-687		
15	IC- 398534	32	Rashmi	49	Imperial GTK	66	Naveen F1		
16	IC- 531896	33	Komal	50	Akhil lattu	67	Pusa Komal		
17	IC- 536536	34	Shiva- 305-F1	51	ES Gola	68	Sudha		

RESULTS

CHAPTER-IV

RESULTS

During the present investigation, the information regarding the occurrence, distribution of the causal virus for bottle gourd mosaic disease in Jammu, Kathua, Samba and Udhampur districts of the subtropical zone of Jammu were undertaken. The identity of the causal virus/viruses associated with the disease was also established. Apart from this an attempt was also made to understand the host range of the virus/ viruses, relationship with insect vector and sources of resistance for the management of the disease.

4.1 Survey

Comprehensive surveys were conducted consecutively for two years i.e. 2020 and 2021, to determine the prevalence of mosaic disease in bottle gourd growing areas of Jammu division. During the period surveys, 55 locations spread across 32 villages were visited and 274 samples were collected, from four sub-tropical districts of Jammu division (Jammu, Kathua, Samba and Udhampur). The per cent disease incidence as well as associated symptoms were recorded (Table 4.1).

During 2020, the incidence of the disease ranged from 6.67 - 50.00 per cent with a mean of 21.75 per cent. In district Jammu, four blocks (R. S. Pura, Marh, Bishnah and Miran Sahib) and 13 villages were surveyed, where the overall range and mean incidence was 6.67 - 50.00 per cent and 26.13 per cent, respectively. The maximum incidence was recorded in the village Bhade- Rode of Marh block with the highest incidence of 50.00 per cent followed by Salaher village (R.S. Pura) with 44.44 per cent incidence. Villages Brahmana Dhamwal (Marh) and Rakh Chatha (Miran Sahib) shared an incidence of 40.00 per cent, similarly Kotli Shah Dhula (R.S. Pura) and Trilok Pur of Marh block were at par with 33.33 per cent incidence respectively. Badyal Brahmana of R.S. Pura block and Ghou Manhasa of Marh were at par with each other as they had an incidence of 26.67 per cent, followed by Deoli (Bishnah) with 23.33 per cent of incidence. The percentage of disease incidence at Rathua (Marh) and Ratwal (Bishnah) was 14.00 per cent respectively, while at Pouni chak (Marh) it was 13.33 per cent. The minimum incidence (6.67%) in district Jammu was observed at the village Laswara of block Bishnah.

In district Kathua, for the year 2020 the overall disease incidence ranged from 6.67-26.67 per cent with mean of 13.46 per cent. Kakhyal village (Nagri block) had the incidence of 26.67 per cent, which was the highest, while the lowest was recorded at village Feruchak (6.67%) of Hiranagar block. Ten per cent disease incidence was observed from the villages Narroha (Nagri) and Patwal (Hiranagar) while the village Dadoli (Nagri) had 14 per cent disease incidence.

An overall range and mean incidence of 10.00-37.78 per cent and 23.73 per cent respectively, was recorded in district Samba. The highest incidence of mosaic was noted in Rajpura (37.78%) of Block Rajpura followed by village Sanoora of block Rajpura (31.10%). However, in Yakh, Koulpur, Supwal and Raiyan villages from Block Vijaypur the incidence of 30.00, 26.67, 23.33 and 17.00 per cent was recorded respectively. Meanwhile, Jenda and Abtal villages of Ramgarh Block had a minimum incidence of 14.00 and 10.00 per cent.

For the year 2020 in district Udhampur (block Chenani), the highest disease incidence (28.89%) was observed at village Chenani followed by Sudh Mahadev and Chohan at 26.67 per cent, Mantalai (23.33%), Kamali (20.00%). The lowest incidence was observed from village Kohsar with 14.00 per cent incidence. Therefore, the overall range of disease incidence in Udhampur was 14.00-28.89 per cent with an overall mean of 23.26 per cent.

During the second year (2021), the overall range of incidence of disease incidence was 0.00 – 62.22 per cent while the overall mean was 25.33 per cent. In district Jammu the overall mean incidence was 28.39 per cent with a range of 0.00-62.22 per cent. The highest incidence was observed in Salaher (62.22%) followed by Rakh Chatha (53.33%), Kotli Shah Dhula (46.67 per cent), Bhad-Rode (43.33%), Trilok Pur (40.00%), Badyal Brahmana (38.33%), Pouni Chak (26.67%).While in,, Rathua (20.33%), Brahmana Dhamwal (20.00%), Ghou Manhasa (20.00%) and Laswara (20.00%) the percentage of disease incidence was at par, however, in Ratwal (0.00%) least incidence of disease was recorded.

In district Kathua, overall range of incidence was 14.00-21.67 per cent while overall mean was 18.60. The highest incidence was observed from Dadoli (21.67%) followed by Kakhyal (20.33%), Patwal (20%), Narroha (17.00%) and the lowest at Feruchak (14.00). For the district Samba, the overall range of disease incidence was 10.00- 43.33 per cent while overall mean was 22.08 per cent. Rajpura had the highest incidence at 43.33 per cent followed by Yakh (40.00%), Raiyan (33.33%), Supwal

Table 4.1: Status of mosaic disease of bottle gourd in Jammu sub tropics during 2020 and 2021

District	Block	Village	Disease incidence (%)		
			2020	2021	Pooled
Jammu	R. S. Pura	Badyal Brahmana	26.67	38.33	32.50
		Kotli Shah Dhula	33.33	46.67	40.00
		Salaher	44.44	62.22	53.33
	Marh	PouniChak	13.33	26.67	20.00
		Brahmana Dhamwal	40.00	20.00	30.00
		Trilok Pur	33.33	40.00	36.67
		Bhad-Rode	50.00	43.33	46.67
		GhouManhasa	26.67	20.00	23.33
		Rathua	14.00	20.33	17.17
	Bisnah	Deoli	23.33	6.67	15.00
		Laswara	6.67	20.00	13.33
		Ratwal	14.00	0.00	7.00
	Miran Sahib	Rakh Chatha	40.00	53.33	46.67
		Mean	26.13	28.39	27.26
		Range	6.67- 50.00	0.00- 62.22	7.00 - 53.33
Kathua	Nagri	Kakhyal	26.67	20.33	23.50
		Narroha	10.00	17.00	13.50
		Dadoli	14.00	21.67	17.83
	Hiranagar	Patwal	10.00	20.00	15.00
		Feruchak	6.67	14.00	10.33
		Mean	13.46	18.60	16.03
		Range	6.67- 26.67	14.00- 21.67	10.33-23.50
Samba	Rajpura	Rajpura	37.78	43.33	40.55
		Sanoora	31.1	13.33	22.22
	Vijaypur	Koulpur	26.67	10.00	18.33
		Supwal	23.33	16.67	20.00
		Raiyan	17.00	33.33	25.17
		Yakh	30.00	40.00	35.00
	Ramgarh	Jenda	14.00	10.00	12.00
		Abtal	10.00	10.00	10.00
		Mean	23.73	22.08	22.91
		Range	10-37.78	10.00- 43.33	10.00- 40.55
Udhampur	Chenani	Chenani	28.89	40.00	34.44
		Mantalai	23.33	40.00	31.67
		Kohsar	14.00	16.67	15.33
		Sudh Mahadev	26.67	33.33	30.00
		Kamali	20.00	23.67	21.83
		Chohan	26.67	15.00	20.83
		Mean	23.26	28.11	25.68
		Range	14.00- 28.89	15.00- 40.00	15.33- 34.44
Overall Mean			**21.75**	**25.33**	**23.54**
Overall range			6.67-50.00	0.00- 62.22	7.00 – 53.33

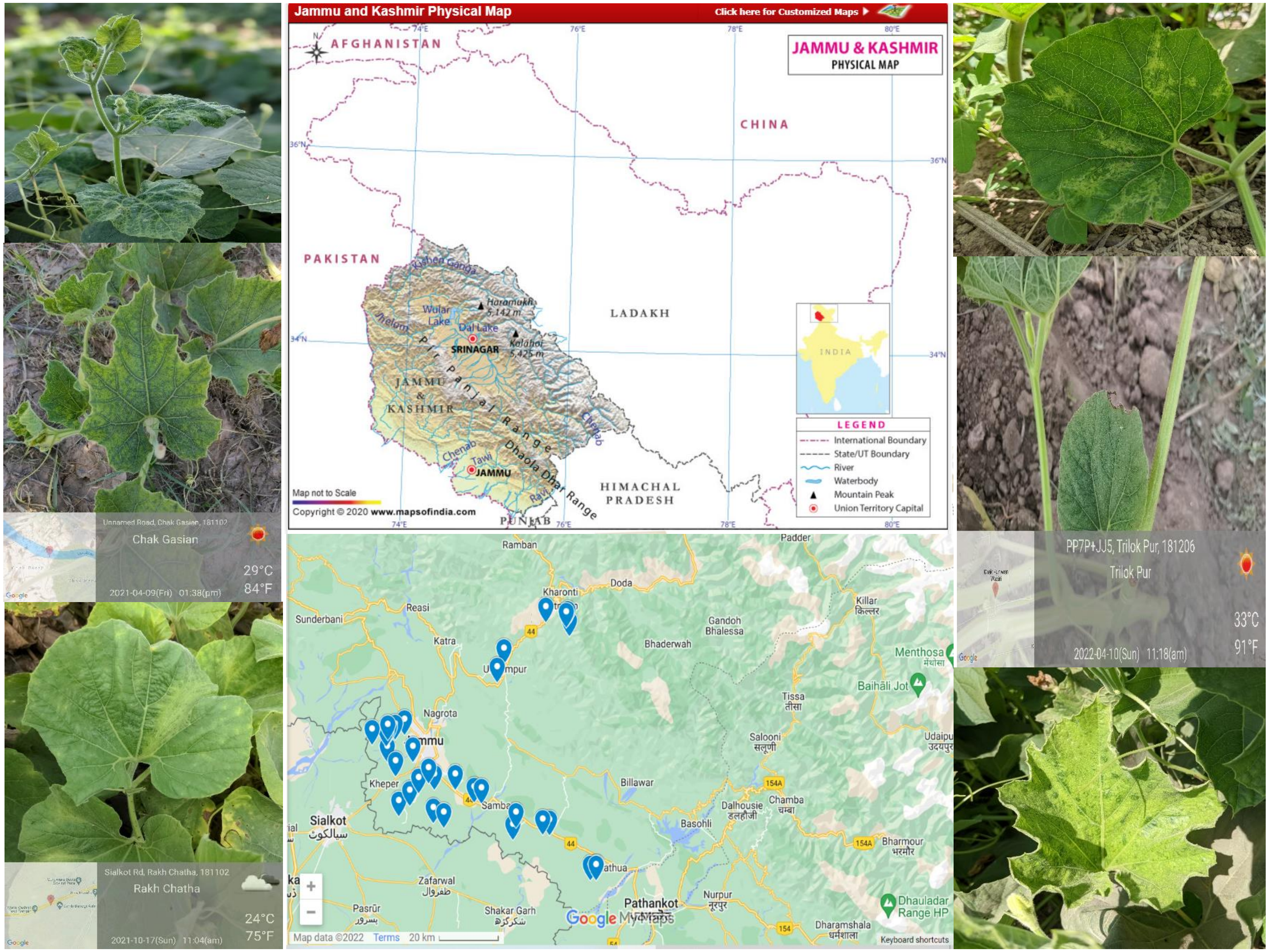
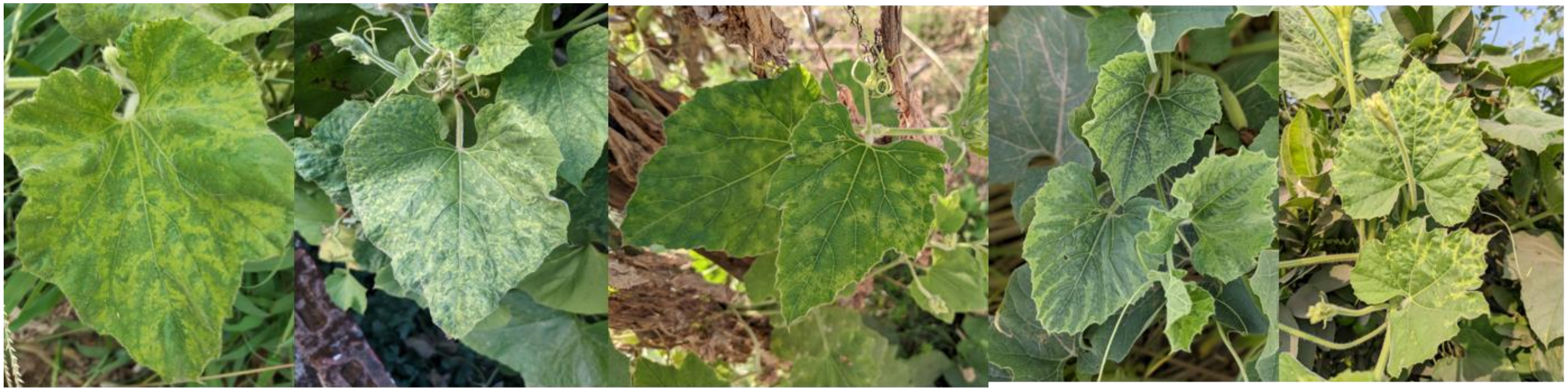


Plate 7: Various symptoms of bottle gourd mosaic disease observed during the survey of various locations of Jammu division



Mosaic **Mottled mosaic** **Mosaic with ringspots** **Vein banding** **Vein clearing**

Leaf Deformation Mosaic with blisters on leaves Veinal thickening

Plate 8: Symptoms on Bottle gourd observed under field conditions

(16.67%), Sanoora (13.33%). Three villages *viz.*, Koulpur, Jenda and Abtal, all had the lowest disease incidence of 10.00 per cent. Udhampur district, had an overall range of 15.00-40.00 per cent disease incidence and overall mean of 28.11 per cent. Both the villages Chenani and Mantalai observed incidence of 40.00 per cent followed by Sudh Mahadev (33.33%), Kamali (23.67%) and Kohsar (16.67%). The lowest incidence was recorded from Chohan (15.00%).

The pooled data of two years revealed that village Salaher, from block R.S. Pura of district Jammu had the highest disease incidence (53.33%) while Ratwal, from block Bishnah of district Jammu had the lowest mean incidence (7.00%). In district Kathua, the highest mean incidence was at village Kakhyal (23.50%) at Nagri block, while the lowest was at Feruchak (10.33%) of Block Hiranagar. In case of Samba district, Rajpura village observed the highest mean incidence (40.55%), while the lowest was at Abtal (Ramgarh) with 10.00 per cent mean incidence. For the district Udhampur, over the two years Chenani village had the highest mean disease incidence (34.44 %) and the lowest was at Kohsar (15.33%).

Year wise comparison revealed that the year 2021 had a higher incidence of mosaic as compared to 2020, whereas, the overall mean for 2021 in four districts was 25.33 per cent which was 21.75 per cent in 2020.

4.2 Symptomatology

To understand the symptomatology, a range of symptoms were recorded under field and artificial conditions. Symptoms predominantly observed during the survey were, “mosaic mottling, mosaic with ringspot and chlorotic spots, mild mosaic, blisters on leaves and fruits”. The symptoms that were associated with fewer samples were mosaic with interveinal yellowing at the center, mosaic with puckering, vein clearing and leaf distortion (Plate 6 and 7).

Under artificially inoculated conditions, the seedlings of susceptible cv. ‘MAHY-1’ showed symptoms after 10-15 days of inoculations and the major symptoms observed were, dark green mosaic mottling, blisters on leaf, vein clearing, puckering and stunting (Plate 8). Generally, vein clearing was observed on the younger leaves, later a mild mosaic or mottled mosaic was observed. With the disease progress, reduction in leaf lamina and symptoms like dark green mosaic mottling, blistering and puckering (in case of few isolates) was observed. The fruits borne on such infected plants were small and deformed.

4.3 Categorization, detection and maintenance of causal viruses/ viral isolates

Samples (274) collected from different locations were brought in the laboratory for further analysis. The samples were categorized into fifty five isolates based on the symptomatology (Plate 9, Table 4.2). They were further subjected to RT-PCR analysis. Coat protein specific primers were used for the detection of a *Tomabovirus* (CGMMV), a *Cucumovirus* (CMV), a *Polerovirus* (ZLCV) and 3 *Potyviruses* (ZYMV, PRSV-W, WMV). Most of the samples were positive for PRSV-W followed by CGMMV and were negative for other viruses, which can be due to the lower incidence of the viruses in other cucurbits. About 16 samples tested negative for all the six viruses (Plate 10, 11, 12 and Table 4.3).

From Jammu district 29 isolates were tested and PRSV-W was observed in 8 isolates while, CGMMV was present in 7 out of 29 isolates as single infections. CGMMV was found as single infection only in district Jammu, in rest three districts it was always found with PRSV-W. Furthermore, 9 isolates were having mixed infections while 5 isolates failed to be detected (Table 4.4). In district Kathua, out of eight isolates, PRSV-W was found only in one sample as single infection whereas, as mixed infection in four isolates. CGMMV was detected in 4 isolates as mixed infections, while it was not detected as single infection. Three isolates out of eight showed no relation to any viruses under investigation. In district Samba, out of the ten isolates tested, only two showed to be positive for PRSV-W while three were positive for mixed infection (CGMMV+PRSV-W). Five out of ten isolates were negative for all the viruses. In Udhampur, PRSV-W was detected from four isolates as mixed infection (CGMMV+PRSV-W) and in one sample as single infection and again five tested negative for all the viral CP primers used in the study.

Therefore, once the identity of the viruses were established through RT-PCR, and categorization based on their identity and symptoms was done, thirty-nine isolates were selected. Out of these isolates, twenty-four belonged to district Jammu, five from Kathua, Samba and Udhampur (Table 4. 5). The isolates were then maintained on the susceptible cv. ‘MAHY-1’ through artificial inoculations, under controlled conditions. The symptoms produced by the isolates were dark green mosaic mottling, interveinal chlorosis, blisters and occasional mosaic with ringspots or chlorotic spots and puckering. The representative samples were also maintained at -20ºC deep freezer for future use.

Table 4.2: List of isolates collected from different locations

District	Block	Village	No. of samples collected	No. of isolates	Sample ID
Jammu	R. S. Pura	Badyal Brahmana	27	4	J-BB-BG1, J-BB-BG2, J-BB-BG3 J-BB-BG4
		Kotli Shah Dhula	12	3	J-KO-BG1, J-KO-BG2, J-KO-BG3
		Salaher	20	5	J-SA-BG1, J-SA-BG2, J-SA-BG3, J-SA-BG4, J-SA-BG5
	Marh	PouniChak	13	1	J-PC-BG1
		Brahmana Dhamwal	9	2	J-BD-BG1, J-BD-BG2
		Trilok Pur	7	1	J-TP-BG1
		Bhad-Rode	21	3	J-BR-BG1, J-BR-BG2, J-BR-BG3
		GhouManhasa	11	1	J-GM-BG1
		Rathua	7	1	J-RT-BG1
	Bisnah	Deoli	8	2	J-DE-BG1, J-DE-BG2
		Laswara	5	1	J-LS-BG1
		Ratwal	10	1	J-RA-BG1
	Miran Sahib	Rakh Chatha	9	4	J-CH-BG1, J-CH-BG2, J-CH-BG3, J-CH-BG4
Kathua	Nagri	Kakhyal	15	2	J-KH-BG1, J-KH-BG2
		Narroha	11	2	J-NA-BG1, J-NA-BG2
		Dadoli	8	2	J-DD-BG1, J-DD-BG2
	Hiranagar	Patwal	7	1	J-PA-BG1
		Feruchak	4	1	J-FE-BG1
Samba	Rajpura	Rajpura	11	3	J-RJ-BG1, J-RJ-BG2, J-RJ-BG3
		Sanoora	4	1	J-SN-BG1
	Vijaypur	Koulpur	5	1	J-KU-BG1
		Supwal	5	1	J-SU-BG1
		Raiyan	8	1	J-RY-BG1
		Yakh	7	1	J-YA-BG1
	Ramgarh	Jenda	4	1	J-JE-BG1
		Abtal	3	1	J-AB-BG1
Udhampur	Chenani	Chenani	8	2	J-CN-BG1, J-CN-BG2
		Mantalai	7	2	J-MN-BG1, J-MN-BG2
		Kohsar	4	1	J-KH-BG1
		Sudh Mahadev	3	1	J-SM-BG1
		Kamali	6	1	J-KM-BG1
		Chohan	5	1	J-CO-BG1
		Total	**274**	**55**	

Table 4.3 Detection of virus causing Bottle gourd mosaic in different isolates by RT-PCR

Sr. no.	Sample ID	Symptoms associated	CMV	CGMMV	PRSV-W	WMV	ZLCV	ZYMV	CGMMV+ PRSV-W
1	J-BB-BG1	Mosaic with ringspot	-	-	+	-	-	-	-
2	J-BB-BG2	Mild mosaic	-	+	-	-	-	-	-
3	J-BB-BG3	Mosaic mottling	-	+	-	-	-	-	-
4	J-BB-BG4	Vein banding	-	+	-	-	-	-	-
5	J-KO-BG1	Mosaic mottling	-	+	-	-	-	-	-
6	J-KO-BG2	Mosaic with interveinal yellowing at center	-	-	-	-	-	-	-
7	J-KO-BG3	Mosaic with leaf distortion	-	-	-	-	-	-	-
8	J-SA-BG1	Mosaic mottling	-	+	-	-	-	-	-
9	J-SA-BG2	Vein banding with mosaic	-	+	-	-	-	-	-
10	J-SA-BG3	Mosaic with ringspots	-	-	+	-	-	-	-
11	J-SA-BG4	Mosaic with blisters on leaves	-	-	-	-	-	-	+
12	J-SA-BG5	Severe mosaic mottling	-	-	-	-	-	-	+
13	J-PC-BG1	Mosaic with ringspots	-	-	+	-	-	-	-
14	J-BD-BG1	Mosaic	-	-	-	-	-	-	+
15	J-BD-BG2	Mosaic mottling	-	-	-	-	-	-	+
16	J-TP-BG1	Mosaic mottling	-	-	-	-	-	-	+
17	J-BR-BG1	Severe mosaic mottling	-	-	-	-	-	-	+
18	J-BR-BG2	Mosaic with blisters on leaves	-	-	-	-	-	-	+

19	J-BR-BG3	Mosaic with ringspot and blisters	-	-	+	-	-	-	-
20	J-GM-BG1	Mosaic	-	-	-	-	-	-	-
21	J-RT-BG1	Mosaic	-	-	-	-	-	-	-
22	J-DE-BG1	Mosaic	-	-	-	-	-	-	-
23	J-DE-BG2	Mosaic	-	-	+	-	-	-	-
24	J-LS-BG1	Mosaic with chlorotic spots	-	+	-	-	-	-	-
25	J-RA-BG1	Mild mosaic	-	-	-	-	-	-	+
26	J-CH-BG1	Vein clearing	-	-	-	-	-	-	+
27	J-CH-BG2	Mosaic mottling	-	-	+	-	-	-	-
28	J-CH-BG3	Mosaic with ringspots	-	-	+	-	-	-	-
29	J-CH-BG4	Mosaic with chlorotic spots	-	+	-	-	-	-	-
30	J-KH-BG1	Mosaic with puckering and yellowing at center	-	-	-	-	-	-	-
31	J-KH-BG2	Mosaic mottling	-	-	+	-	-	-	-
32	J-NA-BG1	Mosaic with crinckling on leaves	-	-	-	-	-	-	+
33	J-NA-BG2	Mosaic with blisters on leaves	-	-	-	-	-	-	+
34	J-DD-BG1	Mosaic with blisters on leaves	-	-	-	-	-	-	+
35	J-DD-BG2	Severe mosaic mottling	-	-	-	-	-	-	+
36	J-PA-BG1	Mosaic with interveinal yellowing at center	-	-	-	-	-	-	-
37	J-FE-BG1	Mosaic with interveinal yellowing at center	-	-	-	-	-	-	-

38	J-RJ-BG1	Mosaic with blisters on leaves	-	-	-	-	-	-	+
39	J-RJ-BG2	Mosaic with interveinal yellowing at center	-	-	-	-	-	-	-
40	J-RJ-BG3	Mosaic with interveinal yellowing at center	-	-	+	-	-	-	-
41	J-SN-BG1	Mosaic mottling	-	-	-	-	-	-	+
42	J-KU-BG1	Mosaic with interveinal yellowing at center	-	-	-	-	-	-	-
43	J-SU-BG1	Mosaic with interveinal yellowing at center	-	-	-	-	-	-	-
44	J-RY-BG1	Mosaic with blisters on leaves	-	-	-	-	-	-	+
45	J-YA-BG1	Mosaic mottling with puckering	-	-	+	-	-	-	-
46	J-JE-BG1	Mosaic	-	-	-	-	-	-	-
47	J-AB-BG1	Mosaic with blisters on leaves	-	-	-	-	-	-	-
48	J-CN-BG1	Mild mosaic	-	-	-	-	-	-	+
49	J-CN-BG2	Mosaic mottling with chlorotic rings	-	-	-	-	-	-	+
50	J-MN-BG1	Mosaic with ringspots	-	-	-	-	-	-	+
51	J-MN-BG2	Mild mosaic	-	-	-	-	-	-	+
52	J-KH-BG1	Mild mosaic	-	-	+	-	-	-	-
53	J-SM-BG1	Mosaic	-	-	-	-	-	-	-
54	J-KM-BG1	Mosaic mottling	-	-	-	-	-	-	-
55	J-CO-BG1	Mild mosaic with chlorosis	-	-	-	-	-	-	-

Table 4.4: Frequency of viruses causing bottle gourd mosaic disease from different districts

	CGMMV	CMV	PRSV-W	WMV	ZLCV	ZYMV	CGMMV+ PRSV-W
JAMMU	7	-	8	-	-	-	9
KATHUA	-	-	1	-	-	-	4
SAMBA	-	-	2	-	-	-	3
UDHAMPUR	-	-	1	-	-	-	4

Table 4.5: Types of symptoms by different isolates

Sr. no	Major symptoms	Isolates	Virus
1	Mosaic	J-BD-BG1	CGMMV+ PRSV-W
		J-DE-BG2	PRSV-W
2	Mild mosaic	J-BB-BG2	CGMMV
		J-RA-BG1, J-MN-BG2, J-MN-BG1, J-KH-BG1, J-CN- BG1	CGMMV+ PRSV-W
3	Mosaic mottling	J-BB-BG3, J-KO-BG1, J-SA-BG1	CGMMV
		J-SA-BG5, J-BD-BG2, J-TP-BG1, J-BR-BG-1, J-DD-BG2, J-SN-BG1	CGMMV+ PRSV-W
		J-CH-BG2, J-KH-BG2	PRSV-W
4	Mosaic / mosaic mottling with ringspots	J-BB-BG1, J-SA-BG3, J-PC-BG1, J-BR-BG3, J-CH-BG3, J-MN-BG1	PRSV-W
5	Mosaic / mosaic mottling with chlorotic spots	J-LS-BG1	PRSV-W
		J-CH-BG4	CGMMV
		J-CN-BG2	CGMMV+ PRSV-W
6	Blisters on leaf with mild mosaic or mosaic mottling	J-SA-BG4, J-BR-BG2, J-NA-BG2, J-DD-BG1, J-RY-BG1	CGMMV+ PRSV-W
7	Interveinal yellowing at center	J-RJ-BG3	PRSV-W
8	Vein banding	J-BB-BG4, J-SA-BG2	CGMMV
9	Vein clearing	J-CH-BG1	CGMMV+ PRSV-W
10	Mosaic/ mosaic mottling with puckering	J-YA-BG1	PRSV-W
11	Mosaic with crinkling	J-NA-BG1	CGMMV+ PRSV-W

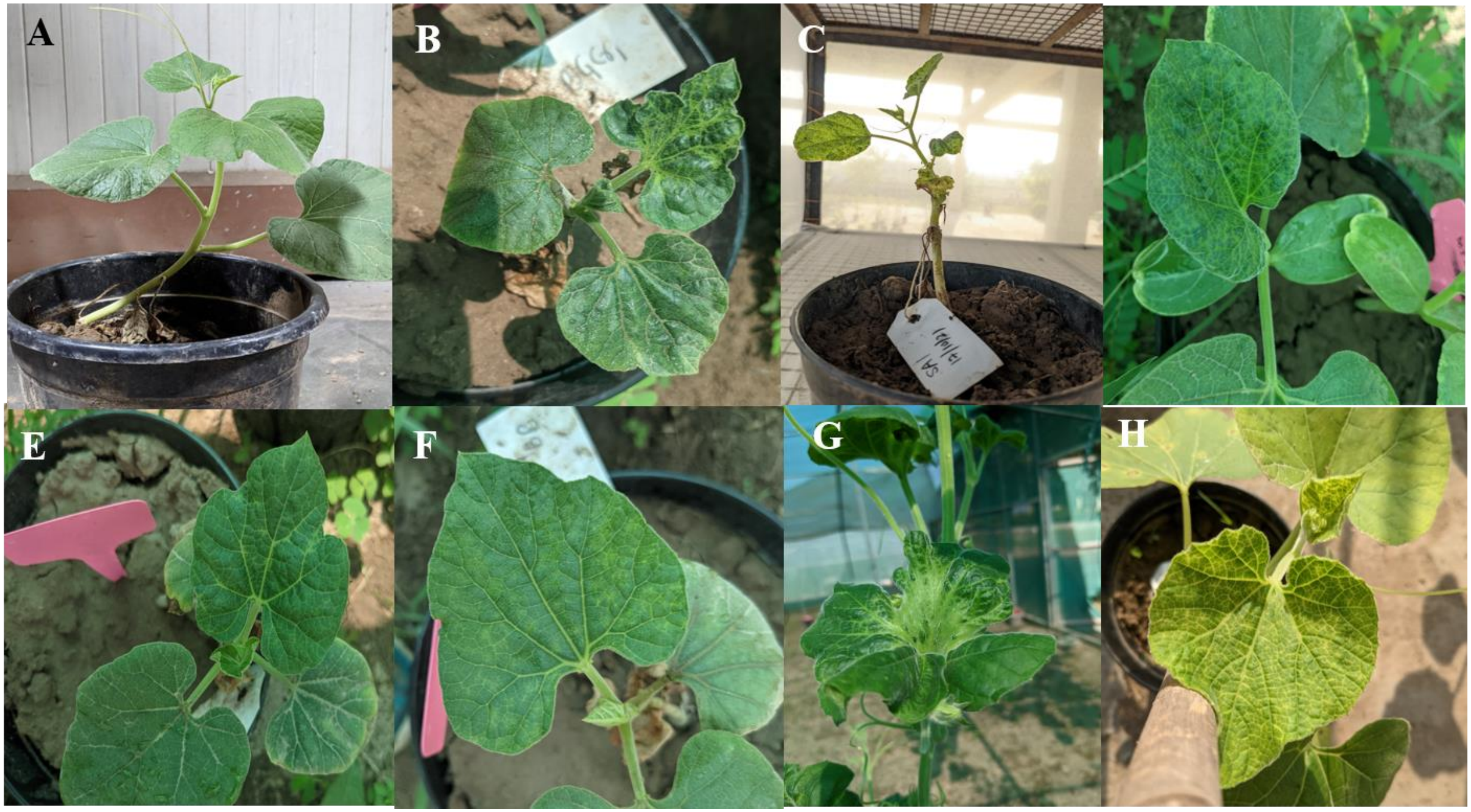


Plate 9: Maintenance of isolates under greenhouse. A: Healthy cv. MAHY- 1, B-H: isolates collected from various locations

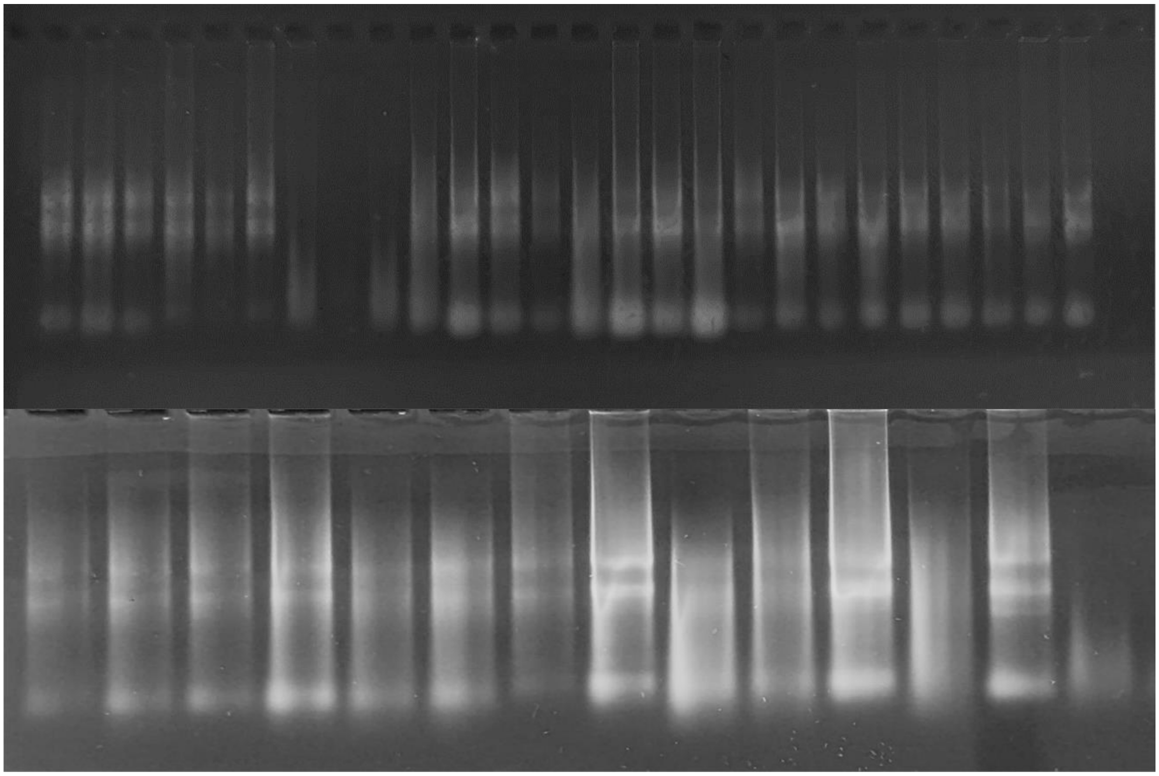


Plate 10: Total RNA isolated from infected bottle gourd mosaic samples

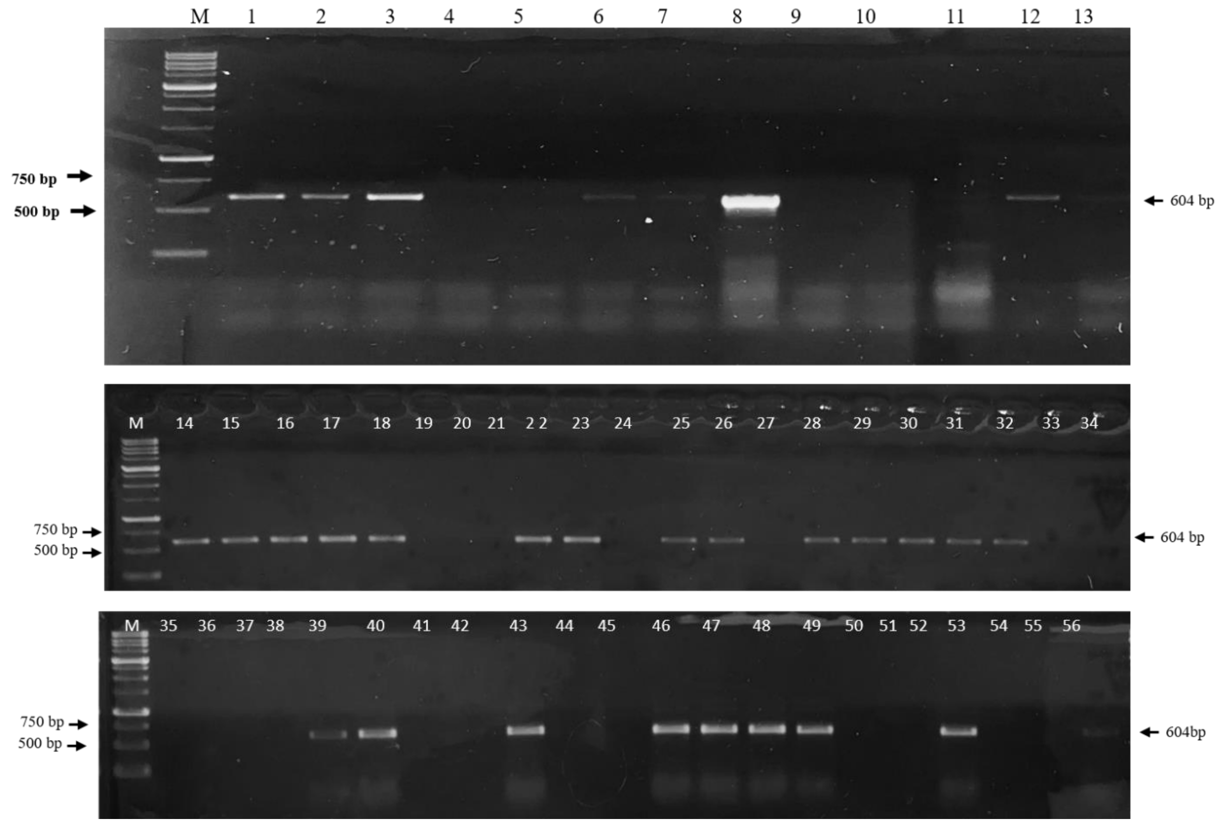


Plate 11: Detection of CGMMV through RT-PCR using coat protein gene specific primers

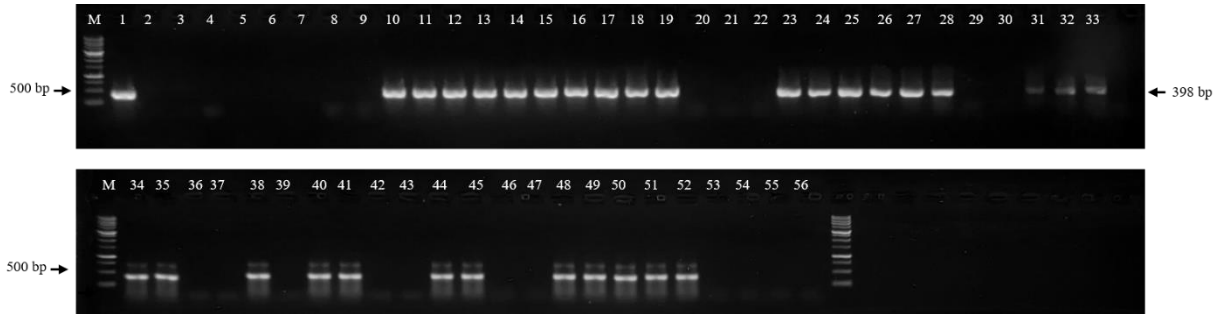


Plate 12: Detection of PRSV-W through RT-PCR using coat protein gene specific primers

4.4 Distribution of viruses

On an average PRSV-W was distributed in all the four districts and had high incidence compared to CGMMV. PRSV-W was detected from 33.33 per cent samples while CGMMV was present in 12.00 per cent samples. In Jammu district, per cent infection of PRSV-W was 23.10, while that of CGMMV was 17.00 per cent. PRSV-W was present in only five per cent samples collected from Kathua and Samba, while in Udhampur it was found in 2.56 per cent of samples. CGMMV was absent from as single infection from Kathua, Samba as well as Udhampur.

About 35 per cent of samples were having mixed (double) infections of PRSV-W and CGMMV. In district Jammu CGMMV+PRSV-W was found in 23.07 per cent samples, while in Kathua it was found only in 7.80 per cent samples. However, in Samba and Udhampur percentage of mixed infection was 7.69 respectively.

4.5 Molecular Characterization of CGMMV

Total RNA was isolated from infected MAHY-1 plants on which different isolates were maintained. RNA was reverse transcribed to cDNA and was amplified using coat protein gene specific primers. The RT-PCR of isolate with CGMMV coat protein gene specific primers produced an amplicon of ~604 bp. The PCR product was purified and send for custom sequencing.

```
atg gct tac aat ccg atc aca cct agc aaa ctt att gcg ttt agt gct tct tat gtt ccc
 M   A   Y   N   P   I   T   P   S   K   L   I   A   F   S   A   S   Y   V   P
gtc agg act tta ctt aat ttt cta gtt gct tca caa ggt acc gct ttc cag act caa gcg
 V   R   T   L   L   N   F   L   V   A   S   Q   G   T   A   F   Q   T   Q   A
gga aga gat tct ttc cgc gag tcc ctg tct gcg tta ccc tcg tct gtc gta gat att aat
 G   R   D   S   F   R   E   S   L   S   A   L   P   S   S   V   V   D   I   N
tct agg ttc cca gat gcg ggt ttt tac gct ttc ctc aac ggt cct gtg ttg agg cct atc
 S   R   F   P   D   A   G   F   Y   A   F   L   N   G   P   V   L   R   P   I
ttc gtt tcg ctt ctc agc tcc acg gat acg cgt aat agg gtc att gag gtt gta gat cct
 F   V   S   L   L   S   S   T   D   T   R   N   R   V   I   E   V   V   D   P
agc aat cct acg act gct gag tcg ctt aat gct gta aag cgt act gat gac gcg tct aca
 S   N   P   T   T   A   E   S   L   N   A   V   K   R   T   D   D   A   S   T
gcc gct agg gct gaa ata gat aat tta ata gag tct att tct aag ggt ttt gat gtt tac
 A   A   R   A   E   I   D   N   L   I   E   S   I   S   K   G   F   D   V   Y
gat agg gct tca ttt gaa gcc gcg ttt tcg gta gtc tgg tca gag gct acc acc tcg aaa
 D   R   A   S   F   E   A   A   F   S   V   V   W   S   E   A   T   T   S   K
gct tag ttt cga ggg tct tct gat ggt ggt gca cac caa
 A   -   F   R   G   S   S   D   G   G   A   H   Q
```

Figure 4.1: Nucleotide and amino acid sequence of CGMMV test isolate

4.5.1 Sequence analysis

The sequence obtained after custom sequencing was Blast analysed to confirm the identity of CGMMV. The isolate sequenced had 520 bp and lacked a poly (A) tail. The base composition of the CP gene calculated using DNA base Composition tool showed frequency of 21.5, 32.11, 23.26 and 23.07 per cent AUGC. The frequency of AU and GC was 53.65 and 46.30 per cent. The sequence was translated into 161 amino acids using ExPASy tool (Figure 4.1).

4.5.2 Comparison of Nucleotide and Protein Sequences of CGMMV

i) Nucleotide sequence analysis

The CP gene based nucleotide sequence were used for the sequences of CGMMV test isolate and other related isolates for multiple alignment, pairwise comparison and phylogenetic analysis (Table 4.6).

The phylogenetic analysis of the test strain revealed it to form cluster with CGMMV isolate CG003 (MH271409) with a per cent identity of 99.80 per cent. The least shared identity was with CGMMV AH-FI197 (KU175639) and CGMMV AH-FX3 isolate (KU175632) i.e. 98.26 and 98.07 per cent respectively (Figure 4.2).

ii) Amino acid sequence analysis

The amino acid sequence was derived from ExPASy translate tools for the twenty-three isolates used in the study and the phylogenetic analysis revealed it to cluster with CGMMV isolates from USA, Greece, Australia, India, China and Japan (AYN80372, AYN80477, AZJ51137, AAP74202, QRM12790, ABA64461 and NP_044580) sharing 100 per cent sequence identity at amino acid level. However, it shared least identity (45.34%) with Cucumber fruit mottle mosaic virus (CFMMV) isolate from Sudan (QSM07163) and Zucchini Green Mottle Mosaic Virus (ZGMMV) from South Korea (NP_624339) (Figure 4.3, Table 4.7).

4.5.3 Recombination analysis

Recombination analysis done using RDP4, revealed that the isolate was not a recombinant as the software could not trace any recombination event.

4.6 Molecular characterization of PRSV-W

Similarly, the RT-PCR of test isolate with PRSV-W coat protein specific primers produced an amplicon of ~398 bp. The PCR product was purified and send for custom sequencing.

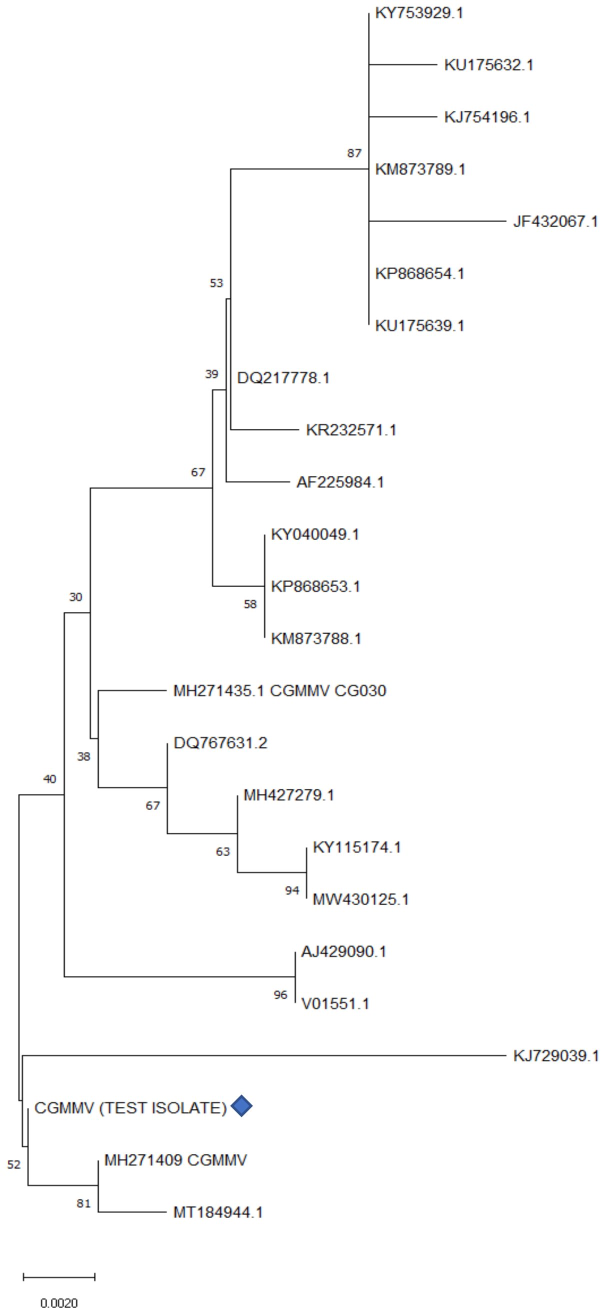


Figure 4.2: Phylogenetic tree inferred using the Neighbor-Joining method, showing relationship between CGMMV (test isolate) and other related strains of CGMMV based on CP nucleotide sequence using MegaX software

Table 4.7: Pairwise similarity between CGMMV and other isolates of CGMMV based on coat protein amino acid sequence using ClustalW program

	CGMMV TEST ISOLATE	MH271409	MH271435	MH427279	DQ767631	MT184944	DQ217778	KR232571	KY040049	KY115174	KP868653	KM873788	MW430125	AF225984	KY753929	KP868654	KM873789	AJ429090	V01551	KJ754196	KJ729039	JF432067	KU175639	KU175632
CGMMV TEST ISOLATE	-	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	99	100	99
MH271409		-	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	99	100	99
MH271435			-	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	99	100	99
MH427279.1				-	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	99	100	99
DQ767631.2					-	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	99	100	99
MT184944.1						-	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	99	100	99
DQ217778.1							-	100	100	100	100	100	100	100	100	100	100	100	100	100	100	99	100	99
KR232571.1								-	100	100	100	100	100	100	100	100	100	100	100	100	100	99	100	99
KY040049.1									-	100	100	100	100	100	100	100	100	100	100	100	100	99	100	99
KY115174.1										-	100	100	100	100	100	100	100	100	100	100	100	99	100	99
KP868653.1											-	100	100	100	100	100	100	100	100	100	100	99	100	99
KM873788.1												-	100	100	100	100	100	100	100	100	100	99	100	99
MW430125.1													-	100	100	100	100	100	100	100	100	99	100	99
AF225984.1														-	100	100	100	100	100	100	100	99	100	99
KY753929.1															-	100	100	100	100	100	100	99	100	99
KP868654.1																-	100	100	100	100	100	99	100	99
KM873789.1																	-	100	100	100	100	99	100	99
AJ429090.1																		-	100	100	100	99	100	99
V01551.1																			-	100	100	99	100	99
KJ754196.1																				-	100	99	100	99
KJ729039.1																					-	97	99	98
JF432067.1																						-	98	98
KU175639.1																							-	98
KU175632.1																								-

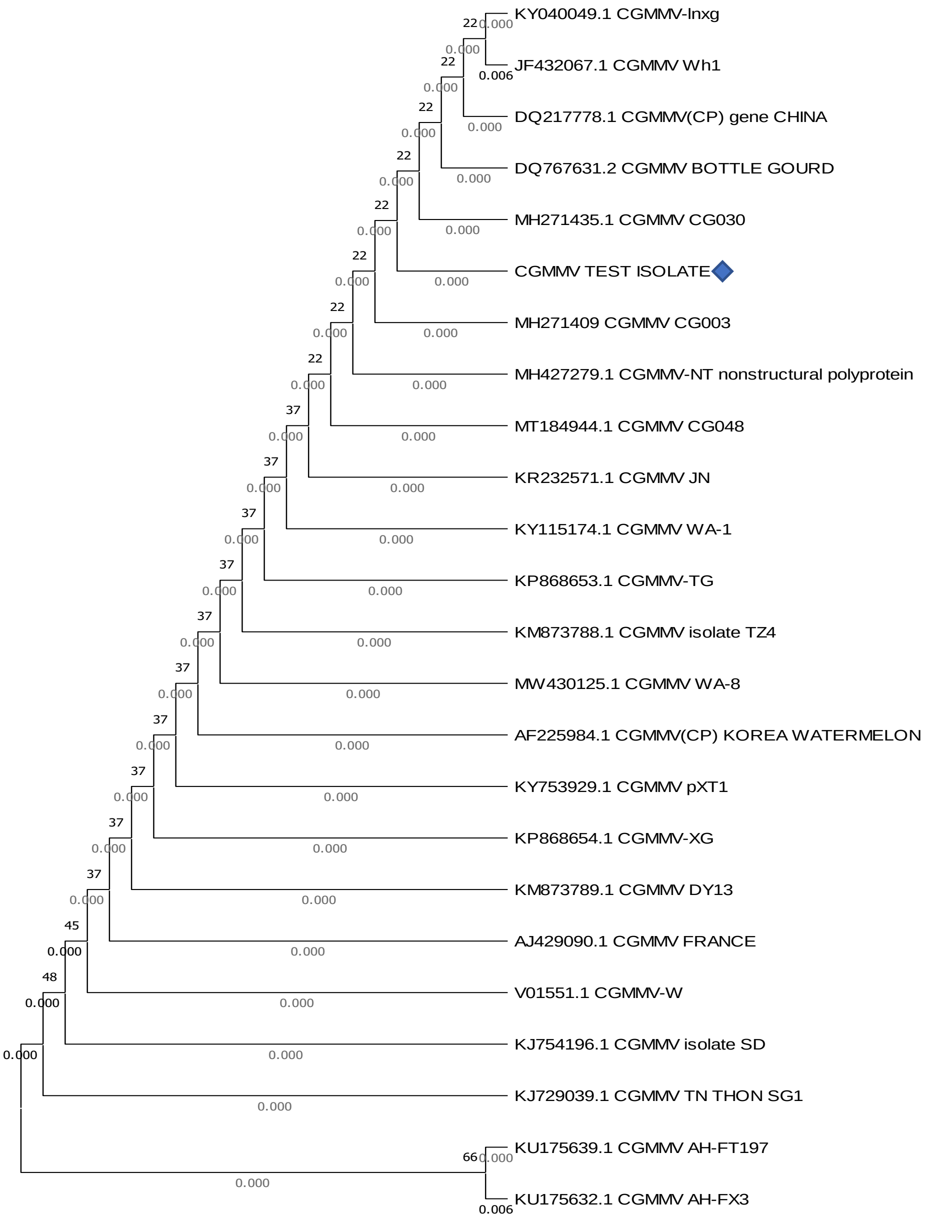


Figure 4.3: Phylogenetic tree inferred using the Neighbor-Joining method, showing relationship between CGMMV (test isolate) and other related strains of CGMMV based on CP amino acid sequence using MegaX software

Table 4.6: Pairwise similarity between CGMMV and other isolates of CGMMV based on coat protein nucleotide sequence using ClustalW program

	TEST ISOLATE	MH271409	MH271435	MH427279	DQ767631	MT184944	DQ217778	KR232571	KY040049	KY115174	KP868653	KM873788	MW430125	AF225984	KY753929	KP868654	KM873789	AJ429090	V01551	KJ754196	KJ729039	JF432067	KU175639	KU175632
CGMMV TEST ISOLATE	-	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	98	98	98	98	98
MH271409		-	98	98	98	99	98	98	97	98	97	98	98	99	98	98	98	95	96	98	97	98	98	98
MH271435			-	98	99	98	99	98	98	98	98	98	98	99	98	98	98	95	95	98	96	98	99	98
MH427279.1				-	99	98	98	98	98	99	98	98	99	99	98	98	98	95	96	98	96	98	98	98
DQ767631.2					-	98	99	98	98	99	98	98	98	99	98	98	98	96	95	98	96	98	99	98
MT184944.1						-	98	97	98	98	98	97	97	99	98	98	97	95	96	98	96	98	98	98
DQ217778.1							-	99	99	98	99	99	97	97	98	99	98	95	98	98	85	87	94	93
KR232571.1								-	99	98	99	98	97	99	99	99	98	95	95	98	96	99	99	99
KY040049.1									-	98	99	99	97	99	99	99	99	95	95	98	96	99	99	99
KY115174.1										-	98	97	99	99	98	98	98	95	96	98	96	98	98	98
KP868653.1											-	99	97	99	99	99	99	96	95	99	96	99	99	99
KM873788.1												-	97	99	99	99	98	96	96	98	96	99	99	99
MW430125.1													-	99	98	97	97	95	95	98	96	98	98	98
AF225984.1														-	99	99	99	98	98	99	88	92	97	97
KY753929.1															-	99	99	95	96	99	96	99	100	99
KP868654.1																-	99	95	95	99	96	99	100	99
KM873789.1																	-	95	95	99	96	99	100	99
AJ429090.1																		-	97	95	96	98	98	97
V01551.1																			-	96	96	98	98	97
KJ754196.1																				-	96	99	99	99
KJ729039.1																					-	96	97	97
JF432067.1																						-	99	99
KU175639.1																							-	99
KU175632.1																								-

4.6.1 Sequence Analysis

A partial coat protein sequence obtained after custom sequencing was Blast analysed to confirm the identity of PRSV-W. The assembled sequence had 398 bases and lacked a poly (A) tail (Figure 4.4).

The base composition of the CP gene calculated using DNA base Composition tool showed frequency of 30.65, 22.36, 24.12 and 22.86 per cent AUGC. The frequency of AU and GC was 53.01 and 47.00 per cent. The sequence was translated into 130 amino acids using ExPASy tool.

```
tgg gtt atg atg gat ggg gat act caa gtc gac tac cca att aaa cca tta att gaa cac
 W   V   M   M   D   G   D   T   Q   V   D   Y   P   I   K   P   L   I   E   H
gca aca cca aca ttc cgt cag att atg gca cac ttc agc aat gca gct gag gca tac att
 A   T   P   T   F   R   Q   I   M   A   H   F   S   N   A   A   E   A   Y   I
gca aaa agg aat gca aca gaa cgg tat atg cct cgt tac ggc ttg aag cga aat ttg act
 A   K   R   N   A   T   E   R   Y   M   P   R   Y   G   L   K   R   N   L   T
gac ata agc ctc gct aga tat gcc ttt gac ttt tat gag atc aac tca aag act cct gat
 D   I   S   L   A   R   Y   A   F   D   F   Y   E   I   N   S   K   T   P   D
cga gca cgt gaa gct cat atg cag atg aag gca gcg gcc ctc cgc aac gct aac agg cgc
 R   A   R   E   A   H   M   Q   M   K   A   A   A   L   R   N   A   N   R   R
tta ttt gga ata gac ggc agt gtc agt aac agg gaa gag aac acg gag aga cac act gtt
 L   F   G   I   D   G   S   V   S   N   R   E   E   N   T   E   R   H   T   V
gag gat gtc gat cgc gac atg cac tct ctc ctg ggt
 E   D   V   D   R   D   M   H   S   L   L   G
```

Figure 4.4: Nucleotide and amino acid sequence of PRSV-W test isolate

4.6.2 Comparison of nucleotide and protein sequences of PRSV-W

i) Nucleotide sequence analysis

The CP gene nucleotide sequence were used for the sequences of the PRSV-W test isolate and other related isolates for multiple alignment, pairwise comparison and phylogenetic analysis (Table 4.8).

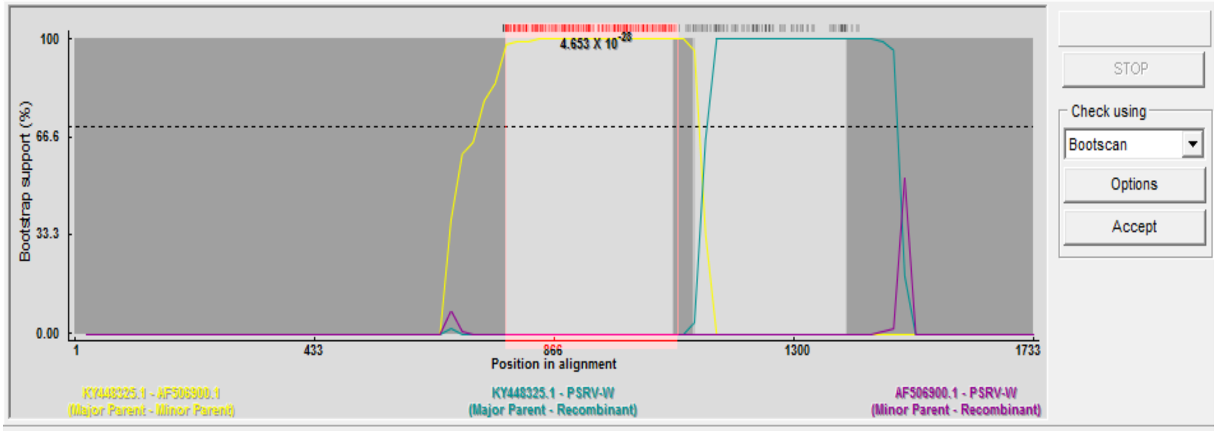
The phylogenetic analysis of the PRSV-W test isolate using MEGAX software based on nucleotide sequence using neighbor joining method revealed that the accession no. KJ 789898 (MWMV KZN31) from South Africa formed an out group from rest of the sequences. The test isolate clustered with PRSV isolate SHK-PM1 (KY448325) which is also sequenced from India. The test isolate shared identity from 67 to 96.23 per cent with other isolates/ virus in study. The test isolate shared the highest per cent identity with PRSV isolate SHK-PM1 (KY448325) i.e. 96.23 followed by PRSV PAK an isolate from Pakistan (AB127935) (92.46%) and this is also reflected in the phylogenetic tree constructed (Figure 4.6, Table 4.8).

ii) Amino acid sequence analysis

The amino acid sequence was derived from ExPASy translate tools for the twenty-six isolates used in the study. The phylogenetic analysis revealed that the test isolate clustered with PRSV isolates from India (KY 448325, LC 457969, LC 457968, KY 4488324 and KY 448332), Myanmar (AB583205, AB583204, AB583212 and AB583214), USA (KF 649240) and Pakistan (AB 127935) sharing 100 per cent sequence identity at amino acid level. It shared the least identity (75.53 %) with the US isolate i.e. PRSV isolate 1116_ 19 (KF649243) (Figure 4.7, Table 4.9).

4.6.3 Recombination analysis

Recombination analysis done using RDP4, revealed that the isolate was a recombinant of PRSV isolate SHK-PM1 (KY448325) and an unknown parent. Based on 32 complete nucleotide sequences of PRSV-W, 11 unique events with 19 recombination signals were obtained from the CP nucleotide sequences of PRSV-W and related strains. The average p-value of 3seq method was 4.653×10^{-28}. The breakage was observed at 616 - 20 nucleotides without gap ending at 311-347, where the minor parent formed a single fragment and the major parent had two fragments. For revealing the recombination event, the most reliable method was PhylPro, which showed 92.7 per cent similarity to major parent (273- 7666 nucleotide) PRSV SHK-PM1 (Figure 4.5, 4.8 and 4.9), interestingly, the phylogenetic analysis and pairwise comparison also had revealed the major parent to be the closest to the test isolate.



Check using: Bootscan

Figure 4.5: A bootscan plot showing the recombination signal. In this case the left and right bounds of the pink region indicate breakpoint positions

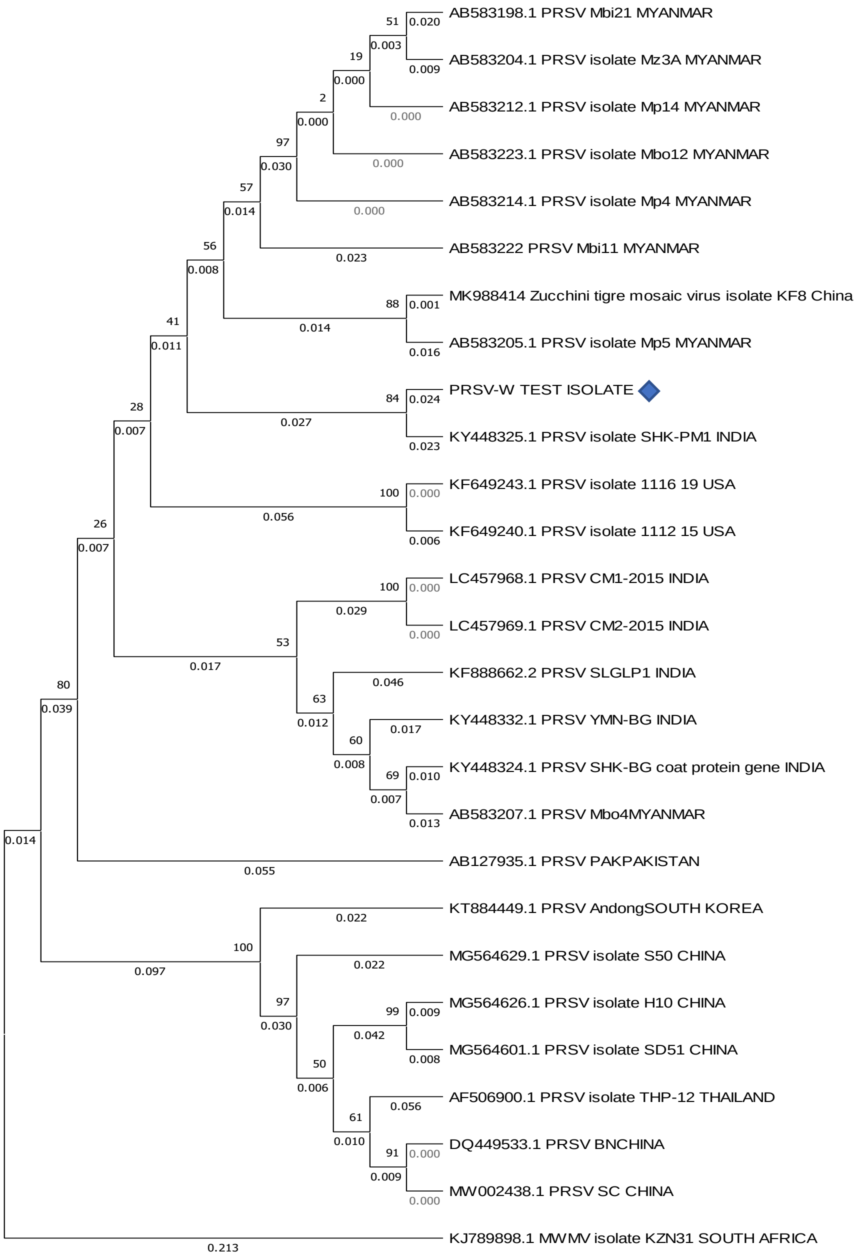


Figure 4.6: Phylogenetic tree inferred using the Neighbor-Joining method, showing relationship between PRSV-W (test isolate) and other related strains of PRSV-W based on CP nucleotide sequence using MegaX software

Table 4.9: Pairwise similarity between PRSV-W and other isolates of PRSV-W based on coat protein amino acid sequence using ClustalW program

	PRSV TEST ISOLATE	MK988414	AB583222	KY448324	AB583223	AB583214	AB583212	AB583198	KY448332	AB583204	AB583207	KF888662	KT884449	MG564629	LC457968	DQ449533	MW002438	MG564626	MG564601	AF506900	KF649243	KF649240	LC457969	KJ789898	KY448325	AB127935	AB583205
PRSV TEST ISOLATE	**-**	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**99**	**96**	**93**	**93**	**93**	**93**	**92**	**90**	**91**	**90**	**75**	**85**	**100**	**82**	**99**	**100**	**99**
MK988414		**-**	**94**	**97**	**93**	**94**	**94**	**94**	**96**	**94**	**96**	**95**	**91**	**90**	**97**	**91**	**93**	**88**	**89**	**89**	**91**	**85**	**100**	**75**	**96**	**96**	**97**
AB583222			**-**	**95**	**96**	**96**	**96**	**96**	**95**	**96**	**95**	**95**	**88**	**88**	**97**	**88**	**91**	**86**	**86**	**87**	**90**	**84**	**98**	**76**	**95**	**95**	**95**
KY448324				**-**	**96**	**92**	**92**	**92**	**96**	**91**	**97**	**97**	**82**	**84**	**100**	**84**	**92**	**82**	**82**	**82**	**91**	**85**	**100**	**67**	**95**	**92**	**95**
AB583223					**-**	**100**	**100**	**99**	**96**	**99**	**96**	**95**	**90**	**90**	**98**	**90**	**92**	**88**	**88**	**88**	**91**	**85**	**100**	**77**	**95**	**95**	**95**
AB583214						**-**	**99**	**96**	**91**	**99**	**94**	**95**	**80**	**82**	**98**	**82**	**92**	**80**	**80**	**80**	**91**	**85**	**100**	**68**	**92**	**92**	**94**
AB583212							**-**	**97**	**91**	**99**	**94**	**95**	**80**	**82**	**98**	**82**	**92**	**80**	**80**	**80**	**91**	**85**	**100**	**68**	**92**	**92**	**94**
AB583198								**-**	**92**	**96**	**94**	**95**	**80**	**82**	**98**	**82**	**92**	**80**	**80**	**79**	**91**	**85**	**100**	**68**	**92**	**91**	**95**
KY448332									**-**	**91**	**98**	**97**	**81**	**84**	**100**	**85**	**92**	**83**	**83**	**81**	**91**	**85**	**100**	**67**	**94**	**92**	**94**
AB583204										**-**	**93**	**95**	**80**	**82**	**98**	**82**	**92**	**80**	**80**	**80**	**91**	**85**	**100**	**68**	**91**	**92**	**94**
AB583207											**-**	**97**	**81**	**83**	**100**	**84**	**91**	**82**	**82**	**81**	**91**	**85**	**100**	**68**	**95**	**92**	**96**
KF888662												**-**	**89**	**89**	**94**	**89**	**88**	**87**	**88**	**86**	**75**	**82**	**97**	**79**	**95**	**96**	**95**
KT884449													**-**	**93**	**94**	**94**	**98**	**92**	**92**	**92**	**87**	**81**	**95**	**65**	**82**	**80**	**83**
MG564629														**-**	**94**	**96**	**98**	**94**	**94**	**93**	**87**	**81**	**95**	**67**	**83**	**82**	**84**
LC457968															**-**	**94**	**88**	**93**	**94**	**91**	**81**	**85**	**94**	**80**	**97**	**98**	**97**
DQ449533																**-**	**100**	**95**	**95**	**92**	**87**	**81**	**95**	**66**	**84**	**82**	**85**
MW002438																	**-**	**96**	**97**	**94**	**71**	**81**	**95**	**77**	**92**	**92**	**93**
MG564626																		**-**	**99**	**92**	**86**	**80**	**94**	**64**	**82**	**80**	**83**
MG564601																			**-**	**92**	**87**	**81**	**95**	**64**	**82**	**80**	**83**
AF506900																				**-**	**84**	**78**	**93**	**64**	**82**	**80**	**82**
KF649243																					**-**	**89**	**77**	**75**	**90**	**91**	**90**
KF649240																	**-**					**-**	**79**	**70**	**84**	**85**	**85**
LC457969																							**-**	**87**	**98**	**100**	**100**
KJ789898																								**-**	**67**	**67**	**68**
KY448325																									**-**	**94**	**94**
AB127935																										**-**	**92**
AB583205																											**-**

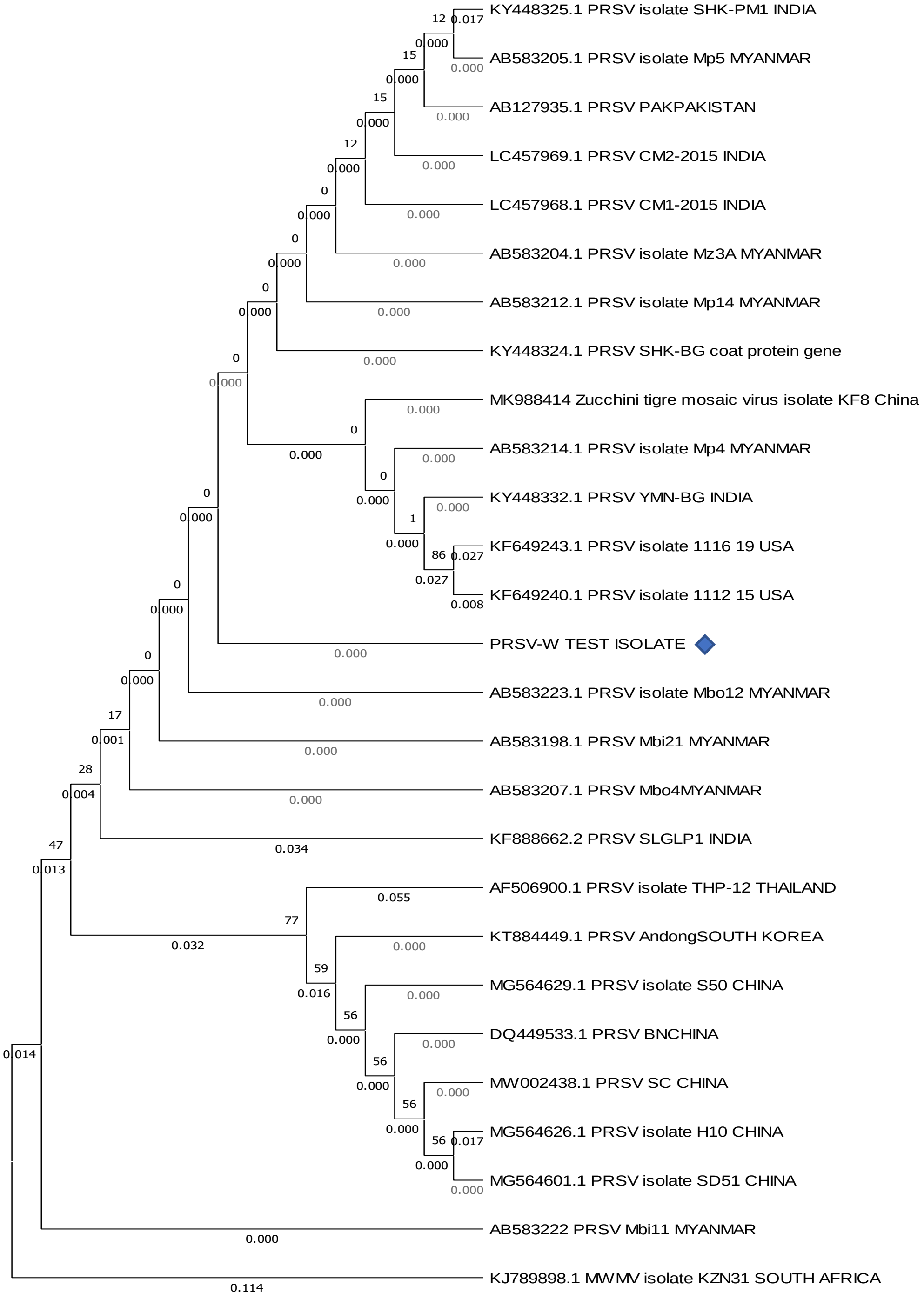


Figure 4.7: Phylogenetic tree inferred using the Neighbor-Joining method, showing relationship between PRSV-W (test isolate) and other related strains of PRSV-W based on CP amino acid sequence using MegaX software

Table 4.8: Pairwise similarity between PRSV-W and other isolates of PRSV-W based on coat protein nucleotide sequence using ClustalW program

	PRSV TEST	MK988414	AB583222	KY448324	AB583223	AB583214	AB583212	AB583198	KY448332	AB583204	AB583207	KF888662	KT884449	MG564629	LC457968	DQ449533	MW002438	MG564626	MG564601	AF506900	KF649243	KF649240	LC457969	KJ789898	KY448325	AB127935	AB583205
PRSV TEST ISOLATE	-	90	90	89	89	89	89	89	88	88	88	87	82	80	84	77	76	77	77	76	72	79	88	67	96	92	92
MK988414		-	91	85	91	91	91	90	85	90	84	88	73	71	88	70	66	69	69	70	86	85	89	53	86	83	94
AB583222			-	76	91	90	91	92	76	90	85	88	67	66	88	66	55	65	65	65	84	83	88	62	77	85	90
KY448324				-	76	84	84	84	94	83	94	94	74	73	94	72	53	72	72	71	83	83	93	61	85	83	84
AB583223					-	98	100	96	76	99	87	88	68	64	88	66	54	65	65	65	86	84	88	63	77	86	90
AB583214						-	98	93	82	98	83	88	73	69	88	69	66	68	68	69	86	84	88	55	84	82	92
AB583212							-	93	82	99	83	88	73	69	88	69	66	68	68	69	86	84	88	55	84	82	91
AB583198								-	83	93	83	88	72	70	88	69	64	68	68	68	84	82	88	53	84	81	89
KY448332									-	82	95	93	74	73	93	73	52	71	71	71	85	83	92	62	84	84	85
AB583204										-	82	87	72	68	87	69	66	68	67	69	84	82	86	55	83	82	91
94AB583207											-	94	74	71	93	72	68	71	71	70	84	83	93	59	84	80	84
KF888662												-	77	77	88	77	75	77	77	75	70	78	91	67	85	86	87
KT884449													-	90	80	90	60	90	90	89	78	76	82	60	75	73	74
MG564629														-	80	94	64	92	92	91	75	75	79	58	73	72	70
LC457968															-	80	74	77	77	76	77	85	95	67	86	88	88
DQ449533																-	66	93	92	92	75	75	79	58	70	70	72
MW002438																	-	62	63	63	60	68	79	53	51	66	66
MG564626																		-	98	90	74	72	77	57	69	68	69
MG564601																			-	90	73	72	77	57	69	68	69
AF506900																				-	72	71	75	57	70	71	69
KF649243																					-	95	73	65	84	84	85
KF649240																	-					-	73	61	81	83	84
LC457969																							-	70	87	88	89
KJ789898																								-	60	56	55
KY448325																									-	89	86
AB127935																										-	84
AB583205																											-

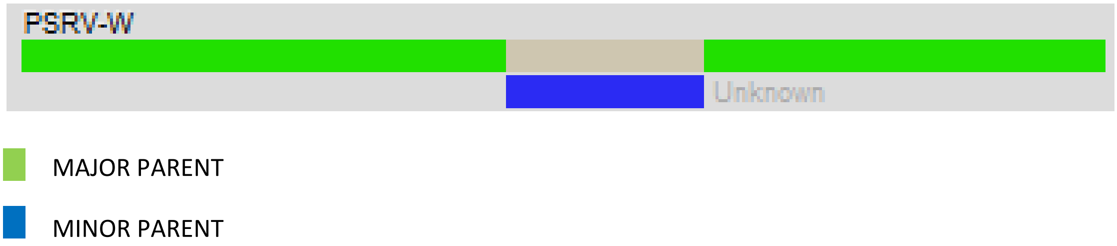


Figure 4.8: The schematic display of recombination scan derived from RDP v.4.80 beta software

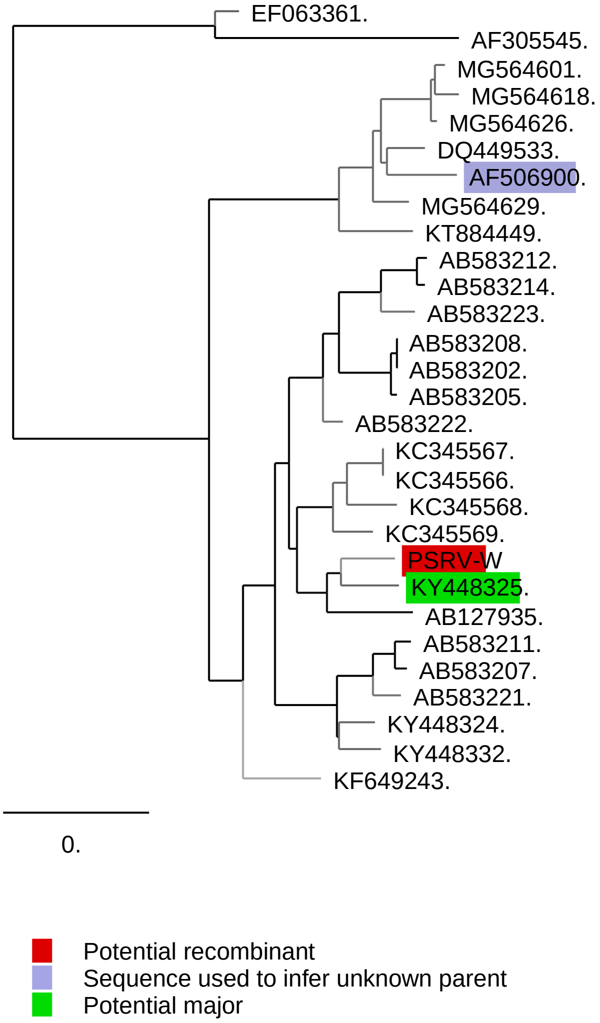


Figure 4.9: Phylogenetic tree showing test isolate PRSV-W as a potential recombinant of PRSV-W isolate SHK-PM1 (KY448325) and unknown parent

4.7 Host range studies

The host range of the bottle gourd mosaic virus was determined using twenty plant species (*Cucumis sativus, Cucurbita pepo, C. melo, Citrullus lanatus, C. maxima, Luffa acutangula, Praecitrullus fistulosus, Momordica charantia, Solanum lycopersicum, S. melongena, Capsicum annum, Abelmoschus aeculentus, Phaseolus vulgaris, Vigna unguiculata, Brassica oleracea* var *capitata, B. oleracea* var *botrytis, Amaranthus* spp. and *Chenopodium* spp.) belonging to six different families (*Cucurbitaceae, Solanaceae, Malvaceae, Fabaceae, Brassicaceae* and *Amaranthaceae),* raised in green house conditions. About ten plants in replication from each species were inoculated mechanically using standard leaf rub method. Inoculated plants were observed for 30-40 days post inoculation (dpi) for studying the symptom expression.

The data presented in the table 4.10 depicts the host range of the virus under study. The virus infected all the members of the Cucurbitaceae i.e. *C. sativus, C. pepo, C. melo, Citrullus lanatus, C. maxima, L. acutangula, P. fistulosus* and *Momordica charantia* and a member of *Amaranthaceae* i.e. *Amaranthus* spp. The plants *S. lycopersicum, S. melongena, C. annum, A. esculentus (L.), Phaseolus vulgaris, Vigna unguiculata, Brassica oleracea* var *capitate, B. oleracea* var *botrytis* and *Chenopodium* spp. were inoculated but did not show any symptoms and failed to infect the susceptible cultivar upon back inoculations. The host that reacted locally was *Amaranthus* spp. while rest showed systemic symptoms (Plate 13).

The virus induced mosaic symptom with 100 per cent transmission both in case of cucumber (*C. sativus)* and pumpkin (*C. pepo*). In case of cucumber the symptoms were expressed after 20-25 days of inoculation while pumpkin exhibited symptoms after 10-15 days. Muskmelon (*C. melo*) showed 100 per cent transmission with symptoms like leaf blisters after 10-15 dpi. In case of watermelon (*C. lanatus*) mosaic symptom was observed (10-15 dpi) followed by yellowing.

In case of summer squash (*C. pepo*) the symptoms were observed earliest after 7-10 days. All the inoculated plants showed symptoms like mosaic, enation, leaf curling and leaf distortion (filiform) in case of severe infection, while in case of winter squash (*C. maxima*) mild mosaic and enation were observed. In *L. acutangula* (ridge gourd) yellowing was observed at 20-25 dpi, while, blisters and mosaic were recorded from the infected leaves of round gourd (*P. fistulosus).* However, mild mosaic was observed in *M. charantia* after 25-30 dpi.

Table 4.10: Observation of bottle gourd mosaic virus in different hosts of various families

FAMILY	SCIENTIFIC NAME	COMMON NAME	TRANSMISSION (%)	DOI	SYMPTOMS OBSERVED
Cucurbitaceae	*Cucumis sativus*	Cucumber	100	20-25	M
	Cucurbita pepo	Pumpkin	100	10-15	M
	Cucumis melo	Muskmelon	100	10-15	B
	Citrullus lanatus	Watermelon	100	10-15	M, Y
	Cucurbita pepo	Summer Squash	100	7-10	M, LD, E
	Cucurbita maxima	Winter Squash	100	10-15	LD, E
	Luffa acutangula	Ridge gourd	100	20-25	Y
	Praecitrullus fistulosus	Round gourd	100	20-25	B, M
	Momordica charantia	Bitter gourd	100	25-30	M
Solanaceae	*Solanum lycopersicum*	Tomato	0	-	-
	Solanum melongena	Brinjal	0	-	-
	Capsicum annuum	Capsicum	0	-	-
	Capsicum annuum	Chilli	0	-	-
Malvaceae	*Abelmoschus esculentus (L.)*	Okra	0	-	-
Fabaceae	*Phaseolus vulgaris*	Rajmash	0	-	-
	Vigna unguiculata	Cow pea	0	-	-
Brassicaceae	*Brassica oleracea* var. *capitata*	Cauliflower	0	-	-
	Brassica oleracea var. *botrytis*	Broccoli	0	-	-
Amaranthaceae	*Amaranthus* spp.	Amaranthus	100	20	LL
	Chenopodium spp.	Chenopodium	0	-	-

DOI- days of inoculation, B- Blisters, E- enation, LD- Leaf distortion, LL- Local Lesion, M- Mosaic, MM- Mild Mosaic, Y- Yellowing, - : No symptom,

MOSAIC (CUCUMBER) ENATION (SUMMER SQUASH) MOSAIC, BITTER GOURD

MILD MOSAIC PUMPKIN MILD MOSAIC, ROUND GOURD YELLOWING, RIDGE GOURD

Plate 13: Different Symptoms observed during host range studies

4.8 Insect- Vector Transmission

During the survey apterous aphid adults were collected from the infected samples and raised as aviruliferous colony after giving them a fasting period. The aphids preserved in 70 per cent ethanol, were used to identify the species of the aphid associated. However, it was observed that the symptoms developed on bottle gourd cv. MAHY-1 plants upon inoculation with aphids, were similar to that produced under natural infection in field.

4.8.1 Identification of aphids:

The microscopic examination of aphid sample showed presence of a dark greenish adult (apterous) along with pale yellow or green nymphs with cornicles twice longer than tongue shaped cauda. The antennal tubercles were weakly developed. Based on the key features, the specie was identified as melon aphid (*A. gossypii)* (Plate 14).

Similarly, the microscopic examination of the other associated aphid species showed a shining black body, no or reduced antennal tubercles. The cornicles were much longer than cauda which was dome shaped and had 4-7 hairs. Based on these features the aphid was identified as black legume aphid (*A. craccivora)* (Plate 15,16).

4.8.2 Aphid transmission

The two species identified were used in the transmission studies. For initial transmission experiment random isolate representing CGMMV and PRSV-W was used. The perusal of the data in Table 4.11 shows that CGMMV failed to be transmitted through both the aphid species. While PRSV-W was successfully transmitted. The symptoms developed were also observed. Later four isolates (J-SA-BG3, J-NA-BG-1, J-RJ-BG3 and J-MN-BG1) each representing PRSV-W isolate from each district (Jammu, Kathua, Samba and Udhampur, respectively) was taken up for determining the transmission efficiencies.

Table 4.11: Transmission of CGMMV and PRSV-W associated with bottle gourd mosaic

Insect species	Virus	Pre-acquisition fasting period	Acquisition Period	No. of insects	Inoculation Period	Incidence	Per cent transmission
A. gossypii	CGMMV	1 h	10 min	10	24 h	0/10	0
	PRSV-W	1 h	10 min	10	24 h	7/10	70
A. *craccivora*	CGMMV	1 h	10 min	10	24 h	0/10	0
	PRSV-W	1 h	10 min	10	24 h	4/10	40

4.8.3 Influence of number of aphids for transmitting PRSV-W isolates

Highest per cent transmission was observed for both the aphid species when at least 15 aphids were released on the healthy plants. In case of *A. gossypii* 87.50 per cent transmission was observed while for *A. craccivora* it was 83.75 per cent mean transmission. The least was observed when only three aphids were released for both *A. gossypii* (23.75%) and *A. craccivora* (18.75%). The transmission increased as the no. of aphids increased from 3 to 15, but after 15 as the no. increased again a decrease in the per cent transmission was observed (Table 4.12 and 4.13).

Table 4.12: Influence of number of aphids (*A. gossypii)* on transmission of PRSV-W

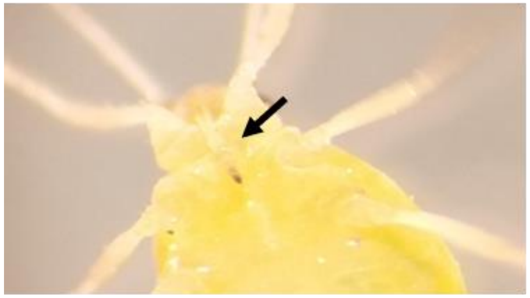
No. of aphids released / plant	**Per cent Transmission**				**Mean transmission (%)**
	J-SA-BG3	**J-NA-BG1**	**J-RJ-BG3**	**J-MN-BG1**	
3	20	35	15	25	23.75
5	35	45	35	35	37.50
10	70	65	75	70	70.00
15	85	85	90	90	87.50
20	75	80	80	75	77.50

Table 4.13: Influence of number of aphids (*A. craccivora)* on transmission of PRSV-W

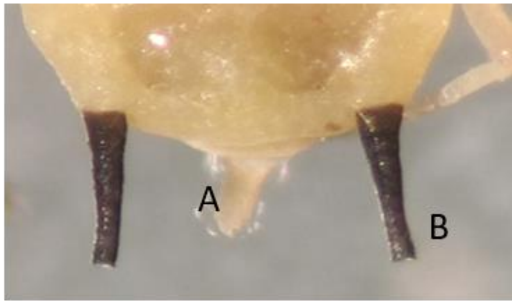
No. of aphids released / plant	**Per cent Transmission**				**Mean transmission (%)**
	J-SA-BG3	**J-NA-BG1**	**J-RJ-BG3**	**J-MN-BG1**	
3	20	15	20	20	18.75
5	35	30	40	35	35.00
10	65	70	70	60	66.25
15	75	90	90	80	83.75
20	70	75	75	75	73.75

4.8.4 Effect of pre- acquisition fasting on aphids transmitting PRSV-W

Average per cent transmission of 78.75 per cent and 56.25 per cent was recorded in *A. gossypii* and *A. craccivora* when they were provided a starvation period of 60 minutes (Table 4.14 and Table 4.15). However, no transmission was observed when starvation period was increased up to 240 minutes (4hrs).

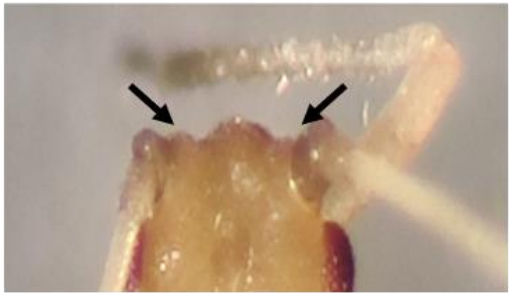


ROSTRUM

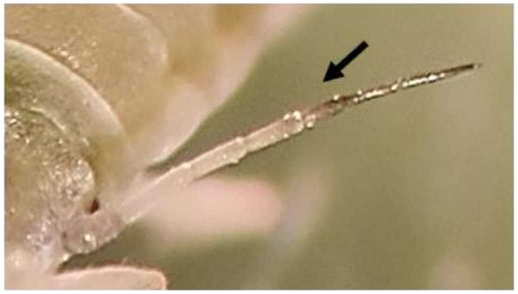


A- CAUDA
B- SIPHUNCULI

Aphis gossypii



ANTENNAL TUBERCLES



ANTENNA

Plate 14: Identification of aphid species (*Aphis gossypii)*

SIPHUNCULUS

CAUDA

Aphis craccivora

ANTENNA

ANTENNAL TUBERCLES

ROSTRUM

Plate 15: Identification of aphid species (*Aphis craccivora*)

Plate no. 16 A: ***Aphis gossypii*** **probing and feeding on infected samples**

B: ***Aphis craccivora*** **feeding on infected samples**

Table 4.14: Influence of pre- acquisition fasting on PRSV-W transmission by *A. gossypii*

Time (min.)	Per cent Transmission				Mean transmission (%)
	J-SA-BG3	J-NA-BG1	J-RJ-BG3	J-MN-BG1	
5	30	30	25	30	28.75
15	45	40	35	40	40.00
30	50	60	60	65	58.75
45	60	60	65	70	63.75
60	75	80	75	85	78.75
240	0	0	0	0	0

Table 4.15: Influence of pre- acquisition fasting on PRSV-W transmission by *A. craccivora*

Time (min.)	Per cent Transmission				Mean transmission (%)
	J-SA-BG3	J-NA-BG1	J-RJ-BG3	J-MN-BG1	
5	15	20	20	20	18.75
15	20	30	25	35	27.50
30	40	40	45	40	41.25
45	45	50	50	45	47.50
60	60	50	50	65	56.25
240	0	0	0	0	0

4.8.5 Influence of Acquisition access period on PRSV-W transmission

For studying the effect of acquisition access period, it was observed that highest mean per cent transmission of 86.25 and 78.75 per cent for *A. gossypii* and *A. craccivora* occurred when the aphids fed for 15 mins on the infected samples which was followed by an acquisition period of 30 minutes (68.75% and 58.75%) for *A. gossypii* and *A. craccivora* respectively. Further, as the acquisition period increased, there was a gradual decrease in the transmission (Table 4.16 and 4.17).

Table 4.16: Influence of Acquisition access period on PRSV-W transmission by *A. gossypii*

Time (min.)	Per cent Transmission				Mean transmission (%)
	J-SA-BG3	J-NA-BG1	J-RJ-BG3	J-MN-BG1	
5	40	35	35	40	37.50
15	85	90	90	80	86.25
30	70	75	65	65	68.75
45	65	60	60	60	61.25
60	50	60	55	55	55.00
240	0	0	0	0	0

Table 4.17: Influence of Acquisition access period on PRSV-W transmission by *A. craccivora*

Time (min.)	Per cent Transmission				Mean transmission (%)
	J-SA-BG3	J-NA-BG1	J-RJ-BG3	J-MN-BG1	
5	30	30	35	25	30.00
15	80	85	75	75	78.75
30	60	60	60	55	58.75
45	50	50	45	50	48.75
60	30	35	30	30	31.25
240	0	0	0	0	0

4.8.6 Influence of Inoculation access period for transmitting PRSV-W

An inoculation access period of 30 minutes was the best for transmitting PRSV-W isolates by *A. gossypii* (77.5%) and *A. craccivora* (75%) followed by mean transmission of 70 per cent at 15 minutes in case of *A. gossypii* and 55 per cent at 45 minutes for *A. craccivora.* A decrease in transmission was noted after 60 minutes for both the aphid species (Table 4.18 and 4.19).

Table 4.18: Influence of Inoculation access period on *A. gossypii* to transmit PRSV-W

Time (min.)	Per cent Transmission				Mean transmission (%)
	J-SA-BG3	J-NA-BG1	J-RJ-BG3	J-MN-BG1	
5	25	30	30	25	27.50
15	70	70	70	70	70.00
30	80	75	75	80	77.50
45	70	65	65	65	66.25
60	65	65	60	55	57.50
240	0	0	0	0	0

Table 4.19: Influence of Inoculation access period on PRSV-W transmission by *A. craccivora*

Time (min.)	Per cent Transmission				Mean transmission (%)
	J-SA-BG3	J-NA-BG1	J-RJ-BG3	J-MN-BG1	
5	20	20	20	20	20.00
15	50	45	50	50	48.75
30	75	75	70	80	75.00
45	55	60	50	55	55.00
60	45	40	35	45	41.25
240	0	0	0	0	0

It was observed that *A. gossypii,* was an efficient vector as compared to *A. craccivora.* However, the symptoms were observed up to 30-35 days on susceptible cv. 'MAHY-1' (Table 4.20). The most common symptoms observed was mosaic mottling and mild blistering of leaves by both the aphids in all isolates. These symptoms resonated with the typical symptoms observed on field during survey.

Table 4.20: **Observation of symptoms development in bottle gourd after aphid inoculation**

Weeks (Post-Inoculation)	Types of symptoms produced
1	No symptoms
2	No symptoms
3	No symptoms
4	Mild mosaic mottled areas
5	Mosaic mottling with mild blistering on younger leaves, reduced leaf size

4.9 Identification of host resistance against mosaic of bottle gourd

An experiment was conducted to identify different sources of resistance in seventy-eight germplasm (20 indigenous accessions and 58 cultivars containing F1 hybrids and OP varieties). These germplasm, were evaluated under both field and greenhouse conditions. The comparative account of disease reaction spectrum of the test genotypes against mosaic virus is given in Table 4.21 and 4.23. The indexing of resistant accessions/cultivars was done through RT-PCR using CP gene specific primers.

4.9.1 Evaluation of Bottle gourd germplasm against mosaic under natural conditions

The experiment was conducted during March- May 2020 and 2021. No variety/ germplasm was found completely resistant against the disease. It was observed that during 2020 seven germplasm (IC-40890, IC- 337077, IC-392192, IC-550725, Pusa Komal, Warad/ MGH-4, PSPL-Sagar) were found moderately resistant, while, fourteen (IC-342081, IC-284965, IC-536536, IC-371675, IC-398534, HY-401, Shiva-305-F1, Imperial G2, Makhmal, Vardan (GI), Ratan, Naveen F1, M-11, HP local-2) were moderately susceptible. Furthermore, forty six (IC-339215, IC-276524, IC-322274, IC-331101, IC-382192, IC-382240,

IC-385816, IC-546147, IC-342077, MGH-1, Bhushan, MHBG 8, Vardan, Sharada, JS-651, GC S27, GC S28, No. 17, Rashmi, Gaurav, Earth Co. lauki, Nirmal lauki, Neo lauki, Lattu Manvik, NE Lattu, Urvashi F1, Pooja, VC-308, Komal Kiran, Kanchan, Imperial GTK, Akhil Lattu, Rs- 101, ES Gola, Uttam, Lata, Rani, Pooja-9, Megha star, Research-10, Kundal BSS-687, Sudha, Akash, HP local-3, JK local-2, Bihar local) were categorized as susceptible whereas, remaining eleven *viz.*, IC-371695, IC-531896, Komal, PSPL-Local, NE Loki, Rs-11, HP local-1, HP local- 4, JK local-1, JK local-3, JK local-4 were highly susceptible (Table 4.21 and 4.22).

However, in the year 2021, six cultivar/ germplasm *viz.*, IC-40890, IC- 337077, IC-392192, IC-550725, Pusa Komal and Warad/ MGH- 4 were moderately resistant while, twelve (IC- 342081, IC-284965, IC- 536536, Bhushan, MHBG 8, Shiva-305-F1, Gaurav, Imperial G2, PSPL-Sagar, Ratan, M-11, HP local-2) were moderately susceptible. Furthermore, fifteen germplasm/ varieties (IC-371695, IC- 339215, IC-531896, MGH-1, Komal, PSPL-Local, NE Loki, NE Lattu, Rs-11, Rs-101, HP local-1, HP local-4, JK local-1, JK local-3, JK local-4) were highly susceptible, whereas remaining forty five (IC-276524, IC-322274, IC-331101, IC-382192, IC-371675, IC- 382240, IC- 385816, IC-398534, IC-546147, IC-342077, Vardan, Sharada, JS-651, GC S27, GC S28, No. 17, HY-401, Rashmi, Earth Co. lauki, Nirmal lauki, Neo lauki, Lattu Manvik, Urvashi F1, Pooja, VC- 308, Komal Kiran, Kanchan, Imperial GTK, Akhil Lattu, ES Gola, Uttam, Lata, Makhmal, Rani, Pooja-9, Vardan (GI), Megha star, Research-10, Kundal BSS-687, Naveen F1, Sudha, Akash, HP local-3, JK local-2, Bihar local) were found susceptible against the mosaic disease under field conditions (Table 4.21 and 4.22).

4.9.2 Evaluation of Bottle gourd germplasm/ varieties against mosaic under artificial condition

The seventy-eight cultivars/ germplasm were also screened against bottle gourd mosaic disease under controlled conditions. The cultivar ‘Warad/ MGH-4’ showed resistant reaction against the virus. However, IC-40890, IC- 337077, IC-550725, Gaurav, Ratan, Pusa Komal were found moderately resistant and twenty-nine cultivars/ germplasm (IC-371695, IC-342081, IC- 371675, IC- 284965, IC- 322274, IC-385816, IC-392192, IC- 536536, IC- 546147, IC-342077, Bhushan, MHBG8, JS-651, No. 17, Shiva- 305-F1, Earth Co lauki, Lattu Manvik, Urvashi F1, Pooja, NE lattu, VC- 038, Komal kiran, Imperial G2, PSPL- Sagar, Lata, Makhmal,

Table 4. 21: Screening of Bottle gourd germplasm for the exploration of resistant sources for mosaic disease under natural conditions

S. No	Genotypes	2020		2021		PCR Detection
		Score	Reaction	Score	Reaction	
1	IC- 371695	5	HS	5	HS	+
2	IC- 342081	3	MS	3	MS	+
3	IC- 40890	2	MR	2	MR	+
4	IC- 371675	3	MS	4	S	+
5	IC- 276524	4	S	4	S	+
6	IC- 284965	3	MS	3	MS	+
7	IC- 322274	4	S	4	S	+
8	IC- 331101	4	S	4	S	+
9	IC- 337077	2	MR	2	MR	+
10	IC- 382192	4	S	4	S	+
11	IC- 339215	4	S	5	HS	+
12	IC- 382240	4	S	4	S	+
13	IC- 385816	4	S	4	S	+
14	IC- 392192	2	MR	2	MR	+
15	IC- 398534	3	MS	4	S	+
16	IC- 531896	5	HS	5	HS	+
17	IC- 536536	3	MS	3	MS	+
18	IC- 546147	4	S	4	S	+
19	IC- 550725	2	MR	2	MR	+
20	IC- 342077	4	S	4	S	+
21	MAHY-1/ MGH-1	4	S	5	HS	+
22	Vardan	4	S	4	S	+
23	Sharada	4	S	4	S	+

24	Bhushan	4	S	3	MS	+
25	MHBG-8	4	S	3	MS	+
26	MGH-4/ Warad	2	MR	2	MR	+
27	JS-651	4	S	4	S	+
28	GC-S27	4	S	4	S	+
29	GC-S28	4	S	4	S	+
30	NO.17	4	S	4	S	+
31	HY- 401	3	MS	4	S	+
32	Rashmi	4	S	4	S	+
33	Komal	5	HS	5	HS	+
34	Shiva- 305- F1	3	MS	3	MS	+
35	Gaurav	4	S	3	MS	+
36	Earth Co. lauki	4	S	4	S	+
37	Nirmal lauki	4	S	4	S	+
38	Neo lauki	4	S	4	S	+
39	PSPL- 101	5	HS	5	HS	+
40	Manviklattu	4	S	4	S	+
41	Urvashi F1	4	S	4	S	+
42	Pooja	4	S	4	S	+
43	NE lauki	5	HS	5	HS	+
44	NE lattu	4	S	5	HS	+
45	VC- 038	4	S	4	S	+
46	Komal Kiran	4	S	4	S	+
47	Kanchan	4	S	4	S	+
48	Imperial G2	3	MS	3	MS	+
49	Imperial GTK	4	S	4	S	+
50	Akhil lattu	4	S	4	S	+
51	ES Gola	4	S	4	S	+

52	Uttam	4	S	4	S	+
53	PSPL Sagar	2	MR	3	MS	+
54	Lata	4	S	4	S	+
55	Makhmal	3	MS	4	S	+
56	Rani	4	S	4	S	+
57	Pooja-9	4	S	4	S	+
58	Vardan (GI)	3	MS	4	S	+
59	Ratan	3	MS	3	MS	+
60	Megha Star	4	S	4	S	+
61	Research-10	4	S	4	S	+
62	RS-11	5	HS	5	HS	+
63	RS-101	4	S	5	HS	+
64	M-11	3	MS	3	MS	+
65	Kundal BSS- 687	4	S	4	S	+
66	Naveen F1	3	MS	4	S	+
67	PusaKomal	2	MR	2	MR	+
68	Sudha	4	S	4	S	+
69	Akash	4	S	4	S	+
70	HP local-1	5	HS	5	HS	+
71	HP local-2	3	MS	3	MS	+
72	HP local-3	4	S	4	S	+
73	HP local-4	5	HS	5	HS	+
74	JK local-1	5	HS	5	HS	+
75	JK local-2	4	S	4	S	+
76	JK local-3	5	HS	5	HS	+
77	JK local-4	5	HS	5	HS	+
78	Bihar local	4	S	4	S	+

Table 4.22: Disease reaction of Bottle gourd germplasm against mosaic disease during 2020-2021

Germplasm/ varieties				Disease Reaction
2020	**No. of entries**	**2021**	**No. of entries**	
IC-40890, IC- 337077, IC-392192, IC-550725, Pusa Komal, Warad/MGH-4, PSPL-Sagar,	7	IC-40890, IC- 337077, IC-392192, IC-550725, Pusa Komal, Warad/ MGH-4	6	Moderately Resistant
IC- 342081, IC- 284965, IC-536536, IC-371675, IC-398534, HY-401, Shiva-305-F1, Imperial G2, Makhmal, Vardan (GI), Ratan, Naveen F1, M-11, HP local-2	14	IC- 342081, IC- 284965, IC-536536, Bhushan, MHBG 8, Shiva-305-F1, Gaurav, Imperial G2, PSPL- Sagar, Ratan, M-11, HP local-2	12	Moderately Susceptible
IC- 339215, IC-276524, IC-322274, IC-331101, IC-382192, IC- 382240, IC- 385816, IC-546147, IC-342077, MGH-1, Bhushan, MHBG 8, Vardan, Sharada, JS-651, GC S27, GC S28, No. 17, Rashmi, Gaurav, Earth Co. lauki, Nirmal lauki, Neo lauki, Lattu Manvik, NE Lattu, Urvashi F1, Pooja, VC-308, Komal Kiran, Kanchan, Imperial GTK, Akhil Lattu, Rs-101, ES Gola, Uttam, Lata, Rani, Pooja-9, Megha star, Research-10, Kundal BSS-687, Sudha, Akash, HP local-3, JK local-2, Bihar local	46	IC-276524, IC- 322274, IC-331101, IC-382192, IC- 382240, IC- 385816, IC-398534, IC-546147, IC-342077, IC-371675Vardan, Sharada, JS-651, GC S27, GC S28, No. 17, HY-401, Rashmi, Earth Co. lauki, Nirmal lauki, Neo lauki, Lattu Manvik, Urvashi F1, Pooja, VC- 308, Komal Kiran, Kanchan, Imperial GTK, Akhil Lattu, ES Gola, Uttam, Lata, Makhmal, Rani, Pooja-9, Vardan (GI), Megha star, Research-10, Kundal BSS-687, Naveen F1, Sudha, Akash, HP local-3, JK local-2, Bihar local	45	Susceptible
IC-371695, IC-531896, Komal, PSPL- Local, NE Loki, Rs- 11, HP local-1, HP local- 4, JK local-1, JK local-3, JK local-4	11	IC-371695, IC- 339215, IC-531896, MGH-1, Komal, PSPL-Local, NE Loki, NE Lattu, Rs-11, Rs- 101, HP local-1, HP local- 4, JK local-1, JK local-3, JK local-4	15	Highly Susceptible

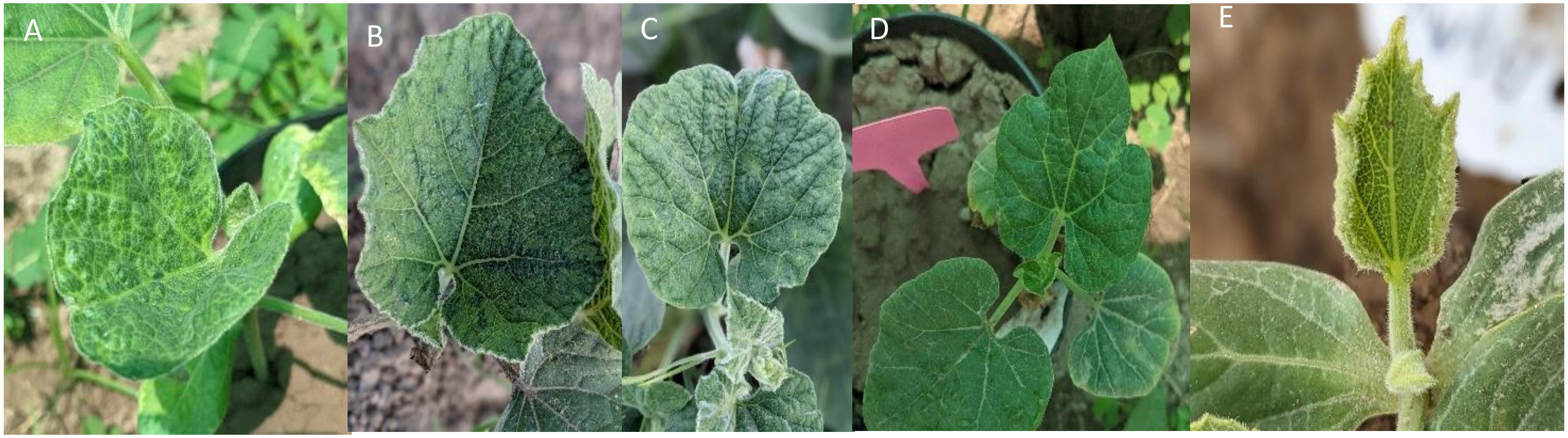


Plate 17: Various symptoms exhibited by the genotypes at 15- 20 dpi. A- mottling with slight blisters, B- mosaic mottling, C- leaf deformation, D- vein clearing in younger leaves

Megha star, M-11, HP local- 2) showed moderately susceptible reaction against the virus. Six cultivars/ germplasm *viz.*, IC-339215, NE lauki, HP Local-1, JK Local-1, JK Local-3, JK Local-4, were highly susceptible, whereas remaining thirty six (IC- 276524,IC-331101, IC- 382192, IC- 382240, IC-398534, IC- 531896, MAHY-1, Vardan, Sharada, GC S27, GC S28, HY-401, Rashmi, Komal, Nirmal Lauki, Neo lauki, PSPL-101, Kanchan, Imperial GTK, Akhil lattu, ES Gola, Uttam, Rani, Pooja-9, Vardan (GI), Research - 10, RS-11, RS-101, Kundal BSS- 687, Naveen F1, Sudha, Akash, HP Local- 3, HP Local-4, JK Local-2, Bihar local) were susceptible to the virus (Table 4.23 and 4.24).

Varied range of symptoms were observed under both field and controlled conditions. The resistant cultivar ‘Warad/ MGH-4’ showed vein clearing symptoms in early 2 leaf stage and later recovered and produced symptoms free leaves and fruits under pot culture conditions, while it was moderately resistant on field where a very mild mosaic was recorded. The plants categorized as moderately resistant had a delayed onset of symptoms. The moderately susceptible plants showed symptoms like blisters on leaves but the effect on the fruit was negligible. The susceptible plants had comparatively early expression of symptoms like mosaic mottling, leaf deformation and substantially deformed fruits. Highly susceptible plants exhibited stunted growth with dark mottled mosaic, distorted leaves (Plate 17). Such vines if produced fruits were deformed. The presence of the virus in infected plants was confirmed using CP primers.

Table 4. 23: Screening of Bottle gourd germplasm for the exploration of resistant sources for mosaic disease under artificial conditions

S. No	Germplasm	Score	Reaction	PCR Detection
1	IC- 371695	3	MS	+
2	IC- 342081	3	MS	+
3	IC- 40890	2	MR	+
4	IC- 371675	3	MS	+
5	IC- 276524	4	S	+
6	IC- 284965	3	MS	+
7	IC- 322274	3	MS	+
8	IC- 331101	4	S	+
9	IC- 337077	2	MR	+
10	IC- 382192	4	S	+
11	IC- 339215	5	HS	+
12	IC- 382240	4	S	+
13	IC- 385816	3	MS	+
14	IC- 392192	3	MS	+
15	IC- 398534	4	S	+
16	IC- 531896	4	S	+
17	IC- 536536	3	MS	+
18	IC- 546147	3	MS	+
19	IC- 550725	2	MR	+
20	IC- 342077	3	MS	+
21	MAHY-1/ MGH-1	4	S	+
22	Vardan	4	S	+
23	Sharada	4	S	+
24	Bhushan	3	MS	+
25	MHBG-8	3	MS	+
26	MGH-4/ Warad	1	R	-
27	JS-651	3	MS	+
28	GC-S27	4	S	+

29	GC-S28	4	S	+
30	NO.17	3	MS	+
31	HY- 401	4	S	+
32	Rashmi	4	S	+
33	Komal	4	S	+
34	Shiva- 305- F1	3	MS	+
35	Gaurav	2	MR	+
36	Earth Co. lauki	3	MS	+
37	Nirmal lauki	4	S	+
38	Neo lauki	4	S	+
39	PSPL- 101	4	S	+
40	Manvik lattu	3	MS	+
41	Urvashi F1	3	MS	+
42	Pooja	3	MS	+
43	NE lauki	5	HS	+
44	NE lattu	3	MS	+
45	VC- 038	3	MS	+
46	Komal Kiran	3	MS	+
47	Kanchan	4	S	+
48	Imperial G2	3	MS	+
49	Imperial GTK	4	S	+
50	Akhil lattu	4	S	+
51	ES Gola	4	S	+
52	Uttam	4	S	+
53	PSPL Sagar	3	MS	+
54	Lata	3	MS	+
55	Makhmal	3	MS	+
56	Rani	4	S	+
57	Pooja-9	4	S	+
58	Vardan (GI)	4	S	+
59	Ratan	2	MR	+
60	Megha Star	3	MS	+

61	Research-10	4	S	+
62	RS-11	4	S	+
63	RS-101	4	S	+
64	M-11	3	MS	+
65	Kundal BSS- 687	4	S	+
66	Naveen F1	4	S	+
67	Pusa Komal	2	MR	+
68	Sudha	4	S	+
69	Akash	4	S	+
70	HP local-1	5	HS	+
71	HP local-2	3	MS	+
72	HP local-3	4	S	+
73	HP local-4	4	S	+
74	JK local-1	5	HS	+
75	JK local-2	4	S	+
76	JK local-3	5	HS	+
77	JK local-4	5	HS	+
78	Bihar local	4	S	+

Table 4.24: Disease reaction of Bottle gourd germplasm against mosaic disease under artificial conditions

Germplasm/ varieties	No. of entries	Disease Reaction
MGH-4 / Warad	1	Resistant
IC-40890, IC- 337077, IC-550725, Gaurav, Ratan, Pusa Komal	6	Moderately Resistant
IC-371695, IC-342081, IC- 371675, IC- 284965, IC-322274, IC-385816, IC-392192, IC- 536536, IC- 546147, IC-342077, Bhushan, MHBG8, JS-651, No. 17, Shiva-305- F1, Earth Co lauki, Lattu Manvik, Urvashi F1, Pooja, NE lattu, VC- 038, Komal kiran, Imperial G2, PSPL-Sagar, Lata, Makhmal, Megha star, M-11, HP local- 2	29	Moderately Susceptible
IC- 276524,IC-331101, IC- 382192, IC- 382240, IC-398534, IC- 531896, MAHY-1, Vardan, Sharada, GC S27, GC S28, HY- 401, Rashmi, Komal, Nirmal Lauki, Neo lauki, PSPL- 101, Kanchan, Imperial GTK, Akhil lattu, ES Gola, Uttam, Rani, Pooja-9, Vardan (GI),Research – 10, RS-11, RS-101, Kundal BSS- 687, Naveen F1, Sudha, Akash, HP Local- 3, HP Local -4, JK Local-2, Bihar local	36	Susceptible
IC-339215, NE lauki, HP Local-1, JK Local-1, JK Local-3, JK Local-4	6	Highly Susceptible

DISCUSSION

CHAPTER- V

DISCUSSION

Bottle gourd (*Lagenaria siceraria*) is one of the most important cucurbitaceous vegetable cultivated in all the tropical and sub-tropical countries. Among various biotic stresses, viral diseases pose considerable threat *via* yield losses. More than 50 viruses are known to infect cucurbits (Nagendran *et al.,* 2015). Hence, investigations on mosaic disease of bottle gourd was carried out on aspects like status, detection, molecular characterization, host range, virus-vector relationship and identification of resistant sources through varietal screening.

Various bottle gourd growing areas of Jammu (R.S. Pura, Marh, Miran Sahib and Bishnah), Kathua (Nagri and Hiranagar), Samba (Rajpura, Vijaypur and Ramgarh) and Udhampur (Chenani) districts were surveyed over the course of two years (2020 and 2021) during the cropping season. An overall mean disease incidence of bottle gourd mosaic in Jammu division was 21.75 and 25.33 per cent during 2020 and 2021 respectively. The pooled data for the two years revealed mean per cent incidence at 23.54 for bottle gourd mosaic disease. In Jammu district four blocks were visited where the overall disease incidence ranged from 6.67 (Laswara) to 50.00 (Bad-Rode) per cent with mean incidence of 26.13 per cent during 2020, while in 2021 the mean incidence was 28.39 per cent with a range of 0.00 (Ratwal) to 62.22 (Salaher) per cent. In Kathua, the two blocks surveyed, had an overall disease incidence ranging from 6.67 (Feruchak) to 26.66 (Kakhyal) per cent during 2020, with an overall mean disease incidence of 13.46 per cent. However, during 2021 the disease incidence ranged from 14.00 (Feruchak) to 21.66 (Dadoli) per cent and the mean disease incidence was 18.60 per cent.

In Samba district the disease incidence ranged from 10.00 (Abtal) to 37.78 (Rajpura) per cent with a mean of 23.73 per cent during 2020 whereas in 2021, the incidence of bottle gourd mosaic ranged between 10.00 (Koulpur, Jenda and Abtal) to 43.33 per cent (Rajpura) with a mean incidence of 22.08 per cent. In Udhampur, during 2020 the disease incidence had a mean of 23.26 per cent ranging from 14.00 (Kohsar) to 28.89 (Chenani) while during 2021 the disease incidence ranged from 15.00 (Chohan) to 40.00 (Chenani and Mantalai) per cent with an overall mean incidence of 28.11 per cent. The data clearly shows that the bottle gourd mosaic disease was prevalent in all the bottle gourd growing areas. It was found that the incidence of the disease during 2021 (25.33%) was slightly high than 2020 (21.75%). The result is in corroboration with the earlier studies. Mantri *et al.* (2005) reported an incidence of 0-100 per cent from Maharashtra. Ali *et al.* (2012) also reported the incidence of CGMMV and PRSV-W along with five more viruses (CMV, ZYMV, MNSV, SqMV, WMV-2 and ZYMV) on the major cucurbit growing areas at Oklahoma, USA. Sharma *et al.* (2012) recorded 25 per cent incidence for CGMMV in bottle gourd in the trans-gangetic plains of India. Nagendran *et al.* (2017a) reported an incidence of 32.10 per cent and 22.20 per cent of PRSV-W and CGMMV in different cucurbits grown in different agroclimates of Tamil Nadu. Khanal *et al.* (2021) reported 59.10 per cent incidence of PRSV-W from various cucurbits. Variation in the incidence of the disease has also been reported by Rao *et al.* (2017), Udavant (2018), Ashfaq *et al.* (2021) and Kumari *et al.* (2021).

Variation in the incidence over locations can be due to various factors *viz.,* cropping pattern, availability of the primary source of infection (infected seed, plant debris), availability of infected alternate or collateral hosts, environmental conditions and availability of insect-vectors for transmission. Jammu district had an overall higher incidence (27.26%) of mosaic disease than other districts, this can be due to the continuous practice of river bed cultivation for cucurbits near the banks of Tawi river especially at Badyal Brahmana, Salaher, Bhad-Rode etc. In this type of cultivation, the contractual farmers go for the monocropping of cucurbits, which in turn helps the inoculum to build up for the next season.

This prevalent disease showed a range of symptoms (mosaic mottling, mosaic with ringspot and chlorotic spots, vein banding, mild mosaic, blisters on leaves and fruits) on infected bottle gourd vines during the two-year survey. Under greenhouse conditions the plants mechanically inoculated showed an array of symptoms (dark green mosaic mottling, blisters on leaf, vein clearing, puckering and stunting). The variation in symptoms under natural conditions were apparent because the cultivars grown location wise varied, the stage of infection can vary depending upon the time of sowing and incidence of insect- vector, strains of a virus. Mixed infections are very common in case of viral diseases and this also leads to development of complex symptom expression which makes it difficult to identify the causal virus solely based on the symptoms. Under controlled conditions although the virus was maintained on same host, different symptoms were obtained as the isolates could vary in the type of virus or strain of the virus. Similar symptoms were recorded by other workers on bottle gourd (Vasudev and Lal, 1943; Capoor and Varma, 1948; Raychaudhuri and Varma, 1975; Mantri *et al.,* 2005; Moradi and Jafarpour, 2011; Ali *et al.,* 2014; Nagendran, 2014; Udavant, 2018).

Studies to identify the causal virus responsible for bottle gourd mosaic disease was carried out under field and laboratory conditions. The identity of the viral isolates of bottle gourd mosaic collected during 2020 and 2021 were identified using coat protein specific primers of six different viruses prevalent in cucurbit growing areas of India. The RT-PCR amplification implicated that out of 55 isolates maintained, two viruses *viz.,* one *Tobamovirus* (CGMMV) and one *Potyvirus* (PRSV-W) was found in 39 isolates representing all the locations visited during the survey. These two viruses were also present as mixed infections in all four districts of Jammu. Mantri *et al.* (2005) detected PRSV in bottle gourd through RT-PCR. However, Roy and Jain (2002) obtained an amplicon of 800 bp from PRSV samples from Andhra Pradesh. Ali *et al.* (2004) detected CGMMV in the samples collected from Pakistan using CP primers. Liu *et al.* (2009) identified CGMMV in watermelon growing areas of China. Nagendran (2014) amplified a region comprising 1263 nucleotides (3’ terminal of Nib gene + CP+ partial 3’ UTR) for detecting PRSV-W, while in case of CGMMV 600 bp amplicon was amplified using CP primers. The detection of CGMMV from different cucurbits like cucumber and watermelon through RT-PCR has been done by various workers (Reingold *et al.*, 2013; Moradi and Jafarpour, 2011)

Both PRSV-W and CGMMV was amplified into an amplicon of 398 bp and 604 bp size respectively, further purified and custom sequenced. The sequence obtained was analysed for the phylogenetic relationship with other isolate sequences available at Gen Bank NCBI.

The CGMMV sequence showed highest per cent identity of 99.80 per cent with CGMMV accession MH271409 at nucleotide level. At amino acid level, the test isolate shared 100 per cent identity with AYN80372, AYN80477, AZJ51137, AAP74202, QRM12790, ABA64461 and NP_044580 while, the least identity was shared with QSM07163 and NP_624339 (45.34%). A phylogenetic tree was constructed using MEGAX programme and based on nucleotide sequence it was found that CGMMV test isolate clustered with MH271409 sharing highest sequence identity. An Indian CGMMV isolate was characterized by Mandal *et al.* (2008) and showed 97 per cent similarity between the isolate and other CGMMV isolates. Ling *et al.* (2014) found 99 per cent similarity of CGMMV isolates with Asian isolates therefore suggesting that Canadian isolates had Asian origin. Nagendran (2014) characterized CGMMV isolate from Snake gourd and found it to share 98.1 per cent sequence identity with Indian bottle gourd isolates at amino acid level. Similar studies were conducted and reported (Ali *et al.,* 2014; Nagendran *et al.,* 2015; Nagendran *et al.,* 2017a; Crespo *et al.,* 2017; Cho *et al.,* 2021; Kehoe *et al.,* 2021)

Similarly, the PRSV-W sequence showed highest (96.23%) similarity with PRSV-W SHK-PM1 isolate (KY448325) and formed a cluster with this accession when the phylogenetic tree was constructed. Nagendran (2014) sequenced ten PRSV-W isolates and the sequence analysis revealed that they shared less identity i.e. 77.1 to 86.6 per cent and were divergent. CP gene sequences was used for grouping the PRSV-W isolates geographically and the CP motifs were studied. The DAG motif was present in all the isolates except TN TDV Coc2. Similarly, various workers have characterized PRSV-W (Chin *et al.,* 2007; Abdalla and Ali, 2012; Ali, 2017; Maina, 2017; Rajbanshi and Ali, 2019).

CP gene is one of the most conserved genes therefore is used for the preliminary identification of the viruses. But this does not shed light on the variability amongst the CGMMV or PRSV-W isolates. For understanding the variability between the isolates collected, whole genome sequencing of the genes representing the variable regions can be undertaken.

The survival and spread of the virus can be determined by host range studies. Therefore, different hosts *viz.,* (*Cucumis sativus, Cucurbita pepo, C. melo, Citrullus lanatus, C. maxima, Luffa acutangula, Praecitrullus fistulosus, Momordica charantia, Solanum lycopersicum, S. melongena, Capsicum annum, Abelmoschus esculentus, Phaseolus vulgaris, Vigna unguiculata, Brassica oleracea* var *capitata, B. oleracea* var *botrytis, Amaranthus* spp., and *Chenopodium* spp.) were screened by sap inoculations in order to deduce the host range of the bottle gourd mosaic virus. All the cucurbits produced systemic symptoms like mild mosaic, puckering, mosaic mottling, and enations. All the symptoms were expressed between 7-10 days in case of summer squash and 20-25 days in bitter gourd. Only *Amaranthus* spp. produced local lesion whereas, all other plants belonging to *Solanaceae, Malvaceae, Fabaceae and Brassicaceae* were non hosts. The study revealed that the causal virus had a narrow host range mostly confined to *Cucurbitaceae*. More refined view on the host range can be achieved if more plant species (especially weeds) of varied families would be involved in such studies.

Investigations of the present study are in confirmation to the findings of Raychaudhuri and Varma (1975) and Hollings *et al.* (1975). However, Sharma (2002) reported the limited host range of CGMMV causing infection in cucumber, watermelon, pumpkin and squash. These plants initially produced chlorotic spots which later led to systemic infection. Local lesions were recorded from *Chenopodium amaranticolor*. The host range of the bottle gourd mosaic virus in different crops like cucurbits, *Nicotiana* spp. were also reported by Mantri *et al.* (2005). Udvant (2018) screened plants of different families (*Cucurbitaceae, Leguminaceae, Solanaceae, Chenopodiaceae, Cruciferae, Malvaceae* and *Poaceae*) against bottle gourd mosaic disease and found that only from *cucurbitaceae* family were susceptible to the disease which is in accordance to our findings.

Different aphid species (*Myzuspersicae*, *Aphis gossypii* and *A. craccivora)* have been reported to transmit bottle gourd mosaic virus by various workers (Singh, 1969; Shukla *et al.,* 1994). The aphid species found in association with infected samples were identified as *A. gossypii* and *A. craccivora*. Through aphid transmission studies it was confirmed that CGMMV was not transmitted by both the species as reported by Capoor and Varma (1948), Inouye *et al.* (1967), Raychaudhuri and Varma (1975) and Rao and Varma (1984) while, PRSV-W isolate was successfully transmitted from infected to healthy plants showing symptoms like mild mosaic and vein clearing. However, four isolates of PRSV-W (J-SA-BG3, J-NA-BG-1, J-RJ-BG3 and J-MN-BG1) were used to study the vector relationship. It was found that *A. gossypii* was an efficient vector than *A. craccivora*.

Minimum, 15 aphids were required for successful transmission of the virus with a minimum of 60 minutes fasting period. Transmission of 78.75 per cent and 56.25 per cent was recorded for *A. gossypii* and *A. craccivora* respectively. Studies on determination of effect of different acquisition access period revealed that a minimum of 15 minutes was necessary for aphids to acquire the virus and showed transmission of 86.25 per cent and 78.75 per cent for *A. gossypii* and *A. craccivora*, respectively.

The per cent transmission decreased with the increase of acquisition access period. The aphids (viruliferous) required an inoculation access period of at least 30 minutes to transmit the virus. *A. gossypii* had a transmission of 77.50 per cent while *A. craccivora* had 75 per cent also the transmission percentage decreased with increase in inoculation access period. The results obtained were in conformity with different workers (Makkouk and Lesemann, 1980; Ilhe *et al.*, 2014; Udavant, 2018).

Therefore, PRSV-W in bottle gourd is transmitted non-persistently by *A. gossypii* and *A. craccivora*. However, *A. gossypii* was found an efficient vector over *A. craccivora*. All the results were in conformity with earlier workers (Quiot-Douine *et al.,* 1990; Shaikh, 1996; Mantri *et al.,* 2005; Biswas and Varma, 2006; Kalleshwaraswamy and Kumar, 2008; Pinto *et al*., 2008; Ilhe *et al.,* 2014 Angelella *et al.,* 2015; Udavant, 2018).

Genetic host resistance is durable and cost-effective measure against plant viruses. Hence resistance breeding has been found as one of the main objectives worldwide and identification of resistant sources is the very initial step in resistance breeding programmes and later its deployment. Screening of different genotypes comprising indigenous accessions, hybrids and OP varieties was done both under natural and controlled conditions during 2020 and 2021. The germplasm were categorized into resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible.

Out of 78 germplasm/ varieties screened, only “Warad/ MGH-4” was found resistant under controlled conditions while six germplasm *viz.,* IC-40890, IC- 337077, IC-392192, IC-550725, Pusa Komal, PSPL- Sagar and Warad were found moderately resistant under field conditions, however, IC-40890, IC- 337077, IC-550725, Gaurav, Ratan and Pusa Komal were moderately resistant under controlled conditions. It was observed that four germplasm/ varieties i.e. IC-40890, IC- 337077, IC-550725, and Pusa Komal were moderately resistant both under field and controlled conditions therefore can be recommended for further utilization in breeding programmes. The identity of the virus was also confirmed later through RT-PCR using CP specific primer.

The disease reaction (resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible) varied under both conditions, and a varied range of symptoms were observed under field conditions over controlled conditions. This was due to presence of more than one virus (mixed infections) which leads to varied disease response and symptoms. Rajamony *et al.* (1990) screened *C. melo* accessions and wild *Cucumis* spp. and found resistance in 6 wild species against CGMMV. Sugiyama *et al.* (2006) found Chang Bougi (*C. melo* var. *makuwa* Makino) resistant to CGMMV. Crespo *et al.* (2018) found *C. anguira* resistant towards CGMMV. Similar studies were conducted against PRSV-W in cucurbits like muskmelon, watermelon, pumpkin, snap melon and bottle gourd under field and controlled conditions against mosaic disease (Baker *et al.*, 1991; Hsiao *et al.*, 1993; Gibb *et al.*, 1994; Strange *et al.*, 2002; Brown *et al.*, 2003; Dhillon *et al.*, 2007; Ling *et al.*, 2013).

SUMMARY AND CONCLUSIONS

CHAPTER-VI

SUMMARY AND CONCLUSIONS

The present investigation entitled "**Identification and characterization of Viruses causing Mosaic of Bottle gourd in Jammu Sub-tropics**" was undertaken to determine the current status of the mosaic disease in bottle gourd, establishing the identity of the causal pathogen, its host range, insect-vector transmission studies and for the identification of resistant sources against the disease.

Extensive survey of bottle gourd growing areas of Jammu division conducted during 2020 and 2021 revealed the disease was prevalent in all the areas. The overall disease incidence during the year 2020 and 2021 ranged from 7.00 to 53.33 per cent with an average incidence of 23.54 per cent. The maximum incidence was observed at Salaher followed by Bhad-Rode and Rakh Chatha. In general, the disease incidence was more in areas where bottle gourd was grown as monocrop every year (river bed cultivation).

Total 274 infected samples showing characteristic symptoms *viz.*, mosaic mottling, mosaic with ringspot and chlorotic spots, mild mosaic, blisters on leaves and fruits were collected and categorized into 55 isolates based on the symptoms expressed and the location from where the sample was collected. The confirmation of the causal virus(es) (CMV, CGMMV, PRSV-W, WMV, ZLCV and ZYMV) was done using RT-PCR based assay exploiting the conserved coat protein (CP). However, the presence of only two viruses (CGMMV and PRSV-W) was confirmed, during the study.

For molecular characterization, total RNA was extracted using RNA isolation Kit. The cDNA was synthesized using oligo$(dT)_{18}$ primer and the PCR amplification was done. In case of CGMMV, an amplicon of 604 bp was generated, while, a 398 bp amplicon was formed for PRSV-W. These amplicons were sequenced using custom services and the Blast analysis revealed CGMMV isolate shared maximum homology with CGMMV viruses while, PRSV-W isolates shared with that of PRSV-W isolates.

Phylogenetic analysis at nucleotide and amino acid level elucidated that the test isolates were close in terms of phylogeny and not much variation was seen at CP region. Furthermore, the recombination analysis showed that PRSV-W was a recombinant of an Indian isolate i.e. PRSV-W SHK PM1 (KY448325) and an unknown parent. Multiple alignment of the CP region for both the viruses at nucleotide and amino acid level showed maximum homology with individual type strains and little homology with the outgroup members.

The host range for bottle gourd mosaic virus was determined by screening of different plants (*Cucumis sativus, Cucurbita pepo, C. melo, Citrullus lanatus, C. maxima, Luffa acutangula, Praecitrullus fistulosus, Momordica charantia, Solanum lycopersicum, S. melongena, Capsicum annum, Abelmoschus esculentus, Phaseolus vulgaris, Vigna unguiculata, Brassica oleracea* var *capitata, B. oleracea* var *botrytis, Amaranthus* spp. and *Chenopodium* spp.) belonging to different families. The percentage of transmission was cent per cent in all the inoculated cucurbits while *Amaranthus* spp. had developed local lesions.

Based on morphology, the aphids affecting bottle gourd were identified as *Aphis gossypii* and *A. craccivora. A. gossypii* (melon aphid) was identified by its tongue like cauda with twice longer cornicles, meanwhile *A. craccivora* (bean aphid) was identified based on dome shaped cauda having 4-7 hairs and shiny black body. *A. gossypii* was found effective in transmission of PRSV-W when compared to *A. craccivora*.

In order to identify potential sources of resistance, seventy-eight bottle gourd accessions and varieties were screened against bottle gourd mosaic under field and was found that no germplasm/ variety was resistant, six were found moderately resistant, twelve were moderately susceptible, forty-five were susceptible while only fifteen were highly susceptible. However, under controlled conditions, only one variety ‘MGH-4’ gave resistant reaction, six were found moderately resistant, twenty-three were found moderately susceptible, forty were susceptible while eight were highly susceptible.

Conclusion

- ✓ The highest incidence of 50 per cent bottle gourd mosaic disease was observed at Bhad-Rode village of Marh Block while the lowest was from Laswara area in Bishnah during 2020 in Jammu district. During 2021, Salaher village of R.S. Pura block witnessed the highest incidence at 62.22 per cent whereas no mosaic disease was observed from Ratwal

- ✓ In Kathua, during 2020 the highest disease incidence i.e. 26.67 per cent was observed in Khakhyal village however in 2021, Dadoli had the highest disease incidence (21.67%). For both the years lowest mosaic incidence was observed in Feruchak with 6.67 and 14.00 per cent incidence, respectively
- ✓ In Samba, the highest incidence was observed at Rajpura during both 2020 and 2021 (37.78 % and 43.33%) with an average highest mean of 40.55 per cent. For both the years lowest mosaic incidence was observed in Abtal with 10.00 per cent incidence
- ✓ In Udhampur, Chenani had the highest incidence of mosaic with 28.89 per cent in 2020 and 40.00 per cent in 2021 while an average incidence of 34.44 per cent was recorded. Over all mean for both the year in Jammu sub tropics was 23.54 per cent
- ✓ Six viruses were reported to cause bottle gourd mosaic disease, but in our study only two viruses i.e. PRSV-W and CGMMV were detected through RT-PCR using coat protein specific primers. The viruses were also present as mixed infections
- ✓ Sequence analysis revealed that CGMMV isolate shared maximum homology (99.80%) with an isolate from USA *viz.*, CGMMV CG003 (MH271409) at nucleotide level while PRSV-W isolate shared the highest per cent identity (96.23%) with another Indian isolate, PRSV- SHK PM1 (KY448325)
- ✓ The host range of the bottle gourd mosaic virus was found confined to *Cucurbitaceae* and *Chenopodiaceae* families only
- ✓ *Aphis gossypii* and *A. craccivora* were found responsible for the transmission of PRSV-W, while CGMMV was not transmitted through aphids thereby establishing strong proof for its identity
- ✓ *Aphis gossypii* in comparison with *A. craccivora* was proved an efficient vector in transmission of bottle gourd mosaic disease under field conditions
- ✓ Out of the seventy eight germplasm screened for bottle gourd mosaic both under field and controlled conditions, MGH-4 gave resistant reaction under controlled conditions while it was moderately susceptible under field conditions. Four germplasm/ variety *viz.,* IC-40890, IC- 337077, IC-550725 and Pusa Komal were found moderately resistant against bottle gourd mosaic disease during both the years

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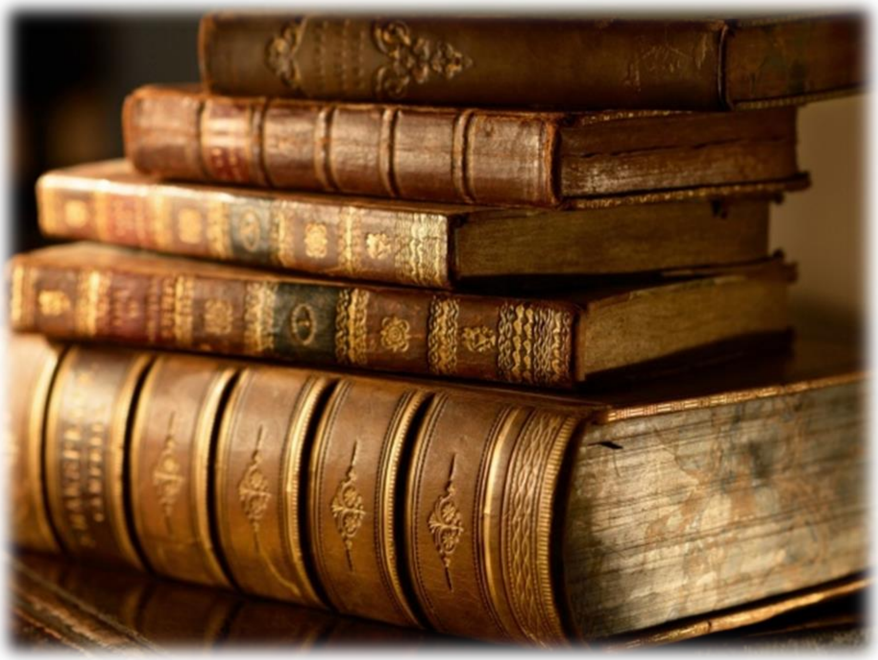

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APPENDICES

APPENDIX-I

Phosphate buffer (0.1 M) (pH = 7.0)

$Na_2HPO_4.2H_2O$	178.05 g/1000 ml
$NaH_2PO_4.2H_2O$	31.21 g/1000 ml

APPENDIX-II

50 X TAE

Tris	242 g
Glacial acetic acid	57.1 ml
EDTA (0.5 M, pH 8.0)	100 ml
Distilled water	to make volume 1000 ml

APPENDIX-III

TE Buffer

Tris	10 mM
EDTA	1 mM

APPENDIX-IV

Multiple alignment of CGMMV test isolate with other CGMMV isolates at nucleotide level using ClustalW

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KM873789.1      CTGAAGAGTCCAGTTCTGTTTCTTTTGAAGATGGCTTACAATCCGATCACACCTAGCAAA
KU175632.1      CTGAAGAGTCCAGTTCTGTTTCTTTTGAAGATGGCTTACAATCCGATCACACCTAGCAAA
KY753929.1      CTGAAGAGTCCAGTTCTGTTTCTTTTGAAGATGGCTTACAATCCGATCACACCTAGCAAA
KJ754196.1      CTGAAGAGTCCAGTTCTGTTTCTTTTGAAGATGGCTTACAATCCGATCACACCTAGCAAA
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KP868654.1      CTGAAGAGTCCAGTTCTGTTTCTTTTGAAGATGGCTTACAATCCGATCACACCTAGCAAA
JF432067.1      CTGAAGAGTCCAGTTCTGTTTCTTTTGAAGATGGCTTACAATCCGATCACACCTAGCAAA
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DQ767631.2      CTGAAGAGTCCAGTTCTGTTTCTTTTGAAGATGGCTTACAATCCGATCACACCTAGCAAA
MH271435.1      CTGAAGAGTCCAGTTCTGTTTCTTTTGAAGATGGCTTACAATCCGATCACACCTAGCAAA
AJ429090.1      CTGAAGAGTCCAGTTCTGTTTCTTTCGACGATGGCTTACAATCCGATCACACCTAGCAAA
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CGMMV           ------------------------------ATGGCTTACAATCCGATCACACCTAGCAAA
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KJ729039.1      GCTGTAAAGCGTACTGATGACGCATCTACAGCCGCTAGGGCTGAAATAGATAATTTAATA
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V01551.1        GAGTCTATTTCTAAGGGTTTTGATGTTTATGATAGGGCTTCATTTGAAGCCGCGTTTTCG
AF225984.1      GAGTCTATTTCTAAGGGTTTTGATGTTTACGATAGGGCTTCATTTGAAGCCGCGTTTTCG
KY115174.1      GAGTCTATTTCTAAGGGTTTTGATGTTTACGATAGGGCTTCATTTGAAGCCGCGTTTTCG
MW430125.1      GAGTCTATTTCTAAGGGTTTTGATGTTTACGATAGGGCTTCATTTGAAGCCGCGTTTTCG
MH427279.1      GAGTCTATTTCTAAGGGTTTTGATGTTTACGATAGGGCTTCATTTGAAGCCGCGTTTTCG
DQ767631.2      GAGTCTATTTCTAAGGGTTTTGATGTTTACGATAGGGCTTCATTTGAAGCCGCGTTTTCG
MH271435.1      GAGTCTATTTCTAAGGGTTTTGATGTTTACGATAGGGCTTCATTTGAAGCCGCGTTTTCG
AJ429090.1      GAGTCTATTTCTAAGGGTTTTGATGTTTATGATAGGGCTTCATTTGAAGCCGCGTTTTCG
KJ729039.1      GAGTCTATTTCTAAGGGTTTTGATGTTTACGATAGGGCTTCTTTTGAAGCCGCGTTTTCG
CGMMV           GAGTCTATTTCTAAGGGTTTTGATGTTTACGATAGGGCTTCATTTGAAGCCGCGTTTTCG
                ***************************** *********** ******************

KM873789.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
KU175632.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
KY753929.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
KJ754196.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
KU175639.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
KP868654.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
JF432067.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
KP868653.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
KY040049.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
KM873788.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
KR232571.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
MH271409        GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
MT184944.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
DQ217778.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
V01551.1        GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
AF225984.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
KY115174.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
MW430125.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
MH427279.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
DQ767631.2      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
MH271435.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
AJ429090.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
KJ729039.1      GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
CGMMV           GTAGTCTGGTCAGAGGCTACCACCTCGAAAGCTTAGTTTCGAGGGTCTTCTGATGGTGGT
                ************************************************************

KM873789.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
KU175632.1      GCACAC------------------------------------------------------
KY753929.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
KJ754196.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
KU175639.1      GCACAC------------------------------------------------------
KP868654.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
JF432067.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
KP868653.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
KY040049.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
KM873788.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
KR232571.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
MH271409        GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
MT184944.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
DQ217778.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
V01551.1        GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
AF225984.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
KY115174.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
MW430125.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
MH427279.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
DQ767631.2      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
MH271435.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
AJ429090.1      GCACACCAAAGTGCA-TAGTGCTTTCCCGTTCACTTAAATCGAACGGTTTGCTCATTGGT
KJ729039.1      GCACACCAAATGGCAATAGTGA--------------------------------------
CGMMV           GCACACCAAA--------------------------------------------------
                ******

KM873789.1      TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
```

```
KU175632.1      ------------------------------------------------------------
KY753929.1      TTGCGGAAACCTCTCACGTGTGACGTCGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
KJ754196.1      TTGCGGAAACCTCTCACGTGTGACGTCGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
KU175639.1      ------------------------------------------------------------
KP868654.1      TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
JF432067.1      TTGCGG------------------------------------------------------
KP868653.1      TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
KY040049.1      TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
KM873788.1      TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
KR232571.1      TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
MH271409        TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
MT184944.1      TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
DQ217778.1      TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
V01551.1        TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
AF225984.1      TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAA-------------
KY115174.1      TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
MW430125.1      TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
MH427279.1      TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
DQ767631.2      TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
MH271435.1      TTGCGGAAACCTCTCGCGTGTGACGCTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
AJ429090.1      TTGCGGAAACCTCTCACGTGTGACGTTGAAGTTTCTATGGGCAGTAATTCTGCAAGGGGT
KJ729039.1      ------------------------------------------------------------
CGMMV           ------------------------------------------------------------


KM873789.1      TCGAATCCCCCCCTTTTCCCCGGGTAGGGGCCCA
KU175632.1      ----------------------------------
KY753929.1      TCGAATCCCCCC--TTTCCCCGGGTAGGGGCCCA
KJ754196.1      TCGAATCCCCCC--TTTCCCCGGGTAGGGGCCCA
KU175639.1      ----------------------------------
KP868654.1      TCGAATCCCCCC-TTTTCCCCGGGTAGGGGCCC-
JF432067.1      ----------------------------------
KP868653.1      TCGAATCCCCCC-TTTTCCCCGGGTAGGGGCCC-
KY040049.1      TCGAAACCCCCC-TTTTCCCCGGGTAGGGGCCCA
KM873788.1      TCGAATCCCCCCCTTTTCCCCGGGTAGGGGCCCA
KR232571.1      TCGAATCCCCCC-TTTCCCCGGGGTAGGGGCCCA
MH271409        TCGAATCCCCCC-TTT-CCCCGGGTAGGGGCCCA
MT184944.1      TCGAATCCCCCC-TTT-CCCCGGGTAGGGGCCCA
DQ217778.1      TCGAATCCCCCC-TTTTCCCCGGGTAGGGGCCCA
V01551.1        TCGAATCCCCCC-TTT-CCCCGGGTAGGGGCCCA
AF225984.1      ----------------------------------
KY115174.1      TCGAATCCCCCC-TTTCCCC-GGGTAGGGGCCCA
MW430125.1      TCGAATCCCCCC-TTTCCCC-GGGTTGATGAGAG
MH427279.1      TCGAATCCCCCC-TTTCCCC-GGGTAGGGGCCCA
DQ767631.2      TCGAATCCCCCC-TTTTCCCTGGGTAAGGGCCCA
MH271435.1      TCGAATCCCCCC-TTTTCCCCGGGTAGGGGCCCA
AJ429090.1      TCGAATCCCCCCCT--------------------
KJ729039.1      ----------------------------------
   CGMMV           ----------------------------------
```

Multiple alignment of CGMMV test isolate with other CGMMV isolates at amino acid level using ClustalW

```
CGMMV           --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
KJ729039.1      ----ILNLQIGGWTLLLKSPVLFLLKMAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
MH427279.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
KR232571.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
KM873788.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
KP868654.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
MH271435.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
KU175639.1      ----------------LKSPVLFLLKMAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
DQ217778.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
KP868653.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
KY753929.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
KJ754196.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
```

```
V01551.1        --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
DQ767631.2      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
KU175632.1      ----------------LKSPVLFLLKMAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
KY040049.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
MW430125.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
KM873789.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
AJ429090.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
AF225984.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
KY115174.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
MT184944.1      --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
MH271409        --------------------------MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
JF432067.1      VVSGILNLQIGGWTLLLKSPVLFLLKMAYNPITPSKLIAFSASYVPVRTLLNFLVASQGT
                                          **********************************

CGMMV           AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
KJ729039.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
MH427279.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
KR232571.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
KM873788.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
KP868654.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
MH271435.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
KU175639.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
DQ217778.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
KP868653.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
KY753929.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
KJ754196.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
V01551.1        AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
DQ767631.2      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
KU175632.1      AFQTQAGRDPFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
KY040049.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
MW430125.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
KM873789.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
AJ429090.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
AF225984.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
KY115174.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
MT184944.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
MH271409        AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTDTRNRV
JF432067.1      AFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIFVSLLSSTGTRNRV
                *********.********************************************.*****

CGMMV           IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
KJ729039.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
MH427279.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
KR232571.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
KM873788.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
KP868654.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
MH271435.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
KU175639.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
DQ217778.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
KP868653.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
KY753929.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
KJ754196.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
V01551.1        IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
DQ767631.2      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
KU175632.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
KY040049.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
MW430125.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
KM873789.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
AJ429090.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
AF225984.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
KY115174.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
MT184944.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
MH271409        IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
JF432067.1      IEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWS
                ************************************************************

CGMMV           EATTSKA------------------------------
```

```
KJ729039.1      EATTSKA-FRGSSDGGAHQMAIV--------------
MH427279.1      EATTSKA------------------------------
KR232571.1      EATTSKA------------------------------
KM873788.1      EATTSKA------------------------------
KP868654.1      EATTSKA------------------------------
MH271435.1      EATTSKA------------------------------
KU175639.1      EATTSKA-FRGSSDGGAH-------------------
DQ217778.1      EATTSKA------------------------------
KP868653.1      EATTSKA------------------------------
KY753929.1      EATTSKA------------------------------
KJ754196.1      EATTSKA------------------------------
V01551.1        EATTSKA------------------------------
DQ767631.2      EATTSKA------------------------------
KU175632.1      EATTSKA-FRGSSDGGAH-------------------
KY040049.1      EATTSKA------------------------------
MW430125.1      EATTSKA------------------------------
KM873789.1      EATTSKA------------------------------
AJ429090.1      EATTSKA------------------------------
AF225984.1      EATTSKA------------------------------
KY115174.1      EATTSKA------------------------------
MT184944.1      EATTSKA------------------------------
MH271409        EATTSKA------------------------------
JF432067.1      EATTSKA-FRGSSDGGAHQSA-CFPVHLNRTVCSLVC
                *******
```

APPENDIX-IV

Multiple alignment of PRSV-W test isolate with other PRSV-W isolates at nucleotide level using ClustalW

```
MG564626.1      ------------------------------------------------------------
MG564601.1      ------------------------------------------------------------
DQ449533.1      ------------------------------------------------------------
MG564629.1      ------------------------------------------------------------
AF506900.1      ------------------------------------------------------------
KT884449.1      ------------------------------------------------------------
KY448332.1      ------------------------------------------------------------
AB583207.1      ------------------------------CAAATCCAAACTACCCGAGCACAGGCTTGA
KY448324.1      ------------------------------------------------------------
KF888662.2      ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
KY448325.1      ------------------------------------------------------------
AB127935.1      ------------------------------------------------------------
AB583214.1      ------------------------------CAAGTCTAAGCTACCTGAGCATAGATTAGA
AB583212.1      ------------------------------CAAGTCTAAGCTACCTGAGCATAGATTAGA
AB583204.1      ------------------------------CAAGTCTAAGCTACCTGAGCATAGATTAGA
AB583223.1      ------------------------------------------------------------
AB583198.1      ------------------------------TAAGTCTAAGCTACCTGAGCATAGACTAGA
AB583222        ------------------------------------------------------------
MK988414        TGAACGCATTGTGGCAATACTTGAATGGGACAAGTCTAAGCTACCTGAGCATAGGCTAGA
AB583205.1      ------------------------------CAAGTCTAAGCTACCTGAGCATAGACTAGA
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
LC457968.1      ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
MW002438.1      ------------------------------------------------------------
KJ789898.1      ------------------------------------------------------------


MG564626.1      ------------------------------------------------------------
MG564601.1      ------------------------------------------------------------
DQ449533.1      ------------------------------------------------------------
MG564629.1      ------------------------------------------------------------
AF506900.1      ------------------------------------------------------------
KT884449.1      ------------------------------------------------------------
KY448332.1      ------------------------------------------------------------
AB583207.1      AGCAATCATGGCTGCCATCATTGAATCGTGGGGTTACGAGGAGCTTACAGACCAAATTCG
KY448324.1      ------------------------------------------------------------
KF888662.2      ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
KY448325.1      ------------------------------------------------------------
AB127935.1      -------------------------------GGCTATGAAGAACTAACAGACCAAATTCG
AB583214.1      AGCGATAATGGCAGCTATTATTGAATCATGGGGCTATGAGGAGCTAACAGATCAAATTCG
AB583212.1      AGCGATAATGGCAGCTATTATTGAATCATGGGGCTATGAGGAGCTAACAGATCAAATTCG
AB583204.1      AGCGATAATGGCAGCTATTATTGAGTCATGGGGCTATGAGGAGCTAACAGATCAAATTCG
AB583223.1      ------------------------------------------------------------
AB583198.1      AGCGATAATGGCAGCCATCATTGAATCATGGGGCTATGAAGAGCTTACAGATCAAATTCG
AB583222        ------------------------------------------------------------
MK988414        AGCGATAATGGCAGCTATCATTGAATCATGGGGCTATGAGGAGCTGACGGATCAAATTCG
AB583205.1      AGCGATAATGGCAGCTATTATTGAATCATGGGGTTATGAGGAGCTAACAGATCAAATTCG
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
LC457968.1      ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
MW002438.1      ------------------------------------------------------------
KJ789898.1      ------------------------------------------------------------


MG564626.1      ------------------------------------------------------------
MG564601.1      ------------------------------------------------------------
```

```
DQ449533.1      ------------------------------------------------------------
MG564629.1      ------------------------------------------------------------
AF506900.1      ------------------------------------------------------------
KT884449.1      ------------------------------------------------------------
KY448332.1      ------------------------------------------------------------
AB583207.1      GAAATTTTACCAATGGGTTTTGGAGCAAGCTCCATACAATGAACTCGCGAAGCAGGGTAA
KY448324.1      ------------------------------------------------------------
KF888662.2      ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
KY448325.1      ------------------------------------------------------------
AB127935.1      GAAGTTCTACCAATGGGTTCTAGAACAAGCTCCATACAATGAGCTTG---ACGACGAGAA
AB583214.1      GAAATTCTACCAGTGGGTGATAGAACAAGCTCCATACAATGAACTTGCGAAGCAAGGTAA
AB583212.1      GAAATTCTACCAGTGGGTGCTAGAACAAGCTCCATACAATGAACTTGCGAAGCAAGGTAA
AB583204.1      GAAATTCTACCAGTGGGTGCTAGAACAAGCTCCATACAATGAACTTGCGAAGCAAGGTAA
AB583223.1      ------------------------------------------------------------
AB583198.1      GAAATTCTACCAGTGGGTTCTAGAACAAGCTCCATACAATGAACTCGCGAAGCAAGGTAA
AB583222        ------------------------------------------------------------
MK988414        GAAATTCTACCAGTGGGTTTTGGAACAAGCTCCGTACAATGAACTTGCGAAGCAGGGTAA
AB583205.1      GAAATTCTACCAATGGGTTCTAGAACAAGCTCCATACAATGAGCTTGCGAAGCAAGGTAA
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
LC457968.1      ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
MW002438.1      ------------------------------------------------------------
KJ789898.1      ------------------------------------------------------------


MG564626.1      ------------------------------------------------------------
MG564601.1      ------------------------------------------------------------
DQ449533.1      ------------------------------------------------------------
MG564629.1      ------------------------------------------------------------
AF506900.1      ------------------------------------------------------------
KT884449.1      ------------------------------------------------------------
KY448332.1      ------------------------------------------------------------
AB583207.1      AGCACCATATGTTTCTGAACTCGGACTTCGAAGACTATACACTAGCACCCGTGGATCCTG
KY448324.1      ------------------------------------------------------------
KF888662.2      ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
KY448325.1      ------------------------------------------------------------
AB127935.1      AGCACCATACGTCTCTGAACTTGGTCTTAGGAGGTTGTATACTAGCACTCGCGGATCCTG
AB583214.1      AGCACCGTACGTTTCTGAACTTGGCCTTCGGAGATTATACACTAGCACTCGCGGCTCCTG
AB583212.1      AGCACCGTACGTTTCTGAACTTGGCCTTCGGAGATTATACACTAGCACTCGCGGCTCCTG
AB583204.1      AGCACCGTACGTTTCTGAACTTGGCCTTCGGAGATTATACACTAGCACTCGCGGCTCCTG
AB583223.1      ------------------------------------------------------------
AB583198.1      AGCACCGTACGTCTCTGAACTCGGCCTTCGGAGATTATACACTAGCGCTCGCGGATCCTG
AB583222        ------------------------------------------------------------
MK988414        AGCACCGTACGTTTCTGAACTTGGCCTTCGGAGATTATACACCAGTACTCGCGGTTCCTG
AB583205.1      AGCACCGTACGTTTCTGAACTTGGCCTTCGGAGATTATACACTAGTACTCGCGGTTCCTG
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
LC457968.1      ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
MW002438.1      ------------------------------------------------------------
KJ789898.1      ------------------------------------------AGCAAGCGCCATACTCTG


MG564626.1      ------------------------------------------------------------
MG564601.1      ------------------------------------------------------------
DQ449533.1      ------------------------------------------------------------
MG564629.1      ------------------------------------------------------------
AF506900.1      ------------------------------------------------------------
KT884449.1      ------------------------------------------------------------
KY448332.1      ------------------------------------------------------------
AB583207.1      TGCAGAGCTTGAAGTATATCTTTCAAAGTACTTCGAGGATGAAAGTGGTGACACACCAGA
KY448324.1      ------------------------------------------------------------
KF888662.2      ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
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KY448325.1        ------------------------------------------------------------
AB127935.1        TGCGGAGTTAGAAGTGTACCTCTCAAAATATTTTGAAGATGAGAGTGGTGACACACCAGA
AB583214.1        TGCAGAGTTGGAAGTATATCTCTCGAAATATTTTGAGGACGAAAGTGGAGACACACCAGA
AB583212.1        TGCAGAGTTGGAAGTATATCTCTCGAAATATTTTGAGGACGAAAGTGGAGACACACCAGA
AB583204.1        TGCAGAGTTGGAAGTGTATCTCTCGAAATATTTTGAGGACGAAAGTGGAGACACACCAGA
AB583223.1        ------------------------------------------------------------
AB583198.1        TGCAGAGCTGGAAGTGTATCTTTCAAAGTATTTTGAGGATGAAAGCGGAGACACACCAGA
AB583222          ------------------------------------------------------------
MK988414          TGCAGAGTTGGAAGTATATCTCTCGAAATATTTTGAGGACGAAAGTGGAGACACACCAGA
AB583205.1        TGCAGAGTTGGAAGTGTATCTCTCGAAATATTTTGAGGACGAAAGTGGAGACACACCAGA
KF649243.1        ------------------------------------------------------------
KF649240.1        ------------------------------------------------------------
LC457968.1        ------------------------------------------------------------
LC457969.1        ------------------------------------------------------------
MW002438.1        ------------------------------------------------------------
KJ789898.1        ACTTAGCTTTGAAGGGTAAAGCACCTTATGTCTCAGAAACAGGACTTAGAAATTTGTACA


MG564626.1        ------------------------------------------------------------
MG564601.1        ------------------------------------------------------------
DQ449533.1        ------------------------------------------------------------
MG564629.1        ------------------------------------------------------------
AF506900.1        ------------------------------------------------------------
KT884449.1        ------------------------------------------------------------
KY448332.1        ------------------------------------------------------------
AB583207.1        GCTTGTAGTATACCATGAGTCTGTGGGCTCGTTGAAACAAACTTTAGCATCCCTACAGAG
KY448324.1        ------------------------------------------------------------
KF888662.2        ------------------------------------------------------------
PRSV-W            ------------------------------------------------------------
KY448325.1        ------------------------------------------------------------
AB127935.1        GCTCGTGGTTTATCATGAGTCTGTGAACCCAATTAAGCAGACTATGGCCTCACTGCAAGG
AB583214.1        ACTTGTAGTTTATCACGAATCTGTGAGCCCAGAGAAGCAGACCGTCGCTTCTTTACAAAG
AB583212.1        ACTTGTAGTTTATCACGAATCTGTGAGCCCAGAGAAGCAGACCGTCGCTTCTTTACAAAG
AB583204.1        ACTTGTAGTTTATCACGAATCTGTGAGCCCAGAGAAGCAGACCGTCGCTTCGTTACAAAG
AB583223.1        ------------------------------------------------------------
AB583198.1        GCTTGTGGTTTATCACGAATCCGTGGGTCCAGTTAAGCAGACCATTGCCTCTTTGCACAG
AB583222          ------------------------------------------------------------
MK988414          ACTCGTAGTTTATCACGAATCTGTAGGCCCAGTTAAGCAGACCGTCGCCTCTTTACAAAG
AB583205.1        ACTTGTAGTTTATCACGAATCTGTGGGCCCAAATAAGCAGACCATCGCTTCTTTACAAAG
KF649243.1        ------------------------------------------------------------
KF649240.1        ------------------------------------------------------------
LC457968.1        ------------------------------------------------------------
LC457969.1        ------------------------------------------------------------
MW002438.1        ------------------------------------------------------------
KJ789898.1        CATCACAGCGAGGTTCTCCACAGGAACTAGAAAAGTACATAACACACTACTTCAGGAGTG


MG564626.1        -------------------TCCAAGAATGAAGCTGTGGAT-GCTGGTCTGAATGAAAAGC
MG564601.1        -------------------TCCAAGAATGAAGCTGTGGAT-GCTGGTTTGAATGAAAAGC
DQ449533.1        ----------------ATGTCCAAAAATGAAGCTGTGGAT-GCTGGTCTGAATGAGAAGC
MG564629.1        -------------------TCCAAGAATGAAGCTGTGGAT-GCTGGTTTAAACGAGAAGC
AF506900.1        --------------------------------------------GGTCTTAATGAGAAGC
KT884449.1        ------------------------------------------------------------
KY448332.1        -------------------TCAAAGAATGAAGCTGTTGAT-GCTGGGGTCAATGCTAAAT
AB583207.1        CAAGAACGTGTTCTATCAATCAAAAGATGGTGCCGTTGAT-GCTGGAGTCAATGCCAAAT
KY448324.1        -------------------TCAAAAGATGGTGCCGTTGAT-GCTGGGGTCAATGCCAAAA
KF888662.2        ------------------------------------------------------------
PRSV-W            ------------------------------------------------------------
KY448325.1        -------------------TCAAAAGATGGCGCTGTGGAT-GCTGGTGTCAACGCTAAGT
AB127935.1        CAAGAATGTGTTCTATCAATCAAAAGATGGCGCTGTGGAT-GCTGGTGTCAATGCTAAGT
AB583214.1        CAAGAACGTATTCTATCAATCAAAGGACGGTGCTGTGGAT-GCTGGAGTTAATGCTAAAC
AB583212.1        CAAGAACGTATTCTATCAATCAAAGGACGGTGCTGTGGAT-GCTGGAGTTAATGCTAAAC
AB583204.1        CAAGAACGTATTCTATCAATCAAAGGACGGTGCTGTGGAT-GCTGGAGTTAATGCTAAAC
AB583223.1        ------------------------------------------------------------
AB583198.1        CAAGAACGTATTCTATCAATCAAAGGATGGCGCTGTGGAT-GCTGGGGTTAATGCTAAAC
AB583222          ------------------------------------------------------------
MK988414          CAAGAACGTATTCTATCAATCAAAGGACGGTGCTGTGGAT-GCTGGAGTTAATGCTAAAT
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AB583205.1          CAAGAACGTATTCTATCAATCAAAGGATGGCGCTGTGGAT-GCTGGAGTTAATGCTAAAT
KF649243.1          ------------------------------------------------------------
KF649240.1          ------------------------------------------------------------
LC457968.1          ------------------------------------------------------------
LC457969.1          ------------------------------------------------------------
MW002438.1          ------------------------------------------------------------
KJ789898.1          AAAGTGGGGATTGTCCAGAGCTAATGGTGTACCACCAAGCAGATGATGCTCGTGATGCAG


MG564626.1          TAAAAGAAAAAGAAAAACAGAGAGAG---AAAGAAAAAGAAAAAGATAAACAAAAAGAGA
MG564601.1          TAAAAGAAAAAGAAAGACAGAGAGAG---AAAGAAAAAGAAAAAGATAAACAAAAAGAGA
DQ449533.1          TCAAAGAGAAAGAAAAACTGAAAGAA---AAAGAAAAAGAAAAA------CAAAAAGAAA
MG564629.1          TCAAAGAAAAAGAAAAACAGAAAG------AAGAAAAAGAAAAA------CAAAAAGAGA
AF506900.1          TCAAAGATAAAGAAAAACAGAAAG------AAGAAAAAGATAAA------CAAAAAGGTA
KT884449.1          ---------------------------------GAATAGAAAAA------CAAAAAGAGA
KY448332.1          TGAAAGAAAAGGAAGAGAAGGAAAAA------GAAAAAGAGAAAGAAAAAGAAAAAGAAA
AB583207.1          TGAAAGAAAAGGAAGAAAAGGAAAAA------GAGAAAGAGAAAGAAAAAGAAAAAGAAA
KY448324.1          TGAAAGAAAAGGAAGAGAAAGAAAAA------GAAAAAGAGAAAGAAAAAGAAAAAGATA
KF888662.2          ------------------------------------------------------------
PRSV-W              ------------------------------------------------------------
KY448325.1          TAAAAGAGAAGGAGGA------GAAGGAAAAAGAAAAAGAGAAAGAAAAAGAAAAGGAGA
AB127935.1          TAAAAGAAAAGGAAGAAAAAGAGAAAGAGAAAGAAAAGGAAAAAGAGAAAGAAAAGGAAA
AB583214.1          TAAAAGAGAAAGAGGAGAAAGAAAAAGAAAAAGAAAAGGAGAAAGAAAGAGAAAAAGAGA
AB583212.1          TAAAAGAGAAAGAGGAGAAAGAAAAAGAAAAAGAAAAGGAGAAAGAAAGAGAAAAAGAGA
AB583204.1          TAAAAGAGAAAGAGGAGAAAGAAAAAGAAAAAGAAAAGGAGAAAGAAAGAGAAAAAGAGA
AB583223.1          ------------------------------------------------------------
AB583198.1          TAAAGGAAAAAGAGGAGAAAGAAAAA------GAAAAGGAGAAAGAAAGAGAAAAAGAGA
AB583222            ------------------------------------------------------------
MK988414            TAAAAGAAAAAAAGGAAAAAGAAAAA------GAAAAAGAAAAAGAAAAAGAAAAAGAAA
AB583205.1          TGAAAGAAAAAGAGGAGAAAGAGAAA------GAAAAAGAAAAGGAGAAGGAAAAAGAAA
KF649243.1          ------------------------------------------------------------
KF649240.1          ------------------------------------------------------------
LC457968.1          ------------------------------------------------------------
LC457969.1          ------------------------------------------------------------
MW002438.1          ------------------------------------------------------------
KJ789898.1          GTCAAGGAGTGGGTGATAAGAAAGAAAAGAAAGAAAAAGAAAAAGAAAAAGAAAAAGAGA


MG564626.1          AAGATAATGATGGAGCTAGTGACGGAAACGATGTGTC-AACTAGCACAAAAACT------
MG564601.1          AAGATAATGATGGAGCTAGTGACGGAAACGATGTGTC-AACTAGCACAAAAACT------
DQ449533.1          AAGATAATGATGGAGCTAGTGACGGAAATGATGTGTC-AACTAGCACAAAAACT------
MG564629.1          AAGACAAAGAAGGAGCTAGTGACGGAAATGATGTGTC-AACTAGCACAAAAACT------
AF506900.1          AAGAAAATAATGAAGCTAGTGACGGAAACAATGTGTC-AACTAGCACAAAAATT------
KT884449.1          AAAATAACAATGAAGCTAGTGACGGAAACGATGTGTC-AACTAGCACAAAAACT------
KY448332.1          AAGAGAAAGAAAAGATTAAAGACGCACCTGAAGCGTCAAGTCAGAAAACAAATTCTGAGA
AB583207.1          AAGAGAAAGAAAAGATTAAGGACGCACCTGAAGCGTCAAGTCAGAAAACAAACTCTGAGA
KY448324.1          AAGAAAAAGAAAAGACTAAGGACGCACCTGAAGCGTCAAGTCAGAAGACAGATTCTGAGA
KF888662.2          ------------------------------------------------------------
PRSV-W              ------------------------------------------------------------
KY448325.1          AAGAAAAAGAGAAGTTAAAAGATGAACCTGAGGCATCTAAACAGAATTTGAACTCAGGAA
AB127935.1          AAGAAAAAGAGAAGGTGAAAGATGAACCTGAAACATCCAAACAAGATCTGAATTCAGGTA
AB583214.1          AGGAAAAGGAAAAGCTGAAGAACGTGCCTGAAACATCGAACCAGGACACGGAATCGATAA
AB583212.1          AGGAAAAGGAAAAGCTGAAGAACGTGCCTGAAACATCGAACCAGGACACGGAATCGATAA
AB583204.1          AGGAAAAGGAAAAGCTGAAGAACGTGCCTGAAACATCGAATCAGGACACGGAATCGATAA
AB583223.1          ------------------------------------------------------------
AB583198.1          AGGAAAAAGAAAAATTGAAGGACGTGCCTGAAACATCTAATCAGGACACGGAATCAATAG
AB583222            ------------------------------------------------------------
MK988414            AGGAAAAAGAAAAACTGAAAGACGTGCCTGAAACATCTAATCAGGAGACGAAGTCAGAGA
AB583205.1          AGGAAAAGGAAAAAGAGAAAGACGTGCCTGAAACATCTAATCAGGATACGAAATCTGAGA
KF649243.1          ------------------------------------------------------------
KF649240.1          ------------------------------------------------------------
LC457968.1          ------------------------------------------------------------
LC457969.1          ------------------------------------------------------------
MW002438.1          ------------------------------------------------------------
KJ789898.1          AAGAAGTGAAAGCTTCAGATGAAACAGGAGGTTCATCATCACAGGAACAAGGCA------
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MG564626.1      -----GGAGAGAGAGATAGAGATGTCAACGCCGGAACTAGTGGAACTTTCACTGTTCCAA
MG564601.1      -----GGAGAGAGAGATAGAGATGTCAACGCCGGAACTAGTGGAACTTTCACTGTTCCAA
DQ449533.1      -----GGAGAGAGAGATAGAGATGTCAATGCCGGAACTAGTGGAACCTTCACTGTTCCGA
MG564629.1      -----GGAGAGAGAGATAGAGATGTCAATGCCGGAACTAGTGGAACTTTCACTGTTCCAA
AF506900.1      -----GGAGAGAGAGATAGAGATGTCAATGCCGGAACTAGTGGTACTTTCACTGTTCCGA
KT884449.1      -----GGAGAGAAAGACAGAGATGTCAATGCTGGAACTAGTGGGACTTTTACTGTTCCGA
KY448332.1      AAGAATGGGAGAGAGATCGAGATGTCAATGCCGGAACAATGGGGAGTTTTACTGTTCCTC
AB583207.1      AAGAAAGGGAGAGAGATCGAGATGTCAATGCTGGGACAATGGGGAGTTTCACTGTTCCTC
KY448324.1      AAGAAAGGGAGAGAGACCGAGATGTCAATGCTGGAACAATGGGGAGTTTCACTGTCCCTC
KF888662.2      ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
KY448325.1      CGGAAAAGGAGAGAGATCGAGATGTTAACGCTGGGACAATGGGGAGTTTTACTGTTCCTC
AB127935.1      CGGAAAGGGAGAGAGATCGAGATGTCAATGCTGGGACAATGGGGAGTTTTACTGTTCCCC
AB583214.1      CAGAAAGGGAGAGAGATCGAGATGTCAATGCTGGGACAATGGGGAGTTTTACTGTTCCCC
AB583212.1      CAGAAAGGGAGAGAGATCGAGATGTCAATGCTGGGACAATGGGGAGTTTTACTGTTCCCC
AB583204.1      CAAAAAGGGAGAGAGATCGAGATGTCAATGCTGGGACAATAGGGAGTTTTACTGTTCCCC
AB583223.1      ----------------------------TGCTGGGACAATGGGGAGTTTTACTGTTCCCC
AB583198.1      CAGAAAGGGAGAGAGATCGAGATGTCAATGCTGGGACAATGGGGAGTTTTACTGTTCCCC
AB583222        ----------------------------TGCTGGGACAATGGGGAGTTTCACTGTTCCCC
MK988414        AAGAAAGGGAGAGAGATCGAGATGTCAACGCTGGGACAATGGGGAGTTTCACTGTTCCCC
AB583205.1      AAGAAAGGGAGAGAGATCGAGATGTCAATGCTGGGACAATAGGGAGTTTCACTGTTCCCC
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
LC457968.1      ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
MW002438.1      ------------------------------------------------------------
KJ789898.1      ---AGAAGGAAAAGGACAAAGACGTTGATGTTGGGACTAAAGGGACCTTCAGCGTTCCAA


MG564626.1      GGATAAAGTCTTTTACTGATAAGATGATTTTACCAAGAATTAAGGGAAGGTCTGTCCTTA
MG564601.1      GGATAAAGTCTTTTACTGATAAGATGATTTTACCAAGAATTAAGGGAAGGTCTGTCCTTA
DQ449533.1      GGATAAAGTCATTTACTGATAAGATGATTTTACCAAGAATTAAGGGAAAGACTGTCCTTA
MG564629.1      GGATAAAGTCTTTTACTGATAAGATGATTTTACCAAGAGTTAGGGGAAAAACTGTCCTTA
AF506900.1      GAATAAAACCATTTACTGACAAAATGATTTTACCAAGAATTAAGGGAAAAACTGTCCTTA
KT884449.1      GAATAAAGTCATTTACTGATAAGATGATTCTACCAAGGATTAAGGGAAAAACTGTCCTTA
KY448332.1      GTATTAAAACATTTACTGATAAAATGATTCTACCTAAGTTCAAAGGAAAGACTGTTTTGA
AB583207.1      GTATTAAAACATTCACTGATAAAATGATTCTACCCAAGTTCAAAGGAAAGACTGTTTTGA
KY448324.1      GGATTAAAACTTTCACTGACAAAATGATACTACCCAAGTTCAAAGGAAAGACTGTTTTGA
KF888662.2      ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
KY448325.1      GAATTAAAACATTCACTGATAAGATGATTCTACCTAAGTTCAAAGGAAAAACTGTTTTGA
AB127935.1      GAATTAAAACATTCACTGATAAGATGGTTTTACCGAAGTTCCAAGGAAAGACTGTTTTGA
AB583214.1      GAATTAAAACATTTACTGACAAAATGATTCTACCTAAGTTCAAAGGAAAGACTGTTTTGA
AB583212.1      GAATTAAAACATTTACTGACAAAATGATTCTACCTAAGTTCAAAGGAAAGACTGTTTTGA
AB583204.1      GAATTAAAACATTTACTGACAAAATGATTCTACCTAAGTTCAAAGGAAAGACTGTTTTGA
AB583223.1      GAATTAAAACATTTACTGACAAAATGATTCTACCTAAGTTCAAAGGAAAGACTGTTTTGA
AB583198.1      GGATTAAAACATTTACTGACAAAATGGTTCTACCTAAGTTCAGAGGAAAGACTGTTTTGA
AB583222        GAATTAAAACATTTACTGATAAAATGGTTTTACCTAAGTTCAAAGGAAAAACTGTTTTGA
MK988414        GAATTAAAACATTTACTGATAAGATGGTTTTACCTAAGTTCAAAGGAAAAACTGTTTTGA
AB583205.1      GAATTAAAACATTTACTGATAAGATGATTCTACCTAAGTTCAAAGGAAAAACTGTTTTGA
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
LC457968.1      ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
MW002438.1      ------------------------------------------------------------
KJ789898.1      AAGTCAAAACTTTCAATGATAAGATGATCTTGCCTAGAGTCAGAGGTAAAATTGCATTGA


MG564626.1      ATTTAAATCATCTTCTTCAGTATAATCCGCAACAAATTGACATCTCAAACACTCGTGCCA
MG564601.1      ATTTAAATCATCTTCTTCAGTATAATCCGCAACAAATTGACATCTCAAACACTCGTGCCA
DQ449533.1      ATTTAAATCATCTTCTTCAGTATAACCCGCAACAAATTGACATCTCAAACACTCGTGCCA
MG564629.1      ATTTAAATCATCTTCTTCAATATAATCCGCAACAAATTGACATCTCAAACACTCGTGCCA
AF506900.1      ATTTAAACCATCTTCTTCAGTATAATCCGCAACAAATAGATATCTCAAACACTCGTGCCA
KT884449.1      ATTTAAATCATCTACTTCAGTATAATCCACAACAAATAGACATCTCTAACACTCGTGCCA
KY448332.1      ACTTAAATCACTTGATTCAGTACAATCCACAGCAAATTGATATCTCAAATACACGTGCCA
AB583207.1      ACTTGAATCACTTGATTCAGTACAATCCACAGCAAATTGATATCTCAAATACACGCGCCA
KY448324.1      ACTTGAATCACTTAATTCAGTACAATCCACAGCAAATTGATATCTCAAATACACGTGCCA
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KF888662.2      ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
KY448325.1      ACTTGAATCATTTGATTCAATACAATCCGCAGCAAATAGACATTTCAAATACACGTGCCA
AB127935.1      ACTTGAACCACTTGATTCAGTACAATCCGCAGCAAATAGATATCTCAAATACACGTGCCA
AB583214.1      ATTTGAATCATTTACTTCAGTATAATCCACTACAGTTCGACATTTCAAACACGCGTGCCA
AB583212.1      ATTTGAATCATTTACTTCAGTATAATCCACTACAGTTCGACATTTCAAACACGCGTGCCA
AB583204.1      ATTTGAATCATTTACTTCAGTATAATCCACTACAGTTCGACATTTCAAACACGCGTGCCA
AB583223.1      ATTTGAATCATTTACTTCAGTATAATCCACTACAGTTCGACATTTCAAACACGCGTGCCA
AB583198.1      ACTTGAATCATTTACTTCAGTATAATCCATTACAGTTTGACATTTCAAACACGCGTGCCA
AB583222        ACTTAAATCATTTAATTCAGTACAATCCATTACAGCTTGACATTTCAAATACACGTGCTA
MK988414        ACTTGAACCATTTAATTCAGTATAACCCTCAGCAGATTGACATTTCAAATACGCGTGCCA
AB583205.1      ACCTAAACCATCTAATTCAGTATAACCCACAACAGATTGACATTTCAAACACGCGTGCCA
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
LC457968.1      ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
MW002438.1      ------------------------------------------------------------
KJ789898.1      ATCTTGAACATCTGTTGCAGTACAATCCTAATCAGATTGACTTGTCAAACACTAGAGCGA


MG564626.1      CTCAATCTCAATTTGAAAAGTGGTATGAGGGAGTGAGGAATGATTATGGTCTTAGTGACA
MG564601.1      CTCAATCTCAATTTGAAAAGTGGTATGAGGGAGTGAGGAATGATTATGGTCTTAGTGATA
DQ449533.1      CTCAATCTCAATTTGAAAAGTGGTATGAGGGAGTGAGGAATGACTATGGTCTTAATGATA
MG564629.1      CTCAATCGCAATTCGAAAAGTGGTATGAGGGAGTGAGGAATGATTATGGTCTGAACGATA
AF506900.1      CTCAATCTCAATTCGAAAAGTGGTATGAGGGAGTGAGGAATGATTACGGTCTTAATGATA
KT884449.1      CTCAGTCACAGTTTGAAAAGTGGTATGAGGGAGTGAGGAATGATTACGGTCTTAATGATA
KY448332.1      CTCAAAGTCAATTTGACAAGTGGTACGGAGGTGTGCGTAACGATTATGGTCTAGATGACA
AB583207.1      CTCAAAGTCAATTTGACAAGTGGTACGAAGGTGTGCGTAACGATTATGGTCTAGATGACA
KY448324.1      CTCAAAGCCAATTTGACAAGTGGTACGAAGGTGTGCGTAATGATTATGGTCTTAATGACA
KF888662.2      ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
KY448325.1      CTCAAAGTCAGTTTGACAAGTGGTATGAAGGTGTGCGTAATGATTACGGGCTCAATGATA
AB127935.1      CTCAAAATCAGTTTGACAAATGGTACGAAGGCGTGCGTAGTGACTACGGGCTTACTGATA
AB583214.1      CTCGGAGTCAGTTTGATAAGTGGTACGAAGGTGTGCGCAATGATTATGGCCTTGATGACA
AB583212.1      CTCGGAGTCAGTTTGATAAGTGGTACGAAGGTGTGCGCAATGATTATGGCCTTGATGACA
AB583204.1      CTCGGAGTCAGTTTGATAAGTGGTACGAAGGTGTGCGCAATGATTATGGCCTTGATGACA
AB583223.1      CTCGGAGTCAGTTTGATAAGTGGTACGAAGGTGTGCGCAATGATTATGGCCTTGATGACA
AB583198.1      CTAGGAGTCAGTTCGATAAATGGTACGAAGGTGTGCGCAATGATTATGGCCTTGATGACA
AB583222        CTCGAAGTCAATTCGATGAATGGTACGTAGGCGTGCGCAATGATTATGGCCTTAATGACA
MK988414        CTCAGAGTCAGTTCGATAAATGGTACGAAGGTGTGCGCAATGATTATGGTCTCAATGACA
AB583205.1      CTCAGAGTCAGTTTGATAAATGGTACGAAGGTGTGCGCAATGATTATGGTCTTAATGACA
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
LC457968.1      ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
MW002438.1      ------------------------------------------------------------
KJ789898.1      CTCAAAATCAGTTTGATAGATGGTACGACGGAGTCAAGAATGACTATGGACTTGATGACG


MG564626.1      ACGAAATGCAAGTGATGTTAAATGGTTTAATGGTTTGGTGTATCGAAAATGGTACATCCC
MG564601.1      ACGAGATGCAAGTGATGTTAAATGGTTTAATGGTTTGGTGTATCGAAAATGGTACATCCC
DQ449533.1      ACGAAATGCAAGTGATGTTGAATGGTTTGATGGTTTGGTGTATCGAAAATGGTACATCTC
MG564629.1      ACGAAATGCAAGTGATGTTAAATGGATTAATGGTTTGGTGTATCGAAAATGGTACATCTC
AF506900.1      ACGAAATGCAAGTGATGTTAAATGGTTTGATGGTTTGGTGCATCGAAAACGGAACATCCC
KT884449.1      ATGAGATGCAAGTGATGCTAAATGGTTTAATGGTATGGTGTATCGAAAATGGTACATCCC
KY448332.1      ATGAGATGCAGGTGATGCTTAACGGTTTAATGGTCTGGTGCATTGAAAATGGAACCTCCC
AB583207.1      ATGAGATGCAGGTGATGCTCAACGGTTTAATGGTCTGGTGCATTGAAAATGGAACCTCCC
KY448324.1      ATGAGATGCAGGTGATGCTTAATGGTTTAATGGTCTGGTGCATCGAAAACGGAACCTCTC
KF888662.2      ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
KY448325.1      GCGAAATGCAGGTGATGCTAAATGGATTAATGGTTTGGTGCATTGAAAACGGCACATCGC
AB127935.1      ACGAAATGCAGGTGATGTTGAATGGACTAATGGTTTGGTGCATTGAAAACGGCACATCTC
AB583214.1      ATGAAATGGCAGTAATGCTAAATGGTCTGATGGTTTGGTGTATTGAAAACGGCACATCAC
AB583212.1      ATGAAATGGCAGTAATGCTAAATGGTCTGATGGTTTGGTGTATTGAAAACGGCACATCAC
AB583204.1      ATGAAATGGCAGTAATGCTAAATGGCCTGATGGTTTGGTGTATTGAAAACGGCACATCAC
AB583223.1      ATGAAATGGCAGTAATGCTAAATGGTCTGATGGTTTGGTGTATTGAAAACGGCACATCAC
AB583198.1      ATGAGATGGCAGTAATGCTGAATGGCTTGATGGTTTGGTGTATTGAAAACGGCACATCAC
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AB583222       ATGAAATGGCAGTGATGTTAAATGGCTTAATGGTTTGGTGCATCGAAAACGGTACGTCAC
MK988414       ACGAAATGCAAGTAATGCTAAATGGCTTGATGGTTTGGTGTATCGAGAATGGTACTTCAC
AB583205.1     ACGAGATGCAAGTAATGTTAAATGGTTTAATGGTTTGGTGTATTGAGAATGGTACTTCAC
KF649243.1     -----------------------------------TGGTGTATTGAGAATGGCACATCCC
KF649240.1     ------------------------------------------------------------
LC457968.1     ------------------------------------------------------------
LC457969.1     ------------------------------------------------------------
MW002438.1     ------------------------------------------------------------
KJ789898.1     AGGAAATGGCAATAGTGCTCAATGGTTTCATGGTATGGTGCATTGAAAATGGCACGTCCC


MG564626.1     CAGACATATCTGGTGTTTGGGTAATGATGGATGGGGAAACCCAGGTTGATTATCCCATCA
MG564601.1     CAGACATATCTGGTGTTTGGGTAATGATGGATGGGGAAACCCAGGTTGATTATCCCATCA
DQ449533.1     CAGACATATCTGGTGTCTGGGTTATGATGGACGGGGAAACCCAAGTCGATTATCCCATTA
MG564629.1     CAGACACATCTGGTGTCTGGGTTATGATGGATGGGGAAACCCAAGTCGACTATCCCATTA
AF506900.1     CAGACATATCTGGTGTCTGGGTGATGATGGATGGGGAAACCCAAGCCGATTATCCCATCA
KT884449.1     CAGACATATCTGGTGTTTGGGTTATGATGGATGGAGAGACTCAAGTTGACTATCCAATTA
KY448332.1     CAGATGTTTCTGGTGTCTGGGTGATGATGGACGGCGACGTTCAAGTAGATTATCCCATCA
AB583207.1     CAGACGTTTCTGGTGTCTGGGTGATGATGGACGGCGACGTTCAGGTGGATTATCCCATCA
KY448324.1     CAGATGTTTCTGGTGTCTGGGTGATGATGGACGGCGACGTCCAAGTGGATTATCCCATCA
KF888662.2     -----------------TGGGTAATGATGGACGGCGACGTTCAAGTGGATTATCCCATCA
PRSV-W         -----------------TGGGTTATGATGGATGGGGATACTCAAGTCGACTACCCAATTA
KY448325.1     CAGATGTATCTGGCGTCTGGGTTATGATGGATGGCGATACGCAAGTTGACTACCCAATTA
AB127935.1     CCGACGTATCTGGCGTCTGGGTCATGATGGATGGCGACACTCAAGTCGACTATCCTATTA
AB583214.1     CAGATGTATCCGGCGTCTGGGTGATGATGGACGGTGATACTCAAGTTGATTATCCCATCA
AB583212.1     CAGATGTATCCGGCGTCTGGGTGATGATGGACGGTGATACTCAAGTTGATTATCCCATCA
AB583204.1     CAGATGTGTCCGGCGTCTGGGTGATGATGGACGGTGATACTCAAGTCGATTATCCCATCA
AB583223.1     CAGATGTATCCGGCGTCTGGGTGATGATGGACGGTGATACTCAAGTTGATTATCCCATCA
AB583198.1     CAGATGTGTCCGGCGTCTGGGTGATGATGGATGGTGATACTCAAGTTGACTATCCCATCA
AB583222       CAGATGTATCCGGCGTCTGGGTGATGATGGACGGAGACACCCAAGTTGACTATCCTATTA
MK988414       CAGATGTGTCTGGCGTCTGGGTAATGATGGACGGCGAAACTCAAGTCGATTATCCTATCA
AB583205.1     CAGATGCGTCTGGCGTCTGGGTAATGATGGATGGCGAGACTCAAGTCGACTATCCTATTA
KF649243.1     CAGATGTATCTGGTATCTGGGTTATGATGGAAGGTGGCAATCAAGTTGATTTTCCCATTA
KF649240.1     --------TCTGGTATCTGGGTTATGATGGA-GGTGGCAATCAAGTTGATTTTCCCATTA
LC457968.1     ------TTTCTGGCGTCTGGGTGATGATGGACGGCGACGTTCAAGTGGATTATCCCATTA
LC457969.1     ------------------------------------------------------------
MW002438.1     -------------------------GATGGATGGGGAAACCCAAGTCGATTATCCCATTA
KJ789898.1     CGAATGTTAATGGAGTTTGGACCATGATGGACAATGGGGAACAAGTTGAATATTTATTGA


MG564626.1     AACCTTTAATTGAACATGCAAATCCTTCATTCAGGCAAATTATGGCTCACTTCAGTAACG
MG564601.1     AACCTTTAATTGAACATGCAACTCCTTCATTCAGGCAAATTATGGCTCACTTCAGTAACG
DQ449533.1     AACCTTTGATTGAACATGCAACTCCTTCGTTTAGGCAAATCATGGCTCACTTCAGTAACG
MG564629.1     AACCTTTGATTGAACACGCAACTCCTTCATTCAGACAAATTATGGCTCACTTCAGTAACG
AF506900.1     AGCCTTTAATCGAACATGCAACTTCTTCGTTCAGGCAAATCATGGCTCACTTCAGTAACG
KT884449.1     AACCTTTAATTGAGCATGCAACTCCTTCATTTAGGCAGATTATGGCTCACTTCAGTAACG
KY448332.1     AGCCGTTAATTGAACATGCAACACCAACATTTCGTCAGATTATGGCGCACTTTAGTAATG
AB583207.1     AGCCGTTAATTGAACATGCAACACCAACGTTTCGTCAGATTATGGCACATTTTAGTAATG
KY448324.1     AGCCATTAATTGAACATGCAACACCAACATTTCGTCAGATTATGGCACACTTTAGTAATG
KF888662.2     AGCCGTTAATTGAACATGCAACACCAACATTCCGCCAGATCATGGCACACTTTAGTAATG
PRSV-W         AACCATTAATTGAACACGCAACACCAACATTCCGTCAGATTATGGCACACTTCAGCAATG
KY448325.1     AACCATTGATTGAACACGCAACACCAACATTTCGACAAATTATGGCACACTTCAGCAACG
AB127935.1     AACCATTAATCGAACACGCAACACCAACATTTCGTCAGATTATGGCACACTTTAGCAACG
AB583214.1     AACCATTAATTGAACATGCAACACCAACATTTCGCCAAATCATGGCGCATTTTAGCAATG
AB583212.1     AACCATTAATTGAACATGCAACACCAACATTTCGCCAAATCATGGCGCATTTTAGCAATG
AB583204.1     AACCATTAATTGAACATGCAACACCAACATTTCGCCAAATCATGGCGCATTTTAGCAATG
AB583223.1     AACCATTAATTGAACATGCAACACCAACATTTCGCCAAATCATGGCGCATTTTAGCAATG
AB583198.1     AACCATTAATTGAACATGCAACACCAACATTTCGCCAAATCATGGCGCATTTTAGCAATG
AB583222       AACCATTAATTGAACATGCGACACCAACATTTCGCCAAATCATGGCGCACTTTAGCAATG
MK988414       AACCATTGATTGAGCATGCAACACCAACATTTCGTCAAATCATGGCGCACTTTAGCAATG
AB583205.1     AACCATTGATTGAACACGCAACACCAACATTTCGTCAAATCATGGCGCACTTTAGCAATG
KF649243.1     AACCATTAATCGAACACGCAACACCAACATTTCGTCAAATCATGGCACACTTTAGCAATG
KF649240.1     AACCATTAATCGAACACGCAACACCAACATTTCGTCAAATCATGGCACACTTTAGCAATG
LC457968.1     AACCATTAATTGAACACGCAACACCGACATTTCGCCAAATCATGGCACACTTTAGCAATG
LC457969.1     ------------------CAACACCGACATTTCGCCAAATCATGGCACACTTTAGCAATG
MW002438.1     AACCTTTGATTGAACATGCAACTCCTTCGTTTAGGCAAATCATGGCTCACTTCAGTAACG
KJ789898.1     AGCCAATGATAGAACATGCGTCTCCGACTCTGCGACAGATCATGGCTCATTATAGTAACG
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                                  *     *  *  *  * ** ** ***** ** *  ** ** *

MG564626.1      CGGCAGAAGCATATATCGCAAAGAGAAATGCAACTGAGAGGTACATGCCGCGGTATGGAA
MG564601.1      CGGCAGAAGCATATATCGCAAAGAGAAATGCAACTGAGAGGTACATGCCGCGGTATGGAA
DQ449533.1      CGGCAGAGGCATACATCGCGAAGAGGAATGCAACTGAGAGGTACATGCCGCGGTATGGAA
MG564629.1      CGGCAGAGGCATACATCGCGAAGAGGAATGCTACTGAGAGGTACATGCCGCGGTATGGAA
AF506900.1      CGGCAGAGGCATATATCGCAAAGAGGAATGCTATTGAGAGGTACATGCCGCGGTTTGGAC
KT884449.1      CGGCAGAGGCATACATTGCAAAGAGAAATGCCACTGAGAGGTACATGCCGCGGTATGGAA
KY448332.1      CAGCTGAGGCATATATTGCAAAGAGGAATGCAACTGAACGATATATGCCTCGGTATGGAT
AB583207.1      CAGCTGAGGCATACATTGCAAAGAGGAATGCAACTGAACGATATATGCCTCGGTATGGAT
KY448324.1      CAGCTGAGGCATACATTGCAAAGAGAAATGCAACTGAACGATATATGCCTCGGTATGGAT
KF888662.2      CAGCTGAAGCATACATTGCAAAGAGGAATGCAACTGAACGATATATGCCTCGGTATGGAT
PRSV-W          CAGCTGAGGCATACATTGCAAAAAGGAATGCAACAGAACGGTATATGCCTCGTTACGGCT
KY448325.1      CAGCTGAGGCATACATTGCAAAAAGAAATGCAACAGAACGATATATGCCCCGTTACGGCT
AB127935.1      CAGCCGAGGCATACATTGCAAAGAGGAATGCGACAGAACGATACATGCCTCGTTATGGTT
AB583214.1      CAGCTGAGGCATACATTGCAAAAAGAAATGCAACGGAGCGATATATGCCCCGTTATGGCT
AB583212.1      CAGCTGAGGCATACATTGCAAAAAGAAATGCAACGGAGCGATATATGCCCCGTTATGGCT
AB583204.1      CAGCTGAGGCGTACATTGCAAAACGAAATGCAACGGAGCGATATATGCCCCGTTATGGCT
AB583223.1      CAGCTGAGGCATACATTGCAAAAAGAAATGCAACGGAGCGATATATGCCCCGTTATGGCT
AB583198.1      CAGCCGAGGCTTACATTGCAAAGAGAAATGCAACGGAGCGATATATGCCCCGTTATGGCT
AB583222        CAGCTGAGGCATACATCGCAAAAAGGAATGCAACGGAGCGATATATGCCTCGTTATGGCT
MK988414        CAGCTGAGGCATACATCGCAAAAAGGAATGCAACAGAACGATACATGCCTCGTTATGGCT
AB583205.1      CAGCTGAGGCATACATCGCAAAAAGGAACGCAACAGAACGATACATGCCTCGTTATGGCT
KF649243.1      CAGCTGAGGCATACATTGCAAGGAGGAATGCAACTGAAAGATACATGCCTCGCTATGGCT
KF649240.1      CAGCTGAGGCATACATTGCAAGGAGGAATGCAACTGAAAGATACATGCCTCGCTATGGCT
LC457968.1      CAGCTGAGGCATACATTGCAAAGAGGAATGCGACTGAACGATATATGCCTCGATATGGAT
LC457969.1      CAGCTGAGGCATACATTGCAAAGAGGAATGCGACTGAACGATATATGCCTCGATATGGAT
MW002438.1      CGGCAGAGGCATACATCGCGAAGAGGAATGCAACTGAGAGGTACATGCCGCGGTATGGAA
KJ789898.1      CAGCAGAGGCGTATATTGCTAAGAGAAATGCAACGGAGCGTTACATGCCTCGATACGGAC
                * ** ** ** ** ** ** *   * ** ** *  **  * ** ***** ** *  **

MG564626.1      TTAAAAGGAATTTGACTGACATTAGCCTCGCTCGATATGCTTTCGACTTCTATGAGGTGA
MG564601.1      TTAAAAGGAATCTGACTGACATTAGCCTCGCTCGATATGCTTTCGATTTCTATGAGGTGA
DQ449533.1      TCAAGAGGAATTTGACTGACATTAGTCTCGCTAGATATGCTTTCGATTTCTATGAGGTGA
MG564629.1      TTAAGAGGAATTTGACTGACATAAGCCTCGCTAGATATGCTTTCGATTTCTATGAGGTGA
AF506900.1      TCAAGAGGAATTTGACTGATATTAGTCTTGCTAGATATGCTTTCGACTTCTATGAGGTGA
KT884449.1      TTAAGAGAAATTTGACTGACATAAGCCTCGCTAGATATGCTTTCGACTTCTATGAGGTTA
KY448332.1      TAAAGAGAAACTTGACTGACATTAGCCTCGCCAGATATGCCTTCGACTTTTATGAGATAA
AB583207.1      TAAAGAGGAATTTGACTGACATTAGCCTCGCCAGATATGCCTTCGACTTTTATGAGATAA
KY448324.1      TAAAGAGGAATTTGACTGACATTAGCCTCGCCAGATATGCCTTCGACTTTTATGAGATTA
KF888662.2      TGAAGAGGAATTTGAATGGCATTAGCCTCGCCAGGTATGCCTTCGATTTTTATGAGATAA
PRSV-W          TGAAGCGAAATTTGACTGACATAAGCCTCGCTAGATATGCCTTTGACTTTTATGAGATCA
KY448325.1      TGAAGCGAAATTTGACTGACATAAGCCTCGCTAGATATGCCTTTGACTTTTGTGAGATCA
AB127935.1      TAAAGCGAAACTTGACTGACATAAGCCTCGCTAGATATGCCTTCGATTTCTATGAGATCA
AB583214.1      TGAAGCGAAATTTGACTGACATAAGCCTCGCCAGATATGCCTTCGATTTCTATGAAATAA
AB583212.1      TGAAGCGAAATTTGACTGACATAAGCCTCGCCAGATATGCCTTCGATTTCTATGAAATAA
AB583204.1      TGAAGCGAAATTTGACTGACATAAGCCTCGCCAGATATGCCTTCGATTTCTATGAAATAA
AB583223.1      TGAAGCGAAATTTGACTGACATAAGCCTCGCCAGATATGCCTTCGATTTCTATGAAATAA
AB583198.1      TGAAGAGAAATTTGACTGACATAAGCCTCGCCAGATATGCCTTCGATTTCTATGAAATAA
AB583222        TGAAGCGAAATTTGACTGACATAAGCCTCGCCAGATATGCCTTTGACTTTTATGAAATAA
MK988414        TGAAGCGAAATTTGACTGACATAAGCCTCGCCAGATATGCCTTCGACTTTTATGAAATAA
AB583205.1      TGAAGCGAAATTTGACTGACATAAGCCTCGCTAGATATGCCTTCGACTTTTATGAAATAA
KF649243.1      TAAAGCGAAATTTGACTGATATAAGCCTCGCCAGATATGCTTTYGACTTTTATGAGATAA
KF649240.1      TAAAGCGAAATTTGACTGATATAAGCCTCGCCAGATATGCTTTCGACTTTTATGAGATAA
LC457968.1      TAAAGAGGAATTTGACTGACATTAGCCTCGCTAGATATGCCTTCGACTTTTATGAGATAA
LC457969.1      TAAAGAGGAATTTGACTGACATTAGCCTCGCTAGATATGCCTTCGACTTTTATGAGATAA
MW002438.1      TCAAGAGGAATTTGACTGACATTAGTCTCGCTAGATATGCTTTCGATTTCTATGAGGTGA
KJ789898.1      AAAAACGAAACCTCAGGGACATCAGCCTGSCCAGATATGCTTTTGATTTTTACGAAATGA
                  **  * **  * *  *  ** ** **  *  * ***** ** ** ** *  **  * *

MG564626.1      ATTCGAAAACACCTGATAGGGCTCGTGAAGCTCATATGCAGATGAAGGCAGCAGCGCTAC
MG564601.1      ATTCGAAAACACCTGATAGGGCTCGTGAAGCTCATATGCAGATGAAGGCAGCAGCGCTAC
DQ449533.1      ATTCAAAAACACCTGATAGGGCTCGTGAAGCTCATATGCAGATGAAGGCTGCAGCGCTGC
MG564629.1      ATTCAAAAACGCCTGATAGGGCTCGTGAAGCTCATATGCAGATGAAGGCTGCAGCGCTGC
AF506900.1      ATTCAAAAACACCTGATAGGGCACGTGAAGCTCATATGCAGATGAAGGCTGCAGCGCTGC
KT884449.1      ACTCAAAGACACCTGATAGAGCTCGTGAGGCTCATATGCAGATGAAAGCTGCAGCTCTGC
KY448332.1      ATTCAAAGACTCCTGATCGAGCTCGAGAAGCACATATGCAGATGAAGGCAGCGGCTCTCC
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AB583207.1      ATTCAAAGACTCCTGATCGAGCTCGAGAAGCACATATGCAGATGAAGGCAGCGGCTCTCC
KY448324.1      ATTCAAAGACTCCTGATCGAGCTCGAGAAGCACATATGCAGATGAAGGCAGCGGCTCTCC
KF888662.2      ATTCAAAGACTCCTGATCGAGCTCGTGAAGCACATATGCAGATGAAGGCAGCGGCTCTCC
PRSV-W          ACTCAAAGACTCCTGATCGAGCACGTGAAGCTCATATGCAGATGAAGGCAGCGGCCCTCC
KY448325.1      ACTCAAAGACTCCTGATCGAGCACGTGAAGCTCATATGCAGATGAAGGCAGCGGCCCTCC
AB127935.1      ATTCAAAAACTCCTGATCGAGCACGTGAAGCTCATATGCAGATGAAGGCAGCGGCCCTCC
AB583214.1      ACTCAAAGACTCCTGATCGAGCTCGTGAGGCACACATGCAGATGAAGGCAGCGGCACTCC
AB583212.1      ACTCAAAGACTCCTGATCGAGCTCGTGAGGCACACATGCAGATGAAGGCAGCGGCACTCC
AB583204.1      ACTCAAAGACTCCTGATCGAGCTCGTGAGGCACACATGCAGATGAAGGCAGCGGCACTCC
AB583223.1      ACTCAAAGACTCCTGATCGAGCTCGTGAGGCACACATGCAGATGAAGGCAGCGGCACTCC
AB583198.1      ACTCAAAGACTCCTGATCGAGCTCGTGAGGCACATATGCAGATGAAGGCAGCGGCACTCC
AB583222        CCTCGAAGACTCCTGATCGAGCTCGTGAGGCACATATGCAGATGAAGGCAGCGGCACTCC
MK988414        ACTCAAAGACTCCTGATCGAGCTCGTGAGGCACATATGCAGATGAAGGCAGCGGCACTCC
AB583205.1      ACTCAAAGACTCCTGACCGAGCTCGTGAGGCACATATGCAGATGAAGGCAGCGGCACTCC
KF649243.1      ACTCAAAGACTCATGCGC------------------------------------------
KF649240.1      ACTCAAAGACTC-CGCGC------------------------------------------
LC457968.1      ACTCAAAGACTCCTGATCGAGCTCGAGAAGCACATATGCAGATGAA--------------
LC457969.1      ACTCAAAGACTCCTGATCGAGCTCGAGAAGCACATATGCAGATGAAAGCCGCAGC-----
MW002438.1      ATTCAAAAACACCTGATAGGGCTCGTGAAGCTCATATGCAGATGAAGGCTGCAGCGCTGC
KJ789898.1      CTTCCAAGACTCCTGAGAGAGCAAGAGAGGCACACATGCAGATGAAAGCAGCAGCGATTA
                  ** ** ** *  *

MG564626.1      GTAACACTAGTCGCAGAATGTTCGGAATGGACGGCAGTGTCAGTAACAAGGAAGAAAACA
MG564601.1      GTAACACTAGTCGCAGAATGTTCGGAATGGACGGCAGTGTCAGTAACAAGGAGGAAAACA
DQ449533.1      GTAATACTGGTCGCAGAATGTTTGGAATAGACGGCAGTGTCAGTAACAAGGAAGAAAACA
MG564629.1      GCAATACTAATCGCAGAATGTTTGGAATGGACGGCAGTGTCAGTAACAAGGAAGAAAACA
AF506900.1      GCAACACTAGTCGCAGAATGTTTGGAATGGACGGCAGTGTCAGTAACAAGGAAGAAAACA
KT884449.1      GAAACACTAATCGCAGAATGTTTGGTATGGACGGCAGTGTCAGTAACAAGGAAGAAAACA
KY448332.1      GCAACGCAAACCGACGCTTATTTGGAATTGACGGCAGTGTCAGTAACAGGGAAGAGAACA
AB583207.1      GAAACGCGAACCGGCGCTTATTTGGAATTGACGGCAGTGTCAGCAACAGGGAAGAGAACA
KY448324.1      GCAACGCAAACCGGCGCTTATTTGGAATTGACGGAAGTGTCAGTAACAGGGAAGAGAACA
KF888662.2      GCAACGCGAACCGGCGCTTATTTGGAATTGACGGCAGTGTCAGTAACAGGGAAGAGAACA
PRSV-W          GCAACGCTAACAGGCGCTTATTTGGAATAGACGGCAGTGTCAGTAACAGGGAAGAGAACA
KY448325.1      GCAACGCCAACAGGCGCTTATTTGGAATAGACGGCAGCGTTAGTAACAGGGAAGAGAACA
AB127935.1      GCAACGCCAACAGGCGTTTATTTGGAATAGACGGTAGCGTCAGTAACAGGGAAGAGAACA
AB583214.1      GTAACGCCAACAGGCGTTTATTTGGAATTGACGGTAGTGTCAGTAACAGGGAAGAGAACA
AB583212.1      GTAACGCCAACAGGCGTTTATTTGGAATTGACGGTAGTGTCAGTAACAGGGAAGAGAACA
AB583204.1      GTAACGCCAACAGGCGTTTATTTGGAATTGACGGTAGCGTCAGTAACAGGGAAGAGAACA
AB583223.1      GTAACGCCAACAGGCGTTTATTTGGAATTGACGGTAGTGTCAGTAACAGGGAAGAGAACA
AB583198.1      GTAACGCCAACAGGCGTTTATTTGGAATTGACGGTAGCGTCAGTAACAGGGAAGAGAACA
AB583222        GCAACGCCAACAGGCGTTTGTTTGGAATTGACGGCAGCGTCAGTAACAGGGAAGAGAACA
MK988414        GCAACGCCAACAGGCGTTTATTTGGAATTGATGGTAGCGTCAGTAACAGGGAAGAGAACA
AB583205.1      GCAACGCCAACAGGCGTTTATTTGGAATTGACGGTAGCGTCAGTAACAGGGAAGAGAACA
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
LC457968.1      ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
MW002438.1      GTAATACTGGTCGCAGAATGTTTGGAATAGACGGCAGTGTCAGTAACAAGGAAGAAAACA
KJ789898.1      GAGGTGCGAACACTCGATTGTTTGGCATTGATGGAAATGTTGGTGGGGGAGAAGAGAACA


MG564626.1      CGGAGAGACACACAGTGGATGATGTCAACAGAGACATGCACTCTCTCCTGGGTATGCGCA
MG564601.1      CGGAGAGACACACAGTGGATGATGTCAACAGAGACATGCACTCTCTCCTGGGTATGCGCA
DQ449533.1      CGGAGAGACACACAGTGGAAGATGTCAACAGAGACATGCACTCTCTCCTGGGTATGCGCA
MG564629.1      CGGAGAGACACACAGTGGAAGATGTCAACAGAGACATGCACTCTCTCCTGGGTATGCGCA
AF506900.1      CGGAGAGACACACAGTGGAAGATGTCAACAGAGACATGCACTCTCTCCTGGGTATGCGCA
KT884449.1      CGGAGAGACACACAGTTGAAGATGTCAATAGAGACATGCACTCTCTCCTGGGTATGCGCA
KY448332.1      CGGAGAGACACACTGTTGAAGATGTTGACCGCGACATGCACTCTCTCCTGGGTATGCGCA
AB583207.1      CGGAGAGACACACTGTTGAGGATGTCGACCGCGACATGCACTCTCTCCTTGGAATGCGTA
KY448324.1      CGGAGAGACACACTGTTGAGGATGTCGACCGCGACATGCACTCTCTCCTGGGTATGCGCA
KF888662.2      CGGAGAGACACACTGTTGAGGATGTCGACCGCGATTTGCACTCCCCCCTTGGAATGCG--
PRSV-W          CGGAGAGACACACTGTTGAGGATGTCGATCGCGACATGCACTCTCTCCTGGGTAT-----
KY448325.1      CGGAGAGACACACTGTTGAGGATGTCGATCGCGACATGCACTCTCTCCTGGGTATGCGCA
AB127935.1      CGGAGAGACACACTGTTGAGGATGTTGATCGCGACATGCACTCTCTCCTTGGAATGCGCA
AB583214.1      CGGAGAGACACACTGTTGAGGACGTCGAACGCGACATGCACTCTCTCCTTGGAATGCGCA
AB583212.1      CGGAGAGACACACTGTTGAGGACGTCGAACGCGACATGCACTCTCTCCTTGGAATGCGCA
AB583204.1      CGGAGAGACACACTGTTGAGGACGTCGAACGCGACATGCACTCTCTCCTTGGAATGCGCA
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AB583223.1      CGGAGAGACACACTGTTGAGGACGTCGAACGCGACATGCACTCTCTCCTTGGAATGCGCA
AB583198.1      CGGAGAGACACACTGTTGAGGATGTCGAACGCGACATGCACTCTCTCCTTGGAATGCGCA
AB583222        CGGAGAGACACACTGTTGAGGATGTCGACCGCGACATGCACTCTCTCCTTGGAATGCGCA
MK988414        CGGAGAGACACACTGTTGAGGATGTCGATCGCGACATGCACTCTCTCCTTGGAATGCGCA
AB583205.1      CGGAGAGACACACTGTTGAGGATGTCGATCGCGACATGCACTCTCTCCTTGGAATGCGCA
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
LC457968.1      ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
MW002438.1      CGGAGAGACACACAGTGGAAGATGTCAACAGAGACATGCACTCTCTCCTGGGTATGCGCA
KJ789898.1      CGGAGAGACACACTGTTGAAGATGTTGATCGCGATATGCATAGCCTCCTGGGCATGMRAA


MG564626.1      ACTAA-------------------------------------------------------
MG564601.1      ATTAA-------------------------------------------------------
DQ449533.1      ACTAA-------------------------------------------------------
MG564629.1      ACTAA-------------------------------------------------------
AF506900.1      AT----------------------------------------------------------
KT884449.1      AC----------------------------------------------------------
KY448332.1      AC----------------------------------------------------------
AB583207.1      AGTGA---------------TGTCGGCTAGGCCTGGCTTAGCCCTGTTCCCCCT----AT
KY448324.1      AC----------------------------------------------------------
KF888662.2      ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
KY448325.1      AC----------------------------------------------------------
AB127935.1      AGTGA---------------TGTTGGCTGGGC-TGGCTTAGCCGTGTTCCCCCT----AT
AB583214.1      AGTGA---------------TGTTGGTTAGGCGTAGCTTAGCCGTGTTCCCCCT----AT
AB583212.1      AGTGA---------------TGTCGGCTAGGCCTGGCTTAGCCGTGTTCCCCCT----AT
AB583204.1      AGTGA---------------TGTCGGCTAGGCCTGGCTTAGCCGTGTTCCCCCT----AT
AB583223.1      AGTGA---------------TGTCGGCTAGGCCTGGCTTAGCCGTGTTCCCCCT----AT
AB583198.1      AGTGA---------------TGTCGGCTAGGCCTGGCTTAGCCGTGTTCCCC-T----AT
AB583222        AGTGA---------------TGTCGGCTAGGCCTGGCTTAGTCGTGTTCCCC-T----AT
MK988414        AGTGA---------------TGTTGGCTAGGCCTAGCTTAGCCGTGTTCCCCTT----AT
AB583205.1      AGTGA---------------TGTTGGTTAGGCGTAGCTTAGCCGTGTTCCCCCT----AT
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
LC457968.1      ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
MW002438.1      ATTGAATACTCGCGCTAGTGTGTTCGTCGGGCCTGGCTCGACCCTGTTTCACCTTATAGT
KJ789898.1      AGTGAG-------------GTCTCGGTTTTAGCCCGCGAAGGAGTCTCTTCTCC-AAAAT


MG564626.1      ------------------------------------------------------------
MG564601.1      ------------------------------------------------------------
DQ449533.1      ------------------------------------------------------------
MG564629.1      ------------------------------------------------------------
AF506900.1      ------------------------------------------------------------
KT884449.1      ------------------------------------------------------------
KY448332.1      ------------------------------------------------------------
AB583207.1      GATATAAGTAACCGTATCTAGTAGTGTGGTTTCACCACCGTATTTACGTTTCAGTGAGGG
KY448324.1      ------------------------------------------------------------
KF888662.2      ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
KY448325.1      ------------------------------------------------------------
AB127935.1      AGTATAAGTAACCGTATCTAATAGTGTGGTCTTACCACCGTATTTACGTTTCAGTGAGGG
AB583214.1      AGTATAAGTAACCGTATCTAATAGTGTGGTTTTACCACCGTATTTACGATTTAGTGAGGG
AB583212.1      AGTATAAGTAACCGTATCTAATAGTGTGGTCATACCACCGTATTTACGTTTTAGTGAGGG
AB583204.1      AGTATAAGTAACCGTATCTAATAGTGTGGTCATACCACCGTATTTACGTTTTAGTGAGGG
AB583223.1      AGTATAAGTAACCGTATCTAATAGTGTGGTCATACCACCGTATTTACGTTT---------
AB583198.1      AGTATAAGTAACCGTACCTAATAGTGTGGTCTTACCACCGTATTTACG-TTTAGTGAGGG
AB583222        AGTATAAGTAACCGTATCTAATAGTGTGGTTTTCCCACCGTATTTACGTTC---------
MK988414        AGTATAAGTAACCGTATCTAATAGTGTGGTCTTACCACCGTATTTACGTTTCAGTGAGGG
AB583205.1      AGTATAAGTAACCGTATCTAATAGTGTGGTTTTACCACCGTATTTACGATTTAGTGAGGG
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
LC457968.1      ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
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MW002438.1      ACTATCATAAGCATTAGAATACAGTGTGGCTGCGCCACCGCTTCTATTTTACAGTGAGGG
KJ789898.1      ACTATAAGGTACACTTATTAGTAGTGAGGTTATACCTCCTTTTATAGTGTTTAGTGAGGG


MG564626.1      ------------------------------------------------------------
MG564601.1      ------------------------------------------------------------
DQ449533.1      ------------------------------------------------------------
MG564629.1      ------------------------------------------------------------
AF506900.1      ------------------------------------------------------------
KT884449.1      ------------------------------------------------------------
KY448332.1      ------------------------------------------------------------
AB583207.1      TGGCCCTCCT--TGTTGCTTGTATTATTCACATTCTCTGAGCTTTGTTTCAGTGTGGGTG
KY448324.1      ------------------------------------------------------------
KF888662.2      ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
KY448325.1      ------------------------------------------------------------
AB127935.1      TGGCCCTCCT--TGTTGCTTATATTATTCGAGTTCTCTAAGCTTTGTTTCAGTGTGGGCG
AB583214.1      TGGCCCTCCT--TGTTGCTTGTATTATTCGAGTTCTCTAAGCTTTGTTCCAGTGTGGGTG
AB583212.1      TTGCCCTCCT--TGTTGCTTATATTATTCGAGTTCTTTAAGCTTTGCTCCAGTGTGGGTG
AB583204.1      TGGCCCTCCT--TGTTGCTTATATTATTCGAGTTCTTTAAGCTTTGCTCCAGTGTGGGTG
AB583223.1      ------------------------------------------------------------
AB583198.1      TGGCCCTCCT--TGTTGCTTATATTATTCGAGTTCTTTAAGCTTTGCTCCAGTGTGGGTG
AB583222        ------------------------------------------------------------
MK988414        TGGCCCTCCT--TGTTGCTTATATTATTCGAGTTCTCTAAGCTTTGTTCCAGGGTGGGTG
AB583205.1      TGGCCCTCCT--TGTTGCTTGTATTATTCGAGTTCTCTAAGCTTTGTTCCAGTGTGGGTG
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
LC457968.1      ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
MW002438.1      TAGCCCTCCG--TGCTTTTAGTATTATTCGAGTTCTCTGAGTCTCCATACAGTGTGGGTG
KJ789898.1      CTTCCCTCCTCTTATCCTTATTAGTATTCGAGCTATCTGTTCCAGTTTCGCGTGAGGCTG


MG564626.1      --------------------------------------------------------
MG564601.1      --------------------------------------------------------
DQ449533.1      --------------------------------------------------------
MG564629.1      --------------------------------------------------------
AF506900.1      --------------------------------------------------------
KT884449.1      --------------------------------------------------------
KY448332.1      --------------------------------------------------------
AB583207.1      TCCCACGTGTACTCAAATCTCTTGGAATGAGAG-----------------------
KY448324.1      --------------------------------------------------------
KF888662.2      --------------------------------------------------------
PRSV-W          --------------------------------------------------------
KY448325.1      --------------------------------------------------------
AB127935.1      TCCCACGTGTACTCAAATCTTTTAGAATGAGAGAAAAAAAAAAAAAAAAAAAAAAA
AB583214.1      TCCCACGTGTGTTCAAATCTTTTAGAATGAGAG-----------------------
AB583212.1      TCCCACGAGTGCTCAAATCTTTTAGAATGAGA------------------------
AB583204.1      TCCCACGAGTGATCAAATCTTTTAGAATGAGAG-----------------------
AB583223.1      --------------------------------------------------------
AB583198.1      GCCCACGAGTGCTCAAATCTTTTAGAATGAGAG-----------------------
AB583222        --------------------------------------------------------
MK988414        TCCCACGTGTGTTCAAATCTTCTAGAATGAGAG-----------------------
AB583205.1      TCCCACGTGTGTTCAAATCTTTTAGAATGAGAG-----------------------
KF649243.1      --------------------------------------------------------
KF649240.1      --------------------------------------------------------
LC457968.1      --------------------------------------------------------
LC457969.1      --------------------------------------------------------
MW002438.1      GCCCACGTGCTATTCGAGC-------------------------------------
KJ789898.1      AGCCTCG-------------------------------------------------
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Multiple alignment of PRSV-W test isolate with other PRSV-W isolates at amino acid level using ClustalW

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KT884449.1      ------------------------------------------------------------
AF506900.1      ------------------------------------------------------------
MG564626.1      ------------------------------------------------------------
MG564601.1      ------------------------------------------------------------
DQ449533.1      ------------------------------------------------------------
MG564629.1      ------------------------------------------------------------
MW002438.1      ------------------------------------------------------------
KY448332.1      ------------------------------------------------------------
LC457968.1      ------------------------------------------------------------
AB583207.1      KSKLPEHRLEAIMAAIIESWGYEELTDQIRKFYQWVLEQAPYNELAKQGKAPYVSELGLR
KY448324.1      ------------------------------------------------------------
KF888662.2      ------------------------------------------------------------
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
AB127935.1      -------------------GYEELTDQIRKFYQWVLEQAPYNELDDEKAPYVSELGLRRL
MK988414        ------------------------------------------------------------
KY448325.1      ------------------------------------------------------------
AB583205.1      KSKLPEHRLEAIMAAIIESWGYEELTDQIRKFYQWVLEQAPYNELAKQGKAPYVSELGLR
AB583212.1      KSKLPEHRLEAIMAAIIESWGYEELTDQIRKFYQWVLEQAPYNELAKQGKAPYVSELGLR
AB583204.1      KSKLPEHRLEAIMAAIIESWGYEELTDQIRKFYQWVLEQAPYNELAKQGKAPYVSELGLR
AB583214.1      KSKLPEHRLEAIMAAIIESWGYEELTDQIRKFYQWVIEQAPYNELAKQGKAPYVSELGLR
AB583223.1      ------------------------------------------------------------
AB583198.1      KSKLPEHRLEAIMAAIIESWGYEELTDQIRKFYQWVLEQAPYNELAKQGKAPYVSELGLR
AB583222        ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
KJ789898.1      --------------------------------------QAPYSDLALKGKAPYVSETGLR


KT884449.1      ------------------------------------------------------------
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MG564626.1      ------------------------------------------------------------
MG564601.1      ------------------------------------------------------------
DQ449533.1      ------------------------------------------------------------
MG564629.1      ------------------------------------------------------------
MW002438.1      ------------------------------------------------------------
KY448332.1      --------------------------------------------------------SKNE
LC457968.1      ------------------------------------------------------------
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KY448324.1      --------------------------------------------------------SKDG
KF888662.2      ------------------------------------------------------------
KF649243.1      ------------------------------------------------------------
KF649240.1      ------------------------------------------------------------
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MK988414        ------------------------------------------------------------
KY448325.1      --------------------------------------------------------SKDG
AB583205.1      RLYTSTRGSCAELEVYLSKYFEDESGDTPELVVYHESVGPNKQTIASLQSKNVFYQSKDG
AB583212.1      RLYTSTRGSCAELEVYLSKYFEDESGDTPELVVYHESVSPEKQTVASLQSKNVFYQSKDG
AB583204.1      RLYTSTRGSCAELEVYLSKYFEDESGDTPELVVYHESVSPEKQTVASLQSKNVFYQSKDG
AB583214.1      RLYTSTRGSCAELEVYLSKYFEDESGDTPELVVYHESVSPEKQTVASLQSKNVFYQSKDG
AB583223.1      ------------------------------------------------------------
AB583198.1      RLYTSARGSCAELEVYLSKYFEDESGDTPELVVYHESVGPVKQTIASLHSKNVFYQSKDG
AB583222        ------------------------------------------------------------
PRSV-W          ------------------------------------------------------------
LC457969.1      ------------------------------------------------------------
KJ789898.1      NLYTSQRGSPQELEKYITHYFRSESGD-----------------------------CPEL


KT884449.1      -QSKTEVDAGLNEKLKEKETQKEKG--IEKQKEKNNNEASDGNDVSTSTKTGEKDRDVNA
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MG564626.1      -SKNEAVDAGLNEKLKEKEKQREKEKEKDKQKEKDNDGASDGNDVSTSTKTGERDRDVNA
MG564601.1      -SKNEAVDAGLNEKLKEKERQREKEKEKDKQKEKDNDGASDGNDVSTSTKTGERDRDVNA
DQ449533.1      MSKNEAVDAGLNEKLKEKEKLKEKEKEK--QKEKDNDGASDGNDVSTSTKTGERDRDVNA
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LC457968.1        ------------------------------------------------------------
AB583207.1        AVDAGVNAKLKEKEEKEKEKEKEKEKEKEKEKIKDA--PEASSQKTNSEKERERDRDVNA
KY448324.1        AVDAGVNAKMKEKEEKEKEKEKEKDKEKEKTKDA--PEASSQKTDSEKERERDRDVNA
KF888662.2        ------------------------------------------------------------
KF649243.1        ------------------------------------------------------------
KF649240.1        ------------------------------------------------------------
AB127935.1        DAGVNAKLKEKEEKEKEKEKEKEKEKEKEKVKDE--PETSKQDLNSGTERERDRDVNA
MK988414          --------------------------------------------------------DVNA
KY448325.1        AVDAGVNAKLKEKEEKEKEKEKEKEKEKEKEKLKDE--PEASKQNLNSGTEKERDRDVNA
AB583205.1        AVDAGVNAKLKEKEEKEKEKEKEKEKEKEKEKEKDV--PETSNQDTKSEKERERDRDVNA
AB583212.1        AVDAGVNAKLKEKEEKEKEKEKEKEREKEKEKEKLKNVPETSNQDTESITERERDRDVNA
AB583204.1        AVDAGVNAKLKEKEEKEKEKEKEKEREKEKEKEKLKNVPETSNQDTESITKRERDRDVNA
AB583214.1        AVDAGVNAKLKEKEEKEKEKEKEKEREKEKEKEKLKNVPETSNQDTESITERERDRDVNA
AB583223.1        -----------------------------------------------------------A
AB583198.1        AVDAGVNAKLKEKEEKEKEKEKE--REKEKEKEKLKDVPETSNQDTESIAERERDRDVNA
AB583222          -----------------------------------------------------------A
PRSV-W            ------------------------------------------------------------
LC457969.1        ------------------------------------------------------------
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AF506900.1        GTSGTFTVPRIKPFTDKMILPRIKGKTVLNLNHLLQYNPQQIDISNTRATQSQFEKWYEG
MG564626.1        GTSGTFTVPRIKSFTDKMILPRIKGRSVLNLNHLLQYNPQQIDISNTRATQSQFEKWYEG
MG564601.1        GTSGTFTVPRIKSFTDKMILPRIKGRSVLNLNHLLQYNPQQIDISNTRATQSQFEKWYEG
DQ449533.1        GTSGTFTVPRIKSFTDKMILPRIKGKTVLNLNHLLQYNPQQIDISNTRATQSQFEKWYEG
MG564629.1        GTSGTFTVPRIKSFTDKMILPRVRGKTVLNLNHLLQYNPQQIDISNTRATQSQFEKWYEG
MW002438.1        ------------------------------------------------------------
KY448332.1        GTMGSFTVPRIKTFTDKMILPKFKGKTVLNLNHLIQYNPQQIDISNTRATQSQFDKWYGG
LC457968.1        ------------------------------------------------------------
AB583207.1        GTMGSFTVPRIKTFTDKMILPKFKGKTVLNLNHLIQYNPQQIDISNTRATQSQFDKWYEG
KY448324.1        GTMGSFTVPRIKTFTDKMILPKFKGKTVLNLNHLIQYNPQQIDISNTRATQSQFDKWYEG
KF888662.2        ------------------------------------------------------------
KF649243.1        ------------------------------------------------------------
KF649240.1        ------------------------------------------------------------
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KY448325.1        GTMGSFTVPRIKTFTDKMILPKFKGKTVLNLNHLIQYNPQQIDISNTRATQSQFDKWYEG
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AB583212.1        GTMGSFTVPRIKTFTDKMILPKFKGKTVLNLNHLLQYNPLQFDISNTRATRSQFDKWYEG
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AB583214.1        GTMGSFTVPRIKTFTDKMILPKFKGKTVLNLNHLLQYNPLQFDISNTRATRSQFDKWYEG
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MW002438.1        --------------------------------------MDGETQVDYPIKPLIEHATPSF
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LC457968.1        --------------------------------SGVWVMMDGDVQVDYPIKPLIEHATPTF
AB583207.1        VRNDYGLDDNEMQVMLNGLMVWCIENGTSPDVSGVWVMMDGDVQVDYPIKPLIEHATPTF
KY448324.1        VRNDYGLNDNEMQVMLNGLMVWCIENGTSPDVSGVWVMMDGDVQVDYPIKPLIEHATPTF
KF888662.2        -----------------------------------WVMMDGDVQVDYPIKPLIEHATPTF
KF649243.1        ---------------------WCIENGTSPDVSGIWVMMEGGNQVDFPIKPLIEHATPTF
KF649240.1        ---------------------------------WYLGYDGGGNQVDFPIKPLIEHATPTF
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KY448325.1      VRNDYGLNDSEMQVMLNGLMVWCIENGTSPDVSGVWVMMDGDTQVDYPIKPLIEHATPTF
AB583205.1      VRNDYGLNDNEMQVMLNGLMVWCIENGTSPDASGVWVMMDGETQVDYPIKPLIEHATPTF
AB583212.1      VRNDYGLDDNEMAVMLNGLMVWCIENGTSPDVSGVWVMMDGDTQVDYPIKPLIEHATPTF
AB583204.1      VRNDYGLDDNEMAVMLNGLMVWCIENGTSPDVSGVWVMMDGDTQVDYPIKPLIEHATPTF
AB583214.1      VRNDYGLDDNEMAVMLNGLMVWCIENGTSPDVSGVWVMMDGDTQVDYPIKPLIEHATPTF
AB583223.1      VRNDYGLDDNEMAVMLNGLMVWCIENGTSPDVSGVWVMMDGDTQVDYPIKPLIEHATPTF
AB583198.1      VRNDYGLDDNEMAVMLNGLMVWCIENGTSPDVSGVWVMMDGDTQVDYPIKPLIEHATPTF
AB583222        VRNDYGLNDNEMAVMLNGLMVWCIENGTSPDVSGVWVMMDGDTQVDYPIKPLIEHATPTF
PRSV-W          -------------------------------------MMDGDTQVDYPIKPLIEHATPTF
LC457969.1      --------------------------------------------------------TPTF
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MK988414        RQIMAHFSNAAEAYIAKRNATERYMPRYGLKRNLTDISLARYAFDFYEINSKTPDRAREA
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MW002438.1      HMQMKAAALRNTGRRMFGIDGSVSNKEENTERHTVEDVNRDMHSLLGMRN
KY448332.1      HMQMKAAALRNANRRLFGIDGSVSNREENTERHTVEDVDRDMHSLLGMRN
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KY448324.1      HMQMKAAALRNANRRLFGIDGSVSNREENTERHTVEDVDRDMHSLLGMRN
KF888662.2      HMQMKAAALRNANRRLFGIDGSVSNREENTERHTVEDVDRDLHSPLGMR-
KF649243.1      --------------------------------------------------
KF649240.1      --------------------------------------------------
AB127935.1      HMQMKAAALRNANRRLFGIDGSVSNREENTERHTVEDVDRDMHSLLGMRK
MK988414        HMQMKAAALRNANRRLFGIDGSVSNREENTERHTVEDVDRDMHSLLGMR-
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AB583205.1      HMQMKAAALRNANRRLFGIDGSVSNREENTERHTVEDVDRDMHSLLGMRK
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LC457969.1      HMQMKAAA------------------------------------------
KJ789898.1      HMQMKAAAIRGANTRLFGIDGNVGGGEENTERHTVEDVDRDMHSLLGMXK
```

CERTIFICATE-IV

Certified that all necessary corrections as suggested by the external examiner and advisory committee have been duly incorporated in the thesis entitled **"Identification and Characterization of viruses causing Mosaic of bottle gourd in Jammu sub-tropics"**, submitted by **Ms. Sneha Choudhary**, Registration No, **J-18-D-350-A**.

Dr. Ranbir Singh
Major Advisor

Place Jammu
Date: 25/08/22

Head
Division of Plant Pathology

VITA

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Qualification	**Month**	**Year**	**School/College**	**Board/University**	**Marks (%)**	**Division**
10th	March	2008	K.V. No 2, Colaba, Mumbai	CBSE, Chennai region	81.20	First
10+2	March	2010	NKSDCP, Ghuggar, Palampur	HPBSE, Dharamshala	83.14	First
B.Sc. Agriculture	July	2014	College of agriculture	CSKHPKV, Palampur	80.07 (8.07)	First
M.Sc. Plant Pathology	November	2016	College of agriculture	CSKHPKV, Palampur	83.50 (8.35)	First
Ph.D. Plant Pathology	June	2022	Faculty of Agriculture	SKUAST- J, Chatha, Jammu	90.5 (9.05)	First

Title of Master's Thesis

"Molecular Characterization of BCMV NL-7n and Yield Loss Assessment in Common Bean"

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- ASPEE gold medalist for the year 2013 - 2014
- ICAR-Senior Research Fellowship during Ph. D. (from 2019 to 2021)
- NET qualified in 2017 and 2018