CHARACTERIZATION OF PAPAYA RING SPOT (PRSV) AND PAPAYA LEAF CURL (PaLCuV) VIRUSES INFECTING PAPAYA, EPIDEMIOLOGY AND MANAGEMENT OF PRSV DISEASE

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AUGUST, 2021

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in

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CERTIFICATE

This is to certify that the thesis entitled **"CHARACTERIZATION OF PAPAYA RING SPOT (PRSV) AND PAPAYA LEAF CURL (PaLCuV) VIRUSES INFECTING PAPAYA, EPIDEMIOLOGY AND MANAGEMENT OF PRSV DISEASE"** submitted in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **PLANT PATHOLOGY** to the University of Horticultural Sciences, Bagalkot, is a record of bonafide research work carried out by **Mr. PREMCHAND U, UHS15PGD141** under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.

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Affectionately Dedicated

to My Beloved Parents,

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1. INTRODUCTION

Carica papaya L. belongs to the family Caricaceae, commonly known as "Papaya". It is also known as paw paw, papaw tree melon, the fruit of angels and poor man's fruit (Aykroyd, 1951). The family of Caricaceae comprised of 34 to 35 species in six genera *i.e.*, *Carica, Cylicomorpha, Horovitzia, Jacaratia, Jarilla* and *Vasconcellea* (Badillo, 1971). In *Carica* genera, only one species is available *i.e.*, *Carica papaya* L. which is the commercially cultivated species and most economically important fruit crop of the tropical and subtropical regions of the world. It is a dicotyledonous, polygamous and diploid species with the geographical origin being southern Mexico and Costa Rica (De Candolle, 1884). It was introduced into India during the 16^{th} century by Portuguese travelers and was spread to the southern part of India (Morton and Miami, 1987). The importance of papaya in the world's economy is demonstrated by its wide distribution and substantial production in tropical countries. It has been cultivated in Brazil, China, India, Indonesia, Jamaica, Mexico, Nigeria, Peru, Philippines, Taiwan, Thailand and USA.

Papaya is mostly cultivated on well drained soil and is mainly propagated by seeds. Plant are herbaceous, but their stature is not that of a typical herbaceous. It is a single stemmed plant that grows up to a height of 9 m and is thus described as a giant herb. The stem is hollow, green or deep purple of about 30 to 40 cm thick and roughened by alternate leaf scars. Leaves develop directly from the upper part of the stem in a spiral manner and horizontally on approximately 30 to 105 cm long petioles and it produces three types of flowers namely, the staminate, pistillate and hermaphrodite flowers. The plants have a rapid growth rate, usually short lived, can bear the fruits within 8 to 9 months after the planting but continue throughout the year (Malo and Campbell, 1986). Fruits are cylindrically long, pear or round shaped, often seen in colors of orange red, yellow green and yellow orange hues with rich sweet and juicy orange pulp when they ripe.

Papaya encompasses most of the desirable qualities of a fruit. Due to that, it is cultivated predominantly as fruits for consumption of flesh, preparation of salads, jams, ice-cream, flavoring, crystallized fruits, marmalade with shreds of orange, candy, pickles, squash and jelly *etc.*, (Amarsing,1996). Apart from that, it is also

grown for milky latex “papain” and almost all the plant parts including leaves, fruits, seeds, latex, bark and roots are used in several ways (Anibijuwon and Udeze, 2009).

Papaya is said to be the third richest fruit for vitamin ‘C’ content after aonla and citrus (Sankat and Maharaj, 1997). It is a powerhouse of nutrient, contains calcium (20 g), phosphorus (16 g), sodium (3 mg), potassium (234 mg), vitamin A (1750 IU), thiamine (0.04 mg), riboflavin (0.4 mg), niacin (0.3 mg) ascorbic acid (56mg), proteins (0.6 g), fat (0.1 g), sugar (10 to13 g) and water (88.7 g). Fruits has 179 to 208 kJ of energy per 100 g fresh weight. Papaya is considered as a multifaceted plant with anti cancerous, anti inflammatory, anti diabetic and anti oxidant properties (Aravind *et al.,* 2013 and Srivastava and Singh, 2016) due to which it is used as raw material in the pharmaceutical industry. It is also utilized in the cosmetic industry (Retuta *et al.,* 2012). The proteolytic enzyme ‘papain’ obtained from raw fruit is used for tenderizing meat, preparation of chewing gum, pre shrinking of wool, degumming natural silk, cosmetics *etc*., Further, its potential applications and uses are yet to be fully explored, derived and understood (Bashra and Tajul, 2013). Production of papaya requires relatively low maintenance costs with fewer needs for pesticides compared to most other tropical fruits and commercially significant production is possible within the first year of planting (Davis and Ying, 1999).

Owing to realizing its importance, the cultivation of papaya has significantly increased across the globe in the recent past. During 1985 the world papaya production was only 3.16 Mt with an area of 2.20 lakh ha having productivity of 14.3 t/ha but its production has increased readily up to 13.74 Mt in an area of 4.62 lakh ha having 29.69 t/ha of productivity in 2019. Similarly, in India during 1985 the production was 0.24 Mt in an area of 0.31 lakh ha having 7.74 t/ha productivity, whereas in 2019 the production is 6.05 Mt with in an area of 1.49 lakh ha having 40.6 t/ha productivity (Anon., 2019). India is the largest and leading producer of papaya in the world which shares 44.04 per cent of global production.

The major papaya growing Indian states are Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Chhattisgarh, West Bengal, Assam, Tamil Nadu and Jharkhand. Karnataka stands 3rd with respect to area and production (Anon., 2018). In Karnataka major papaya growing districts are Ballari, Bidar,

Chamarajanagar, Chitradurga, Mandya, Raichur, Kolar, Chikballapur, Koppal, Ramanagara and Tumkur (Anon., 2018).

At present, the sole cultivation of papaya has become more common after the advent of improved varieties and hybrids. The area under this crop is continuously increasing as farmers prefer its cultivation due to its high yield potential, less water requirement and attractive prices in the market. The crop is emerging as an alternative cash crop to bananas in Karnataka. The most popularly grown varieties in India are Coorg Honey Dew, Pusa Delicious, Pusa Majesty, Pusa Dwarf, Pusa Giant, CO-1, CO-2, CO-3, CO-4, CO-5, CO-6, Surya, Pusa Nanha, Pink flesh sweet, Pant-1, Sunrise Solo, Iceberg, Red Lady Taiwan, Arka Surya, Arka Prabhath *etc*., (Anon., 2018).

Many factors are responsible for the decrease in the papaya production, of which microbial infestation in papaya due to fungi, bacteria, nematode, phytoplasma and viruses are of big concern. These cause considerable losses in yield and deteriorate the quality of fruits. Common bacterial pathogens infecting papaya are *Erwinia* spp. causing cankers and black rot, *Pseudomonads* causing bacterial leaf spot and wilt. Fungal pathogens infecting papaya includes *Colletotrichum gloeosporioides* causing anthracnose; *Asperisporium caricae* causing black spots; *Cercospora papayae, Curvularia, Gloeosporium, Corynespora* causing leaf spots; *Alternaria alternate* and *Stemphylium lycopersici* causing fruit spots; *Fusarium solani, Alternaria, Phomopsis* causing fruit rot; *Guignardia* spp. causing guignardia fruit spots; *Cladosporium* spp., *Alternaria* spp. causing internal blight/internal smut; *Fusarium* spp., *Penicillium* spp., *Verticillium* spp. causing wilt; *Phytophthora palmivora* causing blight, stem rot, fruit rot and root rot; *Oidium caricae* causing powdery mildew; *Pythium* spp. causing pythium root rot; *Rhizopus stolonifer* causing soft rot; *Lasiodiplodia theobromae, Mycosphaerella caricae, Phoma caricae* causing stem end and body rots; *Phomopsis* spp. causing wet fruit rot (Lynne and John, 2008). Two species of nematodes, *viz.,* reniform (*Rotylenchulus* spp.) and root-knot (*Meloidogyne* spp.) have a worldwide distribution in papaya plantations (Luk *et al*., 2005). Papaya yellow crinkling, papaya phyllody and papaya dieback are important phytoplasma diseases in India (Singh, 2003).

Besides these papaya viruses cause diseases of global significance with serious damage in fruit production as well as the devastation of the entire crop (Akhter and Akanda, 2008). More than 29 different important viruses affecting papaya cultivation have been reported worldwide *viz., Alfalfa mosaic virus* (Moreira *et al.*, 2010); *Pepper huasteco yellow vein virus* (Tiznado *et al.*, 2002); *Pepper texas virus* (Tiznado *et al.*, 2002); *Squash yellow mottle virus* (Karkashian *et al.*, 2002); *Papaya leaf curl virus/*Other leaf curl (Saxena *et al.*, 1998a); *Malvestrum leaf curl virus* (Wu and Zhou, 2006); *Cucumber mosaic virus* (Adsuar,1956; Rezende and Costa, 1987); *Papaya comovirus 1* (Alcala-Briseno *et al.*, 2020); *Lettuce chlorosis virus* (Alcala-Briseno *et al.*, 2020); *Tobacco streak virus* (Singh, 2006); *Tobacco ringspot virus* (McLean and Olson, 1962 and Lambe, 1963); *Papaya mosaic virus* (Conover, 1962); *Zucchini yellow mosaic virus* (Ferwerda-Licha, 2002); *Papaw isabela mosaic virus* (Adsuar, 1972); *Papaya leaf distortion mosaic virus* (Yonaha, 1976; Kawano and Yonaha, 1992); *Soybean yellow bud virus* (Rezende and costa, 1987); *Moroccan watermelon mosaic virus* (Arocha *et al.*, 2007); *Papaya ringspot virus* (Jensen, 1949a); *Papaya droopy necrosis virus* (Wan, 1981); *Papaya apical necrosis virus* (Alcala-Briseno *et al.*, 2020); *Papaya nucleorhabdovirus* (Alcala-Briseno *et al.*, 2020); *Lettuce necrotic yellows virus* (Francki *et al.*, 1989); *Papaya lethal yellowing virus* (Loreto *et al.*, 1983); *Papaya meleira virus* (Abreu *et al.*, 2012); *Tobacco rattle virus* (Rezende and Costa, 1987); *Pepper ringspot virus* (Robinson and Harrison, 1989); *Tomato spotted wilt virus* (Kormelink, 2005); *Papaya virus Q* (Cornejo-Franco *et al.*, 2018) and *Papaya mild yellow leaf virus* (Marys *et al.*, 1995). Besides these, several viruses belonging to alfamovirus, begomovirus, cucumovirus, comoviruses, crinivirus, ilarvirus, nepovirus potexvirus, potyvirus, rhabdovirus, sobemovirus, tobravirus, tospovirus, umbravirus and unassigned virus groups have been recorded on papaya.

The *Papaya ringspot virus* (PRSV), *Papaya leaf curl virus* (PaLCuV) and *Papaya mosaic virus* (PMV) have gained global importance in all the papaya growing countries. In India, *Papaya ringspot virus* (PRSV) is one of the greatest concern, potentially causing 100 per cent loss in yield (Sharma and Tripathi, 2014) followed by PaLCuV a begomovirus, cause severe damage but it has a limited distribution and restricted to only a few states.

Papaya ringspot disease was reported for the first time from the island of Ohau in Hawaii State, which was well known for papaya production (Parris, 1938). Later, it

was named as 'Wailu' disease by Linder *et al.* (1945) which was later shown to be of viral nature and named as *Papaya ringspot virus* (PRSV) by Jensen (1949a) from Hawaii. This disease was first reported in India from Pune in 1948 (Capoor and Verma, 1948). Subsequently, PRSV has been recorded from different geographical locations.

PRSV Infected trees produce symptoms within 2 to 3 weeks of infection. Symptoms consist of intense yellow mosaic on leaves, small shoestring like new leaves, dark green and slightly sunken rings on the fruit, numerous oily looking streaks on the stem and stunting of the plant. Fruits produced after infection are usually small, exhibiting lichen like lesions and ring spots with uneven bumps and have an unpleasant taste. Trees infected at a very young age remain stunted and never produce any fruit (Jain *et al.*, 2004a; Kunkalikar *et al.*, 2011 and Reddy *et al.*, 2007). The virus is naturally transmitted by the insect vector aphids in a non persistent manner, from papaya to papaya infecting all plants in an orchard within a few months (Purcifull *et al.*, 1984a). If the transmission occurs before flowering, the flower production will be affected which could cause severe yield loss up to 85.0 to 90.0 per cent (Lokhande *et al.*, 1992, Hussain and Varma, 1994). Singh (2006) reported, 95.0 per cent of disease incidence in eastern U.P and estimated yield loss of about 70.0 per cent. The virions are filamentous, non-enveloped and flexuous measuring 760-800 x 12 nm. Virus particles contain 94.5 per cent protein and 5.5 per cent nucleic acid. The protein component consists of the virus coat protein (CP) with a molecular weight of about 36 kDa. The sedimentation coefficient of purified PRSV is 1.32 g/cm^3 in CsCl. The PRSV genome consists of a unipartite linear single stranded positive sense RNA of 10,326 nucleotides with a 5' terminus, genome linked protein, VPg (Tripathi *et al.*, 2008).

Leaf curl disease in papaya was first reported by Thomas and Krishnaswami (1939). This belongs to the genus begomovirus, a member of the family Geminiviridae. Typically, Geminiviridae comprises of viruses with single stranded circular genome encapsidated in geminate quasi-isometric virion particles of ~20 to 30 nm in size (Harrison, 1985) consisting of two incomplete icosahedral, containing a total of 110 coat protein subunits organized as 22 pentameric capsomers, encapsidating a single stranded circular DNA genome of 2.5 to 2.9 kb (Harrison *et al.*,

1977; Stanley, 1985). This disease was solely transmitted by vector whitefly (*Bemisia tabaci* Gennadius) in a persistent circulative manner (Nariani, 1956; Hull, 2002) but neither transmitted by seed nor by mechanical means (Nariani, 1956). Till now a total of 23 different begomoviruses are reported worldwide, which are also associated with diverse betasatellites (Singh-Pant *et al.*, 2012; Pramesh *et al.*, 2013). Now leaf curl disease causing begomoviruses are of global concern since they cause enormous losses in crop production and productivity. Further, due to their whitefly transmission, they are also difficult to control and no report of leaf curl disease resistant papaya is available to date.

These PRSV and PaLCuV diseases are major limiting biotic factors for papaya production in India. Therefore, considering the research work conducted in India so far and existing research gap, present study has been undertaken pertaining to the prevalence of disease, characterization of viruses, the host range of the virus, epidemiological studies and development of management approaches. The present research work was carried out on the below mentioned objectives.

Objectives:

1. Survey for the prevalence, distribution, collection and characterization of PRSV and PaLCuV diseases associated with papaya in Karnataka
2. Epidemiological studies on the incidence of PRSV disease
3. Integrated management of PRSV disease under field conditions

2. REVIEW OF LITERATURE

The literature about this study was compiled from various available sources and is presented under this chapter**.**

2.1 Virus diseases affecting papaya cultivation

In papaya, severe crop damage is caused due to various virus diseases. The viruses belonging to alfamovirus, begomovirus, cucumovirus, comoviruses, crinivirus, ilarvirus, nepovirus, potexvirus, potyvirus, rhabdovirus, sobemovirus, tobravirus, tospovirus, umbravirus and unassigned virus groups are reported to infect and limit the papaya production in different parts of the world (Table 1). Among these, PRSV belongs to potyviral group which is most widely distributed whereas other viruses have a restricted distribution. In India, PRSV is the most commonly occurring virus followed by PaLCuV which has a limited distribution.

2.2 Survey for the prevalence, distribution, collection and characterization of PRSV and PaLCuV diseases associated with papaya in Karnataka

2.2.1 Survey, distribution and incidence of PRSV

Papaya ringspot disease was reported for the first time from the island of Ohau in Hawaii State by Parris (1938) and coined the name as *Papaya ringspot virus* by Jensen (1949a). Then onwards the distribution and incidence of PRSV is seen in almost all the countries of the world (Table 2). Despite its importance, national economies of many papaya growing nations are jeopardized by the *Papaya ringspot virus* disease, which affects papaya plants at all stages of growth and naturally spreads very quickly, leading to infection of the whole orchard within 3-7 months with severe yield losses up to 100 per cent (Sharma and Tripathi, 2014).

In India, the first report of PRSV was made by Capoor and Verma (1948) whereas, in Karnataka reported by Byadgi *et al*. (1995) from Dharawad. The distribution and incidence of PRSV has been recorded in almost all the parts of India (Table. 3 and 4).

Table 1: List of important virus diseases affecting papaya cultivation

Sl. No.	Virus Groups	Virus infecting papaya	Abbreviation	Place	Symptom	Reference(s)
1.	*Alfamovirus*	*Alfalfa mosaic virus*	AMV	Brazil	M	Moreira *et al.* (2010)
2.	*Begomovirus*	*Pepper huasteco yellow vein virus*	PHYVV	Central Mexico	YM, LD	Tiznado *et al.* (2002)
		Squash yellow mottle virus	SYMoV	Costa Rica	M, C	Karkashian *et al.* (2002)
		Papaya leaf curl virus/Other leaf curl	PaLCuV	Asia	SLC,DLC, VT, VC, DP, D, Y, VE, LR, LL,	Saxena *et al.* (1998a)
		Malvestrum leaf curl virus	MLCuV	China	LC	Wu and Zhou (2006)
3.	*Cucumovirus*	*Cucumber mosaic virus*	CMV	Puerto Rico, Brazil	CM, M, VC, DCL, ILM	Adsuar (1956); Rezende and Costa (1987)
4.	*Comoviruses*	*Papaya comovirus 1*	PCV 1	Chiapas, Mexico	LD	Alcala-Briseno *et al.* (2020)
5.	*Crinivirus*	*Lettuce chlorosis virus*	LCV	Chiapas, Mexico	SM, LD	Alcala-Briseno *et al.* (2020)
6.	*Ilarvirus*	*Tobacco streak virus*	TSV	USA	LD, NL	Singh (2006)
7.	*Nepovirus*	*Tobacco ringspot virus*	TRSV	Luonan	LD	McLean and Olson (1962) and Lambe (1963)
8.	*Potexvirus*	*Papaya mosaic virus*	PapMV	Florida, Venezuela, Sri Lanka, Peru, Bolivia, Mexico	M	Conover (1962)
9.	*Potyvirus*	*Zucchini yellow mosaic virus*	ZYMV	Puerto Rico	LD	Ferwerda-Licha (2002)
		Papaw isabela mosaic virus	PIMV	Puerto Rico	M	Adsuar (1972)
		Papaya leaf distortion mosaic virus	PLDMV	Japan	LD, YM	Yonaha (1976); Kawano and Yonaha (1992) and Maoka and Hataya, (2005)

Contd.……

Sl. No.	Virus Groups	Virus infecting papaya	Abbreviation	Place	Symptom	Reference(s)
		Soyabean yellow bud virus	SYBV	Brazil	YM	Rezende and costa (1987)
		Moroccan watermelon mosaic virus	MWMV	Kenya, Democratic Republic of Congo	M	Arocha *et al.* (2007)
		Papaya ringspot virus	PRSV	Worldwide	M, RS, F	Jensen (1949a)
10.	*Rhabdovirus*	*Papaya droopy necrosis virus*	PDNV	Florida	DN	Wan (1981)
		Papaya apical necrosis virus	PANV	Venezuela, Cuba, Mexico, Brazil, Africa	AN	Alcala-Briseno *et al.* (2020)
		Papaya nucleorhabdovirus	PNRV	Mexico	LD	Alcala-Briseno *et al.* (2020)
		Lettuce necrotic yellows virus	LNYV	US	BLV	Francki *et al.* (1989)
11.	*Sobemovirus*	*Papaya lethal yellowing virus*	PLYV	Brazil	LY	Loreto *et al.* (1983)
		Papaya meleira virus	PMeV	Brazil, Mexico	SLE, N	Abreu *et al.* (2012)
12.	*Tobravirus*	*Tobacco rattle virus*	TRV	Florida	M,C	Rezende and Costa (1987)
		Pepper ringspot virus	PepRSV	Brazil	LD	Robinson and Harrison (1989)
13.	*Tospovirus*	*Tomato spotted wilt virus*	SWV	Hawaii (USA), Australia, Brazil	S, C, N	Kormelink (2005)
14.	*Umbravirus*	*Papaya virus Q*	PpVQ	Ecuador; Brazil	M,C	Cornejo-Franco *et al.* (2018)
15.	Unassigned virus	*Papaya mild yellow leaf virus*	PMYLV	Venezuela	IVY	Marys *et al.* (1995)

Note: AN Apical Necrosis, BLV: Browning of Leaf Veins, C:Chlorosis, CM: Chlorotic mottling, D:Deformation,DLC:Downward Leaf Curling, DP:Distorted Petioles, DN: Droopy Necrosis, F: Filiformy, IVY: Inter Veinal Yellowing, ILM: Irregular Leaf Margins, LD: Leaf Distortion, LL:Leathery Leaf, LR:Leaf Rolling, LY: Lethal Yellowing, M: Mosaic N: Necrosis, NL: Necrotic Lesions, RS: Ringspot, SLC: Severe Leaf Curl, SLE: Sticky Latex Exudation, SM: Severe Mosaic, VC: Vein Clearing, Y:Yellowing, YM: Yellow Mosaic, VE:Vein Enation and VT:Vein Thickening

Table 2: Worldwide scenario on distribution of PRSV on papaya

Continent	Country	Reference(s)
Africa	Côte d'Ivoire	Diallo *et al.* (2007) and Kone *et al.* (2010)
	Egypt	Omar *et al.* (2011)
	Kenya	Ombwara *et al.* (2014)
	Nigeria	Lana (1980)
	Tunisia	Mnari-Hattab *et al.* (2008)
Asia	Bangladesh	Jain *et al.* (2004a) and Begum *et al.* (2018)
	China	Liao *et al.* (2005) and Wu *et al.* (2018)
	Indonesia	Temaja and Darmiati (2015) and Listihani *et al.* (2018)
	India	Capoor and Verma (1948)
	Iran	Pourrahim *et al.* (2003)
	Japan	Maoka *et al.* (1995)
	Lebanon	Katul and Makkouk (1987)
	Malaysia	Mohamed and Wan Kelthom (2005)
	Myanmar	Kim *et al.* (2010)
	Nepal	Shrestha and Albrechtsen (1992)
	Pakistan	Noshad *et al.* (2015)
	Philippines	Opina (1986)
	Sri Lanka	Perera *et al.* (1998)
	Syria	Katul and Makkouk (1987)
	Taiwan	Wang *et al.* (1978)
	Thailand	Yeh and Gonsalves (1994)
	Turkey	Koklu and Yilmaz (2006)
	Vietnam	Bateson *et al.* (1994)
	Yemen	Alhubaishi *et al.* (1987)
Europe	Cyprus	Papayiannis *et al.* (2005)
	France	Pontis Videla (1953)
	Germany	Pontis Videla (1953)
	Italy	Pontis Videla (1953)
	Poland	Hasiow-Jaroszewska *et al.* (2010)

Contd….

Continent	Country	Reference(s)
North America	Bahamas	McMillan *et al.* (1990)
	Costa Rica	Rivera *et al.* (1993)
	Cuba	Cabrera Mederos *et al.* (2019)
	Dominican Republic	Storey and Halliwell. (1969)
	Guatemala	Jeyaprakash *et al.* (2015)
	Honduras	Espinoza and McLeod (1994)
	Jamaica	Chin *et al.* (2008)
	Mexico	Silva-Rosales *et al.* (2000)
	Puerto Rico	Abreu (1994)
	Saint Kitts and Nevis	Chin *et al.* (2008)
	Trinidad and Tobago	Baker (1938)
	United States	Khanal and Ali (2018)
Oceania	Australia	Thomas and Dodman (1993)
	Cook Islands	Davis *et al.* (2005)
	French Polynesia	Davis *et al.* (2005)
	Papua New Guinea	Davis *et al.* (2002)
	Solomon Islands	Davis and Tsatsia (2009)
South America	Argentina	Cabrera Mederos *et al.* (2016)
	Brazil	Gonsalves (1998)
	Colombia	Torres and Giacometti (1966)
	Ecuador	Medina-Salguero *et al.* (2021)
	Paraguay	Esquivel-Farina *et al.* (2020)
	Venezuela	Marys *et al.* (2000)

Table 3: PRSV distribution and incidence on papaya in India

State	Incidence (%)	Reference(s)
Andhra Pradesh	-*	Susan John (1985)
Assam	28.00 - 74.30	Talukdar *et al.* (2013)
	-	Basavaraj *et al.* (2019)
Bihar	-	Mishra and Jha (1955)
	100	Singh *et al.* (2008a)
	-	Prasad and Kudada (2005)
	22.50 - 36.40	Singh *et al.* (2019)
Chhatisgarh	-	Jain *et al.* (2004b)
Goa	-	Basavaraj *et al.* (2019)
Gujarat	-	Parmar (2000)
Haryana	-	Basavaraj *et al.* (2019)
Himachal Pradesh	-	Jain *et al.* (2004b)
	-	Singh *et al.* (2017)
Jharkhand	-	Jain *et al.* (2004b)
	-	Basavaraj *et al.* (2019)
Kerala	35.00 - 66.00	Raj Verma *et al.*(2007)
	25.00 - 99.60	Harish (2018)
Madhya Pradesh	-	Garga (1963)
Maharashtra	75.00 - 100	Lokhande and Moghe (1992b)
	3.00 - 100	Raj verma *et al.* (2007)
	32.00	Sharma *et al.* (2010)
	35.50 - 86.17	Mallikarjun (2009)
	-	Capoor and Verma (1948)
	79.00	Yemewar and Mali (1980)
	-	Verma *et al.* (2014)

Contd.…..

State	Incidence (%)	Reference(s)
Meghalaya	71.70	Babu and Banerjee (2018)
Punjab	-	Cheema and Reddy (1985)
	-	Basavaraj *et al.* (2019)
Rajasthan	-	Sureka *et al.* (1977)
Sikkim	-	Singh *et al.* (2017)
Tamil Nadu	-	Jyoti *et al.* (2005)
Telangana	-	Jyoti *et al.* (2005)
Tripura	51.00	Hemavati *et al.* (2017)
Uttar Pradesh	48.00 - 100	Singh *et al.* (2003)
	95.00	Vimla *et al.* (2005)
	80.00	Ashutosh Singh *et al.* (2005)
	-	Singh *et al.* (2006)
	74.00 - 90.00	Raj verma *et al.* (2007)
	100	Ray (2009)
	75.00 - 100	Khurana and Bhargava (1970)
	-	Basavaraj *et al.* (2019)
West Bengal	-	Jain *et al.* (2004b)
	40.00	Raj Verma *et al.* (2007)
	-	Basavaraj *et al.* (2019)

* Authors are not reported the percent disease incidence but recorded the presence of PRSV

Table 4: PRSV distribution and incidence on papaya in Karnataka

District	Incidence (%)	Reference(s)
Bagalkote	30.00 – 100	Mallikarjun (2009)
	72.00 -100	Ranebennur (2005)
	47.00 - 94.00	Kunkalikar *et al.* (2007)
Bangaluru	75.00 -100	Lakshminarayana Reddy (2000)
	65.38 -100	Jahir Basha (2002)
	93.00 - 53.00	Vinayakumar Reddy *et al.* (2011)
Bangaluru rural	10.00 - 98.00	Mallikarjun (2009)
	35.00 - 57.00	Kunkalikar *et al.* (2007)
	20.00 – 100	Pushpa (2014)
Bangaluru urban	38.00 – 100	Mallikarjun (2009)
	5.00 – 100	Pushpa (2014)
	20.00 - 90.00	Kunkalikar *et al.* (2007)
Belgavi	40.00 -100	Ranebennur (2005)
	30.00 - 95.00	Kunkalikar *et al.* (2007)
	92.50 -100	Shaikh (1996)
	0.00 – 100	Hegde (1998)
	20.00 - 93.00	Mallikarjun (2009)
Bellari	50.00 – 100	Mallikarjun (2009)
	15.00 - 70.00	Ranebennur (2005)
	0.00 - 86.00	Kunkalikar *et al.* (2007)
Bidar	40.00 – 10.00	Mallikarjun (2009)
	90.00 – 100	Kunkalikar *et al.* (2007)
Chamarajanagara	15.00 - 25.00	Pushpa (2014)
	0.00 - 46.00	Kunkalikar *et al.* (2007)
	30.00 - 90.00	Mallikarjun (2009)
	34.72	Vinayakumar Reddy *et al.* (2011)
Chikmagalur	0.00 - 44.00	Kunkalikar *et al.* (2007)
	10.00 - 70.00	Mallikarjun (2009)
	19.08	Vinayakumar Reddy *et al.* (2011)
Chikballapur	0.00 – 100	Pushpa (2014)
Chitradurga	29.90 - 30.00	Jahir Basha (2002)
	30.00 -100	Mallikarjun (2009)
	52.12	Vinayakumar Reddy *et al.* (2011)
	0.00 - 20.00	Kunkalikar *et al.* (2007)

Contd…..

District	Incidence (%)	Reference(s)
Dakshin Kannada	0.00 - 80.00	Kunkalikar *et al.* (2007)
	34.38	Vinayakumar Reddy *et al.* (2011)
	10.00 - 30.00	Mallikarjun (2009)
Davanagere	33.50 - 94.10	Jahir Basha (2002)
	0.00 - 12.00	Kunkalikar *et al.* (2007)
	10.00 - 50.00	Mallikarjun (2009)
Dharwad	0.00 – 100	Byadgi *et al.* (1995)
	0.00 – 100	Shaikh (1996)
	50.00 -100	Ranebennur (2005)
	0.00 – 100	Kunkalikar *et al.* (2007)
	30.00 – 100	Mallikarjun (2009)
Gadag	30.00 – 100	Mallikarjun (2009)
	20.00 - 70.00	Ranebennur (2005)
	0.00 - 41.00	Kunkalikar *et al.* (2007)
Hasan	10.00 - 40.00	Mallikarjun (2009)
	-*	Kunkalikar *et al.* (2007)
	-	Kalleshwaraswamy and Krishna Kumar (2008)
	-	Vinayakumar Reddy *et al.* (2011)
	0.00 - 15.00	Pushpa (2014)
Haveri	20.00 - 100	Mallikarjun (2009)
	24.00 - 70.00	Ranebennur (2005)
	30.00 - 95.00	Kunkalikar *et al.* (2007)
	0.00 -100	Hegde (1998)
Kalaburagi	15.00 - 100	Mallikarjun (2009)
	100	Kunkalikar *et al.* (2007)
Kodagu	-	Kunkalikar *et al.* (2007)
	-	Kalleshwaraswamy and Krishna Kumar (2008)
	10.00 - 40.00	Mallikarjun (2009)
	-	Pushpa (2014)
Kolar	75.89	Vinayakumar Reddy *et al.* (2011)
	0 - 40.00	Kunkalikar (2003)
Koppal	20.00 - 80.00	Mallikarjun (2009)
	5.00 - 30.00	Kunkalikar *et al.* (2007)

Contd…..

District	Incidence (%)	Reference(s)
Mandya	0.00 - 1.00	Kunkalikar *et al.* (2007)
	18.45	Vinayakumar Reddy *et al.* (2011)
	0.00 - 30.00	Pushpa (2014)
Mysore	0.00 - 23.00	Kunkalikar *et al.* (2007)
	10.00 - 75.00	Mallikarjun (2009)
	5.00 - 57.00	Pushpa (2014)
Raichur	10.00 - 75.00	Mallikarjun (2009)
	0.00 - 55.00	Kunkalikar *et al.* (2007)
Ramanagara	0.00 - 72.00	Pushpa (2014)
Shimoga	0.00 - 11.00	Kunkalikar *et al.* (2007)
	14.16	Vinayakumar Reddy *et al.* (2011)
	10.00 - 40.00	Mallikarjun (2009)
Tumkur	10.00 - 50.00	Mallikarjun (2009)
	56.60	Vinayakumar Reddy *et al.* (2011)
	0.00 - 73.00	Pushpa (2014)
	0.00 - 16.00	Kunkalikar *et al.* (2007)
Udupi	-	Kunkalikar *et al.* (2007)
	0.00 - 10.00	Mallikarjun (2009)
	-	Vinayakumar Reddy *et al.* (2011)
	-	Kalleshwaraswamy and Krishna Kumar (2008)
Uttar Kannada	0.00 – 100	Hegde (1998)
	0.00 - 70.00	Kunkalikar *et al.* (2007)
	10.00 - 75.00	Mallikarjun (2009)
Vijayapura	55.00 - 100	Mallikarjun (2009)
	63.00 -100	Ranebennur (2005)
	85.00 - 100	Kunkalikar *et al.* (2007)

*Authors are not reported the percent disease incidence but recorded the presence of PRSV

2.2.2 Characterization of PRSV

The Potyviruses belong to the family potyviridae which are single-stranded, positive-sense (+) RNA genome. They are flexuous filamentous particles of 680 to 900 nm long and 11 to 20 nm width having average size of 9.7 kb. It is largest family of RNA plant viruses and the second largest plant virus family after geminiviridae.

2.2.2.1 Taxonomy and Nomenclature of PRSV

PRSV belongs to Domain: Virus; Group: "Positive sense ssRNA viruses"; Group: "RNA viruses"; Family: Potyviridae; Genus: Potyvirus; Species: *Papaya ringspot virus*. Two strains of *Papaya ringspot virus* (PRSV) are recognized, PRSV-type P and PRSV-type W. These have been classified as strains of the same virus based on morphological and serological similarities (Yeh and Gonsalves, 1984). They also have a high percentage of nucleotide and amino acid sequence homologies on the NIb and coat protein genes (Quemada *et al.*, 1990; Bateson and Dale, 1992; Wang and Yeh, 1992; Jain *et al.*, 1998; Wang *et al.*, 1998). They are distinguished only by the host range. PRSV-P infects papaya and cucurbits but causes a destructive disease only on papaya. PRSV-W infects cucurbits, but not papaya.

PRSV-P belongs to the family potyviridae, genus potyvirus which typically has flexuous, filamentous particles, 760-800 nm long and 12 nm in diameter (Herold and Weibel, 1962; Purcifull *et al.*, 1984b). Type W, which causes severe damage to cucurbits, was previously referred to as *Watermelon mosaic virus* 1 (WMV-1) (Van Regenmortel, 1971). The International Committee on Taxonomy of Viruses still lists WMV-1 as a synonym for PRSV (Fauquet and Martelli, 1995).

2.2.2.3 Genome organization of PRSV

Potyvirus consists of non enveloped flexuous filamentous particles which consist of a positive sense, single stranded, unipartite RNA genome encapsidated by coat protein (CP). The genomic RNA of PRSV is ~10,326 nucleotides which encoded P1 (Protein 1), HC-Pro (Helper Component-Protease), P3 (Protein 3), 6K1 (6-kDa peptide 1), CI (Cylindrical Inclusion protein), 6K2 (6-kDa peptide 2), VPg (Viral Protein genome-linked), NIa-Pro (Nuclear inclusion A-protease), NIb (Nuclear inclusion B) and followed by a tract of polyA sequence (An). Details of genomic organization given in table 5.

Table 5: Description of potyviridae proteins and their functions

Sl. No.	Protein	Nuclieotide length (bp)	Molecular weights (kDa)	Functions	Reference(s)
1	P1 (Protein 1)	~86-1727 (~1641)	~63kDa	Participates in translation; Modulator of RNA replication; N terminal part of P1 is hypervariable, Dispensable for virus replication; Implicated in adaptation to new hosts; Host defense responses; Host-dependent pathogenicity; Symptom determinant; Proteinase; Cell-to-cell movement	Valli *et al.* (2007), Rohozkova and Navratil (2011), Maliogka *et al.* (2012), Pasin *et al.* (2014) and Revers and Garcia (2015)
2	HC-Pro (Helper Component-Protease)	~1728-3098 (~1370)	~52 kDa	RNA silencing suppressors; Promote virus susceptibility by interfering with antiviral defense; Gene silencing suppressor; Symptoms development; Vector transmission; Cell-to-cell movement	Torres-Barcelo *et al.* (2008), Garcia-Ruiz *et al.* (2010), Murray *et al.* (2013) and Revers and Garcia (2015)
3	P3 (Protein 3)	~3099-4105 (~1006)	~46 kDa	Virus replication; Host range; Symptom development; Plant pathogenicity	Urcuqui-Inchima *et al.* (2001), Gonsalves *et al.* (2007) and Wen *et al.* (2011)
4	6K1 (6-kDa peptide 1)	~4106-4289 (~183)	~6 kDa	Replication, Regulation; cell-to-cell movement; virulence; Inhibition of NIa nuclear translocation	Urcuqui-Inchima *et al.* (2001) and Gonsalves *et al.* (2007)
5	CI (Cylindrical Inclusion protein)	~4290-6194 (~1904)	~72 kDa	ATPase/RNA helicase; Cell-to-cell movement, Long-distance movement, Development of symptoms, accumulates in inclusion bodies in the cytoplasm and Breaking the host resistance	Urcuqui-Inchima *et al.* (2001) and Gonsalves *et al.* (2007)
6	6K2 (6-kDa peptide 2)	~6195-6365 (~170)	~6 kDa	Anchoring the viral replication complex to ER; Long-distance movement; Development of systemic infection; Inhibition of NIa nuclear translocation	Urcuqui-Inchima *et al.* (2001) and Gonsalves *et al.* (2007)

Contd…..

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Sl. No.	Protein	Nuclieotide length (bp)	Molecular weights (kDa)	Functions	Reference(s)
7	VPg (Viral Protein genome-linked)	~6365-6905 (~540)	~21 kDa	Viral RNA translation; Silencing suppression; RNA replication; Cell-to-cell and systemic virus movement	Revers and Garcia (2015) and Cheng and Wang (2017)
8	NIa-Pro (Nuclear inclusion A-protease)	~6906-7646 (~740)	~27 kDa	NIa-Pro cysteine proteinase is responsible for the cleavage of host DNA Cellular localization;	Adams *et al.* (2005), Adams *et al.* (2011) and Sochor *et al.* (2012)
9	NIb (Nuclear inclusion B)	~764-9197 (~1550)	~59 kDa	RNA-dependent-RNA polymerase; RNA replication	Sochor *et al.* (2012) and Revers and Garcia (2015)
10	CP (Coat capsid protein)	~9198-10121 (~923)	~35 kDa	Virion assembly; Cell-to-cell and long-distance movement; Aphid transmission.	Lopez-Moya *et al.* (1999), Dombrovsky *et al.* (2005) and Ivanov *et al.* (2014)

2.2.2.4 Transmission and propagation of PRSV

Transmission of PRSV between host plants within a field or between distant fields is often dependent on aphid vector. To infect a plant, a virus must physically penetrate the cell wall barrier, which may result from mechanical inoculation carried out by humans, or natural transmission by animals and insects (Khan and Dijkstra, 2002; Trigiano *et al.*, 2003). Bayot *et al.* (1990) published the single report of transmission of PRSV through papaya seed, which suggest the seed transmission is in traces (about 0.0015 %). However, there is no further report on seed transmissibility of PRSV (Tennant *et al.*, 2007).

2.2.2.5 Symptomatology

PRSV infects all parts of the papaya plants at all stages. Leaves exhibit a variety of peculiar symptoms depending upon the stage of the plant, viral load and environmental conditions. Linder *et al.* (1945) observed mosaic patterns on leaves and yellow rings on fruits. Later, different symptoms like puckering of leaf tissues between veins and veinlets of young leaves and mosaic pattern on expanded leaves (Holmes *et al.*, 1948). Swelling of leaf tissues between veins resulting in upward curling of leaves followed by chlorotic mottling, blistering of leaf tissue were recorded by Jensen (1949b). The other symptoms like chlorotic ringspot, mosaic pattern, shoestring on leaves, blistering and distortion of leaves in severe cases, lobed and semi apocarpous conditions of fruits and overcrowding of leaves were also noticed (Bhandari, 1952).

Ringspot on fruits, hollow stem and malformed fruits were seen prominent in severely infected orchards (Khurana and Bhargava, 1970; Yemewar and Mali, 1980). In the early infested and abandoned orchards, bare plants without any fruit set with no flowering, stunted growth, beheaded appearance, tapered canopy and death of the plant were recorded (Lokhande *et al.*, 1992; Thomas and Dodman, 1993; Hussain and Varma, 1994; Shaikh, 1996; Dahal *et al.*, 1997; Kunkalikar, 2003; Ranebennur, 2005). Apart from these, Singh *et al.* (2003) recorded downward turning of leaf margins, elongation and distortion of leaves with scorched appearance. The symptoms on the fruit consist of dark green concentric rings or spots or C-shaped markings, often slightly sunken in the fruit are diagnostic (Gonsalves, 1998). Fruits often show

uneven bumps, especially those fruits that develop after a tree is infected. The number of rings in fruits can be variable and the rings become less distinct as the fruit matures.

Vigour of trees and fruit sets are usually reduced depending on the age of the plant when infected. Fruit quality, particularly flavor is also adversely affected. Varying degrees of deformation, abnormalities on fruits, stunted growth and collapsing nature of plants in advanced stages of infection were noticed by Lokhande and Moghe (1992), Thomas and Dodman (1993), Hussain and Verma (1994), Shaikh (1996), Dahal *et al.* (1997), Lakshminarayana Reddy (2000), Kunkalikar (2003), Ranebennur (2005), Raj Verma *et al.* (2007), Mallikarjun (2009) and Vinayakumar Reddy *et al.* (2011).

2.2.2.6 Characterization of PRSV

Genetic variability of PRSV is related to the geographical origin of the isolates. Evolution and molecular epidemiology of PRSV-P by sequencing CP genes of both PRSV-P and W type isolates from Vietnam, Thailand, India and the Philippines was reported by Bateson *et al.* (2002). The homology of twelve Brazilian isolates of PRSV was studied by Lima *et al.* (2002). Hema and Prasad (2004) compared the CP gene of south Indian strain PRSV-INP-UAS with other sequences. The CP sequences of eleven PRSV isolates originating from different locations in India were compared with other isolates of PRSV by Jain *et al.* (2004a). Bag *et al.* (2007) studied the sequence diversity in the CP of 28 PRSV isolates originating from different locations in India. The complete sequencing of the New Delhi Indian isolate of PRSV (PRSVDEL) from IARI was done by Parameswari *et al.* (2007). Gibbs *et al.* (2008) provided evidence of the Asian origin of PRSV and proposed that the introduction of the virus to the America occurred about 300 years ago (Chin *et al.*, 2007; Zambrana-Echevarria *et al.*, 2016).

The genetic diversity of PRSV populations is highest in India (Jain *et al.*, 2004b). Srinivasulu and Sai Gopal (2011) evaluated the PRSV isolates from different geographical locations in the Indian subcontinent. Partial characterization of isolate BUH-1 by CP gene showed the highest homology of 98.0 per cent with south Indian isolates by Pushpa (2014). A Phylodynamic analysis was performed with the partial

coat protein gene from the American continent and the Caribbean islands by Mederos *et al.* (2019).

2.2.3 Survey, distribution and incidence of PaLCuV

Distribution and per cent incidence of begomovirus is limited as compaired to PRSV infection. The litreture showed that (Table 6) around 23 different begomoviruses are characterized and reported worldwide and their distribution is majourly in Asian in countries with overall 0 to 80.0 per cent incidence.

2.2.4 Characterization of PaLCuV

PaLCuV belongs to geminiviridae which is having small single stranded circular DNA plant viruses with distinctive geminate (paired) particles (20 × 38 nm) consisting of two incomplete icosahedral, containing a total of 110 coat protein subunits organized as 22 pentameric capsomers, encapsidating a single stranded circular DNA genome of 2.5-2.9 kb (Harrison *et al.*, 1977; Stanley, 1985, Zerbini *et al.*, 2017; Varsani *et al.*, 2017). Among the nine genera in geminiviridae, begomovirus is the largest one comprising about 322 species. The Begomovirus derives its name from the type member, *Bean golden mosaic virus* (BGMV) that causes golden mosaic disease in beans in Central America.

Begomovirus is having a broad range of host crops and causes substantial crop losses worldwide (Morales and Anderson, 2001; Mansoor *et al.*, 2003). Having a small genome, geminiviruses utilize a strategy of overlapping genes in different frames to efficiently code the proteins needed for replication, control of gene expression, encapsidation and movement.

PaLCuV disease caused by begomovirus in papaya has limited in distribution, the available litreture showed that incidence of this disease is restricted manly in the countries of Asian continent *viz.*, Bangladesh, China, India, Iran, Korea, Nepal, Oman, Pakistan and Taiwan (Saxena *et al.*, 1998b; Nadeem *et al.*, 1997; Chang *et al.*, 2003; Wang *et al.*, 2004; Khan *et al.*, 2012; Shahid *et al.*, 2013; Bananej *et al.*, 2016; Byun *et al.*, 2016; Hamim *et al.*, 2019). Apart from this, recent reports showed that begomoviral distribution in papaya also been seen in countries of North-American continent like USA and Mexico with 40.0 to 100 per cent (Alabi *et al.*, 2016; Alcala-Briseno *et al.*, 2020) (Table 6).

Table 6: Worldwide distribution of begomovirus infecting papaya

Sl. No.	Continent/ Country	Virus identified	Abbreviation	Reference(s)
I	**Asia**			
1.	Bangladesh	*Tomato leaf curl Bangladesh virus*	ToLCBV	Hamim *et al.* (2019) and Hamim *et al.* (2020)
2.		*Tomato leaf curl Joydebpur virus*	ToLCJoV	Hamim *et al.* (2020)
3.		*Tomato leaf curl New Dehli virus*	ToLCNDV	Hamim *et al.* (2020)
4.	China	*Ageratum yellow vein virus*	AYVV	Shen *et al.* (2014)
5.		*Papaya leaf curl China virus*	PaLCuCNV	Wang *et al.* (2004), Cai *et al.* (2005) and Cai *et al.* (2007)
6.		*Papaya leaf curl Guangdong virus*	PaLCuGDV	Wang *et al.* (2004) and Lubin *et al.* (2005)
7.	India	*Ageratum enation virus*	AEV	Singh-Pant *et al.* (2012)
8.		*Chilli leaf curl virus*	ChiLCuV	Singh-Pant *et al.* (2012), Dwivedi *et al.* (2016) and Kumar *et al.* (2021)
9.		*Cotton leaf curl Multan virus*	CLCuMV	Sinha *et al.* (2013) and Sinha (2015)
10.		*Croton yellow vein mosaic virus*	CYVMV	Krishnareddy *et al.* (2010), Singh-Pant *et al.* (2012) and Sinha *et al.* (2016)
11.		*Papaya leaf crumple virus,*	PaLCrV	Singh-Pant *et al.* (2012)
12.		*Papaya leaf curl Coimbatore virus*	PaLCCoV	Krishnareddy *et al.* (2010)
13.		*Papaya leaf curl virus*	PaLCuV	Thomas and Krishnaswami (1939), Sen *et al.* (1946), Nariani (1956), Govindu (1956), Govindu (1964), Sureka *et al.* (1977), Pandey and Marathe (1986), Verma (1996), Saxena *et al.* (1998a), Saxena *et al.* (1998c), Patel (2006), Singh (2006), Krishnareddy *et al.* (2010), Usharani *et al.* (2013), Singh-Pant *et al.* (2012), Dubey *et al.* (2015), Saxena *et al.* (2016) and Singh and Awasthi (2017)

Contd.……

Sl. No.	Continent/ Country	Virus identified	Abbreviation	Reference(s)
14.	India	*Papaya yellow leaf curl virus*	PaLCuV	Nehra *et al.* (2019)
15.		*Pedilanthus leaf curl virus*	PeLCV	Singh-Pant *et al.* (2012)
16.		*Tomato leaf curl Gujarat virus*	ToLCuGuV	Varun and Saxena (2018)
17.		*Tomato leaf curl Joydebpur virus*	ToLCJoV	Singh-Pant *et al.* (2012)
18.		*Tomato leaf curl Karnataka virus*	ToLCKV	Singh-Pant *et al.* (2012)
19.		*Tomato leaf curl New Delhi virus*	ToLCuNDV	Raj *et al.* (2008), Krishnareddy *et al.* (2010) and Singh-Pant *et al.* (2012)
20.		*Tomato leaf curl virus*	TLCV	Singh-Pant *et al.* (2012) and Kumar *et al.* (2021)
21.	Indonesia	*Begomovirus*		Sutrawati *et al.* (2021)
22.	Iran	*Okra enation leaf curl virus*	OELCuV.	Bananej *et al.* (2016)
23.	Iran	*Cotton leaf curl Gezira virus*	CLCuGeV	Bananej *et al.* (2021)
24.	Korea	*Papaya leaf curl virus*	PaLCuV	Byun *et al.* (2016)
25.	Nepal	*Ageratum yellow vein virus*	AYVV	Shahid *et al.* (2013)
26.	Oman	*Cotton leaf curl Gezira virus*	CLCuGeV	Khan *et al.* (2012)
27.	Oman	*Tomato leaf curl Albatinah virus*	ToLCABV	Ammara *et al.* (2015) and Haq *et al.* (2018)
28.	Pakistan	*Papaya Leaf Curl virus*	PaLCuV	Nadeem *et al.* (1997)
29.	Taiwan	*Papaya Leaf Curl virus*	PaLCuV	Chang *et al.* (2003)
30.	Vietnam	*Tomato leaf curl Hainan virus*	ToLCHnV	Ha *et al.* (2011)
II	**North-America**			
31.	USA	*Tomato yellow leaf curl virus-Israel*	TYLCV-IL	Alabi *et al.* (2016)
32.	Mexico	*Papaya begomovirus* 1	PapBV	Alcala-Briseno *et al.* (2020)

The first report of papaya leaf curl disease is from India in 1939 by Thomas and Krishnaswami (Thomas and Krishnaswami, 1939). Although this disease is reported in early 20th century, very less studies have been done to confirm the causal agent and disease transmiting vecter. Saxena *et al.* (1998a,b,c) conducted an exclusive stuies and conformed the causal agent as *Papaya leaf curl virus* (PaLCuV) and transmiting vector as whitefly (*Bemisia tabaci*).

2.2.4.1 Begomoviruses infecting papaya

Begomoviruses has limited in worldwide distribution, but high in their numbers of viruses infecting papaya. This makes it as second most important viral disease infecting papaya. Until now worldwide a total of 23 begomoviruses are reported *viz., Ageratum enation virus, Ageratum yellow vein virus, Chilli leaf curl virus, Cotton leaf curl Gezira virus, Cotton leaf curl Multan virus, Croton yellow vein mosaic virus, Okra enation leaf curl virus, Papaya begomovirus 1, Papaya leaf crumple virus, Papaya leaf curl China virus, Papaya leaf curl Coimbatore virus, Papaya leaf curl Guangdong virus, Papaya leaf curl virus, Papaya yellow leaf curl virus, Pedilanthus leaf curl virus, Tomato leaf curl Albatinah virus, Tomato leaf curl Bangladesh virus, Tomato leaf curl Gujarat virus, Tomato leaf curl Joydebpur virus, Tomato leaf curl Karnataka virus, Tomato leaf curl New Delhi virus, Tomato leaf curl virus* and *Tomato yellow leaf curl virus-Israel* (Table 6).

2.2.4.2 Genome organization of begomovirus

The begomoviruses associated with papaya are distinct species and most of them consist of a monopartite genome organization, unlike several begomoviruses which have a bipartite genome. There are closely related begomovirus infecting papaya which have single stranded genome occurs as a covalently closed circular DNA molecule of approximately 2.5 to 3.0 kb that is housed in a geminate isometric icosahedral capsid (18 × 20 nm) constructed with 27 to 30 kD protein subunits (Howarth and Goodman, 1982; Lazarowitz, 1992; Zhang *et al.*, 2001). The five or six proteins encoding proteins for replication, transcription, encapsidation and movement are on the single DNA component of the monopartite begomoviruses or the two DNA components of the bipartite begomoviruses (Saxena *et al.*, 1998a).

Begomoviral DNA components contain protein coding regions in the viral strand and the complementary strand. DNA-A codes for proteins responsible for coat protein (CP) such as AV1 (Hohnle *et al.*, 2001; Kheyr-Pour *et al.*, 2000; Noris *et al.*, 1998), Replication Initiation Protein (Rep) such as AC1 (Fontes *et al.*, 1994; Gutierrez, 2003; Hanley-Bowdoin *et al.*, 1999) Transcriptional Activator (TrAP) AC2 (Sunter and Bisaro, 1992; Voinnet *et al.*, 1999; Van Wezel *et al.*, 2002a; Sunter and Bisaro, 2003), Replication Enhancer (REn) such as AC3 (Settlage *et al.*, 1996; Settlage *et al.*, 2001) and Hypersensitive Response-like reaction initiated by Rep such as AC4 (Van Wezel *et al.*, 2002b). The genes, BV1 and BC1 located in DNA-B encode two proteins involved in intracellular virus movement (Nuclear Shuttle Protein -NSP) (Noueiry *et al.*, 1994; Sanderfoot and Lazarowitz, 1995; Sanderfoot *et al.*, 1996) and intercellular virus movement (Movement Protein-MP) (Ingham *et al.*, 1995; Schaffer *et al.*, 1995; Jeffrey *et al.*, 1996; Lazarowitz and Beachy, 1999) (Table 7).

Three categories of novel satellite-like ssDNA molecules (DNA-1, DNAβ and defective interfering DNA) have been found associated with some monopartite begomoviruses and they depend upon their helper viruses for encapsidation, replication, movement and insect transmission (Briddon *et al.*, 2008).

2.2.4.3 Symptomatology

Symptoms of the different begomovirus infection on papaya are almost similar. The papaya leaf curl disease shows the characteristic symptoms as severe leaf curl on leaf. Saxena *et al.* (1998a) observed the downward leaf curling, vein thickening and clearing symptoms. Sometimes affected leaves also become rubbery, fragile and with distorted petioles (Byun *et al.*, 2016).

Chang *et al.* (2003) reported the symptoms of curling, deformation, and yellowing of young leaves which are known typical symptoms of *Papaya leaf curl virus* (PaLCuV), along with twisted petioles, vein enation, and stunting. Severe infection results in distortion of leaves associated with curling and rolling of leaves, leathery leaf, vein zigzag and reduction in the size of petioles (Singh and Awasthi, 2017).

Table 7: Description of begomovirus gene/proteins and their functions

Virus genome component	Gene	Protein	Function	Reference(s)
DNA-A	AV1	CP	Whitefly-mediated transmission and virion capsid	Noris *et al.* (1998), Kheyr-Pour *et al.* (2000), Hohnle *et al.* (2001)
	AV2	-	Viral movement	Hanley-Bowdoin *et al.* (2000)
	AV3	-	RNA silencing suppressor	Gong *et al.* (2021)
	AC1	Rep	Viral DNA replication	Fontes *et al.* (1994), Hanley-Bowdoin *et al.* (1999), Gutierrez (2003)
	AC2	TrAP	Transcriptional activator for the virus-sense genes, suppresses RNA silencing and other host defense responses	Sunter and Bisaro (1992), Voinnet *et al.* (1999), Van-Wezel *et al.* (2002a) and Sunter and Bisaro (2003)
	AC3	REn	Increases viral replication	Settlage *et al.* (1996) and Settlage *et al.*(2001)
	AC4	-	Hypersensitive response-like reaction initiated by Rep	Van-Wezel *et al.* (2002b)
	AC5	PTGS	Post-transcriptional gene silencing	Li *et al.* (2015)
	AC6	-	Uncharacterized open reading frame	Verma and Saxena (2017) and Muthupandi *et al.* (2019)

CP: Coat protein, Rep: Replication-associated protein, TrAP: Transcriptional activator protein, REn: Replication enhancer protein, PTGS: Post-transcriptional gene silencing The products encoded by ORFs AV2, AV3, AC4, AC5 and AC6 yet to be named

Fruits from infected plants are reduced in number and never develop to full maturity, often falling from trees prematurely (Saxena *et al.*, 1998a). In addition, diseased fruits are stunted and distorted, along with shortening of internodes and main shoot (Nadeem *et al.*, 1997).

2.2.5 Studies on host range and virus indexing against PRSV

Host range studies of virus and the symptoms it produces often provide an important clue to its identity. It also helps to know the best host for propagation, assaying and maintenance of virus isolates. A useful range of test plants for virus diagnosis has been listed by Hollings (1983). PRSV-P has limited in host rang, it is predominantly restricted to two families other than Caricacea *viz.,* Cucurbitaceae and Chenopodioideae but recent reports showed that PRSV-P can also transmit to few crop species of Amaranthaceae, Asteraceae, Euphorbiaceae and Solanaceae which can also act as alternative host for the PRSV in off-season (Chavan *et al.*, 2010; Singh *et al.*, 2017). Detailed list of host crop species against PRSV is given in table 8.

2.3 Epidemiological studies on incidence of PRSV

Epidemiological studies are more relevant as they reveal dynamics of virus disease incidence in relation to climate. This helps to design the management strategies to focus on reducing virus transmission by vectors. The rate of spread of the virus has often been shown to depend on the season, age of the plant, vereity, weather, vector population, size of the cropped area, its shape and surounding host crops (Taylor, 1986).

2.3.1 Effect of different months of planting on the incidence of PRSV disease, growth and yield parameters of papaya at different stage of growth

The spatial and temporal distribution and progress of PRSV as well as aphid populations were monitored in papaya cultivated areas in the state of Veracruz, Mexico. The aphid population had a bimodal distribution with the highest population peak in December to February and a secondary peak in August to September and illustrated the change in disease incidence was influence of aphid population echelon of the previous month (Mora-Aguilera *et al.*, 1992).

Table 8: List of host species reported for PRSV-P

Sl. No.	Family	Species	Symptoms	Reference(s)
1.	Amaranthaceae	*Alternanthera sessilis*	VC	Chavan *et al.* (2010)
2.		*Gomphrena globosa*	VC	Singh *et al.* (2017)
3.	Asteraceae	*Xanthium indicum Koern.*	VC	Chavan *et al.* (2010)
4.	Chenopodioideae	*Chenopodium amaranticolor*	LL	Purcifull *et al.* (1984), Perera *et al.* (1998), Parmar (2000), Kunkalikar (2003), Tripathi *et al.* (2008), Kumar *et al.* (2014), Limkar (2017), Navanath *et al.* (2017) and Harish (2018)
5.		*Chenopodium quinoa*	LL	Purcifull *et al.* (1984), Yeh and Gonsalves (1984), Perera *et al.* (1998), Parmar (2000), Lakshminarayana Reddy (2000), Tripathi *et al.* (2008), Kelaniyangoda and Madhubashini (2010), Limkar (2017), Singh *et al.* (2017) and Navanath *et al.* (2017)
6.		*Spinacia oleracea*	M, VC	Parmar (2000)
7.	Cucurbitaceae	*Bennicasa hispida*	CS	Shaikh (1996), Dahal *et al.* (1997) and Navanath *et al.* (2017)
8.		*Citrullus lanatus*	VC	Zettler *et al.* (1968), Thomas and Dodman (1993), Lakshminarayana Reddy (2000), Singh *et al.* (2003) and Singh *et al.* (2017)
9.		*Citrullus vulgaris*	VC, Myc, CS	Conover (1962), Zettler *et al.* (1968), Thomas and Dodman (1993), Dahal *et al.* (1997), Roy *et al.* (1999), Parmar (2000), Kunkalikar (2003) and Singh *et al.* (2017)
10.		*Coccina indica*	VC	Parmar (2000)
11.		*Cucumis anguria*	VC	Kunkalikar (2003) and Singh *et al.* (2017)

Contd…..

Sl. No.	Family	Species	Symptoms	Reference(s)
12.	Cucurbitaceae	*Cucumis melo*	VC	Conover (1962), Zettler *et al.* (1968), Thomas and Dodman (1993), Dahal *et al.* (1997), Perera *et al.* (1998), Parmar (2000), Kunkalikar (2003), Chavan *et al.* (2010) and Singh *et al.* (2017)
13.		*Cucumis metuliferus*	VC, CS	Yeh and Gonsalves (1984), Tripathi *et al.* (2008) and Singh *et al.* (2017)
14.		*Cucumis sativus*	VC, MyS, CS	Conover (1962), Zettler *et al.* (1968), Thomas and Dodman (1993), Dahal *et al.* (1997), Parmar (2000); Kunkalikar (2003), Kumar *et al.* (2014), Limkar (2017), Singh *et al.* (2017), Navanath *et al.* (2017) and Harish (2018)
15.		*Cucurbita maxima*	VC	Dahal *et al.* (1997)
16.		*Cucurbita moschata*	VC, CS	Parmar (2000), Kunkalikar (2003), Kumar *et al.* (2014), Limkar (2017), Singh *et al.* (2017) and Harish (2018)
17.		*Cucurbita pepo*	VC, CS	Conover (1962), Zettler *et al.* (1968), Storey and Halliwell (1969), Thomas and Dodman (1993), Dahal *et al.* (1997), Roy *et al.* (1999); Kunkalikar (2003), Tripathi *et al.* (2008), Singh *et al.* (2017), Limkar (2017); Navanath *et al.* (2017), Babu and Banerjee (2018)
18.		*Lagenaria siceraria*	Mys, CS	Dahal *et al.* (1997); Parmar (2000); Kunkalikar (2003), Kumar *et al.* (2014), Limkar (2017), Singh *et al.* (2017) and Navanath *et al.* (2017)
19.		*Luffa acutangula*	CS	Shaikh (1996), Dahal *et al.* (1997), Kunkalikar (2003), Kumar *et al.* (2014), Limkar (2017), Singh *et al.* (2017) and Navanath *et al.* (2017)
20.		*Luffa aegyptica*	CS	Parmar (2000)

Contd…..

Sl. No.	Family	Species	Symptoms	Reference(s)
21.	Cucurbitaceae	*Luffa cylindrical*	CS	Shaikh (1996), Limkar (2017) and Singh *et al.* (2017)
22.		*Memordica charantia*	CS	Dahal *et al.* (1997), Parmar (2000), Kunkalikar (2003), Limkar (2017), Singh *et al.* (2017), Navanath *et al.* (2017) and Harish (2018)
23.		*Sechium edule*	CS	Dahal *et al.* (1997)
24.		*Trichosanthes anguina*	CS	Kunkalikar (2003)
25.		*Triohosanthes cucumeria*	CS	Dahal *et al.* (1997), Kumar *et al.* (2014), Navanath *et al.* (2017) and Harish (2018)
26.		*Zucchini*	CS	Navanath *et al.* (2017)
27.	Euphorbiaceae	*Ricinus communis*	CS	Singh *et al.* (2017)
28.	Fabaceae	*Cassia tora*	CS	Chavan *et al.* (2010)
29.	Solanaceae	*Physalis minima*	CS	Chavan *et al.* (2010)

CS: Chlortic Spot, LD-Leaf Distortion LL- Local Lesions M- Mosaic MMo- Mosaic Mottling Mys: Mosaic Yellow Spot. SM- Severe Mosaic and VC- Vein Clearing

Thiara *et al.* (2003) studied the effect of four dates of sowing *viz.* May 16, May 31, June 16 and June 30 during 2000-01 on the disease index and incidence of *Soybean yellow mosaic* (SoYMV) on two soybean varieties. Maximum disease was observed in May 16 sown crops, which decreased with subsequent sowing dates. Pod and seed weight per plant were highest on May 31 and June 16 sown crops.

Trial was conducted during 2003-04 on planting dates from May 1st to July 1st at 15 days intervals in three commercial varieties of cotton. Maximum *Cotton leaf curl virus* (CLCuV) percentage was recorded in June 1st planting. It was found that the CLCuV percentages rapidly increase in the first week of August in all the planting dates. It was also concluded that planting of cotton should be done from May 1st to June 1st to avoid the severe losses of CLCuV (Tahir *et al.*, 2004).

Effect of planting date on pest infestation and viral plant disease for winter wheat (*Triticum aestivum)* in south Dakota and the northern Great Plains was studied. It is observed that, the incidence of *Barley yellow dwarf virus* (BYDV) declined with late planting and was correlated with the autumnal abundance of cereal aphids, with greater incidence in early and middle plantings (Hesler *et al.*, 2005).

A field trial was conducted to study PRSV incidence and populations of aphid on papaya during the lean period of the aphid vectors population (spring season) at IARI, Regional Station, Pune. The PRSV infection was delayed till monsoon, by which time plants had crossed the fruit bearing stage. PRSV infection till flowering was minimum on plants transplanted in early spring (18.25 %), followed by mid (22.91 %) and late (33.33 %) spring by Sharma *et al.* (2010).

Singh and Shukla (2011) performed studies on PRSV disease severity and yield of the papaya at different months. Inoculations were done in pre-*kharif* season (March to April) and *kharif* season (July to August). The symptoms were very severe in seedlings inoculated in *kharif* and the yield of the plants was recorded only 63.9 %. However, seedlings of the pre-*kharif* season showed mild mottling and only a slight reduction. This is because the symptoms were masked at high temperatures resulting in a mild incidence of the disease. Whereas dring low temperatures severe expression of symptoms and a heavy yield loss was observed. It is concluded that, disease severity in the seedlings inoculated during *kharif* season was high due to the increased population of aphids.

Chandrashekar *et al*. (2015) studied the effect of the aphid population on the incidence of PRSV-P by planting papaya during different months of the year (2010-12) at ICAR-IARI Regional Station, Pune. The population monitoring of aphid vectors of PRSV indicated a peak population of aphids in January and a low population from March onwards till September. Papaya planted from February to April showed significantly less incidence of PRSV compared to those planted from September to January. Virus incidence recorded at the flowering time was high (100 %) in September to January plantation, while it was about 50.0 to 80.0 per cent when planted in February to April. PRSV incidence on papaya planted in different months showed a positive correlation with the aphid population.

Studies were conducted to understand the influence of weather factors on the aphid population and the incidence of PRSV. It was observed that the crop planted during August to December recorded minimum PRSV incidence (10 to 20 %) compared to February and March planted crop that succumbed to PRSV incidence at 60 days after planting. Avoiding planting of papaya during February to April months could reduce PRSV (Thiribhuvanamala *et al.*, 2016).

An experiment was conducted by Kone *et al.* (2017) to assess the effect of planting date on the incidence of viral diseases, severity and susceptibility of the cucumber and zucchini cultivars under field condition during May-July 2014, September-November 2014 and February to April 2015. Results demonstrated the effect of planting dates on cultivars was significantly different at the different growing stages.

An experiment was undertaken to evaluate the impact of planting date on *Chilli leaf curl virus* (ChiLCV) disease development and its vector whitefly abundance on chilli in Jessore during 2019 and 2020. Of the three chilli planting dates, early planting (Januvary 1) significantly suppressed ChiLCV incidence, severity and its vector whitefly abundance over mid (February 1) and late planting (March 1) dates (Das *et al.*, 2021)

Pallavi *et al.* (2021) studied the influence of different sowing months on PDI and mite population, age of host on disease development of *Sterility mosaic virus* disease incidence in pigeonpea. The early stage crop has less disease incidence and a

gradual increase in incidence was recorded at later stages of crop growth. Cent per cent incidence was observed at 90 DAS in almost all months. The incidence was lesser in the early stage due to invasion of less number of mites and population build up as the plant grew vigorous in the later stage of the crop which could result in attaining maximum incidence.

2.3.2 Identification of susceptible stage of papaya for PRSV infection

Pio-Ribeiro *et al.* (1978) reported that *Cowpea aphid-borne mosaic virus* (CABMV) and *Cucumber mosaic virus* interact synergistically to produce cowpea stunt, a disease characterized by severe stunting and yields loss when infection occurred early. Niblett and Claflin *et al.* (1978) and Uyemoto *et al.* (1981) demonstrated that *Maize dwarf mosaic virus* (MDMV) in maize (*Zea mays*) induce the necrosis disease which resulted in up to 91 per cent yield loss and death of many plants especially when infection occurred early. Bosque-Perez *et al.* (1998) conducted field experiments to assess the effects of *Maize streak virus* (MSV) disease on the growth and yield of maize having, found that plant age at the time of virus challenge had significant effects on yield and growth characters.

Booker *et al.* (2005) demonstrated that *Cowpea severe mosaic virus* (CPSMV) inoculations at early log phase (seedling stage) 12 days after seeding (DAS) had the greatest impact (50 to 85 % yield loss) compared to those inoculated during the exponential growth phase (24 DAS; 22 to 66 % yield loss) or linear growth phase (35 DAS; 2 to 36 % yield loss) and concluded that control measures for CPSMV should be aimed at delaying infection by CPSMV to minimize the impact on cowpea yield.

Kareem and Taiwo (2007) demonstrated that inoculation of cowpea with *Cowpea aphid borne mosaic virus* (CABMV), *Cowpea mottle virus* (CMeV) and *Southern bean mosaic virus* (SBMV) alone and in mixed infection at 10 days after planting resulted in fewer leaves and reduced plant height than inoculation at 30 days after planting.

Inoculation of each *Potato virus X* (PVX) and *Tomato mosaic virus* (ToMV) after 14 days tomato germination induced an acute symptomatic response and eventual death of over 90.0 per cent of the plant population in 15 days after

inoculation. But when inoculated to 28 days old plants has only a synergistic disease response but no deaths in plant population (Balogun, 2008).

Levy and Lapidot, (2008) determined the effects of plant age on the expression of genetic resistance against *Tomato yellow leaf curl virus* (TYLCV) by inoculating TYLCV at different days after sowing and recorded maximum yield loss in plants inoculated at early stage *i.e.* 14 DAS followed by 28 DAS. Further delayed inoculation recorded least yield loss (45 DAS). Thus they showed the occurrence of age related (or mature plant) resistance in tomato plants against TYLCV.

Aliyu *et al.* (2010) conducted screenhouse study to evaluate the influence of yellow mosaic disease on age of cowpea seedling. It was found that inoculation of virus at 7 DAG subsequently led to the most severe symptoms and eventual poor growth and yield attributes compared with 21 DAG which had the highest yield attributes.

Onwughalu *et al.* (2011) demonstrated that inoculation of *Rice yellow mottle virus* (RYMV) at early growth phase (seedling and booting) is most vulnerable phase to virus infection in rice. De Breuil *et al.* (2012) reported that CMV on peanut severly reducess seed yield (30 %) with greater number of immature pods when inoculated at early (V4-6) growth stage. Olobashola *et al.* (2017) established that *Cucumber mosaic virus* (CMV) disease on sweet pepper was more devastating in early infection than late infection.

2.4 Integrated management of PRSV disease under field conditions

2.4.1 Insecticides

Gourmet *et al.* (1996) demonstrated that treatment of imidacloprid is effective in controlling of barley yellow dwarf and increasing the yield up to 112 per cent in oat and wheat

Ahmed *et al.* (2001) reported that two applications at four rates of confidor, an imidacloprid insecticide (47.6, 71.4, 95.2, and 119 g a.i./ha) indirectly controlled *Tomato yellow leaf curl virus* (TYLCV) in field plantings of tomato. Similarly, Csinos *et al.* (2001) reported that imidacloprid (Admire 2F) at 67.2 g a.i./7,000 plants is

significantly effective in reducing the *Tomato spotted wilt virus* (TSWV) disease on tobacco.

Prabhaker *et al.* (2005) demonstrated that treatment of imidacloprid and thiamethoxam prevented whitefly population their by suppressing incidence of *Cucurbit yellow stunting disorder virus* (CYSDV). Mowry (2005) reported that use of esfenvalerate, imidacloprid, methamidophos, oxamyl, pymetrozine and thiamethoxam were effective in reducing the transmission of *Potato leafroll virus* (PLRV) by *Myzus persicae*. Khan *et al.* (2012) evaluated the chemicals against *Mungbean yellow mosaic virus* (MBYMV) and whitefly. The chemical imidacloprid was most effective to control whitefly population their by reducing the incidence of MBYMV.

Chavan *et al.* (2015) conducted PRSV-P disease management study through vector control using chemical pesticides on papaya under field condition. The number of aphids collected on traps in different treatments was significantly less compared to control plots along with lowest disease incidence in fortnightly alternate sprays of dimethoate 30 EC (0.05%) and azadirachtin 0.03 EC (4 ml/l) followed by sprays of thiamethoxam 25 WG (0.005%) and buprofenzin 25% SC (0.025%).

Mahalakshmi *et al.* (2015) recorded the significant reduction in whiteflies population and incidence of *Yellow mosaic virus* in blackgram when treated with neonicotiniods such as imidacloprid 200 SL @ 0.3 ml/l, thiamethoxam 25 WG @ 0.2 g/land thiacloprid 21.7 SC @ 1.25 ml/l. Further a study conducted by Patel *et al.* (2018) reported that dinotefuron @ 0.2% concentration were significantly effective in controlling the insect vector population (94%) and decreasing the rice tungro disease infection to 75% under glasshouse condition.

Field experiments were conducted by Kakati and Nath (2019) during 2013-15 in Jorhat, Assam to evolve a suitable management strategy against *Pentalonia nigronervosa* vector of *Banana bunchy top virus* disease. They found that foliar spraying of imidacloprid @ 0.1% at 60, 90, 120 and 150 days after planting showed no disease incidence, zero insect vector population count in all the two cropping seasons.

Ambarish *et al.* (2020) demonstrated the effect of insecticides on vector whitefly and the incidence of yellow mosaic disease in greengram. Two years of pooled data revealed that thiamethoxam of 25 WG and imidacloprid 17.8 SL recorded lowest population of whiteflies (0.80 and 1.16) per trifoliate leaf and lowest per cent disease incidence of 2.28 and 2.48 respectively.

2.4.2 Bio rationals

In an attempt to combat the disease, a field trial was conducted to manage PRSV through an integrated approach using seven different treatments. The most effective treatment was an application of neem oil 1% + dimethoate 1.05 % with the least disease incidence 6.66 per cent and 41.66 per cent respectively at 60 and 150 DAP. This was followed by the application of dimethoate 1.05 % with 16.33 and 56.66 per cent PRSV incidence while the untreated (control) plants registered 68.33 and 90 per cent incidence respectively at 60 and 150 DAP (Singh *et al.* 2008b).

A combination of reflective row cover, mineral oil (2 Tin oil) @ 0.1 % and imidacloprid @ 0.0053 % (alternative fortnightly) spray was recorded as the most effective treatment in delaying the PRSV infection on papaya and significantly higher number of marketable papaya fruits per plant (Kalleshwaraswamy *et al.*, 2009).

Hansen and Nielsen (2012) reported that mineral oil combined with insecticide (Karate 2.5 WG, lambda-cyhalothrin) was effective in controling aphid virus vectors to reduce *Potato virus Y* infections in seed potatoes. Jimenez *et al.* (2011) reported that treatment of plants with seaweeds showed a significant reduction of symptoms caused by *Tobacco mosaic virus* (TMV) in tobacco plants.

Pushpa *et al.* (2018) reported that foliar application of aqueous sea weed extract (*Kappaphycus alvarezii*) at 4 ml/l on papaya against PRSV disease at every 15 days interval recorded the delay and relatively lesser per cent incidence with less symptomatic and well formed fruits than the control plants. Moreover, plants that were treated with sea weed extract were relatively taller in height, dense in foliar canopy compared to those which are untreated. The average number of fruits in case of the treated plants was higher than those in the untreated group. The average number of fruits per plant was 30 for treated group while the plants in the untreated group showed only 15 fruits per plant.

Kavyashri (2019) demonstrated that red seaweed extract (*Kappaphycus alvarezii*) is significantly effective in reducing per cent disease incidence (30.35 and 39.48 %) of *Cucumber mosaic virus* (CMV) infection in Chilli *var*. Pusa Jwala during *kharif* 2016-17 and *kharif* 2017-18 respectively under field conditions

A study was carried out during 2016-17 and 2017-18 at ACHF, Navsari and assessed the effect of silicon and seaweed extract on papaya *cv*. 'Red Lady'. The best results concerning growth parameters *i.e.* plant height, stem girth, number of leaves per plant and leaf area were obtained by spraying plants with potassium silicate at 0.4 % + seaweed extract at 4 % by Patel *et al.* (2020).

3. MATERIAL AND METHODS

The present investigation was carried out at the Department of Plant Pathology, College of Horticulture Bagalkot, UHS Bagalkot, Karnataka during the year 2019-20 and 2020-21. The details of the materials used and methods followed are elaborated below.

3.1 Survey for the prevalence, distribution, collection and characterization of PRSV and PaLCuV diseases associated with papaya in Karnataka.

3.1.1 Survey for the prevalence and distribution of viral diseases in Karnataka

An intensive roving survey was conducted from November 2019 to April 2021 to know the viral disease incidence in major papaya growing districts of Karnataka *viz.*, Bagalkote, Belagavi, Ballari, Chitradurga, Gadag, Haveri, Kalaburagi, Koppal, Vijayapura and Yadgiri

The disease diagnosis in the field was based on the symptoms of virus on plants. In each field, virus infected samples were collected in polythene bags, sealed with proper labeling. Observations were recorded with respect to area cultivated, cultivar used, crop stage, the previous crop, surrounding crop and typical symptoms on plant. In each plot random samples were made, from which the number of plants affected over the total number of plants was counted and expressed as disease incidence in percentage using the formula.

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

3.1.2 Collection of virus samples, molecular detection and characterization of viruses associated with papaya

3.1.2.1 Collection of virus samples

The leaf samples showing typical symptoms of ringspot (PRSV) and leaf curl (PaLCuV) were collected separately and labeled. Characterization of virus from the different locations during survey showing typical symptoms on leaves were subjected to both total DNA and total RNA extraction.

3.1.2.1 Extraction of total DNA using cetyl trimethyl ammonium bromide (CTAB) method from papaya leaf samples

The genomic DNA was extracted from the healthy and virus infected papaya leaf samples using the CTAB method (Lodhi *et al.*, 1994) as described in appendix I-A and appendix II-A and stored at -20º C for further use.

3.1.2.2 Phenol-chloroform extraction of DNA purification

Purification of nucleic acids was done by extraction with phenol-chloroform method as described in appendix II-B.

3.1.2.3 Extraction of total RNA from virus infected papaya leaves

Extraction of total RNA from healthy and virus infected papaya leaf sample was done by using Spectrum™ Plant Total RNA Kit from Sigma-Aldrich (Takara Bio, Catalog No. STRN50) method as described in the appendix II-C.

3.1.2.4 Quantification of total DNA and RNA

Total DNA and RNA extracted from papaya leaf samples was quantified using Thermo Fisher scientific Nanodrop TM 2000 spectrophotometer. For quantification, 1µl of total DNA or RNA extract was loaded on nanodrop by taking $T_{10}E_1$ or elusion buffer as blank.

3.1.2.5 Reverse transcription (cDNA Synthesis) of RNA

Extracted total RNA from healthy and virus infected samples were quantified and diluted to 1000 ng/µl. The diluted RNA was taken for reverse transcription for synthesizing cDNA using PrimeScript™ 1st strand cDNA Synthesis Kit (Takara Bio, Catalog No. #6110A). The method is described in appendix II-D.

3.1.2.6 Polymerase chain reaction and reverse transcription-PCR (RT-PCR) mediated amplification

The DNA or cDNA synthesized by reverse transcription was subjected to PCR. The PCR reaction mixture and procedure are described in appendix II-E. Specific primers were used to amplify the DNA and RNA viruses are mentioned in table 9.

Table 9: PCR primers and amplification condition used for detection and characterization of viruses infecting papaya

Primer	Oligonucleotides (5'-3')	Length (nt)	PCR condition						Remarks	Reference
			Initial-denaturation	No. of cycles	Denaturation	Annealing	Extension	Final extension		
Papaya ringspot virus primers										
MB 11A	GGATCCATGTCCAAAAATGAAGCTGTGGATGCT	33	94°C for 5 min	35	94°C for 2 min	55°C for 1 min	72°C for 1 min	72°C for 10 min		Bateson, *et al*. (1994)
MB 11B	TCAATGGCGCATACCCAGGAGAGT	24								
PRSV878S –F	CACCTGGCACTCGAAGTTGGCAATGAAA	28	94°C for 5 min	35	94°C for 30 Sec	55°C for 30 Sec	72°C for 2 min	72°C for 7 min.		Ortiz-Rojas and Chaves-Bedoya (2017)
PRSV2746A-R	TTTCTTAGCTATGTACATTGAATCAGAC	28								
PRSV2289S –F	TCACTTCAACCCAGCTCTTACGTGTGAC	28								
PRSV3926A-R	TGCTTCAATTGTATATGCTCTGTGG	25								
PRSV4199S –F	TCGACGTGGACAAGAGTGACTGTGTTTA	28								
PRSV5924A–R	TCACTTTCAATCAGTTTATCAATCGTTA	28								
PRSV8961S –F	GTCATGGGGATATGGGGAGTTAACACA	27								
PRSVPOLYT-R	TTTTTTTTCTCTCATTCTAAGAGGCTCG	28								
PRSV3310S –F	GTTTTAGTAGAGCAATTATGTCGGAAAG	28	94°C for 5 min	35	94°C for 30 Sec	50.9°C for 30 Sec	72°C for 2 min	72°C for 7 min.		
PRSV4850A–R	TTTTCAATCGCGTTCCCCTCCATCAAA	27								
PRSV5355S –F	TGGAAGACATAAAGAGGGAATTGCACTA	28								
PRSV7195A–R	TTGCTCTGCTGTGGAATGGTGGAAAGTC	28								
PRSV1S -F	AAATAAAACATCTCAACACAACACAATTCAAAG	33	94°C for 5 min	35	94°C for 30 Sec	60°C for 30 Sec	72°C for 2 min	72°C for 7 min.		
PRSV1780A-R	TGCACACATGATCCGTTGGTTTTCT	25								
PRSV6777S –F	TGATTTAACACCACATAACCCACTCAAG	28								
PRSV8041A–R	TTTGTAAAACTTTGTCATGCCAACAGTC	28								

Contd……

Primer	Oligonucleotides (5'-3')	Length (nt)	PCR condition						Remarks	Reference
			Initial-denaturation	No. of cycles	Denaturation	Annealing	Extension	Final extension		
PRSV7497S –F	CAGGCAGATTGATGATTTAACTTGGAGC	28								
PRSV9389A–R	TCTCTATCTCTCTCTCCAGTTTTTGTGC	28								
Milk vetch dwarf virus primers										
M-F	CTAGTCAGCCATCCAATGGTG	21	94°C for 3 min	35	94°C for 30 Sec.	58°C for 30 Sec.	72°C for 1 min	72°C for 10 min.		Lal *et al.* (2020)
M-R	GTGCAGGGTTTGATTGTCTGC	21								
Papaya lead distortion virus primers										
PLDMV355-F	GGCATGTGGTTTATGATGCAAGGG	24	94°C for 3 min	35	94°C for 30 Sec.	55°C for 30 Sec.	72°C for 1 min.	72°C for 5 min.		Tuo *et al.* (2014)
PLDMV355-R	GCTCCGTGTTCTCAGTCGCATT	22								
Papaya mosaic virus primers										
PapMV205-F	CCAAATTTGCCGCGTTCGACT	21	94°C for 3 min	35.	. 94°C for 30 Sec.	55°C for 30 Sec	72°C for 1 min.	72°C for 5 min		Tuo *et al.* (2014)
PapMV205-R	GACCCAGAAATTTGGCCTTTGGTGATG	27								
Zucchini yellow mosaic virus primers										
ZYMV-F (CPF-NocI)	CCATGGTCAGGCACTCARCCAACT	24	94°C for 5 min	35	94°C for 30 Sec	55°C for 30 Sec	72°C for 2 min	72°C for 7 min.		Villamor *et al.* (2003)
ZYMV-R (CPR-NocI)	CCATGGCTGCATTGRTTCACACCTAGYAAG	30								
Degenerative primers for detecting Begomovirus										
PAL1v1978	GCATCTGCAGGCCCACATYGTCTTYCCNGT	30	94°C for 3 min	30	94°C for 1 min.	55°C for 2 min.	72°C for 2 min.	72°C for 10 min.	DNA-A (Top half)	Rojas *et al.* (1993)
PAR1c496	AATACTGCAGGGCTTYCTRTACATRGG	27								
PAL1v1978	GCATCTGCAGGCCCACATYGTCTTYCCNGT	30	94°C for 3 min	30	94°C for 1 min.	55°C for 2 min	72°C for 2 min	72°C for 10 min.	DNA-A (Top half)	

Contd.……

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Primer	Oligonucleotides (5'-3')	Length (nt)	PCR condition						Remarks	Reference
			Initial-denaturation	No. of cycles	Denaturation	Annealing	Extension	Final extension		
PARIc715b	GATTTCTGCAGTTDATRTTYTCRTCCATCCA	31								
PARIv722b	ATATCTGCAGGGNAARATHTGGATGGA	27	94°C for 3 min	30	94°C for 1 min	60°C for 2 min.	72°C for 2 min.	72°C for 10 min.	DNA-A (Bottom half)	
PALIc1960b	TGGACTGCAGACNGGNAARACNATGTGGGC	30								
Av494	GCCYATRTAYAGRAAGCCMAG	24	94°C for 3 min	30	94°C for 1 min.	60°C for 2 min.	72°C for 2 min.	72°C for 10 min.	DNA-A (Coat protine)	Wyatt and Brown (1996)
Ac1048	GGRTTDGARGCATGHGTACATG	23								
PBL1v2040	GCCTCTGCAGCARTGRTCKATCTTCATACA	30	94°C for 3 min	30	94°C for 1 min.	50°C for 1 min.	72°C for 2 min.	72°C for 10 min.	DNA-B	Rojas *et al*. (1993)
PCRc1	CTAGCTGCAGCATATTTACRARWATGCCA	29								
beta-DNA primers										
BETA01	GGTACCACTACGCTACGCAGCAGCC	25	94°C for 3 min	30	94°C for 1 min.	50°C for 1 min.	72°C for 3 min.	72°C for 10 min		Briddon *et al*. (2002)
BETA02	GGTACCTACCCTCCCAGGGGTACAC	25								
alpha-DNA primers										
DNA101	CTGCAGATAATGTAGCTTACCAG	23	94°C for 5 min	35	94°C for 1 min.	50°C for 1 min.	72°C for 1.5 min.	72°C for 15 min.		Bull *et al*. (2003)
DNA102	CTGCAGATCCTCCACGTGTATAG	23								
UN101	AAGCTTGCGACTATTGTATGAAAGAGG	27	94°C for 5 min	5	94°C for 1 min.	45°C for 1 min.	72°C for 1.5 min.	72°C for 10 min		
UN102	AAGCTTCGTCTGTCTTACGAGCTCGCTG	28		30	94°C for 1 min.	50°C for 1 min.	72°C for 1.5 min.			

*Nucleotides at degenerate positions are represented by a single letter of the IUPAC ambiguity code: D=A,G,T; H=A,C,T; K=G,T; M=A,C; N=A,C,G,T; R=A,G; W=A,T; Y=C,T.. Degeneracy is the product of the numbers that designate the number of bases or nucleotides that may occur at each position in that primer.

3.1.2.7 RT-PCR conditions used to detect and characterization of RNA viruses

The RT-PCR amplification was carried out for detecting and characterizing the PRSV and other RNA virus infections in papaya leaf samples using the specific primers. Detection of PRSV in surveyed samples was done by using a set of primer MB 11A/MB 11B (Bateson *et al.*, 1994) given in table 9. Characterization and amplification of full length genome of PRSV was done using nine overlapping primer pairs designed by Ortiz-Rojas and Chaves-Bedoya (2017) given in table 9.

Detection for the presence or association of RNA virus infections (apart from PRSV) like *Papaya mosaic virus, Papaya leaf distortion virus, Papaya milk vetch dwarf virus* and *Zucchini yellow mosaic virus* was done by using respective virus specific primers with suitable PCR conditions as given in table 9.

3.1.2.8 PCR conditions used to amplify the components (DNA-A and DNA-B) of begomovirus

The PCR amplification was carried out for detecting the presence of begomovirus (ssDNA virus) infection in papaya leaf samples along with the DNA extracted from chilli (showing leaf curl symptoms) and ridge gourd (showing yellow mosaic symptoms) plants collected from the field as a positive control (Plate 1a and 1b).

Amplification of DNA-A/DNA-B components was carried out by using the different sets of degenerative overlapping forward and reverse primers in a thermal cycler with recommended PCR conditions given in table 9.

3.1.2.9 Detection of the beta and alpha satellites in infected samples

The PCR amplification for detecting the presence of beta and alpha satellites in papaya was carried out along with positive control sample (chilli leaf curl). Specific primers were used to amplify the beta and alpha DNAs and PCR conditions are given in table 9.

3.1.2.10 Analysis of PCR and RT-PCR products by agarose gel electrophoresis

The reagents for the preparation of agarose gel are mentioned in appendix I-B and the procedure for agarose gel electrophoresis are given in appendix II-F.

Plate 1: Chili plant showing leaf curl symptoms (A) and ridge gourd plants showing yellow mosaic symptoms (B) used as +ve control in amplification of DNA-A and DNA-B components of isolated PaLCuV respectively

3.1.2.11 Elution of amplified PCR fragments from agarose gel

After confirmation of desired fragment size of PCR product, it is purified using NucleoSpin® Gel and PCR Clean-up kit (Takara Bio, Catalog No. # 740986.50) according to the manufacturer instructions (Appendix II-G). The purified product was checked by agarose gel electrophoresis along with 1 kb DNA ladder (StepUp™ 1 kb DNA Ladder, 50 µg, SKU: MBD20).

3.1.2.12 Cloning and ligation

The cloning and ligation was carried out for complete genome sequencing of representative samples. A sample collected from Bagalkote, Karnataka was used for full length genome sequencing of PRSV and 13 PaLCuV representative samples (Table 10) collected from different surveyed locations were subjected for complete genome sequencing. The PCR amplified fragments were cloned into the plasmid vector pMD20-T vector (Takara Bio Mighty TA-cloning Kit, Takara Bio Catalog No. #6028) (Fig. 1) according to the instructions (Appendix II-H).

3.1.2.13 Preparation of competent cells and transformation

Competent cells of *Escherichia coli* strain DH5α were prepared by the calcium chloride method as described by Sambrook and Russel (2001). The media and buffers used in the preparation of competent cells are mentioned in appendix I-C and transformation protocols in appendix II-I.

3.1.2.14 Patch up of the transformed colonies

The white and blue colonies were obtained in plates. Only white colonies are individually picked and patched up into the new plates containing LB agar medium containing ampicillin and incubated at 37^0 C overnight.

3.1.2.15 Screening of transformants through colony PCR

From the patched up plate, the colonies from respective plates were selected, picked and used as templates in the colony PCR by using specific primers.

Table 10: Representative PaLCuV samples of papaya used for full length genome sequencing

Sl. No.	Name assigned	Location		
		Village	Taluk	District
1.	PaLCuV -1	Bangwhad	Gokak	Belagavi
2.	PaLCuV -2	Ghataprabha	Gokak	Belagavi
3.	PaLCuV -3	Tulsigeri	Bagalkote	Bagalkote
4.	PaLCuV -4	Honakatti	Bilgi	Bagalkote
5.	PaLCuV -5	Thornur	Ramadurga	Belagavi
6.	PaLCuV -6	Banihatti	Hungund	Bagalkote
7.	PaLCuV -7	Hiremagi	Hungund	Bagalkote
8.	PaLCuV -8	Anoor	Afzalpur	Kalaburagi
9.	PaLCuV -9	Tavarkeda	Afzalpur	Kalaburagi
10.	PaLCuV -10	Farahatabad	Kalaburagi	Kalaburagi
11.	PaLCuV -11	Hadagalarathi	Kalaburagi	Kalaburagi
12.	PaLCuV -12	Nidagundi	Muddebihal	Vijayapura
13.	PaLCuV -13	Kancharagatti	Haveri	Haveri

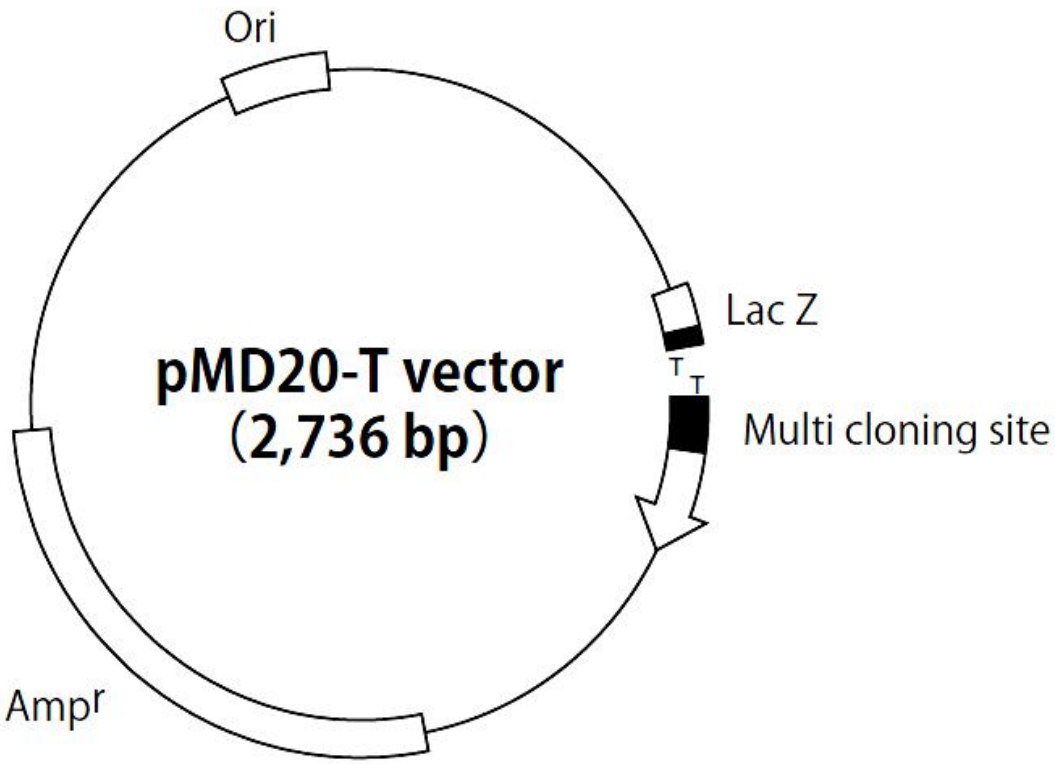

Fig. 1: Vector Map of pMD20-T

3.1.2.16 Preparation of plasmid DNA by alkaline lysis with sodium dodecyl sulfate (SDS)

The solutions and procedure for plasmid isolation is mentioned in appendix I-D and procedure in appendix II-J.

3.1.2.17 Visualization of plasmid DNA by agarose gel electrophoresis

Electrophoresis of plasmid DNA from clones done in 1.0 per cent agarose gel. The plasmid DNA from the clones of each fragment was subjected to another confirmation test for the presence of the cloned DNA fragment such as PCR analysis of recombinant plasmid.

3.1.2.18 PCR analysis of recombinant plasmids

PCR amplification of recombinant plasmid was carried out by using the specific primers as described earlier and the amplified products were visualized in gel documentation unit after agarose gel electrophoresis.

3.1.2.19 Sequencing of the cloned insert

After successful confirmation of the presence of the expected insert in the clone, the plasmid DNA was isolated in large quantity by using the alkaline lysis method and sequenced in both directions using universal M13 forward and reverse primers at Sakala Enterprises, Bangalore, India.

3.1.2.20 Sequence analysis

The selected potyvirus and begomovirus sequences were downloaded from the NCBI database and used for the comparison analysis. The name of virus, accession number with its acronym were given in table 11 (PRSV) and table 12 (PaLCuV).

3.1.2.21 Finding genes and open reading frames (ORFs)

To find the location of each gene/ORF, analysis was carried out by using the online software ‘ORF finder’ at www.ncbi.nlm.nih.gov. The ORF finder is a graphical analysis tool which finds all open reading frames of a selectable minimum size in a sequence. This tool identifies all open reading frames using the standard or alternative

Table 11: GenBank accession numbers of selected potyvirus sequences from database used in this study for analysis of PRSV

Sl. No.	Organisum	Accession No.	Acronym
1.	*Papaya ringspot virus*-[Papua New Guinea:16A:Papaya:2016]	MH404261	PRSV-[PNG:16A:Pap:16]
2.	*Papaya ringspot virus*-[Papua New Guinea:22A:Papaya:2016]	MH404264	PRSV-[PNG:22A:Pap:16]
3.	*Papaya ringspot virus*-[Papua New Guinea:12B:Papaya:2016]	MH404260	PRSV-[PNG:12B:Pap:16]
4.	*Papaya ringspot virus*-[India:Meghalaya:Umiam:Papaya:2015]	MF356497	PRSV-[IN:Meg:Umi:Pap:15]
5.	*Papaya ringspot virus*-[India:West Bengal:WB:Papaya:2017]	LC482263	PRSV-[IN:WB:Pap:17]
6.	*Papaya ringspot virus*-[Mexico:PRSV.CD-PS:Papaya:2014]	MN203187	PRSV-[MX:PRSV.CD-PS:Pap:14]
7.	*Papaya ringspot virus*-[India:Telangana:Hyderabad:HYD:Papaya:2015]	KP743981	PRSV-[IN:TS:Hyd:HYD:Pap:15]
8.	*Papaya ringspot virus*-[India:Maharashtra:PM-H:Papaya:2015]	MF405295	PRSV-[IN:MH:PM-H:Pap:15]
9.	*Papaya ringspot virus*-[India:Maharashtra:PM-I:Papaya:2015]	MF405296	PRSV-[IN:MH:PM-I:Pap:15]
10.	*Papaya ringspot virus*-[India: Maharashtra:PS3-H:Papaya:2015]	MF405297	PRSV-[IN:MH:PS3-H:Pap:15]
11.	*Papaya ringspot virus*-[India:Maharashtra:PS3-H:Papaya:2015]	MF405298	PRSV-[IN:MH:PS3-H:Pap:15]
12.	*Papaya ringspot virus*-[Mexico:PRSV.CD-VA:Papaya:2014]	MN203186	PRSV-[MX:PRSV.CD-VA:Pap:14]
13.	*Papaya ringspot virus*-[China:HN-1:Papaya:2010]	HQ424465	PRSV-[CN:HN-1:Pap:10]
14.	*Papaya ringspot virus*-[China:Hainan:Papaya:2011]	KF734962	PRSV-[CN:Hai:Pap:11]
15.	*Papaya ringspot virus*-[China:Hainan:Dongfang:HN-DF:Papaya:2012]	KT895257	PRSV-[CN:Hai:Dongfang:HN-DF:Pap:12]
16.	*Papaya ringspot virus*-[Taiwan:Taichung:Papaya:2005]	X67673	PRSV-[TW:TXG:Pap:05]
17.	*Papaya ringspot virus*-[Taiwan:Ping-tong:Papaya:2006]	DQ340769	PRSV-[TW:Ping-tong:Pap:06]
18.	*Papaya ringspot virus*-[Taiwan:Ping-tong:Papaya:2005]	DQ340770	PRSV-[TW:Ping-tong:Pap:05]
19.	*Papaya ringspot virus*-[Taiwan Ping-tong:Papaya:2005]	DQ340771	PRSV-[TW:Ping-tong:Pap:05]

Contd.……

Sl. No.	Organisum	Accession No.	Acronym
20.	*Papaya ringspot virus*-[Taiwan:prT$_3$-AX-N-Ad6:Papaya:2012]	JX448369	PRSV-[TW:prT$_3$-AX-N-Ad6:Pap:12]
21.	*Papaya ringspot virus*-[Taiwan:prT$_3$-AX-N-Ph:Papaya:2012]	JX448370	PRSV-[TW:prT$_3$-AX-N-Ph:Pap:12]
22.	*Papaya ringspot virus*-[Taiwan:prT$_3$-AX-M-Ph:Papaya:2012]	JX448371	PRSV-[TW:prT$_3$-AX-M-Ph:Pap:12]
23.	*Papaya ringspot virus*-[Taiwan:pfT$_3$-AX-D-Ph:Papaya:2012]	JX448372	PRSV-[TW:pfT$_3$-AX-D-Ph:Pap:12]
24.	*Papaya ringspot virus*-[Taiwan:pFT$_3$-NP:Papaya:2012]	JX448373	PRSV-[TW:pFT$_3$-NP:Pap:12]
25.	*Papaya ringspot virus*-[China:Hainan:Haikou:PRSV-HNVb:Papaya:2013]	KF791028	PRSV-[CN:Hai:Haikou:PRSV-HNVb:Pap:13]
26.	*Papaya ringspot virus*-[India:PRSVR3:Papaya:2013]	KJ755852	PRSV-[IN:PRSVR3:Pap:13]
27.	*Papaya ringspot virus*-[Colombia:Villa del Rosario:PRSV_VR:Papaya:2014]	KT275937	PRSV-[CO:VDR:PRSV_VR:Pap:14]
28.	*Papaya ringspot virus*-[Australia:53C:Papaya:2015]	KX655866	PRSV-[AU:53C:Pap:15]
29.	*Papaya ringspot virus*-[China:Papaya:2016]	KY933061	PRSV-[CN:Pap:16]
30.	*Papaya ringspot virus*-[India:Maharashtra:Pune:Papaya:2015]	MH311882	PRSV-[IN:MH:Pun:Pap:15]
31.	*Papaya ringspot virus*-[USA:Hawaii:P/mutant HA 5-1:Papaya:1984]	MT470188	PRSV-[US:HI:P/mutantHA5-1:Pap:84]
32.	*Papaya ringspot virus*-[China:Hainan:Lingshui:PRSV-LM:Papaya:2015]	KT633943	PRSV-[CN:Hai:Lin:PRSV-LM:Pap:15]
33.	*Papaya ringspot virus*-[Bangladesh:BD-2:Papaya:2016]	MH397222	PRSV-[BD:BD-2:Pap:16]
34.	*Papaya ringspot virus*-[Ecuador:Bab-Ec:Papaya:2017]	MH974109	PRSV-[EC:Bab-Ec:Pap:17]
35.	*Papaya ringspot virus*-[Ecuador:PRSV_Pap_Ec:Papaya:2018]	MH974110	PRSV-[EC:PRSV_Pap_Ec:Pap:18]
36.	*Papaya ringspot virus*-[Thailand:Papaya:2000]	AY010722	PRSV-[TH:Pap:00]
37.	*Papaya ringspot virus*-[Thailand:Papaya:2002]	AY162218	PRSV-[TH:Pap:02]
38.	*Papaya ringspot virus*-[China:P:Papaya:2006]	EF183499	PRSV-[CN:P:Pap:06]
39.	*Papaya ringspot virus*-[Thailand:Samutsakhon:SMK:Papaya:2013]	MT470190	PRSV-[TH:Sam:SMK:Pap:02]

Contd.……

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Sl. No.	Organisum	Accession No.	Acronym
40.	*Papaya ringspot virus*-[Mexico:Veracruz:Paso de Ovejas:Mex-VrPO:2003]	AY231130	PRSV-[TH:Ver:PDO:Mex-VrPO:Pap:13]
41.	*Papaya ringspot virus*-[USA:Hawaii:PG:Papaya:2007]	EU126128	PRSV-[US:HI:PG:Pap:07]
42.	*Papaya ringspot virus*-[Taiwan:P-5-19:Papaya:2007]	EU882728	PRSV-[TW:P-5-19:Pap:07]
43.	*Papaya ringspot virus*-[USA:PRSV-PTX:Papaya:2014]	KY271954	PRSV-[US:PRSV-PTX:Pap:14]
44.	*Papaya ringspot virus*-[India:New Delhi:DEL:Papaya:2006]	EF017707	PRSV-[IN:ND:DEL:Pap:06]
45.	*Papaya ringspot virus*-[Viet Nam:Tien Giang:PRSV- TG5:Papaya:2015]	MT470189	PRSV-[VN:TG:PRSV-TG5:Pap:15]
46.	*Papaya ringspot virus*-[Papua New Guinea:17B:Papaya:2016]	MH404262	PRSV-[PNG:17B:Pap:16]
47.	*Papaya ringspot virus*-[Papua New Guinea:18B:Papaya:2016]	MH404263	PRSV-[PNG:18B:Pap:16]
48.	*Papaya ringspot virus*-[Papua New Guinea:8A:Papaya:2016]	MH404259	PRSV-[PNG:8A:Pap:16]
49.	*Papaya ringspot virus*-[Bangladesh:BD-1:Papaya:2016]	MH444652	PRSV-[BD:BD-1:Pap:16]
50.	*Papaya ringspot virus*-[Mexico:PRSV.PC-PS:Papaya:2014]	MN203185	PRSV-[MX:PRSV.PC-PS:Pap:14]
51.	*Papaya ringspot virus*-[Mexico:PRSV.PC-VA:Papaya:2014]	MN203183	PRSV-[MX:PRSV.PC-VA:Pap:14]
52.	*Papaya ringspot virus*-[Mexico:PRSV.PC-OS:Papaya:2014]	MN203184	PRSV-[MX:PRSV.PC-OS:Pap:14]
53.	*Papaya ringspot virus*-[Colombia:Campo Hermoso:PRSV_CH:Papaya:2014]	KT275938	PRSV-[CO:CH:PRSV_CH:Pap:14]
54.	*Papaya ringspot virus*-[Taiwan:Taichung:Papaya:2018]	NC_001785	PRSV-[TW:TXG:Pap:18]
55.	*Papaya ringspot virus*-[Taiwan:YK:Papaya:2008]	X97251	PRSV-[TW:YK:Pap:08]
56.	*Papaya ringspot virus*-[USA:Hawaii:HA:Papaya:1993]	S46722	PRSV-[US:Hi:HA:Pap:93]
57.	*Papaya ringspot virus*-[India:VC:Papaya:2015]	MF405299	PRSV-[IN:VC:Pap:15]
58.	*Papaya ringspot virus*-[India:HimachalPradesh:Palampur:Papaya:2018]	MW030522	PRSV-[IN:HP:Pal:Pap:18]

Table 12: GenBank accession numbers of selected begomovirus sequences from database used in this study for analysis of PaLCuV

Sl. No.	Organisum	Accession No.	Acronym
1.	*Ageratum enation virus*-[India:Rajasthan:CN2:Papaya:2014]	KP725057	AEV-[IN:Raj:CN2:Pap:14]
2.	*Ageratum yellow vein virus*-[China: Sanya: Papaya:2013]	KM051844	AYVV-[CN:Sanya:Pap:13]
3.	*Bhendi yellow vein Bhubhaneswar virus*-[India: Orissa:Okra:03]	FJ589571	BYVBV-[IN:OD:Bhu:Okra:03]
4.	*Catharanthus yellow mosaic virus*-[Pakistan:Faisalabad:ZF-16:Papaya:2009]	LN864815	CaYMV-[PK:Fsd:ZF-16:Pap:10]
5.	*Chayote yellow mosaic virus*-[Cameroon:Papaya-20-14:Papaya:2014]	KT454822	ChaYMV-[CR:Pap-20-14:Pap:14]
6.	*Chilli leaf curl India virus*-[India: Meerut:Papaya:2017]	MF574143	ChiLCINV-[IN:Mer:Pap:17]
7.	*Chilli leaf curl virus*-[Indi:Shahahanpur:PSB-21:Papaya:2014]	MH765693	ChiLCV-[IN:Sha:PBS-21:Pap:14]
8.	*Chilli leaf curl virus*-[India:Amritsar:Papaya:2009]	GU136803	ChiLCV-[IN:Amr:Pap:09]
9.	*Chilli leaf curl virus*-[India:Karnataka:Raichuru:Chilli:2017]	MK161454	ChiLCV-[IN:Kar:Rai:Chi:17]
10.	*Chilli leaf curl virus*-[India:Uttar Pradesh: Faizabad: PSB-45:Papaya:2014]	MH765698	ChiLCV-[IN:UP:Faz:PSB-45:Pap:14]
11.	*Chilli leaf curl virus*-[India:Uttar Pradesh:Mahoba:PSB-42:Papaya:2014]	MH765697	ChiLCV-[IN:UP:Mah:PSB-42:Pap:14]
12.	*Chilli leaf curl virus*-[Pakistan:WA19:Papaya:2018]	MN839535	ChiLCV-[PK:WA19:Pap:18]
13.	*Chilli leaf curl virus*-DU [India:New Delhi:DU:Papaya:2009]	HM140364	ChiLCV-DU[IN:ND:DU:Pap:09]
14.	*Chilli leaf curl virus*-HD [India:New Delhi:HD:Papaya:2007]	HM140365	ChiLCV-HD[IN:ND:HD:Pap:07]
15.	*Chilli leaf curl virus*-India [India: New Delhi: AD: Papaya:2005]	DQ989326	ChiLCV-IN[IN:ND:AD:Pap:05]
16.	*Chilli leaf curl virus*-Najafgarh2 [India:New Delhi:Papaya:2009]	HM140370	ChiLCV-Naj2[IN:ND:Pap:09]
17.	*Chilli leaf curl virus*-Noida [India:Uttar Pradesh:Noida:Papaya:2009]	HM140371	ChiLCVN-[IN:UP:Noi:Pap:09]

Contd…..

Sl. No.	Organisum	Accession No.	Acronym
18.	*Chilli leaf curl virus*-Panipat1 [India:Panipat:Papaya:2008]	HM140366	ChiLVCP1[IN:Pan:Pap:08]
19.	*Cotton leaf curl Burewala virus*-[India: LK_2N:Papaya:2011]	KX302707	CLCuBuV-[IN:LK_2N:Pap:11]
20.	*Croton yellow vein mosaic virus*-[India:Hariyana:Acalypha:07]	FN645901	CYVMV-[IN:HR:Acl:07]
21.	*Croton yellow vein mosaic virus*-[India:New Delhi:croton:2007]	JN817516	CYVMV-[IN:ND:Cro:08]
22.	*Croton yellow vein mosaic virus*-[India:Panjab:Acalypha:2007]	FN645926	CYVMV-[IN:Pun:Acl:07]
23.	*Croton yellow vein mosaic virus*-[India:Ranchi:PSB-38:Papaya:2014]	MH765696	CYVMV-[IN:Ran:PSB-38:Pap:14]
24.	*Duranta leaf curl virus*-[India: New Delhi:PSB-63:Papaya:2016]	MH807202	DLCV-[IN:ND:PSB-63:Pap:16]
25.	*Malvastrum leaf curl virus*-[China:Guangxi:G100:Papaya:2006]	AM260699	MaLCuV-[CN:GX:G100:Pap:06]
26.	*Mungbean yellow mosaic India virus*-[India:New Delhi: :2005]	DQ389153	MYMIV-[IN:ND:Cp:05].DQ389153
27.	*Okra enation leaf curl virus*-[Iran:Bahu Kalat: OELCuV_IR_P7_2010:Papaya:2010]	KJ397529	OELCuV-[IR:BK:OELCuV_IRP72010:Pap:10]
28.	*Papaya leaf crumple virus*-[India: Mohali:Pap-Moh7:Papaya:2011]	KR052159	PaLCrV-[IN:Moh:Pap-Moh7:Pap:11]
29.	*Papaya leaf crumple virus*-[India:Bhopal:B2_5N:Papaya:2011]	KX302712	PaLCrV-[IN:Bho:B2_5N:Pap:11]
30.	*Papaya leaf crumple virus*-[India:Bhopal:PSB-66:Papaya:2016]	MH807203	PaLCrV-[IN:Bho:PSB-66:Pap:16]
31.	*Papaya leaf crumple virus*-[India:Hajipur:B2_3N:Papaya:2011]	KX302710	PaLCrV-[IN:Haj:B2_3N:Pap:11]
32.	*Papaya leaf crumple virus*-[India:Jabalpur:PSB-32:Papaya:2016]	MH674437	PaLCrV-[IN:Jab:PSB-32:Pap:11]
33.	*Papaya leaf crumple virus*-[India:Kolkata:B2_4N:Papaya:2012]	KX302711	PaLCrV-[IN:Kol:B2_4N:Pap:12]
34.	*Papaya leaf crumple virus*-[India:Lalitpur:PSB-47:Papaya: 2015]	MH807200	PaLCrV-[IN:Lal:PSB-47:Pap:15]
35.	*Papaya leaf crumple virus*-[India:Lucknow:B2_2N:Papaya:2012]	KX302709	PaLCrV-[IN:Luc:B2_2N:Pap:12]

Contd…..

Sl. No.	Organisum	Accession No.	Acronym
36.	*Papaya leaf crumple virus*-[India:Mohali:3B2_2:Papaya:2012]	KX302708	PaLCrV-[IN:Moh:3B2_2:Pap:12]
37.	*Papaya leaf crumple virus*-[India:New Delhi:PSB-60:Papaya:2016]	MH807201	PaLCrV-[IN:ND:PSB-60:Pap:16]
38.	*Papaya leaf crumple virus*-[Pakistan:Lahore:KN6:Papaya:2011]	HE580236	PaLCrV-[PK:Lah:KN6:Pap:11]
39.	*Papaya leaf crumple virus*-Najafgarh1[India:New Delhi:Papaya:2008]	HM140369	PaLCrV-Naj1[IN:ND:Pap:08]
40.	*Papaya leaf crumple virus*-Nirulas[India:New Delhi:Papaya:2007]	HM140368	PaLCrV-Nirulas[IN:ND:Pap:07]
41.	*Papaya leaf crumple virus*-[India: Mohali:New Delhi:croton:2008]	JN817517	PaLCrV-[IN:ND:Cro:08]
42.	*Papaya leaf crumple virus*-Panipat 8 [India:Panipat:Papaya:2008]	HM140367	PaLCrV-Panipat8[IN:Pan:Pap:08]
43.	*Papaya leaf crumple virus*-Panipat8[India:Haryana:Panipat:Papaya:2008]	NC_014707	PaLCrV-Panipat8[IN:Har:Pan:Pap:08]
44.	*Papaya leaf curl China virus*-[China:Guangxi:G2:Papaya:2004]	AJ558123	PaLCuV-[CH:Gua:G2:Pap:04]
45.	*Papaya leaf curl China virus*-[China:Guangxi:G4:Papaya:2004]	AJ811914	PaLCuV-[CH:Gua:G4:Pap:04]
46.	*Papaya leaf curl Guandong virus*-[China:Guangxi:GD2:Papaya: 2004]	AJ558122	PaLCuGdV-[CH:Gua:GD2:Pap:04]
47.	*Papaya leaf curl Guandong virus*-[China:Guangxi:GD2:Papaya:2004]	NC_005844	PaLCuGdV-[CH:Gua:GD2:Pap:04]
48.	*Papaya leaf curl virus*-[India: Tamil Nadu:Wellington:WB2&5:Papaya:2010]	KX302713	PaLCuV-[IN:TN:Wel:WB2&5:Pap:10]
49.	*Papaya leaf curl virus*-[India::Rajasthan:MM1:Papaya:2019]	MN529626	PaLCuV-[IN:Raj:MM1::Pap:09]
50.	*Papaya leaf curl virus*-[India:Gujarat:Jamnagar:PSB-34:Papaya:2015]	MH807205	PaLCuV-[IN:Guj:Jam:PSB-34:Pap:15]
51.	*Papaya leaf curl virus*-[India:Karnataka:Madikeri:Candela:2018]	MK087120	PaLCuV-[IN:Kar:Madi:Cal:18]
52.	*Papaya leaf curl virus*-[India:Madhya Pradesh:Pap:ND:13:Papaya:2012]	KF307208	PaLCuV-[IN:MP:Pap:ND:13:Pap:12]
53.	*Papaya leaf curl virus*-[India:New Delhi:Papaya:2016]	KY800906	PaLCuV-[IN:ND:Pap:16]

Contd…..

Sl. No.	Organisum	Accession No.	Acronym
54.	*Papaya leaf curl virus*-[South Korea: Korea:Papaya:2014]	KT266873	PaLCuV-[SK:Korea:Pap:14]
55.	*Papaya yellow leaf curl vieus*-[India:New Delhi:Radish:2018]	FJ593629	PaYLCuV-[IN:ND:Rad:18]
56.	*Papaya yellow leaf curl virus*-[India: Hyderabad:PSB-51:Papaya:2016]	MH807204	PaYLCuV-[IN:Hyd:PSB-51:Pap:16]
57.	*Papaya yellow leaf curl virus*-[India: Rajasthan:DP2:Papaya:2015]	KX353622	PaYLCuV-[IN:Raj:DP2:Pap:15]
58.	*Pedilanthus leaf curl virus*-[India: Gujarat:Ahmedabad:PSB37:Papaya:2015]	MH765695	PeLCV-[IN:Guj:Amd:PSB37:Pap:15]
59.	*Tomato leaf curl Bangladesh virus*-[Bangladesh:Gazipur:Papaya:2016]	MH380003	ToLCBV-[BD:Gaz:Pap:16]
60.	*Tomato leaf curl Gujarat virus*-[India:Lucknow:Papaya:2014]	MG757245	ToLCuGuV-[IN:LukPap:14]
61.	*Tomato leaf curl Karnataka virus*-India [India:Gujarat:Tomato:2008]	MH5770301	ToLCKV-[IN:GJ:AHM:Tom:16]
62.	*Tomato leaf curl Karnataka virus*-India [India:Karnataka:Banglore:Chilli:2008]	HM007094	ToLCKV-[IN:Kar:Ben:Chi:08]
63.	*Tomato leaf curl Karnataka virus*-India [India:TamilnaduTomato:2012]	KF551579	ToLCKV-[IN:TN:Com:Tom:12]
64.	*Tomato leaf curl virus*-[India:Karnataka:Bengaluru:Chrysanthamum:2017]	MG758145	ToLCV-[In:Kar:Ben:Chr:17]
65.	*Tomato leaf curl virus*-[India:Tamilnadu:Tomato:2012]	KC713784	ToLCV-[IN:TN:Com:Tom:12]
66.	*Tomato yellow leaf curl viru Israel* -[USA:P3-T1-3:Papaya:2014]	KX024639	TYLCV-[USA:P3-T1-3:Pap:14]

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genetic codes. The deduced amino acid sequence were saved in various formats and searched against the sequence database using the BLAST server (http://www.ncbi.nlm.nih.gov/gorf/gorf.html).

3.1.2.22 Nucleotide sequence identity analysis

The nucleotide sequences present in the genomes were aligned in FASTA format and analyzed through online software CLUSTALW multiple alignment tools. The homology percentage and the sequence identities were calculated.

3.1.2.23 Phylogenetic analysis of full length genomes of PRSV and PaLCuV

Sequences obtained were identified in terms of closest homology sequence using BLAST. Multiple and pairwise alignment of all sequences was carried out by CLUSTALW. Then neighbor joining tree was generated using MEGA software (version 11) and used for the phylogenetic analysis (Tamura *et al.*, 2013). The tree was generated using the neighbor joining tree with the 60.0 per cent cut off value and 1,000 bootstrap replicates.

3.1.2.24 Recombination analysis of complete nucleotide sequence of isolated PRSV and PaLCuV

Recombination analyses were carried out using the Recombination Detection Program (RDP), GENECOV, Bootscan, Max Chi, Chimera, Si Scan and 3Seq which are integrated in RDP.4 to detect the recombination break points (Martin *et al.*, 2020). These programs can identify the recombination sites among different viral strains, different viral species, and even between the virus and the host (Chare and Holmes, 2006). Default RDP settings with 0.05 p-value cut-off throughout and standard Bonferroni correction was used.

3.1.2.25 Species demarcation tool (SDT) of complete nucleotide sequence of DNA-A component of isolated begomoviruses with other begomoviruses

Complete nucleotide sequences of full length genomes of DNA-A component of isolated begomovirus were employed to align with other begomoviruses by using the CLUSTALW and percentage pairwise nucleotide identity matrix plot was made using SDT version 1.2 program (http://web.cbio.uct.ac.za/~brejnev/) (Chandrakant, 2014 and Premchand, 2015).

3.1.3 Virus indexing/identification of reservoir hosts through host range study.

Virus indexing of PRSV to identify reservoir hosts was done by using eight plant species belonging to four families as indicator host. The seeds of indicator hosts were collected from ICAR- National Bureau of Plant Genetic Resources, New Delhi. The details of indicator hosts used along with stage of inoculation is given in table 13.

The healthy host plants were artificially inoculated with PRSV infected plant sap under the controlled condition at a different stage of the crop. Mechanical sap inoculation was done by using a 0.005 M phosphate buffer (Appendix I-K).

3.2 Epidemiological studies on the incidence of PRSV disease

Epidemiological studies were carried out to determine the effect of months of planting on PRSV disease incidence and identification of the susceptible stage of papaya for PRSV infection

3.2.1 Effect of different months of planting on the incidence of PRSV disease, growth and yield parameters of papaya at different stage of growth

The experiment on the effect of different months of planting on incidence of PRSV disease, growth and yield of papaya at different growth stages was conducted by laying out field experiment at Haveli Farm, College of Horticulture Bagalkot, UHS, Bagalkot during 2019 to 2021. Planting has been done at the monthly interval and details of treatments are given in table 14 and plate 2.

3.2.1.1 Observations recorded

The observations on plant height, plant girth, number of leaves per plant, internodal length, days taken for first flowering, number of flowers per plant, number of days from flowering to first fruit set, number of days to fruit harvest after fruit set, fruit length, fruit breadth, fruit diameter, cavity diameter, yield per plant and yield per hectare were recorded at 30, 60, 90, 120, 150, 180, 210, 240, and 270 (Days after transplanting) and data analyzed statistically.

Table 13: Details of host plant and inoculation stage of the crop

Family	Plant species	Stage of inoculation
Solanaceae	*Datura stramonium*	6th leaf stage
	Datura metel	6th leaf stage
	Capsicum annuum cv. California Wonder	8-10th leaf stage
	Nicotiana tabacum	6th leaf stage
	Nicotiana glutinosa	6th leaf stage
Fabaceae	*Phaseolus vulgaris*	2nd leaf stage
Chenopodiaceae	*Chenopodium quinoa*	4-8th leaf stage
Cucurbitaceae	*Cucumis sativus*	Primordial leaf stage (2nd leaf stage)

Table 14: Details of experiment on planting of papaya at different months

Location	Haveli Farm, COH, Bagalkot
Year	2019-20 and 2020-21
Design	Randomized Block Design (RBD)
Crop	Papaya
Cultivar	Red Lady
Number of treatments	10
Number of replications	03
Number of plants per replications	12
Plot size	4.5 m x 3 m
Spacing	1.5 m x 1.5 m
Treatment details	
Treatment	**Month of Planting**
T_1	1st week of June
T_2	1st week of July
T_3	1st week of August
T_4	1st week of September
T_5	1st week of October
T_6	1st week of November
T_7	1st week of December
T_8	1st week of January
T_9	1st week of February
T_{10}	1st week of March

N

R1	R2	R3
T9R3	T7R2	T1R1
T8R3	T6R2	T2R1
T7R3	T9R2	T10R1
T6R3	T8R2	T4R1
T5R3	T3R2	T5R1
T4R3	T10R2	T6R1
T3R3	T1R2	T7R1
T2R3	T4R2	T8R1
T10R3	T2R2	T3R1
T1R3	T5R2	T9R1

Plate 2: Layout of experiment on planting of papaya at different months

* Papaya plants (12 plants/ replication)

Observations on the population dynamics of aphids were regularly monitored by setting yellow sticky traps. Traps were installed at two different places (50 m apart) in the experimental plot. The number of aphids collected was recorded at weekly and monthly by cumulative aphid population was calculated.

3.2.2 Identification of susceptible stage of papaya for PRSV infection

This experiment was laid out under net house conditions at Haveli Farm of College of Horticulture Bagalkot, UHS, Bagalkot during 2019-20 to find out susceptible growth stage of papaya for infection (Table 15).

For challenge inoculation, healthy aphid (*Aphis craccivora*) colonies were maintained under insect proof net house on cotton which was collected from field (Plate 3 A). These healthy aphid colonies were allowed to feed on virus infected papaya seedling by giving 24 hours of acquisition access period. Further, these viruliferous aphids were released to healthy papaya plants (15/plant) by giving 24 hrs of inoculation access period. The plants were inoculated through aphids at different growth stages (Plate 3 B,C,D). Plants in each replication of the treatments were covered with insect proof net to avoid the spreading of inoculated viruliferous aphids (Plate 4).

3.2.2.1 Observations recorded

The observations on per cent transmission, days taken for symptom expression post inoculation (dpi), typical symptoms, plant height, plant girth, number of leaves per plant, internodal length, days taken for first flowering, number of flowers per plant, number of days from flowering to first fruit set, number of days from fruit set to harvest after fruit set, fruit length, fruit breadth, fruit diameter, cavity diameter, yield per plant and yield per hectare were recorded and data analyzed statistically.

3.2.2.2 Quantification of PRSV viral titer using qRT-PCR

qRT-PCR was performed to find the PRSV titer in the inoculated plants at the different growth stages. Leaf samples were collected at 15 days after inoculation in each treatment. To prevent the degradation of nucleic acid, the leaf is flash frozen

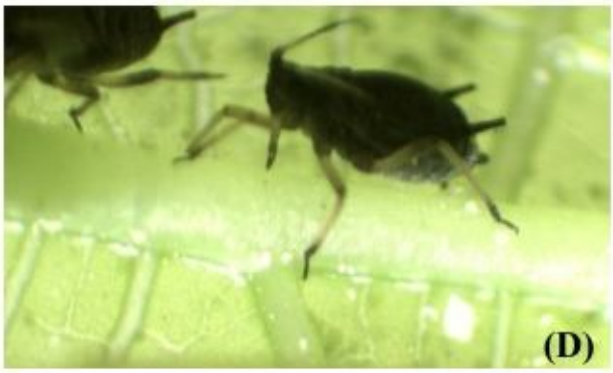
Table 15: Details of the experiment on identification of susceptible stage of papaya for PRSV infection

Location	Net house, Haveli Farm, COH, Bagalkot
Year	2019 to 2020
Design	Randomized Block Design (RBD)
Crop	Papaya
Cultivars	Red Lady
Date of planting	15.06.2019
Number of treatments	10
Number of replications	03
Number of plants per replications	04
Plot size	2 x 2 m^2
Spacing	1.5 m x 1.5 m
Treatment details	
Treatment	**Stage of plant growth at which inoculated**
T_1	30 DAT
T_2	60 DAT
T_3	90 DAT
T_4	120 DAT
T_5	150 DAT
T_6	180 DAT
T_7	210 DAT
T_8	240 DAT
T_9	270 DAT
T_{10}	Control (Un inoculated)

(A)

(B)

(C)



(D)

(E)

Plate 3 A & B: Aphid (*Aphis craccivora*) colonies maintained on cotton C. Nymphs D. Wingless aphids E. Winged aphids.

immediately in liquid nitrogen and then stored at -80° C. At this temperature, nucleases remain inactive and nucleic acid stable.

These leaves from each treatment we subjected to total RNA extraction, quantification, reverse transcription (cDNA) syntheses and subjected to qRT-PCR reaction mentioned in appendix II-C, D, E.

3.2.2.3 Primers for qRT-PCR assay

The qRT-PCR assay to find the viral load of PRSV in the leaf samples inoculated at different crop growth stage was performed using the specific primers (PRSV2F and PRSV2R). These primers were designed using the sequence data obtained after amplification of HC-Pro region of Bagalkote isolate (Appendix II-L) along with candidate reference gene, TATA-binding protein 2 (TBP2F and TBP2R) designed by Zhu *et al.* (2012). Details of primer are given in table 16.

3.2.2.4 qRT-PCR conditions

The qRT-PCR assay was performed in Applied StepOnePlus Real-Time PCR System at Molecular lab, Department of Plant Pathology, College of Horticulture Bagalkot, UHS Bagalkot. The PCR conditions used are given in table 16.

3.3 Integrated management of PRSV disease under field conditions

The investigation was done to study the effect of insecticides, bio rationals (plant based oils and seaweed extract) against PRSV under field conditions. The experiment was laid out in Haveli farm, College of Horticulture Bagalkot, UHS Bagalkot during 2019-20 and 2020-21. The treatments and experimental details are mentioned in the below table 17a and 17b, Plate 5.

Further, three integrated disease management (IDM) modules were designed using two seasons of experimental results conducted on the effect of insecticides, bio rationals and their combination against PRSV disease on papaya. The three modules were evaluated along with package of practices of UHS Bagalkot as check under natural disease pressure during 2020-21. The designed IDM modules were laid out in

Table 16: Primers used in a qRT-PCR assay for the detection of viral load

Primer	Oligonucleotides (5'-3')	Initial denaturation	PCR reaction	Amplicon length(bp)	Reference
PRSV2F	GGCACACTTAGATGGAGGACC	Cycles: 1 95^0 30 sec	Cycles: 40 95^0C for 5 sec 60^0C for 30 - 34 sec Dissociation Stage	136	Designed
PRSV2R	ACCAGGAAGGTGGTAGGTTCA				
TBP2F	TGTGAATACTGGTGCTGAG			104	Zhu *et al.* (2012)
TBP2R	GGCATGAGACAAGACCTATA				

Table 17a: Details of the experiment on integrated management of PRSV disease

Location	Haveli Farm, COH, Bagalkot
Year	2019-20 and 2020-21
Design	Randomized Block Design (RBD)
Crop	Papaya
Cultivars	Red Lady
Date of planting	01.06.2019
Number of treatments	09
Number of replications	03
Number of plants per replications	12
Plot size	4.5 m x 3 m
Spacing	1.5 m x 1.5 m
Number of sprays	8 Sprays at 30 day interval

R1	R2	R3			
T1R1	T6R1	T5R2	T7R2	T9R3	T3R3
T2R1	T7R1	T8R2	T2R2	T4R3	T7R3
T3R1	T8R1	T10R2	T1R2	T6R3	T10R3
T4R1	T9R1	T4R2	T9R2	T8R3	T2R3
T5R1	T10R1	T6R2	T3R2	T1R3	T5R3

Plate 4: Layout of experiment on identification of susceptible stage of papaya for PRSV infection

* Papaya plants (4 plants/ replication)

R1	R2	R3
T9R3	T7R2	T1R1
T8R3	T6R2	T2R1
T7R3	T9R2	T3R1
T6R3	T8R2	T4R1
T5R3	T3R2	T5R1
T4R3	T2R2	T6R1
T3R3	T1R2	T7R1
T2R3	T4R2	T8R1
T1R3	T5R2	T9R1

Plate 5: Layout of experiment on integrated management of PRSV disease

* Papaya plants (12 plants/ replication)

Table 17b: Details of the treatment combination of insecticides and bio rationals for the management of PRSV under field conditions

Treatment	1st Spray at 30 DAT*	2nd Spray at 60 DAT	3rd Spray at 90 DAT	4th Spray at 120 DAT	5th Spray at 150 DAT	6th Spray at 180 DAT	7th Spray at 210 DAT	8th Spray at 240 DAT
T_1	Tolfenpyrad 15% EC 1 ml/l followed by micronutrients**	Imidacloprid 17.8% SL 0.2 ml/l followed by micronutrients	Thiacloprid 21.7 SC 1 ml/l followed by micronutrients	Dinotefuran 20 % SG 0.5g/l followed by micronutrients	Tolfenpyrad 15% EC 1 ml/l followed by micronutrients	Imidacloprid 17.8% SL 0.2 ml/l followed by micronutrients	Thiacloprid 21.7 SC 1 ml/l followed by micronutrients	Dinotefuran 20 % SG 0.5g/l followed by micronutrients
T_2	1% Neem oil 10 ml/l followed by micronutrients	Pongamia oil 10 ml/l followed by micronutrients	Groundnut oil 10 ml/l followed by micronutrients	Mineral oil 10 ml/l followed by micronutrients	1% Neem oil 10 ml/l followed by micronutrients	Pongamia oil 10 ml/l followed by micronutrients	Groundnut oil 10 ml/l followed by micronutrients	Mineral oil 10 ml/l followed by micronutrients
T_3	Seaweed extract 4ml/l followed by micronutrient	Seaweed extract 4ml/l followed by micronutrient	Seaweed extract 4ml/l followed by micronutrient	Seaweed extract 4ml/l followed by micronutrient	Seaweed extract 4ml/l followed by micronutrient	Seaweed extract 4ml/l followed by micronutrient	Seaweed extract 4ml/l followed by micronutrient	Seaweed extract 4ml/l followed by micronutrient
T_4	Tolfenpyrad 15% EC 1 ml/l followed by micronutrients	1% Neem oil 10 ml/l followed by micronutrients	Imidacloprid 17.8% SL 0.2 ml/l followed by micronutrients	Pongamia oil 10 ml/l followed by micronutrients	Thiacloprid 21.7 SC 1 ml/l followed by micronutrients	Groundnut oil 10 ml/l followed by micronutrients	Dinotefuran 20 % SG 0.5g/l followed by micronutrients	Mineral oil 10 ml/l followed by micronutrients
T_5	Tolfenpyrad 15% EC 1 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	Imidacloprid 17.8% SL 0.2 ml/l followed by micronutrients	Seaweed extrac-4ml/l followed by micronutrients	Thiacloprid 21.7 SC 1 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	Dinotefuran 20 % SG 0.5g/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients
T_6	1% Neem oil 10 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	Pongamia oil 10 ml/l followed by micronutrients	Seaweed extract 1gm/l followed by micronutrients	Groundnut oil 10 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	Mineral oil 10 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients
T_7	Tolfenpyrad 15% EC 1 ml/l followed by micronutrients	1%Neem oil 10 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	Imidacloprid 17.8% SL 0.2 ml/l followed by micronutrients	Groundnut oil 10 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients
T_8	Micronutrients*	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients
T_9	Control (No spray)							

*DAT: Day After Transplanting, ** Zn-3.0%, Fe-2.0%, Mn-1.0%, B-0.50%, Mg-2.0%

farmer field at Mannikeri village of Bilgi of taluk in Bagalkote district of Karnataka. The modules designed and evaluated are given in table 18a and 18b.

3.3.1 Observations recorded

The observations on disease incidence, plant height, plant girth, number of leaves per plant, internodal length, days taken for first flowering, number of flowers per plant, number of days from flowering to first fruit set, number of days from fruit set to harvest, fruit length, fruit breadth, fruit diameter, cavity diameter, yield per plant and yield per hectare were recorded at 30, 60, 90, 120, 150, 180, 210, 240 and 270 DAT and data analyzed statistically.

3.3.2 Benefit Cost ratio

The Economics of the experiment was worked out taking into consideration the cost of individual treatments, total cost and income got in returns Benefit Cost ratio (B:C) and Incremental Benifit Cost Ratio (ICBR) and net returns were calculated as follows.

Net returns (Rs.): Total returns-Total cost

$$\text{B: C: } = \frac{\text{Total returns (Rs. /ha)}}{\text{Total cost (Rs. /ha)}}$$

$$\text{ICBR} = \frac{\text{Additional returns over control (Rs. /ha)}}{\text{Additional cost over control (Rs. /ha)}}$$

Table 18a: Details of the experiment on evaluation of modules for integrated management of PRSV disease

Location	Mannikeri, Bilgi taluk, Bagalkot
Year	2020-21
Crop	Papaya
Cultivars	Red Lady
Date of planting	01.08.2020
Plot size	10.5 m x 13.5 m
Spacing	1.5 m x 1.5 m
Number of modules	04
Number of plants per modules	72
Number of sprays	12 Sprays at 20 day interval

Table 18b: Details of the IDM modules for integrated management of PRSV under field conditions

Treatment	1st Spray at 20 DAT*	2nd Spray at 40 DAT	3rd Spray at 60 DAT	4th Spray at 80 DAT	5th Spray at 100 DAT	6th Spray at 120 DAT
M_1	Tolfenpyrad 15EC 1 ml/l followed by micronutrients **	Imidacloprid 17.8% SL 0.2 ml/l followed by micronutrients	Thiacloprid 21.7 SC 1 ml/l followed by micronutrients	Dinotefuran 20 % SG 0.5g/l followed by micronutrients	Tolfenpyrad 15EC 1 ml/l followed by micronutrients	Imidacloprid 17.8% SL 0.2 ml/l followed by micronutrients
M_2	Tolfenpyrad 15EC 1 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	Imidacloprid 17.8% SL 0.2 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	Thiacloprid 21.7 SC 1 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients
M_3	Tolfenpyrad 15EC 1 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	Imidacloprid 17.8% SL 0.2 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	Thiacloprid 21.7 SC 1 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients
M_4 (UHS POP)	Dimethoate 30 % EC 1.7ml/l followed by 1% neem oil 10 ml/l	Neam Leaf extract 1500 ppm 2 ml/l	Oxydemeton methyl 25% EC 1.5ml/l followed by 1% groundnut oil 10 ml/l	Neam leaf extract 1500 ppm 2 ml/l	Imidacloprid 17.8% SL 0.25ml/l followed by 1% neem oil 10 ml/l	Neam leaf extract 1500 ppm 2 ml/l

Treatment	7th Spray at 140 DAT	8th Spray at 160 DAT	9th Spray at 180 DAT	10th Spray at 200 DAT	11th Spray at 220 DAT	12th Spray at 240 DAT
M_1	Thiacloprid 21.7 SC 1 ml/l followed by micronutrients	Dinotefuran 20 % SG 0.5g/l followed by micronutrients	Tolfenpyrad 15EC 1 ml/l followed by micronutrients	Imidacloprid 17.8% SL 0.2 ml/l followed by micronutrients	Thiacloprid 21.7 SC 1 ml/l followed by micronutrients	Dinotefuran 20 % SG 0.5g/l followed by micronutrients
M_2	Dinotefuran 20 % SG 0.5g/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	Tolfenpyrad 15EC 1 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	Imidacloprid 17.8% SL 0.2 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients
M_3	Dinotefuran 20 % SG 0.5g/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	1%Neem oil 10 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients	Groundnut oil 10 ml/l followed by micronutrients	Seaweed extract 4ml/l followed by micronutrients
M_4 (UHS POP)	Thiamethoxam 25% Wg 0.20 ml/l followed by 1% groundnut oil 10 ml/l	Neam leaf extract 1500 ppm 2 ml/l	Acephate 75 % SP 1.0 gm/l followed by spray 1%Neem oil 10 ml/l	Neam Leaf extract 1500 ppm 2 ml/l	Dimethoate 30 % EC 1.7ml/l followed by 1% groundnut oil 10 ml/l	Neam leaf extract 1500 ppm 2 ml/l

*DAT: Day After Transplanting, ** Zn-3.0%, Fe-2.0%, Mn-1.0%, B-0.50%, Mg-2.0%

4. EXPERIMENTAL RESULTS

The present investigation pertaining to characterization of *Papaya ringspot virus* (PRSV) and *Papaya leaf curl virus* (PaLCuV) infecting papaya in Karnataka and epidemiology and management of PRSV disease was carried out to know the prevalence and distribution of viruses, molecular detection and characterization of causal agent, host indexing, epidemiological studies and integrated disease management of PRSV disease at Department of Plant Pathology, College of Horticulture Bagalkot, UHS Bagalkot during 2019-20 to 2020-21.

4.1 Survey for the prevalence, distribution, collection and characterization of PRSV and PaLCuV diseases associated with papaya in Karnataka

4.1.1 Survey for the prevalence and distribution of PRSV and PaLCuV diseases

The roving survey was carried out from November 2019 to April 2021in major papaya growing districts of Karnataka *viz.*, Bagalkote, Belagavi, Ballari, Chitradurga, Gadag, Haveri, Kalaburagi, Koppal, Vijayapura and Yadgiri to determine the prevalence and distribution of viral diseases on papaya as well as for collection of virus samples. Disease diagnosis in field was based on symptoms expressed on leaves, petioles, stems and fruits. The data on area cultivated, cultivar used, age of the crop, type of symptoms observed on different parts of the plant, previous crop, surrounding crop and disease incidence of PRSV and PaLCuV (Plate 6) were determined and presented in table 19.

Table 19 revealed the incidence of PRSV in different parts of Karnataka. The average disease incidence ranged from 50.5 to 100 per cent. The minimum disease incidence was recorded in Gadag (50.05 %) and Kalaburagi (53.53 %) districts. The district wise average disease incidence of the PRSV on papaya was highest in Koppal (100 %) and Yadgir (100 %), followed by Haveri (91.47 %), Bagalkote (85.81 %), Ballari (70.26 %), Belagavi (76.12 %), Vijayapura (66.97 %), Chitradurga (61.93 %), Kalaburagi (53.53 %) and Gadag (50.50 %) (Table 19 and Fig. 2).

The typical symptoms of PRSV infected papaya plants were green mosaic, yellow mosaic, leaf curling, rings spot, stunted growth, puckering of leaves, mottling,

Table 19: Status of PRSV and PaLCuV diseases in major papaya growing districts of Karnataka

Sl. No.	Taluk	Village	Name of Variety	Area (Acre)	Crop Stage (Months)	Previous crop	Surrounding Crop	PRSV Disease Incidence (%)	PRSV symptoms in field	No. of PaLCuV infected papaya plants	PaLCuV symptoms in field
I	**District : Bagalkote**										
1.	Bagalkote	Kolakachi	Ice Berg	1.00	04	Sugarcane	Tomato	0.67	GM	-	-
2.		Tulsigeri	RedLady	1.50	16	Brinjal	Pomegranate	100	GM, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	03	SLC, LR, ZV, VT, VCL, DP, D, Y,VE, LL
3.	Bilgi	Honakatti	RedLady	2.33	06	Brinjal	Sugarcane	100	GM, B, LC, SS, YM, Mo, LP, LD, OS	02	SLC, LR, VT, VCL, DP, VE, LL
4.		Bilgi	RedLady	2.00	16	Sugarcane	Sugarcane	100	GM, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-
5.	Hungund	Bannihatti	RedLady	1.08	09	Tomato	Maize	100	GM, B, LC, SS, Mo, LP, BF, LD, OS, S, RS	02	SLC, DLC, VT, VCL, DP, VE, LR, LL
6.		Hiremagi	RedLady	2.00	10	Maize	Chilli	91.67	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S	02	SLC, DLC, ZV, VT, VCL, DP, Y, VE, LL
7.		Hiremagi	RedLady	1.42	12	Maize	Brinjal	94.12	GM, B, LC, ST, Mo, LP, BF, LD, OS, S, RS, CS, DF	03	SLC, LR, ZV, VT, VCL, DP, D, Y, VE, LL
8.		Hiremagi	RedLady	1.25	09	Tomato	Pomegranate	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	02	SLC, DLC, ZV, VT, VCL, DP, D, Y, LL
							Average	**85.81**			

Contd…..

Sl. No.	Taluk	Village	Name of Variety	Area (Acre)	Crop Stage (Months)	Previous crop	Surrounding Crop	PRSV Disease Incidence (%)	PRSV symptoms in field	No. of PaLCuV infected papaya plants	PaLCuV symptoms in field
II	**District : Ballari**										
9.	Ballari	Belagal	RedLady	1.25	12	Papaya	Sapota	6.67	GM, B, LC, Mo	-	-
10.	Hagari bomanahalli	Kannehalli	RedLady	1.00	13	Maize	Banana	8.33	GM, B, LC, Mo	-	-
11.		Gaddikeri	RedLady	1.83	07	Maize	Sapota	100	GM, B, LC, SS, Mo, LP, BF, LD, OS, S	-	-
12.		Mailara	RedLady	1.42	08	Banana	Banana	47.06	GM, LC, SS, YM, Mo, LP, B, OS, S	-	-
13.	Sndur	Yelubenchi	Ice Berg	1.83	9	Maize	Banana	100	GM, B, LC, ST, Mo, LP, BF, LD, OS, S, RS	-	-
14.		Bilakudi	RedLady	2.50	10	Maize	Pomegranate	100	GM, B, LC, ST, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-
15.		Basavanadurga	RedLady	1.00	08	Maize	Guava	100	GM, B, LC, ST, Mo, LP, BF, LD, OS, S,	-	-
16.		Vysapura	RedLady	0.92	11	Maize	Banana	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS	-	-
							Average	**70.26**			
III	**District : Belagavi**										
17.	Gokak	Bangwhad	RedLady	0.83	24	Capsicum	Chilli	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	01	SLC, DLC, ZV, VT, VCL, DP, Y, VE, LL
18.		Bangwhad	RedLady	1.25	17	Maize	Turmeric	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-

Contd…..

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Sl. No.	Taluk	Village	Name of Variety	Area (Acre)	Crop Stage (Months)	Previous crop	Surrounding Crop	PRSV Disease Incidence (%)	PRSV symptoms in field	No. of PaLCuV infected papaya plants	PaLCuV symptoms in field
19.	Gokak	Bangwhad	RedLady	1.00	05	Marigold	Sugarcane	8.33	GM	-	-
20.		Ghataprabha	RedLady	1.25	17	Tomato	Turmeric	80.00	GM, B, LC, SS, YM, Mo, LP, BF, OS, S, RS, CS, DF	01	SLC, DLC, LR, ZV, VT, VCL, D, Y, VE, LL
21.		Ghataprabha	RedLady	1.25	04	Marigold	Sugarcane	5.20	GM	-	-
22.	Ramadurga	Mudakavi	RedLady	1.00	24	Sugarcane	Sugarcane	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-
23.		Vasavinagar	RedLady	1.67	24	Maize	Sugarcane	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-
24.		Vasavinagar	RedLady	1.25	24	Maize	Sugarcane	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-
25.		Thornur	RedLady	0.83	09	Groundnut	Sugarcane	100	GM, B, LC, SS, Mo, LP, BF, LD, OS, S, RS	-	-
26.		Thornur	RedLady	1.50	09	Cotton	Sugarcane	100	GM, B, LC, SS, YM, Mo, LP, B, LD, OS, S, RS	01	SLC, DLC, LR, VT, VCL, DP, D, Y, VE,, LL
27.		Ramdurga	RedLady	0.83	3	Mulberry	Mulberry	0.00	-	-	-
28.	Savadatti	Benakhati	RedLady	3.75	05	Sugarcane	Sugarcane	0.44	GM	-	-
29.		Kadavi Shivapura	RedLady	0.42	17	Brinjal	Banana	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-
30.		Yargheri	RedLady	0.83	17	Maize	Sugarcane	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-

Contd…..

Sl. No.	Taluk	Village	Name of Variety	Area (Acre)	Crop Stage (Months)	Previous crop	Surrounding Crop	PRSV Disease Incidence (%)	PRSV symptoms in field	No. of PaLCuV infected papaya plants	PaLCuV symptoms in field
31.	Savadatti	Yargheri	RedLady	0.67	17	Maize	Sugarcane	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-
32.		Yargheri	RedLady	0.75	17	Maize	Sugarcane	100	GM, LC, SS, YM, Mo, LP, B, LD, OS, S, RS, CS, DF	-	-
33.		Yargheri	RedLady	1.67	17	Maize	Sugarcane	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-
							Average	**76.12**			
IV	**District : Chitradurga**										
34.	Challakere	Renukapura	RedLady	1.08	05	Chilli	Pomegranate	76.92	GM, LC, SS, Mo, LP, BF, OS, S	-	-
35.		Ydalagatte	RedLady	1.25	03	Tomato	Pomegranate	1.47	GM	-	-
36.		Katandevarakote	RedLady	1.00	02	Groundnut	Pomegranate	83.33	GM, LC, SS, Mo	-	-
37.		Renukapura	RedLady	1.67	06	Groundnut	Pomegranate	100	GM, LC, SS, YM, Mo, LP, B, LD, OS, S, RS, CS, DF	-	-
38.		Katandevarakote	Ice Berg	1.00	05	Groundnut	Pomegranate	100	GM, LC, SS, YM, Mo, LP, LD, OS, S, RS, CS, DF	-	-
39.		Katandevarakot	RedLady	0.83	05	Ridge Gourd	Pomegranate	8.00	GM	-	-
40.		Dhoderri	RedLady	1.25	07	Tomato	Pomegranate	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S	-	-

Contd…..

Sl. No.	Taluk	Village	Name of Variety	Area (Acre)	Crop Stage (Months)	Previous crop	Surrounding Crop	PRSV Disease Incidence (%)	PRSV symptoms in field	No. of PaLCuV infected papaya plants	PaLCuV symptoms in field
41.	Challakere	Bharamasagara	RedLady	1.25	06	Groundnut	Pomegranate	80.00	GM, LC, SS, Mo, LP, B, OS, S	-	-
42.		Bharamasagara	RedLady	1.08	06	Groundnut	Pomegranate	7.69	GM	-	-
							Average	**61.93**			-
V	**District : Gadag**										
43.	Gadag	Harthi	RedLady	2.00	07	Banana	Banana	1.58	GM, LC	-	-
44.		Hollalapura	RedLady	2.50	14	Cotton	Maize	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-
45.		Kiratageri	RedLady	1.25	17	Chickpea	Banana	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-
46.		Harthi	RedLady	1.92	08	Banana	Banana	0.43	GM	-	-
							Average	**50.50**			
VI	**District : Haveri**										
47.	Haveri	Basapura	RedLady	1.42	07	Maize	Chilli	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S	-	-
48.		Basapura	RedLady	1.00	08	Brinjal	Chilli	66.67	GM, B, LC, SS, Mo, LP, BF, OS, S, RS	-	-
49.		Jangamanakoppa	RedLady	2.42	12	Chilli	Maize	96.55	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-
50.		Kancharagatti	RedLady	1.33	09	Maize	Maize	93.75	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, CS, RS	-	-

Contd…..

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Sl. No.	Taluk	Village	Name of Variety	Area (Acre)	Crop Stage (Months)	Previous crop	Surrounding Crop	PRSV Disease Incidence (%)	PRSV symptoms in field	No. of PaLCuV infected papaya plants	PaLCuV symptoms in field
51.	Haveri	Kancharagatti	RedLady	1.50	14	Maize	Tomato	100	GM, B, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	01	SLC, LR, ZV, VT, VCL, DP, D, VE, LL
52.		Bommanakatti	RedLady	1.00	08	Maize	Maize	83.33	GM, B, LC, SS, Mo, LP, BF, OS, S, RS,	-	-
53.		Bommanakatti	RedLady	1.17	10	Maize	Maize	100	GM, B, LC, SS, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-
							Average	**91.47**			
VII	**District : Kalaburagi**										
54.	Afzalpur	Anoor	RedLady	1.17	11	Watermelon	Sugarcane	100	GM, LC, SS, Mo, LP, BF, LD, OS, S, RS, CS, DF	04	SLC, DLC, ZV, VT, VCL, DP, Y, VE, LL
55.		Anoor	Ice Berg	2.00	02	Watermelon	Grapes	0.83	GM	-	-
56.		Kallur	RedLady	3.75	07	Banana	Grapes	0.00	-	-	-
57.		Tavarkeda	RedLady	1.00	11	Sugarcane	Watermelon	100	GM, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS	03	SLC, LR, ZV, VT, DP, D, Y, VE, LL
58.	Aland	Moga (K)	RedLady	3.17	08	Banana	Grapes	100	GM, LC, SS, Mo, LP, BF, LD, OS, S	-	-
59.	Kalaburagi	Mulkunda (K)	RedLady	1.08	02	Watermelon	Watermelon	0.38	GM	-	-
60.		Mulkunda (K).	RedLady	1.25	02	Watermelon	Sugarcane	0.53	GM	-	-
61.		Gobur	RedLady	1.42	05	Watermelon	Watermelon	100	GM, LC, SS, Mo, LP, B, LD, OS, S	-	-

Contd…..

Sl. No.	Taluk	Village	Name of Variety	Area (Acre)	Crop Stage (Months)	Previous crop	Surrounding Crop	PRSV Disease Incidence (%)	PRSV symptoms in field	No. of PaLCuV infected papaya plants	PaLCuV symptoms in field
62.	Kalaburagi	Farahatabad	RedLady	1.00	16	Banana	Watermelon	100	GM, LC, SS, YM, Mo, LP, B, LD, OS, S, RS, CS, DF	03	SLC, LR, ZV, VT, VCL, DP, D, VE, LL
63.		Hadagalarathi	RedLady	0.67	03	Toor Dal	Watermelon	100	GM, LC, SS, Mo,	-	-
64.		Kalangraga	RedLady	1.08	04	Watermelon	Watermelon	100	GM, LC, SS, Mo, LP, B, LD, OS	-	-
65.		Hadagalarathi	RedLady	1.00	08	Watermelon	Maize	100	GM, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS,	03	SLC, DLC, R, ZV, F, DP, D, Y, VE, LL
66.		Mulkunda (K)	RedLady	2.42	06	Watermelon	Maize	0.17	GM, LC	-	-
67.		Mulkunda (K)	RedLady	2.50	07	Watermelon	Watermelon	0.93	GM, LC	-	-
68.		Sirsangi	RedLady	1.50	02	Banana	Watermelon	0.17	GM, LC	-	-
							Average	**53.53**			
VIII	**District : Koppal**										
69.	Koppal	Achar Thimapura	RedLady	0.83	05	Pomegranate	Guava	100	GM, LC, SS, Mo, LP,, LD, OS, S,	-	-
70.		Halawarthi	RedLady	5.00	09	Banana	Mango	100	GM, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS	-	-
71.		Achar Thimapura	RedLady	3.75	09	Banana	Banana	100	GM, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-
72.		Methagal	RedLady	2.50	08	Pomegranate	Pomegranate	100	GM, LC, SS, Mo, LP, BF, LD, OS, S	-	-
							Average	**100**			

Contd…..

Sl. No.	Taluk	Village	Name of Variety	Area (Acre)	Crop Stage (Months)	Previous crop	Surrounding Crop	PRSV Disease Incidence (%)	PRSV symptoms in field	No. of PaLCuV infected papaya plants	PaLCuV symptoms in field
IX	**District : Vijayapura**										
73.	Muddebihal	Nidagundi	RedLady	2.83	15	Watermelon	Tomato	100	GM, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	01	SLC, DLC, ZV, VT, VCL, DP, Y, VE, LL
74.		Vanahalli	RedLady	1.42	15	Watermelon	Banana	100	GM, LC, SS, YM, Mo, LP, BF, LD, OS, S, RS, CS, DF	-	-
75.		Kavadimatti	RedLady	1.67	08	Banana	Maize	0.90	GM	-	-
							Average	**66.97**			-
X	**District: Yadgiri**										
76.	Surapura	Narayanpur	Ice Berg	3.17	09	Watermelon	Maize	100	GM, LC, SS, Mo, LP, BF, LD, OS, S, RS	-	-
							Average	**100**			

*B: Blistering, BF: Bumps on fruits, CS: Chlorotic spot od fruits, D: Deformation, DF: Distorted Fruit, DLC: Downward Leaf Curling, DP: Distorted Petioles, GM: Green Mosaic, LC: Leaf Curling, LD: Leaves distortion, LL: Leathery Leaf, LP: Leaves Puckering, LR: Leaf Rolling, Mo: Mottling, OS: Oily Streak, SLC: Severe Leaf Curl, RS: Rings Spot, S: Stunted Growth, SS: Shoestring, VCL Vein Clearing, VE: Vein Enation, VT: Vein Thickening, Y: Yellowing, YM: Yellow Mosaic and ZV: Zigzag Vein

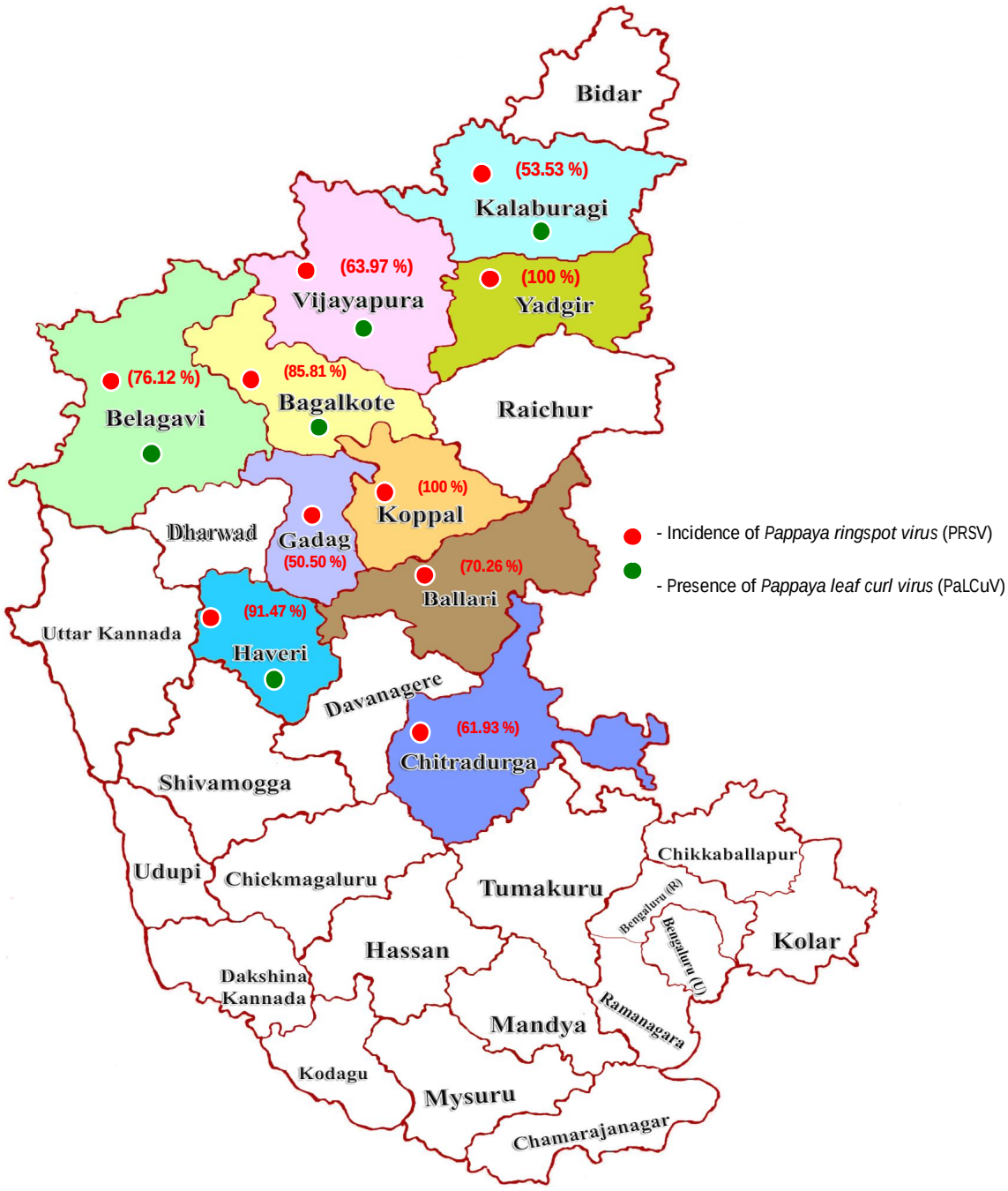

Fig. 2: Status of PRSV and PaLCuV diseases on major papaya growing districts of Karnataka

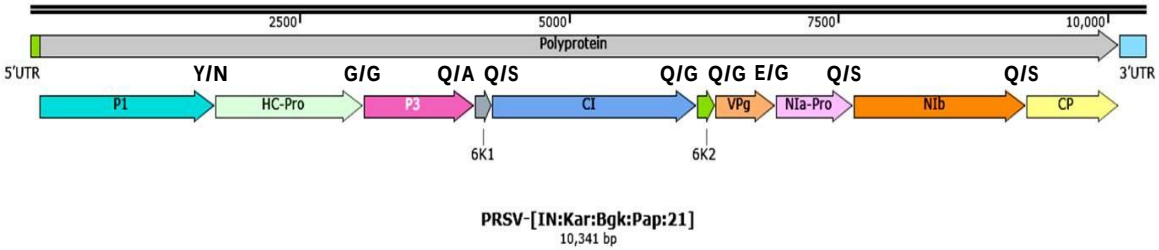

Fig. 3: Genome organization PRSV isolate

Plate 6: Field view of papaya plot infected by severe PRSV

blistering, distortion of leaves, blistering on leaves, shoestring, oily streak, chloretic spots and distorted fruit. Green mosaic was present at all the locations irrespective age of the plant (Plate 7). Younger plants (< 5 month) recorded only green mosaic. However, the symptoms like yellow mosaic, leaf curling, stunted growth, puckering, mottling and blistering on leaves and shoestring symptoms were common in above 5 months old plants. Wherever the fruiting was seen, oily spots were common symptom, while distorted fruits were seen at 28 surveyed locations. Further, distortion of leaves was seen only in those locations were PRSV incidence was more than 90.0 per cent.

Apart from PRSV incidence, PaLCuV infection on papaya plants was also observed in some of the surveyed districts of Karnataka *viz.*, Bagalkote, Belagavi, Haveri, Kalaburgi and Vijayapura (Fig. 2). The typical symptoms expressed by PaLCuV infected plants were severe leaf curl, downward leaf curling, vein thickening, vein clearing, distorted petioles, deformation, yellowing, vein enation, leaf rolling, leathery leaf and the zigzag vein (Plate 8).

4.1.2 Collection of virus isolates

During survey, 75 papaya leaf samples expressing typical symptoms of PRSV were collected from surveyed field for detection and characterization. Further, 32 leaf samples in plants expressing typical PaLCuV disease symptoms were also collected from different districts of Karnataka.

4.1.3 Molecular detection of PRSV and PaLCuV in papaya

4.1.3.1 Detection of coat protein component of PRSV

RT-PCR was employed to detect PRSV infection in surveyed samples by using a set of primer MB 11A/MB 11B which specifically amplify the coat protein gene of PRSV. Out of 107 samples tested, 75 samples which recorded typical PRSV symptoms showed desired amplification (~905 bp) confirming PRSV infection (Plate 9). No amplification was found in RNA extracted from the remaining 32 samples which were having leaf curl symptoms.

Plate 7: PRSV infected papaya plants showing different symptoms on field

A. Green Mosaic, B. Leaf Curling, C. Shoestring, D. Yellow Mosaic, E. Mottling, F. Blistering of leaves, G. Leaves Puckering, H. Leaves distortion, I. Oily Streak, J.Stunted Growth, K. Rings Spot and L. Bumps and Distorted Fruit

Plate 8: PaLCuV infected papaya plants showing different symptoms on field

A. Severe Leaf Curl,B: Leaf Rolling, C. Downward Leaf Curling, D. Zigzag Vein, E. Vein Thickening, F. Vein Clearing, G Distorted Petioles, H and I Deformation, J. Yellowing, K. Vein Enation and L. Leathery Leaf.

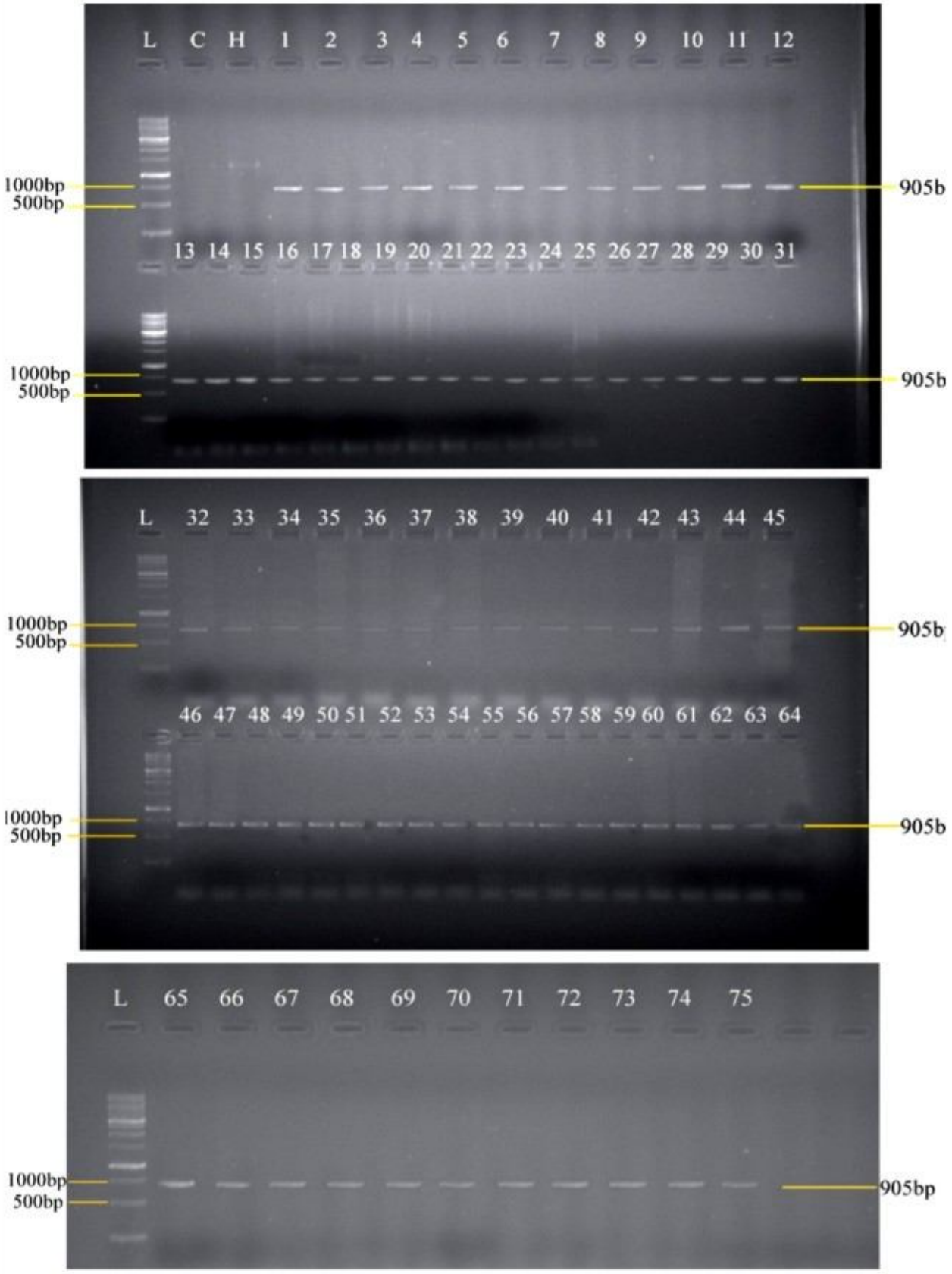

Plate 9: Agarose gel photograph showing amplification of coat protein fragment of PRSV by using a set of primer MB 11A/MB 11B (~905 bp)

Lane L: 1 kb lader (StepUp™ 1 kb DNA Ladder), Lane C: Water control, Lane H: Healthy leaf, Lane 1-75 samples from different location.

4.1.3.2 Detection for the presence of other RNA virus

To determine the presence or association of any other RNA viral infections along with the PRSV in surveyed samples. RT-PCR was performed using primers *viz.,* M-F/M-R, PLDMV355-F/PLDMV355-R, PapMV205-F/PapMV205-R and ZYMV-F (CPF-Nocl)/ZYMV-R (CPR-Nocl) which are specific to detect *Papaya milk vetch dwarf virus, Papaya leaf distortion virus, Papaya mosaic virus* and *Zucchini yellow mosaic virus* respectively. During RT-PCR amplification none of the samples tested positive for these viral infections. This result confirming that these tested RNA viruses was not associated with PRSV in surveyed samples of Karnataka.

4.1.3.3 Detection for DNA-A component of begomovirus

All the 107 surveyed samples were subjected for DNA isolation and PCR was employed to detect and amplify DNA-A fragment of begomovirus using a set of overlapping degenerative primers PAL1v1978/PARIc715b (~1.5 kb); PAL1v1978/ PAR1c496 (~1.2 kb); PARIv722b / PALIc1960b (~1.2 kb); Av494/ Ac1048 (~0.6 kb) (Plate 10 and Plate 11). The 32 samples having typical leaf curl symptoms in the field have showed positive amplification along with positive control *Chilli leaf curl* isolate, while no amplification was observed in healthy and remaining 75 samples out of 107 samples.

4.1.3.4 Detection for DNA-B component of begomovirus

PCR was employed for surveyed samples to detect and amplify a fragment of DNA-B using PBL1v2040/PCRc1 (~0.6 kb) primers set. Positive amplification was found in *Ridge gourd yellow mosaic* (positive control) and no amplification was observed in surveyed samples (Plate 12). This result indicated a lack of bipartite nature.

All 32 leaf curl samples showed positive amplification for only the DNA-A component and not for DNA-B. These results confirm that begomovirus infecting papaya showing leaf curl symptoms in surveyed regions of Karnataka is monopartite.

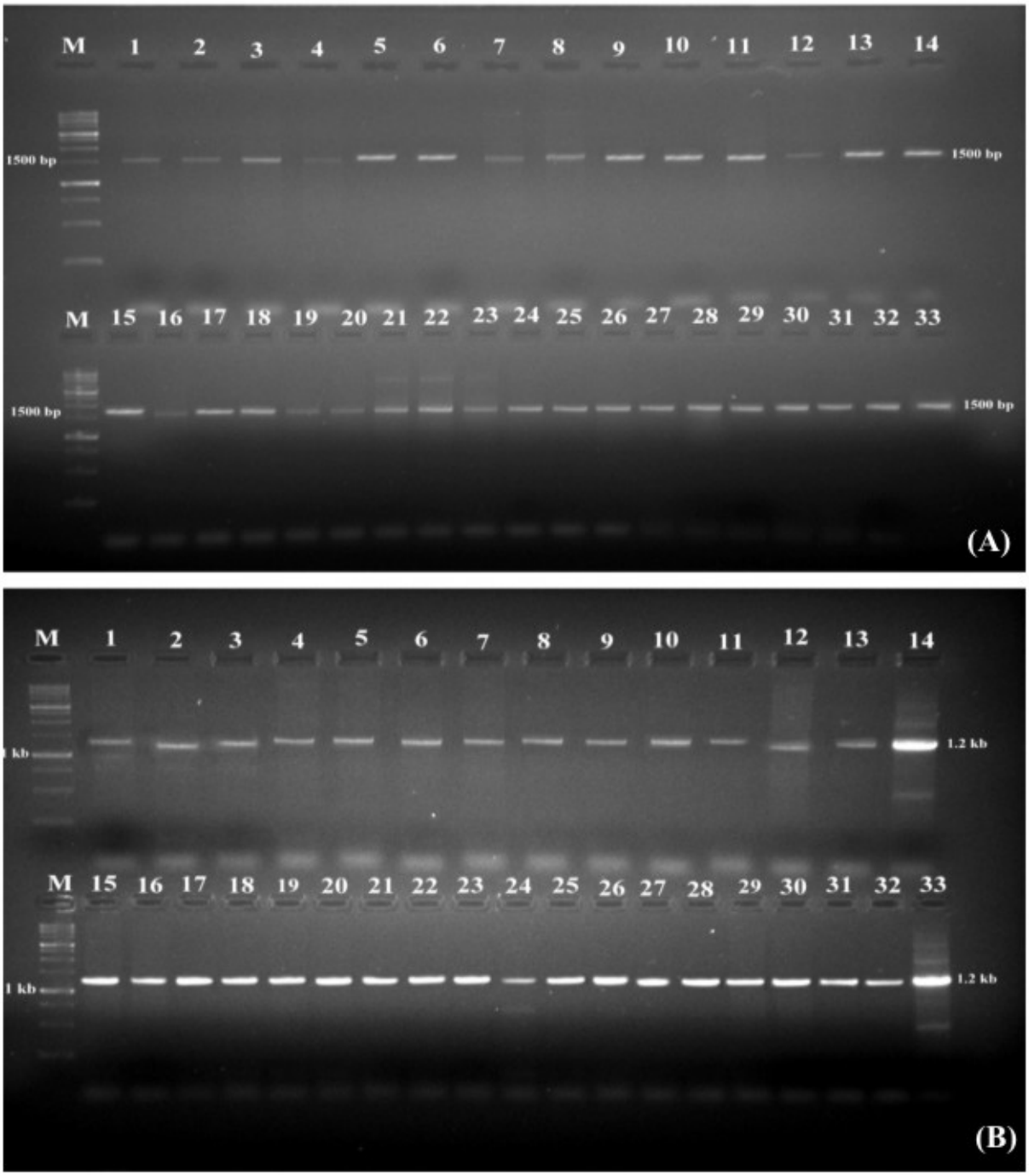

Plate 10: Agarose gel photograph showing amplification of DNA-A fragment of PaLCuV by using a set of primer PAL1v1978/PARIc715b (~1.5 kb) (A) and PAL1v1978/PAR1c496 (~1.2 kb) (B)

Lane M: 1 kb lader (StepUp™ 1 kb DNA Ladder), Lane 1-32 samples from different location and Lane 33: +ve control(*Chili leaf curl virus*).

90

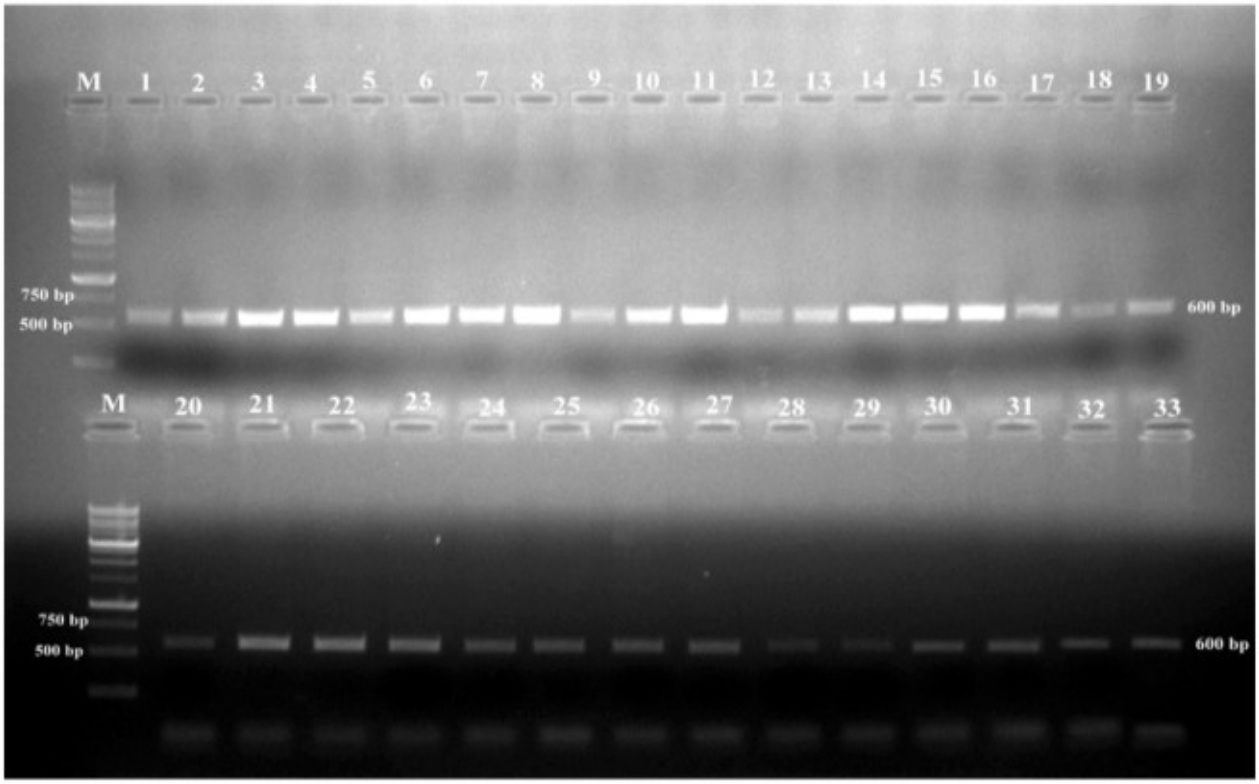

Plate 11: Agarose gel photograph showing amplification of DNA-A fragment of PaLCuV by using a set of primer Av494/Ac1048 (~0.6 kb)

Lane M: 1 kb lader (StepUp™ 1 kb DNA Ladder). A. Lane 1-32 samples from different location and Lane 33: +ve control (*Chili leaf curl virus)*.

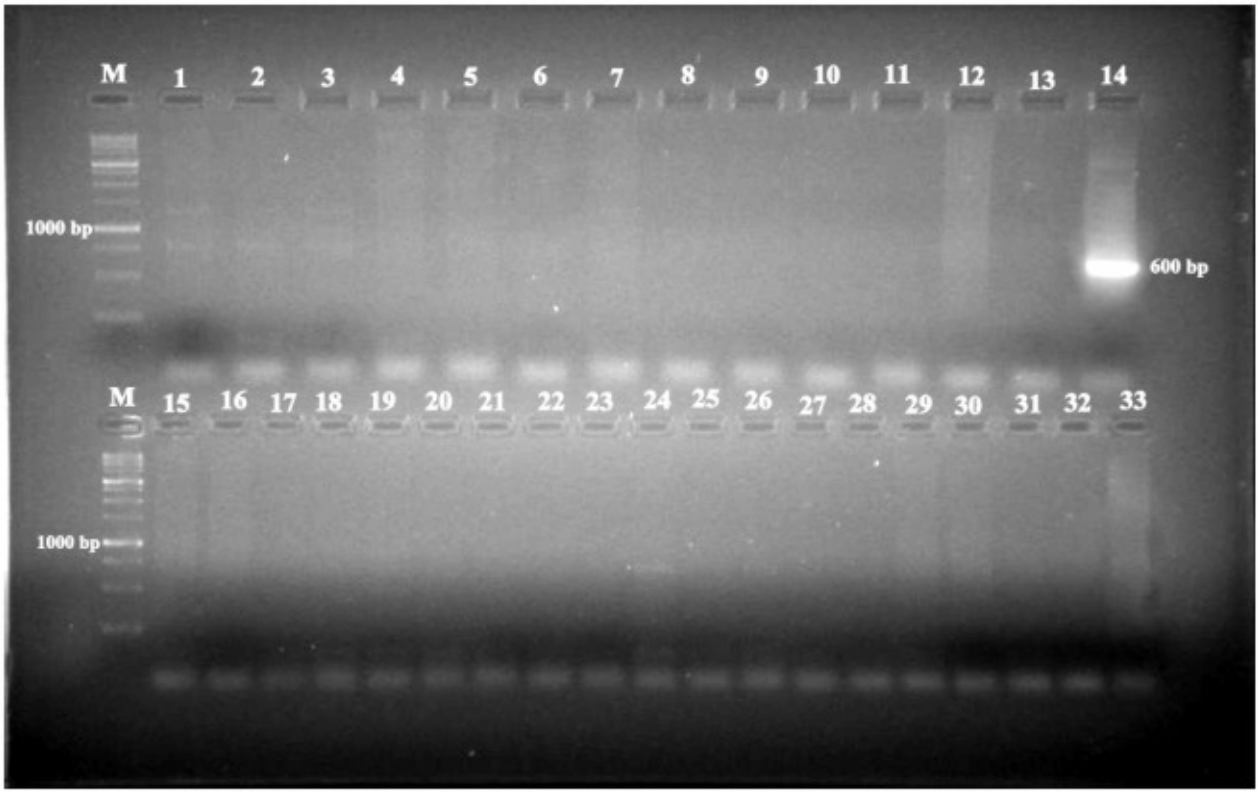

Plate 12: Agarose gel photograph showing amplification of DNA-B fragment of PaLCuV by using a set of primer PBL1v2040/PCRc1 (~0.6 kb)

Lane M: 1 kb lader (StepUp™ 1 kb DNA Ladder). A. Lane 1-33 samples from different location and Lane 14: +ve control (*Ridge gourd yellow mosaic*)

4.1.3.5 Detection of the beta and alpha satellites in infected samples

PCR was employed for detecting the beta and alpha DNA satellites in 32 leaf curl samples showing leaf curl disease of papaya along with *Chilli leaf curl virus* (positive control) using specific primers of beta-DNA (BETA01/BETA02) and alpha-DNA (DNA101/DNA102). The PCR resulted in successful amplification of beta satellite in all the 32 samples (~700 bp) along with positive control (~1.3 kb) and no amplification in healthy samples. The amplification of the beta satellite fragment at ~700 bp signifies the presence of defective satellite associated with begomovirus infecting papaya in the Karnataka region (Plate 13). However, alphasatalite was not found association in any of the surveyed and test samples except with the positive control (~1.3 kb).

4.1.3.6 Molecular characterization of the PRSV and PaLCuV diseases associated with in Karnataka

4.1.3.6.1 Cloning of PRSV components

A representative surveyed sample PRSV-BGK collected from, Bagalkote district of Karnataka is subjected for cloning, sequencing and sequence analysis.

RT-PCR was performed using nine overlapping primer pairs designed by Ortiz-Rojas and Chaves-Bedoya (2017). These primers sequentially amplify the entire components of PRSV (~10.341 kb), combining amplified fragments of PRSV1S –F/PRSV1780A–R (~1.8 kb), PRSV878S–F/PRSV2746A–R (~1.8 kb); PRSV2289S–F/PRSV3926A–R (~1.6 kb), PRSV3310S–F/PRSV4850A–R (~1.5 kb), PRSV4199S –F/PRSV5924A–R (~1.8 kb), PRSV5355S–F/PRSV7195A–R (~1.8 kb), PRSV6777S–F/PRSV8041A–R (~1.3 kb), PRSV7497S–F/PRSV9389A–R (~1.8 kb) and PRSV8961S–F/ PRSVPOLYT–R (~1.4 kb) (Plate 14).

PRSV genomic components like P1 (Protein 1), HC-Pro (Helper Component-Protease), P3 (Protein 3), 6K1 (6-kDa peptide 1), CI (Cylindrical Inclusion protein), 6K2 (6-kDa peptide 2), VPg (Viral Protein genome-linked), NIa-Pro (Nuclear inclusion A-protease), NIb (Nuclear inclusion B) and CP (Coat protein) were obtained.

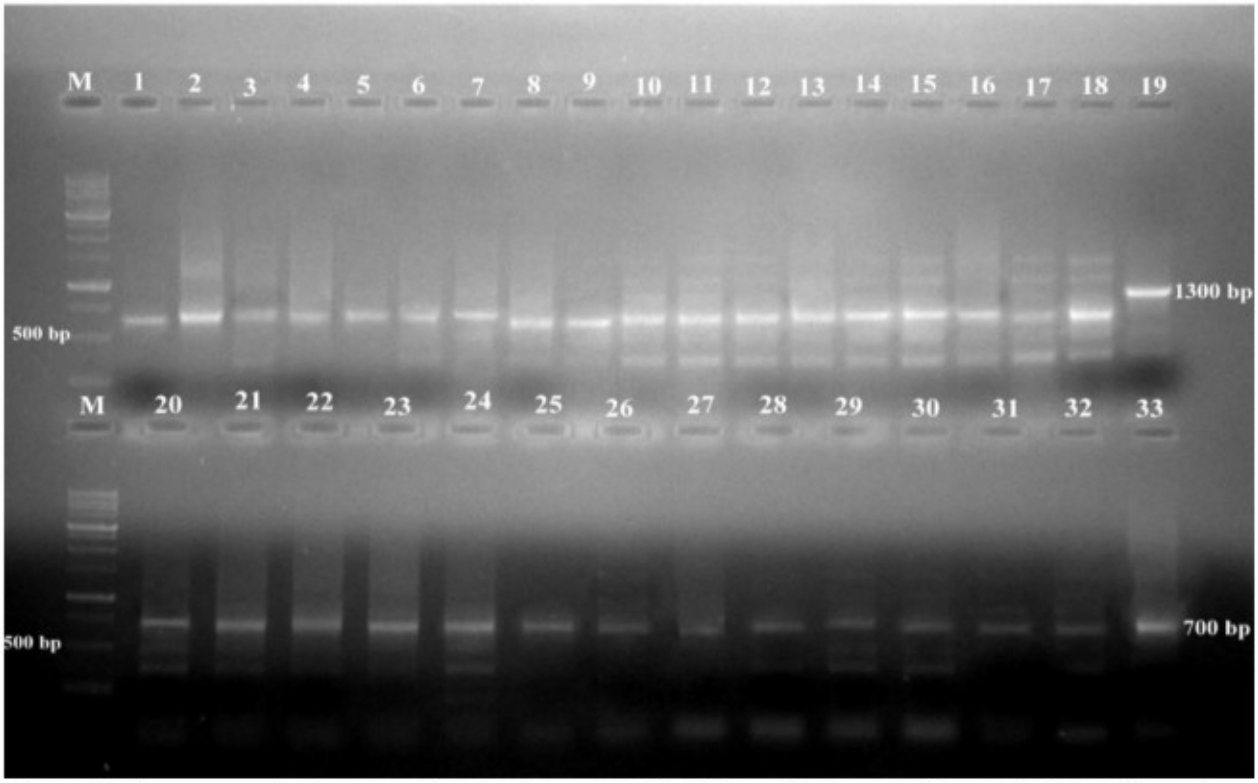

Plate 13: Agarose gel photograph showing amplification of beta satellite of PaLCuV by using beta-DNA specific primers BETA01/BETA02 (~0.7 kb and ~1.3 kb)

Lane M: 1 kb lader (StepUp™ 1 kb DNA Ladder). A. Lane 1-33 samples from of different location (~0.7 kb) and Lane 14: +ve control (*Chili leaf curl virus*) (~1.3 kb).

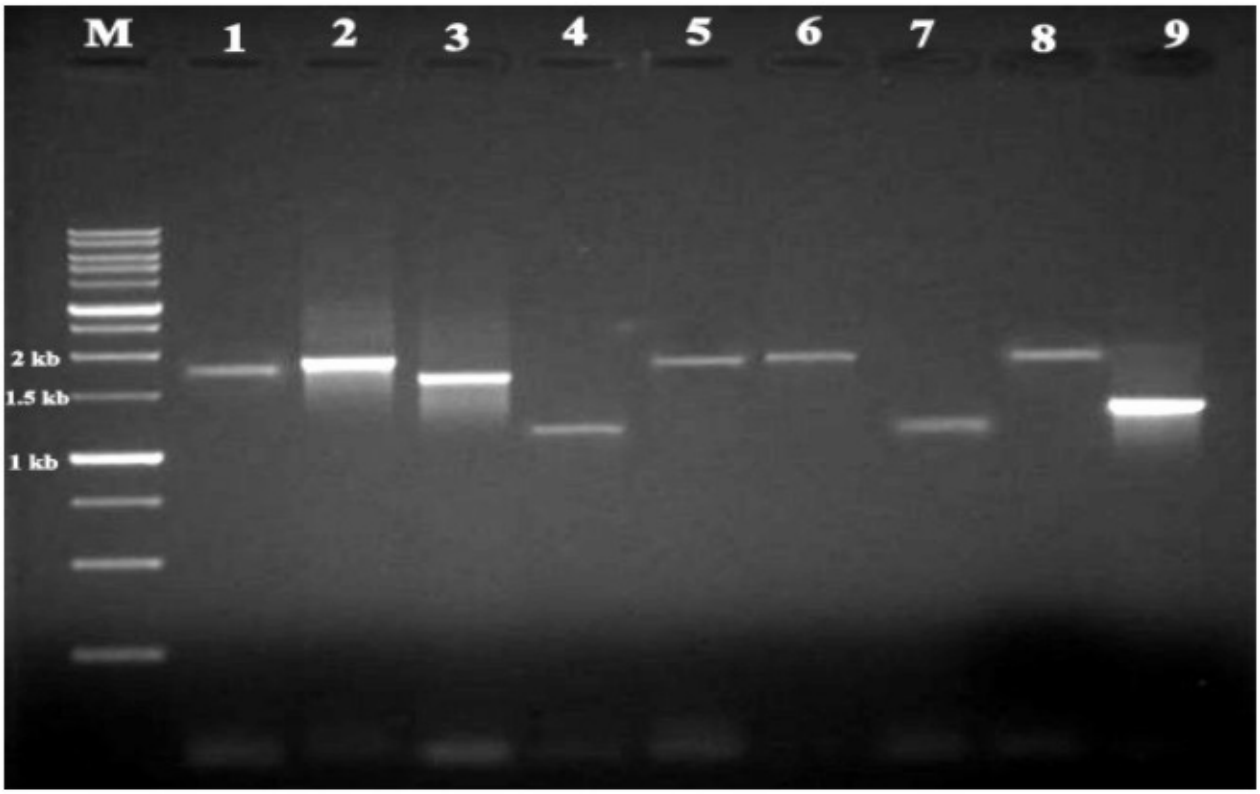

Plate 14: Agarose gel photograph showing amplification of entire components of PRSV by using the overlapping primers

PRSV1S F/PRSV1780AR (~1.8 kb) (Lane 1), PRSV878SF/PRSV2746AR (~1.8 kb) (Lane 2); PRSV2289SF/PRSV3926AR (~1.6 kb) (Lane 3), PRSV3310SF/PRSV4850AR (~1.5 kb) (Lane 4), PRSV4199S F/PRSV5924AR (~1.8 kb) (Lane 5), PRSV5355SF/PRSV7195AR (~1.8 kb) (Lane 6), PRSV6777SF/PRSV8041AR (~1.3 kb) (Lane 7), PRSV7497SF/PRSV9389AR (~1.8 kb) (Lane 8) and PRSV8961SF/ PRSVPOLYTR (~1.4 kb) (Lane 9) in PRSV infected papaya leaf surveyed samples, Lane M: 1 kb lader (StepUp™ 1 kb DNA Ladder)

RT-PCR amplified products were purified from excised gel pieces and then ligated into cloning vector pMD20-T. All the components were transformed into *E.coli* DH 5α strain. The transformed clones obtained were sub cultured on an LB agar media containing ampicillin and checked for the presence of insert through colony PCR.

4.1.3.6.2 Confirmation of PRSV clones through colony PCR

By using transformed white colonies, colony PCR was conducted by using the colony as template DNA. The PCR was employed by using respective specific primers to check the presence of insert. The colony PCR results revealed the presence of respective nine cloned fragments of PRSV which corresponded to the original RT-PCR fragments.

4.1.3.6.3 Confirmation of PRSV clones through PCR analysis of recombinant plasmids

The clones which were showing positive with colony PCR for nine fragments of PRSV were grown in the LB broth and plasmids were isolated from each colony of different fragments respectively. The isolated plasmids from the transformants were used as template DNA for PCR analysis by using PRSV fragment specific primers and confirming the presence of the clones in plasmids.

4.1.3.6.3 Cloning of DNA-A components of begomovirus

Among 32 begomovirus detected samples, 13 representative samples (Table 10) were subjected to cloning, sequencing and sequence analysis. The Intergenic region (IR), coat protein region (AV1), partial coat protein (AV2), transcriptional activator protein (TrAP), replication enhancer protein (Ren), nuclear shuttle protein (NSP), replication-associated protein (Rep) and movement protein (MP) gene respectively were obtained.

PCR amplified products were purified from excised gel pieces and then ligated into cloning vector pMD20-T. All the components were transformed into *E.coli* DH 5α strain. The transformed clones obtained were subcultured on an LB agar media containing ampicillin and checked for the presence of insert through colony PCR.

4.1.3.6.4 Confirmation of DNA-A clones through colony PCR

By using transformed white colonies as a template DNA, colony PCR was carried. The reaction was carried out by using respective DNA-A specific degenerative primers to check the presence of insert. The colony PCR results revealed the presence of cloned fragments of 1.5 kb, 1.2 kb and 0.5 kb of DNA-A which corresponded to the original PCR fragments.

4.1.3.6.5 Confirmation of DNA-A clones through PCR analysis of recombinant plasmids

The clones which were showing positive with colony PCR were grown in the LB broth and plasmids were isolated. The isolated plasmids from the transformants were used as template DNA for PCR analysis by using respective degenerative primers and confirmed the presence of fragments (Plate 15).

4.1.3.7 Sequence analysis

Sequences were obtained by sequencing at Sakala Enterprises (Pvt. Ltd.), Bangalore, India, and subjected for analysis.

4.1.3.8 Finding genes/ORFs of PRSV isolated from papaya

The software package of NCBI "ORF finder" at www.ncbi.nlm.nih.gov/ was used to detect the genes/ORFs present on the PRSV genome of Bagalkote isolate and recorded the single major polyprotine containing open reading frame (ORF) located at +ve strand of the genome (Table 20).

4.1.3.9 Genome organization of full length PRSV isolated from papaya

The complete genome of PRSV-BGK isolate (Bagalkote, Karnataka) consisted of 10,341 nt in length (Appendix III-A). The genome starts from 5' untranslated region (UTR) code from 1st nt to 85th nt and contain conserved terminal regions 5'-AAATAAAACATCT. The region from 86th nt to 10,114th nt position (start-stop codon) codes for translated large polyprotein having the length of 10,029 nt coding for 3,342 aa with a protein weight of 380.24 kDA and it contains ten mature peptides resides on a virion (+ve) sense strand in clockwise direction. The protease peptid (P1)

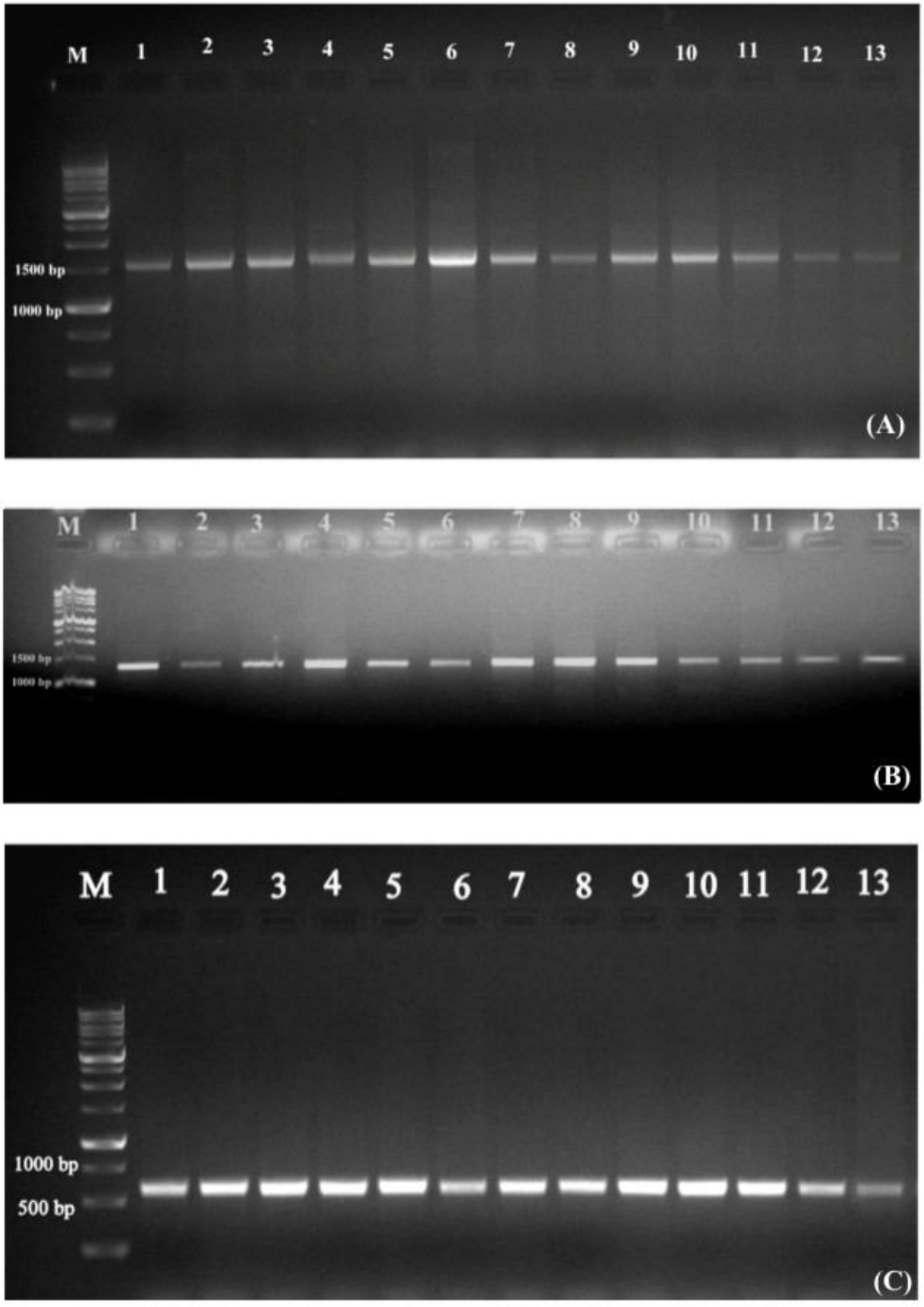

Plate 15: Agarose gel photograph of colony PCR showing the screening of transformants for inserts by using the primers P AL1v1978/PARIc715b (~1.5 kb) (A), PAL1v1978/PAR1c496 (~1.2 kb) (B) and Av494/Ac1048 (~0.6 kb) (C).

Lane M: 1 kb lader (StepUp™ 1 kb DNA Ladder) and Lane 1-13: Representative PaLCuV infected surveyed samples.

Table 20: Genome organization of PRSV-BGK isolate

Sl. No.	Untranslated regions and functional regions	Position in Genome (Start-Stop Codon)	Predicted ORFs Size (nt)	Predicted size of protein (No. of amino acids)	Predicted protein weight (kDa)	Predicted cleavage site	Strand orientation	Direction with start codon
1	Genome length (nt)	1-10,341	-	-	-	-	-	-
2	Polyprotein	86-10,114	10,029	3,342	380.24	-	+ve	Clockwise
3	5'UTR[a]	1–85	85	-	-	-	-	-
4	P1[a]	86–1,723	1,638	546	62.3	MEQY/N	+ve	Clockwise
5	HC-Pro[a]	1,724–3,094	1,371	457	52.12	HYIVG/G	+ve	Clockwise
6	P3	3,095–4,129	1,035	345	39.9	VIHQ/A	+ve	Clockwise
7	6K1	4,130–4,285	156	52	6.01	VYHQ/S	+ve	Clockwise
8	CI[a]	4,286–6,190	1,905	635	71.35	VYHQ/G	+ve	Clockwise
9	6K2	6,191–6,361	171	57	6.36	VFHQ/G	+ve	Clockwise
10	VPg[a]	6,362–6,928	567	189	21.33	VHHE/G	+ve	Clockwise
11	NIa-Pro[a]	6,929–7,642	714	238	26.51	VFEQ/S	+ve	Clockwise
12	NIb[a]	7,643–9,253	1,611	537	61.82	VYHQ/S	+ve	Clockwise
13	CP[a]	9,254–10,114	861	286	32.72	-	+ve	Clockwise
14	3'UTR	10,115–10,341	227	-	-	-	-	-
15	Name assigned based on NSI	*Papaya ringspot virus*-[India:Karnataka:Bagalkote:Papaya:2021]						
16	Virus acronyms	PRSV-[IN:Kar:Bgk:Pap:21]						

[a] UTR: Untranslated region, P1: Protease, HC-Pro: Helper Component protease, CI: Cylindrical Inclusion, VPg: Viral Protein genome linked, NIa-Pro: Nuclear Inclusion a protease, NIb: Nuclear Inclusion b and CP: Coat Protein

is having length of 1,638 nt (start codon at 86th nt and stop codon at 1,723rd nt position) which codes for 546 aa with a protein weight of 62.3 kDa. Helper component protease (HC-Pro) is containing of 1,371 nt in length (start codon at 1,724th nt and stop codon at 3,094th nt position) with 457 aa of 52.12 kDa. P3 ORF having start codon from 3,095th nt to stop codon at 4,129th nt position with a total of 1,035 nt length containing 345 aa with 39.9kDa. 6K1 having short nucleotide length of 156 nt which is starting from start codon at 4,130th nt to stop codon 4,285th nt position contain 52 aa with 6.01 kDa. Cylindrical inclusion (CI) start with start codon at 4,286th nt to stop codon 6,190th nt position containg 1,905 nt length having 635 aa with 71.35 kDa. 6K2 is containg 171 nt length (start codon at 6,191st nt to stop codon at 6,361st nt position) having 57 aa with 6.36 kDa. Viral protein genome linked (VPg) coding from start codon at 6,362nd nt to stop codon 6,928th nt position with a length of 567 nt having 189 aa with 21.33 kDa. Nuclear inclusion a protease (NIa-Pro) consistes of 714 nt, start from start codon at 6,929th nt to stop codon at 7,642nd nt position having 238 aa with 26.51 kDa. Nuclear inclusion b (NIb) containing 1611 nt with start codon at 7,643rd to stop codon 9,253rd having 537 aa with 61.82 kDa. Coat protine (CP) contains 861 nt length which start at start codon 9,254th nt to stop codon 10,114th nt having 286 aa with 32.72 kDa and finaly terminal region of the genome contains 3' untranslated region (UTR) from 10,115th to 10,341st with conserved sequence of -CTCTTAGAATGAG-3'(Table 20 and Fig, 3).

The potential cleavage sites around polyproteins are also been identified in the PRSV-BGK isolate, a total of nine cleavage site between each peptides. MEQY/N is the cleavage site identified between P1/HC-Pro region; HYIVG/G between HC-Pro/P3 region; VIHQ/A between P3/6K1 region; VYHQ/S between 6K1/ CI region; VYHQ/G between CI/6K2 region; VFHQ/G between 6K2/VPg region; VHHE/G between VPg/NIa-Pro region; VFEQ/S between NIa-Pro/ NIb region and VYHQ/S between NIb/CP region.

Further, well characterized motifs were also located in this genome, such as KITC in the HC-Pro region for aphid transmission, AVGSGKST in the CI region for helicase function, GDD in the NIb region for replicase activity, and DAG in the CP region for aphid transmission, were also located.

4.1.3.10 Comparison of complete nucleotide sequence of PRSV-BGK isolate with other potyviruses sequences

Comparison done for the nucleotide sequence of full length PRSV isolated from papaya with other homologous potyvirus sequences (Table 11). The full length nucleotide sequence comparison revealed that PRSV-BGK isolate has recorded maximum nucleotide sequence identity of 95.84 per cent with PRSV-[IN:TS:Hyd:HYD:Pap:15] (KP743981) which is reported from Telangana state, India followed by PRSV-[IN:MH:Pun:VC:Pap:15] (MF405299) (having 95.54 %) and PRSV-[IN:MH:PS3-H:Pap:15] (MF405297) of 93.89 per cent reported from Maharashtra, India. The least sequence identity was with PRSV-[BD:BD-1:Pap:16] (MH444652) (70.20 %) which is infecting papaya from Bangladesh (Table 21). Based on ICTV poty virus species demarcation criteria this PRSV-BGK isolate is demarcated as variant and based on sampling location and host the it has been given a descriptor as PRSV-[INDIA:Karnataka:Bagalkote:Papaya:2021]. It is designated as PRSV-[IN:Kar:Bgk:Pap:21].

4.1.3.11 Phylogenetic analysis of full length genome of PRSV

The complete genome sequence of PRSV-BGK isolate was aligned with those of other selected potyviruses available in the database (Table 11). The phylogenetic tree was generated from multiple alignment and pairwise alignment by using the CLUSTALW having 60 per cent cut-off value with 1,000 bootstrap replicates in MEGA.11 version. Phylogenetic tree based on alignment of complete nucleotide sequences of other selected potyviruses with the present study virus revealed that the PRSV-BGK isolate clustered with Indian isolates and formed the distinct branch with PRSV-[IN:TS:Hyd:HYD:Pap:15] infecting papaya from Telangana (Fig. 4), Thus the PRSV-BGK isolate is conformed as new variant of PRSV-[IN:TS:Hyd:HYD:Pap:15]. This result also supported by nucliotide sequence identity (Table 21).

4.1.3.12 Recombination analysis of complete nucleotide sequence of isolated PRSV with other PRSV sequences

Table 22 summarizes the results obtained using the computational tests performed for detecting the past recombination events using Recombination Detection Program (RDP), GENECOV, Bootscan, Max Chi, Chimera, Si Scan and 3Seq based

Table 21: Pair wise percent nucleotide sequence identity between the PRSV isolated from papaya with other selected potyvirus

Sl. No.	Potyvirus	Accession No.	Percentage nucleotide sequence identity (%)
1.	PRSV-[IN:TS:Hyd:HYD:Pap:15]	KP743981	95.84
2.	PRSV-[IN:MH:Pun:VC:Pap:15]	MF405299	95.54
3.	PRSV-[IN:MH:PS3-H:Pap:15]	MF405297	93.89
4.	PRSV-[IN:MH:PM-H:Pap:15]	MF405295	93.09
5.	PRSV-[IN:MH:PM-I:Pap:15]	MF405296	92.32
6.	PRSV-[IN:MH:PS3-H:Pap:15]	MF405298	91.20
7.	PRSV-[IN:ND:DEL:Pap:06]	EF017707	89.03
8.	PRSV-[TW:TXG:Pap:05]	X67673	85.28
9.	PRSV-[TH:Ver:PDO:Mex-VrPO:Pap:13]	AY231130	85.28
10.	PRSV-[TW:TXG:Pap:18]	NC_001785	85.28
11.	PRSV-[US:Hi:HA:Pap:93]	S46722	85.28
12.	PRSV-[US:HI:P/mutantHA5-1:Pap:84]	MT470188	85.22
13.	PRSV-[US:HI:PG:Pap:07]	EU126128	84.99
14.	PRSV-[PNG:16A:Pap:16]	MH404261	84.96
15.	PRSV-[US:PRSV-PTX:Pap:14]	KY271954	84.95
16.	PRSV-[PNG:12B:Pap:16]	MH404260	84.91
17.	PRSV-[PNG:18B:Pap:16]	MH404263	84.76
18.	PRSV-[EC:Bab-Ec:Pap:17]	MH974109	84.70
19.	PRSV-[PNG:17B:Pap:16]	MH404262	84.69
20.	PRSV-[EC:PRSV_Pap_Ec:Pap:18]	MH974110	84.63
21.	PRSV-[CO:CH:PRSV_CH:Pap:14]	KT275938	84.61
22.	PRSV-[CO:VDR:PRSV_VR:Pap:14]	KT275937	84.58
23.	PRSV-[AU:53C:Pap:15]	KX655866	84.58
24.	PRSV-[PNG:8A:Pap:16]	MH404259	84.57
25.	PRSV-[PNG:22A:Pap:16]	MH404264	84.50
26.	PRSV-[MX:PRSV.PC-VA:Pap:14]	MN203183	83.91
27.	PRSV-[MX:PRSV.PC-PS:Pap:14]	MN203185	83.81
28.	PRSV-[MX:PRSV.CD-VA:Pap:14]	MN203186	83.69
29.	PRSV-[MX:PRSV.PC-OS:Pap:14]	MN203184	83.66
30.	PRSV-[MX:PRSV.CD-PS:Pap:14]	MN203187	83.62

Contd.……

Sl. No.	Potyvirus	Accession No.	Percentage nucleotide sequence identity (%)
31.	PRSV-[IN:MH:Pun:Pap:15]	MH311882	81.18
32.	PRSV-[TW:pfT3-AX-D-Ph:Pap:12]	JX448372	80.84
33.	PRSV-[TW:prT3-AX-M-Ph:Pap:12]	JX448371	80.78
34.	PRSV-[TW:Ping-tong:Pap:05]	DQ340771	80.76
35.	PRSV-[TW:Ping-tong:Pap:06]	DQ340769	80.74
36.	PRSV-[TW:pFT3-NP:Pap:12]	JX448373	80.69
37.	PRSV-[TW:YK:Pap:08]	X97251	80.69
38.	PRSV-[TW:P-5-19:Pap:07]	EU882728	80.68
39.	PRSV-[TH:Pap:02]	AY162218	80.53
40.	PRSV-[TH:Sam:SMK:Pap:02]	MT470190	80.43
41.	PRSV-[TH:Pap:00]	AY010722	80.40
42.	PRSV-[TW:Ping-tong:Pap:05]	DQ340770	80.38
43.	PRSV-[VN:TG:PRSV-TG5:Pap:15]	MT470189	80.38
44.	PRSV-[IN:PRSVR3:Pap:13]	KJ755852	80.26
45.	PRSV-[CN:Hai:Dongfang:HN-DF:Pap:12]	KT895257	80.24
46.	PRSV-[TW:prT3-AX-N-Ph:Pap:12]	JX448370	80.22
47.	PRSV-[TW:prT3-AX-N-Ad6:Pap:12]	JX448369	80.18
48.	PRSV-[CN:HN-1:Pap:10]	HQ424465	80.16
49.	PRSV-[IN:Meg:Umi:Pap:15]	MF356497	79.83
50.	PRSV-[CN:P:Pap:06]	EF183499	79.82
51.	PRSV-[CN:Hai:Pap:11]	KF734962	79.63
52.	PRSV-[CN:Hai:Lin:PRSV-LM:Pap:15]	KT633943	79.59
53.	PRSV-[CN:Hai:Haikou:PRSV-HNVb:Pap:13]	KF791028	79.56
54.	PRSV-[IN:WB:Pap:17]	LC482263	78.65
55.	PRSV-[BD:BD-2:Pap:16]	MH397222	78.56
56.	PRSV-[IN:HP:Pal:Pap:18]	MW030522	78.39
57.	PRSV-[CN:Pap:16]	KY933061	77.94
58.	PRSV-[BD:BD-1:Pap:16]	MH444652	70.20

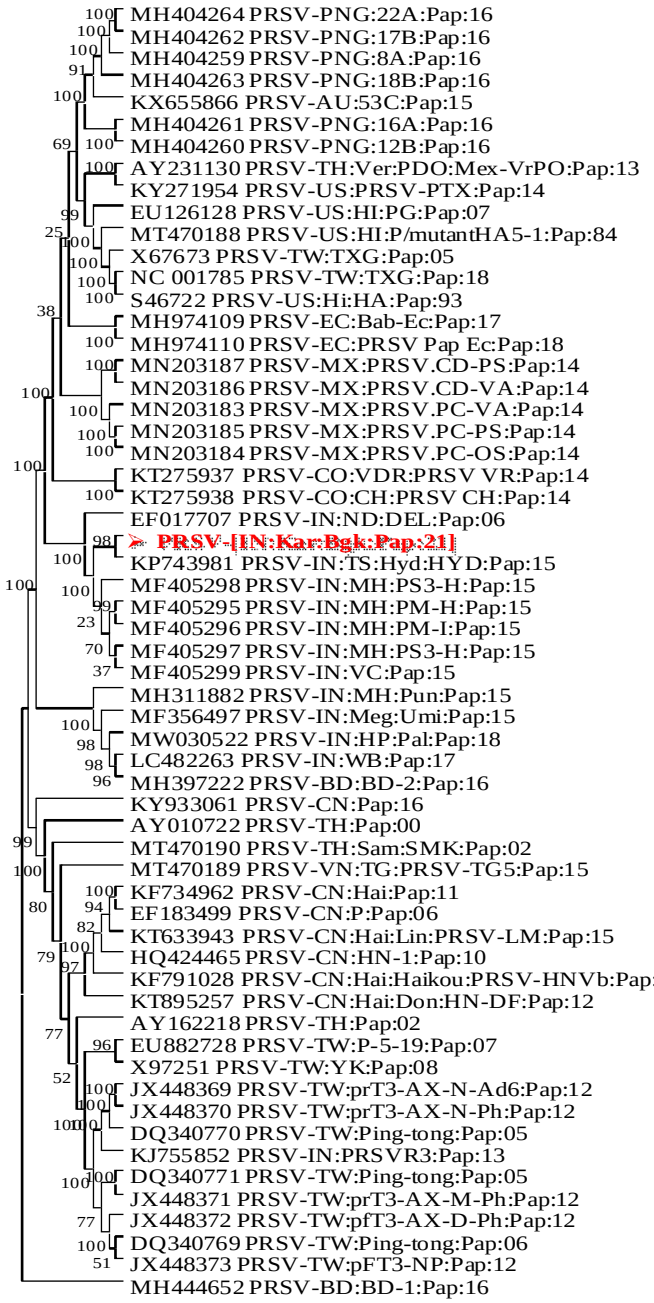

Fig. 4: Phylogenic tree showing the relationship of PRSV isolated from papaya with other selected potyviruses based on their full length genome nucleotide sequence (*The number of each node indicates=60 percentage bootstrap value with 1000 replicates)

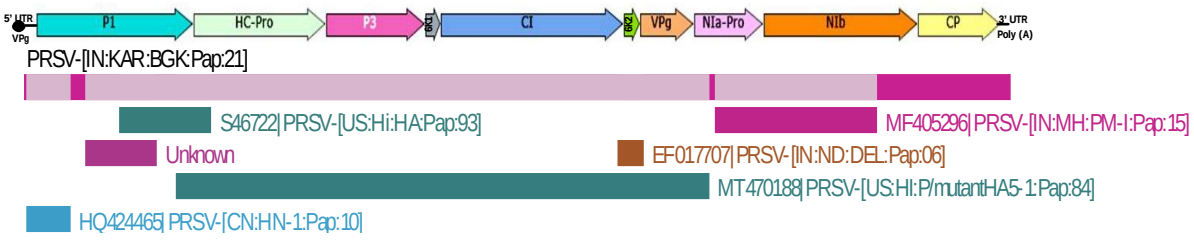

Fig. 5: Recombination analysis of breakpoint and its putative parental sequences of isolated PRSV-BGK isolate

[The recombinant fragments are shown as shaded bars with the origin (parental virus species)]

Table 22. Recombination analysis of breakpoint and its putative parental sequences of PRSV-BGK isolate

Virus		Break point (nt)		Recombination parents		p-value					
	Events	Begin	End	Major parent	Minor parent	RDP	Geneconv	MaxChi	Chimera	SiScan	3Seq
PRSV-[IN:KAR:BGK:Pap:21]	1	14	512	PRSV-[AU:53C:Pap:15] KX655866	PRSV-[CN:HN-1:Pap:10] HQ424465	$1.155X10^{-02}$	-*	$5.468 X 10^{-03}$	$1.608 X 10^{-04}$	$2.596 X 10^{-14}$	-
	2	642	1429	PRSV-[TW:Ping-tong:Pap:05] DQ340770	PRSV-[PNG:8A:Pap:16] MH404259	$2.669 X10^{-03}$	-	$4.965 X 10^{-04}$	$3.800 X 10^{-04}$	$2.201 X 10^{-08}$	$2.487 X10^{-04}$
	3	1021	1990	PRSV-[TW:prT3-AX-N-Ph:Pap:12] JX448370	PRSV-[US:Hi:HA:Pap:93] S46722	$3.358 X10^{-06}$	-	$1.629 X 10^{-05}$	$1.919 X 10^{-04}$	$3.661 X 10^{-16}$	$3.084 X 10^{-09}$
	4	1604	7290	PRSV-[TH:Pap:00] AY010722	PRSV-[US:HI:P/mutantHA5-1:Pap:84] MT470188	$3.589 X 10^{-04}$	-	$1.178 X 10^{-05}$	$7.676 X10^{-05}$	$2.026 X 10^{-18}$	$4.286 X 10^{-07}$
	5	6306	6578	PRSV-[IN:TS:Hyd:HYD:Pap:15] KP743981	PRSV-[IN:ND:DEL:Pap:06] EF017707	-	$1.241 X 10^{-09}$	-	-	-	$2.236 X 10^{-06}$
	6	7332	9066	PRSV-[IN:TS:Hyd:HYD:Pap:15] KP743981	PRSV-[IN:MH:PM-I:Pap:15] MF405296	$2.669 X 10^{-03}$	-	$4.965 X 1^{0-04}$	$3.800 X 10^{-04}$	$2.201 X 10^{-08}$	$2.487 X 10^{-04}$

*Non significant value

upon statistical likelihood (p-values) of strand swapping among parental genotypes (irrespective of inter and intragenic exchange or number of crossovers/ recombinational breakpoints) using RDP4 package. The recombination analysis provided evidence for the presence of past six recombination events in PRSV-[IN:Kar:Bgk:Pap:21] isolate at different location of the genome (Fig 5).

The 1st recombination event recorded between 14th nt to 512th nt position (between 3'UTR and P1 region) with major parent PRSV-[AU:53C:Pap:15] (KX655866) and minor parent PRSV-[CN:HN-1:Pap:10] (HQ424465), 2nd event recorded at 642nd nt to 1,429th nt position (P1 region) having major parent PRSV-[TW:Ping-tong:Pap:05] (DQ340770) and minor parent PRSV-[PNG:8A:Pap:16] (MH404259), 3rd event recorded exactly between at 1,021st nt to 1,990th nt position (between P1 and HC-Pro region) with major parent PRSV-[TW:prT3-AX-N-Ph:Pap:12] (JX448370) and minor parent PRSV-[US:Hi:HA:Pap:93] (S46722), 4th event recorded was the longest recombination event between 1,604th nt to 7,290th nt position (from P1 to NIa-Pro regions) with major parent PRSV-[TH:Pap:00] (AY010722) and minor parent PRSV-[US:HI:P/mutantHA5-1:Pap:84] (MT470188), 5th event recorded at 6,306th nt to 6,578th nt position (between 6K2 and VPg region) with major parent PRSV-[IN:TS:Hyd:HYD:Pap:15] (KP743981) and minor parent PRSV-[IN:ND:DEL:Pap:06] (EF017707) and finally 6th recombination event recorded at 7,332nd nt to 9,066th nt position (between NIa-Pro and NIb region) with major parent PRSV-[IN:TS:Hyd:HYD:Pap:15] (KP743981) and minor parent PRSV-[IN:MH:PM-I:Pap:15] (MF405296).

4.1.3.13 Finding genes/ORFs of begomoviruses isolated from papaya

The software package of NCBI "ORF finder" at www.ncbi.nlm.nih.gov/ was used to detect the genes/ORFs present on the DNA-A component of 13 begomovirus isolates (Table 10). In three different frames the location of the different genes/ORFs of the genome were presented (+ ve and -ve strands). Two genes *i.e.* coat protein (AV1) and pre coat protein (AV2) were found on the virion (+ve) strand and five [Rep (AC1), TrAP (AC2), REn (AC3), AC4 and AC5 (only in PaLCuV-3, PaLCuV-11, PaLCuV-13)], were found on the complementary (-ve) strand of DNA-A.

4.1.3.14 Genome organization of full length DNA-A component of begomoviruses isolated from papaya

The 13 begomoviruses isolated from papaya are monopartite viruses which was confirmed through the amplification of DNA-A genomes. The genome of all isolates contain intergenic region which has stem loop structure of nonanucleotide sequence "TAATATTAC" which is non coding sequence present in all the begomoviruses which is also called as common region that is highly conserved. The genome contains six to seven genes/ORFs *viz.*, AV1 (known as (CP) coat protein), AV2 (known as pre coat protein), AC1 (known as replication initiator (Rep) protein), AC2 known as transcriptional activator protein (TrAP) and AC3 replication enhancer (REn) protein. AC4 and AC5 (in few viruses) have not yet been named. Detail genome organization is given in table 23 and fig. 6.

The PaLCuV-1 isolate consisted of 2,763 nt, its genome starts with AV2 gene at start codon of 145th nt and stops at 491th nt position which resides on viron (+ve) stand that runs in clockwise direction having the length of 348 nt coding for 115 aa. AV1 gene resides on virion (+ve) sense strand in clockwise direction with start codon at 304th nt and stop codon at 1074th nt position having the length of 771 nt coding for 256 aa. AC1gene resides on the complementary (-ve) sense strand that runs anticlockwise having the length of 1,086 nt with 361 aa which is larger among all genes and it has start codon at 2611th nt and stop codon at 1526th nucleotide. The ORF AC2 having length of 402 nt, start codon at 1623th nt and stop codon at 1233th nt codes for 133 aa that runs in anticlockwise direction. The ORF AC3 is located on the complementary (-ve) sense strand located anticlockwise with start codon at 1484th nt and stop codon at 1077th nt position having length of 408 nt which codes for 135 aa. ORF AC4 starts at 2454th nt and stops at 2161st nt position, which is found on the complementary (-ve) strand that runs in anticlockwise direction, having length of 294 nt which code for 97 aa. ORF AC5 is found on complementary (-ve) strand in anticlockwise direction with a start codon at 612th nt and stop codon at 295th nt position with length of 318 nt which codes for 105 aa.

The PaLCuV-2 isolate consisted of 2,760 nt, its genome starts with AV2 gene at start codon of 147th nt and stops at 503th nt position which resides on viron (+ve) stand that runs in clockwise direction having the length of 357 nt coding for 118 aa.

Table 23: Genome organization of DNA-A component of begomoviruses isolated from papaya

ORFs		PaLCuV-1	PaLCuV - 2	PaLCuV - 3	PaLCuV - 4	PaLCuV - 5	PaLCuV - 6	PaLCuV - 7	PaLCuV - 8	PaLCuV - 9	PaLCuV - 10	PaLCuV - 11	PaLCuV - 12	PaLCuV - 13
Nucleotide length (bp)		2763	2760	2757	2773	2763	2762	2765	2763	2763	2773	2750	2772	2737
AV2[b]	SSC*	144-491	147-503	145-501	146-502	147-503	146-502	146-502	147-503	147-503	146-502	146-502	147-502	120-458
	PO*	348	357	357	357	357	357	357	357	357	357	357	357	339
	PP*	115	118	118	118	118	118	118	118	118	118	118	118	112
AV1(CP)[b]	SSC	304-1074	307-1077	305-1075	306-1076	307-1077	306-1076	306-1076	307-1077	307-1077	306-1076	306-1076	306-1076	280-1050
	PO	771	771	771	771	771	771	771	771	771	771	771	771	771
	PP	256	256	256	256	256	256	256	256	256	256	256	256	256
AC1(Rep)[b]	SSC	2611-1526	2608-1526	2615-1530	2616-1525	2611-1526	2610-1523	2613-1525	2611-1526	2611-1526	2616-1531	2616-1531	2613-1525	2584-1499
	PO	1086	1083	1086	1092	1086	1086	1089	1086	1086	1086	1086	1089	1086
	PP	361	360	361	363	361	361	362	361	361	361	361	362	361
AC2 (TrAP)[b]	SSC	1623-1222	1623-1219	1627-1223	1628-1224	1623-1219	1622-1218	1622-1218	1623-1219	1623-1219	1628-1224	1628-1224	1628-1224	1596-1192
	PO	402	405	405	405	405	405	405	405	405	405	405	405	405
	PP	133	134	134	134	134	134	134	134	134	134	134	134	134
AC3 (REn)[b]	SSC	1484-1077	1478-1074	1482-1078	1483-1079	1478-1074	1477-1073	1477-1073	1478-1056	1478-1074	1483-1079	1483-1079	1483-1079	1457-1047
	PO	408	405	405	405	405	405	405	423	405	405	405	405	411
	PP	135	134	134	134	134	134	134	140	134	134	134	134	136
AC4[b]	SSC	2454-2161	2457-2158	2458-2120	2459-2202	2454-2161	2459-2160	2462-2163	2460-2161	2460-2161	2459-2202	2459-2202	2459-2202	2433-2251
	PO	294	300	339	258	294	300	300	300	300	258	258	258	183
	PP	97	99	112	85	97	99	99	99	99	85	85	85	60
AC5a[b]	SSC	612-295		1144-740								1145-741		791-531
	PO	318		405								405		261
	PP	105		134								134		86
AC5b[b]	SSC			613-296								614-291		
	PO			318								324		
	PP			105								107		

***** bp: Base pairSSC: Start-Stop codon (nt), PO: Predicted size of ORFs (nt), PP: Predicted size of protein (no. of amino acids)

[a]Open reading frames, [b]Genes are indicated as AV1: Coat protein (CP), AV2: Pre coat protein, AC1:Replication-associated protein (Rep), AC2: Transcriptional activator protein (TrAP) and AC3: Replication enhancer (REn). The products encoded by ORFs AC4 and AC5 have yet to be named.

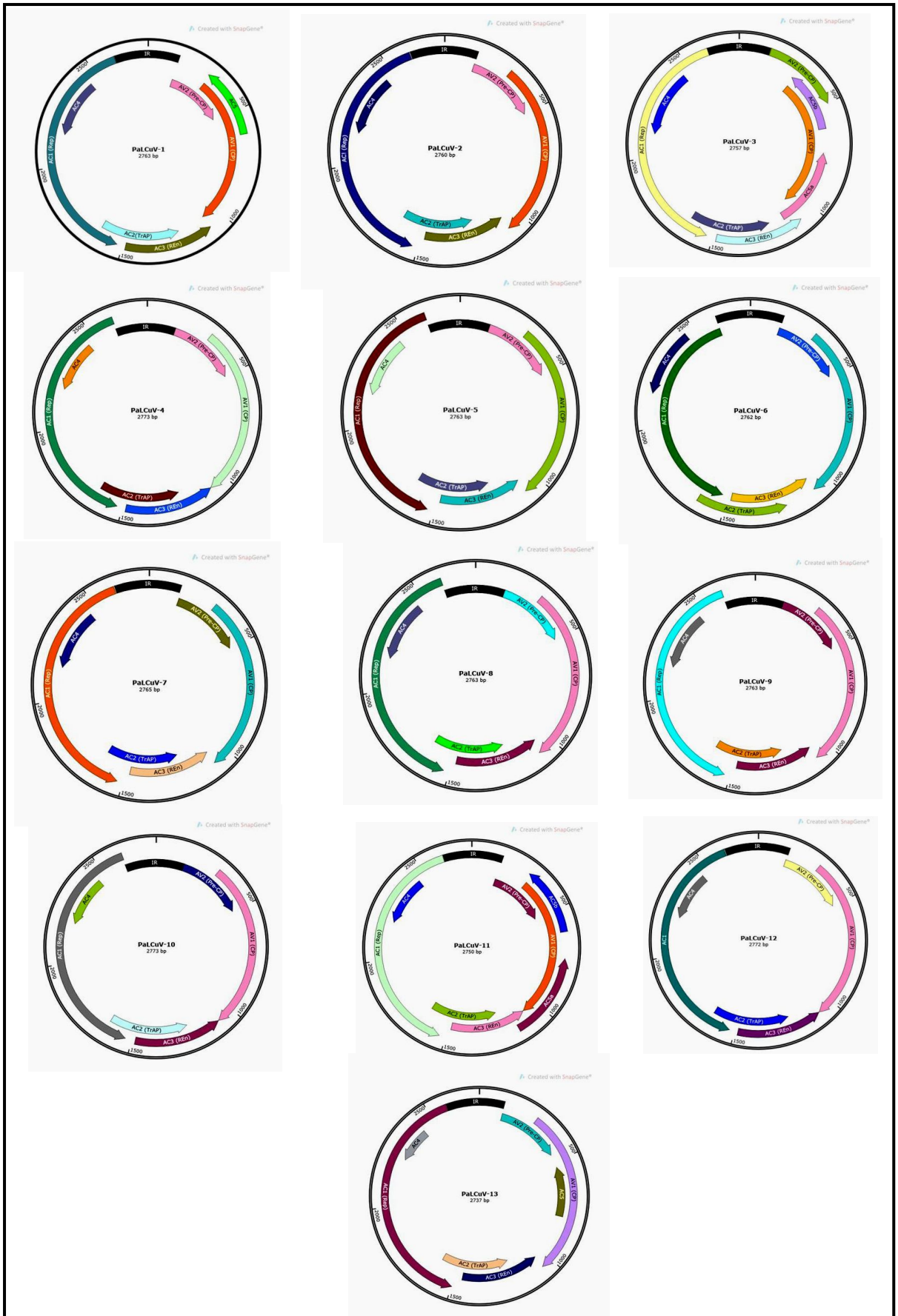

Fig. 6: Genome organization of DNA-A component of begomoviruses isolated from papaya

AV1 gene resides on a virion (+ve) sense strand in clockwise direction with start codon at 307th nt and stop codon at 1077th nt position having the length of 771 nt coding for 256 aa. AC1 gene resides on the complementary (-ve) sense strand that runs anticlockwise having the length of 1083 nt with 360 aa, which is larger among all genes and it has start codon at 2608th nt and stop codon at 1526th nucleotide. The ORF AC2 having length of 405 nt, start codon at 1623th nt and stop codon at 1219th nt codes for 134 aa that runs in anticlockwise direction. The ORF AC3 is located on the complementary (-ve) sense strand located anticlockwise with start codon at 1478th nt and stop codon at 1047th nt position having length of 405 nt which codes for 134 aa. ORF AC4 starts at 2457th nt and stops at 2158st nt position, which is found on the complementary (-ve) strand that runs in anticlockwise direction, having length of 300 nt which code for 99 aa.

The PaLCuV-3 isolate consisted of 2,757 nt, its genome starts with AV2 gene at start codon of 145th nt and stops at 501th nt position which resides on viron (+ve) stand that runs in clockwise direction having the length of 357 nt coding for 118 aa. AV1 gene resides on a virion (+ve) sense strand in clockwise direction with start codon at 305th nt and stop codon at 1075th nt position having the length of 771 nt coding for 256 aa. AC1gene resides on the complementary (-ve) sense strand that runs anticlockwise having the length of 1086 nt with 361 aa which is larger among all genes and it has start codon at 2615th nt and stop codon at 1530th nucleotide. The ORF AC2 having length of 405 nt, start codon at 1627th nt and stop codon at 1223th nt codes for 134 aa that runs in anticlockwise in direction. The ORF AC3 is located on the complementary (-ve) sense strand located anticlockwise with start codon at 1482th nt and stop codon at 1078th nt position having length of 405 nt which codes for 134 aa. ORF AC4 starts at 2458th nt and stop at 2120th nt position, which is found on the complementary (-ve) strand that runs in anticlockwise direction, having length of 339 which code for 112 aa. ORF AC5a is found on complementary (-ve) strand in anticlockwise direction with a start codon at 1144th nt and stop codon at 740th nt position with length of 405 nt which codes for 134 aa. ORF AC5b is found on complementary (-ve) strand in anticlockwise direction with a start codon at 613th nt and stop codon at 296th nt position with length of 318 nt which codes for 105 aa.

The PaLCuV-4 isolate consisted of 2,773 nt, its genome starts with AV2 gene at start codon of 146th nt and stops at 502th nt position which resides on viron (+ve) stand that runs in clockwise direction having the length of 357 nt coding for 118 aa. AV1 gene resides on a virion (+ve) sense strand in clockwise direction with start codon at 306th nt and stop codon at 1076th nt position having the length of 771 nt coding for 256 aa. AC1gene resides on the complementary (-ve sense strand that runs anticlockwise having the length of 1092 nt with 363 aa which is larger among all genes and it has start codon at 2616th nt and stop codon at 1525th nucleotide. The ORF AC2 having length of 405 nt it is start codon at 1628th and stop codon at 1224th nt which codes for 134 aa that runs in anticlockwise in direction. The ORF AC3 is located on the complementary (-ve) sense strand located anticlockwise with start codon at 1483th nt and stop codon at 1079th nt position having length of 405 nt which codes for 134 aa. ORF AC4 starts at 2459th nt and stop at 2202nd nt position, which is found on the complementary (-ve) strand that runs in anticlockwise direction, having length of 258 nt which code for 85 aa.

The PaLCuV-5 isolate consisted of 2,763 nt, its genome starts with AV2 gene at start codon of 147th nt and stops at 503th nt position which resides on viron (+ve) stand that runs in clockwise direction having the length of 357nt coding for 118 aa. AV1 gene resides on a virion (+ve) sense strand in clockwise direction with start codon at 307th nt and stop codon at 1077th nt position having the length of 771 nt coding for 256 aa. AC1 gene resides on the complementary (-ve) sense strand that runs anticlockwise having the length of 1086 nt with 361 aa which is larger among all genes and it has start codon at 2611th nt and stop codon at 1526th nucleotide. The ORF AC2 having length of 405 nt it is having start codon at 1623th nt and stop codon at 1219th nt which codes for 134 aa that runs in anticlockwise in direction. The ORF AC3 is located on the complementary (-ve) sense strand located anticlockwise with start codon at 1478th nt and stop codon at 1074th nt position having length of 405 nt which codes for 134 aa. ORF AC4 starts at 2454th nt and stop at 2161st nt position, which is found on the complementary (-ve) strand that runs in anticlockwise direction, having length of 294 nt which code for 97 aa.

The PaLCuV-6 isolate consisted of 2,762 nt, its genome starts with AV2 gene at start codon of 146th nt and stops at 502th nt position which resides on viron (+ve)

stand that runs in clockwise direction having the length of 357 nt coding for 118 aa. AV1 gene resides on a virion (+ve) sense strand in clockwise direction with start codon at 306th nt and stop codon at 1076th nt position having the length of 771 nt coding for 256 aa. AC1 gene reside on the complementary (-ve) sense strand that runs anticlockwise having the length of 1086 nt with 361 aa which is larger among all genes and it has start codon at 2610th nt and stop codon at 1523th nucleotide. The ORF AC2 having length of 405 nt it is having start codon at 1622th nt and stop codon at 1218th nt which codes for 134 aa that runs in anticlockwise in direction. The ORF AC3 is located on the complementary (-ve) sense strand located anticlockwise with start codon at 1477th and stop codon at 1073th nt position having length of 405 nt which codes for 134 aa. ORF AC4 starts at 2459th nt and stop at 2160th nt position, which is found on the complementary (-ve) strand that runs in anticlockwise direction, having length of 300 nt which code for 99 aa.

The PaLCuV-7 isolate consisted of 2,765 nt, its genome starts with AV2 gene at start codon of 146th nt and stops at 502th nt position which resides on viron (+ve) stand that runs in clockwise direction having the length of 357 nt coding for 118 aa. AV1 gene resides on a virion (+ve) sense strand in clockwise direction with start codon at 306th nt and stop codon at 1076th nt position having the length of 771 nt coding for 256 aa. AC1 gene resideon the complementary (-ve) sense strand that runs anticlockwise having the length of 1089 nt with 362 aa which is larger among all genes and it has start codon at 2613th nt and stop codon at 1525th nucleotide. The ORF AC2 having length of 405 nt it is having start codon at 1622th nt and stop codon at 1218th nt which codes for 134 aa that runs in anticlockwise in direction. The ORF AC3 is located on the complementary (-ve) sense strand located anticlockwise with start codon at 1477th and stop codon at 1073th nt position having length of 405 nt which codes for 134 aa. ORF AC4 starts at 2462th nt and stop at 2163th nt position, which is found on the complementary (-ve) strand which runs in anticlockwise direction, having length of 300 nt which code for 99 aa.

The PaLCuV-8 isolate consisted of 2,763 nt, its genome starts with AV2 gene at start codon of 147th nt and stops at 503th nt position which resides on viron (+ve) stand that runs in clockwise direction having the length of 357 nt coding for 118 aa. AV1 gene resides on a virion (+ve) sense strand in clockwise direction with start

codon at 307th nt and stop codon at 1077th nt position having the length of 771 nt coding for 256 aa. AC1gene residing on the complementary (-ve) sense strand that runs anticlockwise having the length of 1086 nt with 361 aa which is larger among all genes and it has start codon at 2611th nt and stop codon at 1526th nucleotide. The ORF AC2 having length of 405 nt it is having start codon at 1623th nt and stop codon at 1219th nt which codes for 134 aa that runs in anticlockwise in direction. The ORF AC3 is located on the complementary (-ve) sense strand located anticlockwise with start codon at 1478th nt and stop codon at 1056th nt position having length of 405 nt which codes for 140 aa. ORF AC4 starts at 2460th nt and stop at 2161st nt position, which is found on the complementary (-ve) strand that runs in anticlockwise direction, having length of 300 nt which code for 99 aa.

The PaLCuV-9 isolate consisted of 2,763 nt, its genome starts with AV2 gene at start codon of 147th nt and stops at 503th nt position which resides on viron (+ve) stand that runs in clockwise direction having the length of 357 nt coding for 118 aa. AV1 gene resides on a virion (+ve) sense strand in clockwise direction with start codon at 307th nt and stop codon at 1077th nt position having the length of 771 nt coding for 256 aa. AC1 gene reside on the complementary (-ve) sense strand that rums anticlockwise having the length of 1086 nt with 361 aa which is larger among all genes and it has start codon at 2611th nt and stop codon at 1526th nucleotide. The ORF AC2 having length of 405 nt it is having start codon at 1623th nt and stop codon at 1219th nt which codes for 134 aa that runs in anticlockwise in direction. The ORF AC3 is located on the complementary (-ve) sense strand located anticlockwise with start codon at 1478th and stop codon at 1074th nt position having length of 405 nt which codes for 134 aa. ORF AC4 starts at 2460th nt and stop at 2161st nt position, which is found on the complementary (-ve) strand that runs in anticlockwise direction, having length of 300 nt which code for 99 aa.

The PaLCuV-10 isolate consisted of 2,773 nt, its genome starts with AV2 gene at start codon of 146th nt and stops at 502th nt position which residing on viron (+ve) stand that runs in clockwise direction having the length of 357 nt coding for 118 aa. AV1 gene resides on a virion (+ve) sense strand in clockwise direction with start codon at 306th nt and stop codon at 1076th nt position having the length of 771 nt coding for 256 aa. AC1gene residing on the complementary (-ve) sense strand that

runs anticlockwise having the length of 1086 nt with 361 aa which is larger among all genes and it has start codon at 2616th nt and stop codon at 1531th nucleotide. The ORF AC2 having length of 405 nt, it is having start codon at 1628th nt and stop codon at 1224th nt which codes for 134 aa that runs in anticlockwise in direction. The ORF AC3 is located on the complementary (-ve) sense strand located anticlockwise with start codon at 1483th nt and stop codon at 1079th nt position having length of 405 nt which codes for 134 aa. ORF AC4 starts at 2459th nt and stop at 2202nd nt position, which is found on the complementary (-ve) strand which runs in anticlockwise direction, having length of 258 nt which code for 85 aa.

The PaLCuV-11 isolate consisted of 2,750 nt, its genome starts with AV2 gene at start codon of 146th nt and stops at 502th nt position which resides on viron (+ve) stand that runs in clockwise direction having the length of 357 nt coding for 118 aa. AV1 gene resides on a virion (+ve) sense strand in clockwise direction with start codon at 306th nt and stop codon at 1076th nt position having the length of 771 nt coding for 256 aa. AC1 gene reside on the complementary (-ve) sense strand that runs anticlockwise having the length of 1086 nt with 361 aa which is larger among all genes and it has start codon at 2616th nt and stop codon at 1531th nucleotide. The ORF AC2 having length of 405 nt it is having start codon at 1628th nt and stop codon at 1224th nt which codes for 134 aa that runs in anticlockwise in direction. The ORF AC3 is located on the complementary (-ve) sense strand located anticlockwise with start codon at 1483th and stop codon at 1079th nt position having length of 405 nt which codes for 134 aa. ORF AC4 starts at 2459th nt and stop at 2202st nt position, which is found on the complementary (-ve) strand which runs in anticlockwise direction, having length of 258 nt which code for 85 aa. ORF AC5a is found on complementary (-ve) strand in anticlockwise direction with a start codon at 1145th nt and stop codon at 741th nt position with length of 405 nt which codes for 134 aa. ORF AC5b is found on complementary (-ve) strand in anticlockwise direction with a start codon at 614th nt and stop codon at 291th nt position with length of 324 nt which codes for 107 aa.

The PaLCuV-12 isolate consisted of 2,772 nt, its genome starts with AV2 gene at start codon of 147th nt and stops at 502th nt position which resides on viron (+ve) stand that runs in clockwise direction having the length of 357 nt coding for 118

aa. AV1 gene resides on a virion (+ve) sense strand in clockwise direction with start codon at 306th nt and stop codon at 1076th nt position having the length of 771 nt coding for 256 aa. AC1 gene reside on the complementary (-ve) sense strand that runs anticlockwise having the length of 1089 nt with 362 aa which is larger among all genes and it has start codon at 2613th nt and stop codon at 1525th nucleotide. The ORF AC2 having length of 405 nt it is having start codon at 1628th nt and stop codon at 1224th nt which codes for 134 aa that runs in anticlockwise in direction. The ORF AC3 is located on the complementary (-ve) sense strand located anticlockwise with start codon at 1483th and stop codon at 1079th nt position having length of 405 nt which codes for 134 aa. ORF AC4 starts at 2459th nt and stop at 2202nd nt position, which is found on the complementary (-ve) strand which runs in anticlockwise direction, having length of 258 nt which code for 85 aa.

The PaLCuV-13 isolate consisted of 2,737 nt, its genome starts with AV2 gene at start codon of 120th nt and stops at 458th nt position which resides on viron (+ve) stand that runs in clockwise direction having the length of 339 nt coding for 112 aa. AV1 gene resides on a virion (+ve) sense strand in clockwise direction with start codon at 280th nt and stop codon at 1050th nt position having the length of 771 nt coding for 257 aa. AC1 gene reside on the complementary (-ve) sense strand that runs anticlockwise having the length of 1086 nt with 361 aa which is larger among all genes and it has start codon at 2584th nt and stop codon at 1499th nucleotide. The ORF AC2 having length of 405 nt it is having start codon at 1596th and stop codon at 1192th nt which codes for 134 aa that runs in anticlockwise in direction. The ORF AC3 is located on the complementary (-ve) sense strand located anticlockwise with start codon at 1457th and stop codon at 1047th nt position having length of 411 nt which codes for 136 aa. ORF AC4 starts at 2433th nt and stop at 2251st nt position, which is found on the complementary (-ve) strand which runs in anticlockwise direction, having length of 183 nt which code for 60 aa. ORF AC5 is found on complementary (-ve) strand in anticlockwise direction with a start codon at 791th nt and stop codon at 531th nt position with length of 261 nt which codes for 86 aa. Full length genome sequence of all the isolates (PaLCuV 1 to PaLCuV 13) is given in appendix III-B to N.

4.1.3.15 Comparison of complete nucleotide sequence of DNA-A component of isolated begomoviruses with other begomoviruses

Comparison done for the nucleotide sequence of full length DNA-A component of 13 begomoviruses isolated from papaya with other homologous begomovirus sequences given in table 12 and per cent nucleotide sequence identity is given in appendix IV-A.

The nucleotide sequence compression revealed that the PaLCuV-1 isolate showed high nucleotide sequence identity range of 69.6-88.6 per cent with *Tomato leaf curl virus*. Next close association was with *Papaya leaf curl virus* (71.5-87.1%) and *Croton yellow vein mosaic virus* (80.5-85.7 %). The lowest nucleotide sequence identity was found with *Mungbean yellow mosaic India virus* (62.2 %), followed by *Okra enation leaf curl virus* (70.7%) (Table 24). Further, PaLCuV-1 isolate has recorded maximum per cent nucleotide sequence identity of 88.6 % with ToLCKV-[IN:GJ:AHM:Tom:16] (MH5770301) (Table 25) which is *Tomato leaf curl Karnataka virus* infecting tomato in Gujarat, India. This result along with SDT color code matrix (Fig. 7) indicated that PaLCuV-1 isolate is different species of ToLCKV, based on the present ICTV begomovirus species demarcation criterion of < 91.0 per cent nucleotide sequence identity for demarcating the virus as different species. Based on sampling location and host the PaLCuV-1 isolate has been given a descriptor as *Papaya leaf curl Bagalkote virus*-[India:Karnataka:Belagavi:Papaya:2021] Gokak. It is designated as PaLCuBKV-[IN:Kar:Bel:Pap:21].

The PaLCuV-2 isolate showed high nucleotide sequence identity range of 73.8-96.3 per cent with *Chilli leaf curl virus*, followed by *Papaya leaf curl virus* (72.9-95.3 %) and *Tomato leaf curl virus* (71.6-84.2 %). The lowest nucleotide sequence identity was found with *Mungbean yellow mosaic India* virus (61.6 %), followed by *Okra enation leaf curl virus* (70.8 %) (Table 24). Further, PaLCuV-2 isolate has recorded maximum per cent nucleotide sequence identity of 96.3% with ChiLCV-DU[IN:ND:DU:Pap:09] (HM140364) (Table 25) which is *Chilli leaf curl virus* infecting papaya in New Delhi, India. This result along with SDT color code matrix (Fig. 7) indicated that PaLCuV-2 isolate is a variant of ChiLCV. Based on present ICTV (2021) begomovirus species demarcation criterion of 94.0 to 100 per cent

Table 24: Per cent sequence identity range of DNA-A component between begomoviruses isolated from papaya with other selected isolates of begomoviruses

Sl. No.	Begomovirus	Per cent sequence identity range												
		PaLCuV - 1	**PaLCuV - 2**	**PaLCuV - 3**	**PaLCuV - 4**	**PaLCuV - 5**	**PaLCuV - 6**	**PaLCuV - 7**	**PaLCuV - 8**	**PaLCuV - 9**	**PaLCuV - 10**	**PaLCuV - 11**	**PaLCuV - 12**	**PaLCuV - 13**
1.	AEV [1][a]	78.6	79.6	78.1	81.0	79.4	80.9	80.9	80.7	79.2	81.0	76.8	78.4	72.8
2.	AYVY [1][a]	72.6	73.6	72.3	74.1	73.8	75.0	75.2	75.0	74.2	73.7	69.9	72.1	70.1
3.	BYVBV [1][a]	84.2	78.5	85.0	88.6	78.5	80.0	80.1	79.6	81.7	87.9	81.9	84.3	72.2
4.	CaYMV [1][a]	72.1	74.9	72.7	73.4	74.9	76.3	76.5	76.3	74.1	76.4	78.5	76.1	69.2
5.	ChaYMV [1][a]	66.4	68.1	66.1	68.5	68.2	69.3	69.5	69.6	69.3	69.1	65.9	67.5	66.2
6.	ChiLCV [13][a]	72.1-78.5	73.8-96.3	72.6-78.6	73.3-80.9	73.7-96.5	75.1-98.5	75.2-98.5	74.9-98	73.4-93.5	75.6-78.8	74.6-79.7	74.6-76.8	68.5-80.8
7.	CLCuV [1][a]	74.7	75.6	74.6	76.8	75.7	77.2	77.2	77.1	74.9	75.8	72.2	74.0	71.9
8.	CYMV [4][a]	80.5-85.7	73.4-75.9	81.2-84.8	81.1-85.2	73.5-76.4	74.7-77.6	74.9-77.7	74.9-77.4	77.7-80.9	84.8-89.3	87.7-93.5	84.4-88.8	67.5-70.0
9.	DLCV [1][a]	71.9	73.5	71.9	72.7	73.5	74.6	74.6	74.9	72.9	75.0	75.3	73.7	69.8
10.	MaLCuV [1][a]	74.6	73.5	74.9	76.2	73.6	74.9	75.0	74.8	76.5	76.0	73.3	74.3	707.0
11.	MYMIV [1][a]	62.2	61.6	62.2	63.3	61.7	62.4	62.5	62.2	61.8	61.0	58.4	59.9	59.6
12.	OELCuv [1][a]	70.7	70.8	70.2	72.3	70.9	72.3	72.5	72.2	71.1	71.6	68.3	70.7	68.7
13.	PaLCrV [15][a]	70.0-72.7	72.8-75.5	70.0-72.5	72.6-74.8	75.0-75.4	74.0-76.8	74.2-76.9	74.2-76.9	72.5-75	72.4-74.6	68.2-70.1	70.8-72.7	72.3-74.5
14.	PaLCuV [15][a]	71.5-87.1	72.9-95.3	70.7-85.4	72.6-87.9	73.2-94.1	74.5-95.9	74.4-96.0	74.5-96.7	73.6-92.0	72.9-92.4	69.3-91.9	79.0-93.0	67.9-79.9
15.	PeLCV [1][a]	75.3	77.9	75.2	76.5	77.7	79.4	79.4	79.3	77.3	76.7	74.0	74.5	71.2
16.	ToLCV [8][a]	69.6-88.6	71.6-84.2	69.0-84.7	70.9-86.6	71.3-84.1	72.7-86.0	72.9-86.0	72.8-85.7	71.1-83.9	70.1-84.9	67.0-82.1	69.5-84.2	69.7-87.2

aNumber of sequences from the databases used in the comparisons

AEV: *Ageratum enation virus*, AYVY: *Ageratum yellow vein virus*, BYVBV: *Bhendi yellow vein Bhubhaneswar virus*, CaYMV: *Catharanthus yellow mosaic virus*, ChaYMV: *Chayote yellow mosaic virus*, ChiLCV: *Chilli leaf curl virus*, CLCuV: *Cotton leaf curl*, CYMV: *Croton yellow vein mosaic virus*, DLCV: *Duranta leaf curl virus*, MaLCuV: *Malvastrum leaf curl virus*, MYMIV: *Mungbean yellow mosaic India virus*, OELCuV: *Okra enation leaf curl virus*, PaLCrV: *Papaya leaf crumple virus*, PaLCuV: *Papaya leaf curl virus*, PeLCV: *Pedilanthus leaf curl virus*, ToLCV: *Tomato leaf curl virus*

Table 25: Maximum percentage nucleotide sequence identity of DNA-A component of begomoviruses isolated from papaya with other selected isolates of begomoviruses and acronyms assigned for the isolates

Sl. No.	Begomovirus Isolate	Maximum NSI (%)	Begomovirus	Name assigned based on NSI	Virus acronyms
1.	PaLCuV -1	88.6	ToLCKV-[IN:GJ:AHM:Tom:16]-MH5770301	*Papaya leaf curl Bagalkote virus*-[India:Karnataka:Belagavi:Papaya:2021]	PaLCuBKV-[IN:Kar:Bel:Pap:21]
2.	PaLCuV -2	96.3	ChiLCV-DU[IN:ND:DU:Pap:09]-HM140364	*Chilli leaf curl virus*-[India:Karnataka:Belagavi:Papaya:2021]	ChiLCV-[IN:Kar:Bel:Pap:21]
3.	PaLCuV -3	85.4	PaLCuV-[IN:Kar:Madi:Cal:18]-MK087120	*Papaya leaf curl Bagalkote virus*-[India:Karnataka:Bagalkote:Papaya:2021]	PaLCuBKV-[IN:Kar:Bgk:Pap:21]
4.	PaLCuV -4	88.6	BYVBV-[IN:OD:Bhu:Okra:03]-FJ589571	*Papaya leaf curl Bagalkote virus* -[India:Karnataka:Bagalkote:Papaya:2021]	PaLCuBKV-[IN:Kar:Bgk:Pap:21]
5.	PaLCuV -5	96.5	ChiLCV-[IN:Kar:Rai:Chi:17]-MK161454	*Chilli leaf curl virus*-[India:Karnataka:Belagavi:Papaya:2021]	ChiLCV-[IN:Kar:Bel:Pap:21]
6.	PaLCuV -6	98.5	ChiLCV-[IN:Kar:Rai:Chi:17]-MK161454	*Chilli leaf curl virus*-[India:Karnataka:Bagalkote:Papaya:2021]	ChiLCV-[IN:Kar:Bgk:Pap:21]
7.	PaLCuV -7	98.5	ChiLCV-[IN:Kar:Rai:Chi:17]-MK161454	*Chilli leaf curl virus*-[India:Karnataka:Bagalkote:Papaya:2021]	ChiLCV-[IN:Kar:Bgk:Pap:21]
8.	PaLCuV -8	98.0	ChiLCV-DU[IN:ND:DU:Pap:09]-HM140364	*Chilli leaf curl virus*-[India:Karnataka:Kalaburagi:Papaya:2021]	ChiLCV-[IN:Kar:Kal:Pap:21]
9.	PaLCuV-9	93.5	ChiLCV-DU[IN:ND:DU:Pap:09]-HM140364	*Chilli leaf curl virus*-[India:Karnataka:Kalaburagi:Papaya:2021]	ChiLCV-[IN:Kar:Kal:Pap:21]
10.	PaLCuV -10	92.4	PaLCuV-[IN:Kar:Madi:Cal:18]-MK087120	*Papaya leaf curl virus*-[India:Karnataka:Kalaburagi:Papaya:2021]	PaLCuV-**[**IN:Kar:Kal:Pap:21]
11.	PaLCuV -11	93.5	CYVMV-[IN:ND:Cro:08]-JN817516	*Croton yellow vein mosaic virus*-[India:Karnataka:Kalaburagi:Papaya:2021]	CYVMV-**[**IN:Kar:Kal:Pap:21],
12.	PaLCuV -12	93.0	PaLCuV-[IN:Kar:Madi:Cal:18]-MK087120	*Papaya leaf curl virus*-[India:Karnataka:Vijayapura:Papaya:2021]	PaLCuV-[IN:Kar:Vij:Pap:21]
13.	PaLCuV -13	87.2	ToLCV-[In:Kar:Ben:Chr:17]-MG758145	*Papaya leaf curl Haveri virus*-[India:Karnataka:Haveri:Papaya:2021]	PaLCuHV-[IN:Kar:Hav:Pap:21]

NSI: Nucleotide Sequence Identity

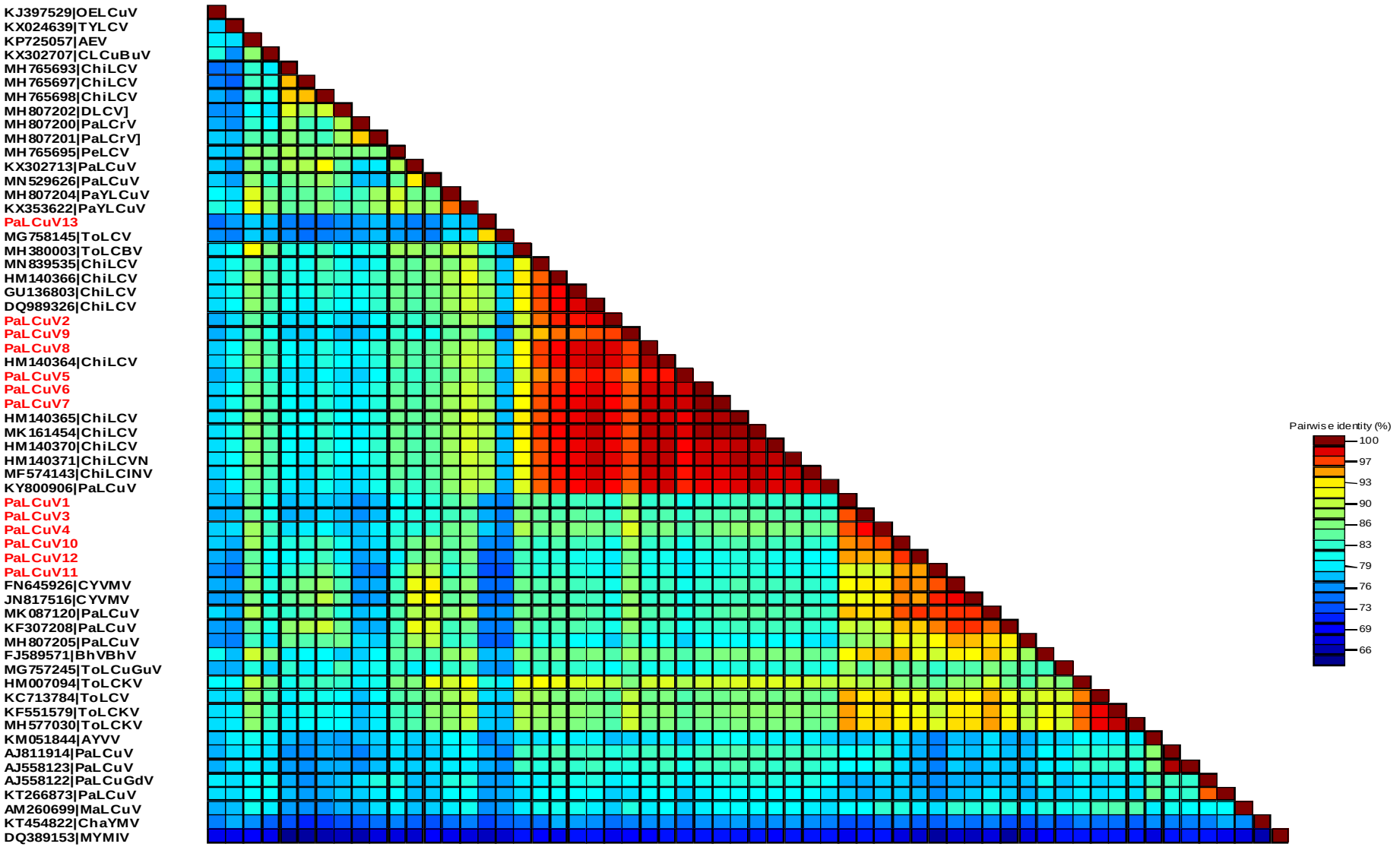

Fig. 7: Graphical representation of per cent pair wise genome scores and nucleotide identity plot of full length genome of DNA-A component of isolated begomoviruses (*Prepared using the SDTv1.2 (Species Demarcation Toll))

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nucleotide sequence identity is demarcates as ‘‘variants’’ and based on sampling location and host the PaLCuV-2 isolate has been given a descriptor as *Chilli leaf curl virus*-[India:Karnataka:Belagavi:Papaya:2021] Gokak variant. It is designated as ChiLCV-[IN:Kar:Bel:Pap:21].

The PaLCuV-3 isolate showed high nucleotide sequence identity range of 70.7-85.4 per cent with *Papaya leaf curl virus*, followed by *Croton yellow vein mosaic virus* (81.2-84.8 %) and *Tomato leaf curl virus* (71.6-84.2 %). The lowest nucleotide sequence identity was found with *Mungbean yellow mosaic India* virus (62.2 %), followed by *Okra enation leaf curl virus* (70.2 %) (Table 24). Further, PaLCuV-3 isolate has recorded maximum per cent nucleotide sequence identity of 85.4 per cent with PaLCuV-[IN:Kar:Madi:Cal:18] (MK087120) (Table 25) which is *Papaya yellow leaf curl virus* infecting calandula in Madikeri, Karnataka, India. This result along with SDT color code matrix (Fig. 7) indicated that PaLCuV-3 isolate is different species of PaYLCuV. Based on the ICTV (2021) begomovirus species demarcation criterion of < 91.0 per cent nucleotide sequence identity demarcates the virus as different species and based on sampling location and host the PaLCuV-3 isolate has been given a descriptor as *Papaya leaf curl Bagalkote virus*-[India:Karnataka:Bagalkote:Papaya:2021] Bagalkote. It is designated as PaLCuBKV-[IN:Kar:Bgk:Pap:21].

The PaLCuV-4 isolate showed high nucleotide sequence identity range of 88.6 per cent with *Bhendi yellow vein Bhubhaneswar virus*, followed by *Papaya leaf curl virus* (72.6-87.9 %) and *Tomato leaf curl virus* (70.9-86.6 %) (Table 24). The lowest nucleotide sequence identity was found with *Mungbean yellow mosaic India* virus (63.3 %), followed by *Okra enation leaf curl virus* (72.3 %). Further, PaLCuV-4 isolate has recorded maximum per cent nucleotide sequence identity of 85.4 per cent with BYVBV-[IN:OD:Bhu:Okra:03] (FJ589571) (Table 25) which is *Bhendi yellow vein Bhubhaneswar* virus a begomovirus infecting okra in Odisha, India. This result along with SDT color code matrix (Fig. 7) indicated that PaLCuV-4 isolate is different species of BYVBV and demarcates this virus as different species and based on sampling location and host the PaLCuV-4 isolate has been given a descriptor as *Papaya leaf curl Bagalkote virus*-[India:Karnataka:Bagalkote:Papaya:2021] Bilgi. It is designated as PaLCuBKV-[IN:Kar:Bgk:Pap:21].

The PaLCuV-5 isolate showed high nucleotide sequence identity range of 73.7-96.5 per cent with *Chilli leaf curl virus*, followed by *Papaya leaf curl virus* (73.2-94.1 %) and *Tomato leaf curl virus* (71.3-84.1 %) (Table 24). The lowest nucleotide sequence identity was found with *Mungbean yellow mosaic India* virus (61.7 %), followed by *Okra enation leaf curl virus* (70.9 %). Further, PaLCuV-5 isolate has recorded maximum per cent nucleotide sequence identity of 96.5 per cent with ChiLCV-[IN:Kar:Rai:Chi:17] (MK161454) (Table 25) which is *Chilli leaf curl virus* a begomovirus infecting to chilli in Raichur, Karnataka, India. This result along with SDT color code matrix (Fig. 7) indicated that PaLCuV-4 isolate is variant of ChiLCV and based on sampling location and host the PaLCuV-5 isolate has been given a descriptor as *Chilli leaf curl virus*-[India:Karnataka:Belagavi:Papaya:2021] Ramadurga variant. It is designated as ChiLCV-[IN:Kar:Bel:Pap:21].

The PaLCuV-6 isolate showed high nucleotide sequence identity range of 75.1-98.5 per cent with *Chilli leaf curl virus*, followed by *Papaya leaf curl virus* (74.5-95.9 %) and *Tomato leaf curl virus* (72.7-86.0 %). The lowest nucleotide sequence identity was found with *Mungbean yellow mosaic India* virus (62.4 %), followed by *Okra enation leaf curl virus* (72.3 %) (Table 24). Further, PaLCuV-6 isolate has recorded maximum per cent nucleotide sequence identity of 98.5 per cent with ChiLCV-[IN:Kar:Rai:Chi:17] (MK161454) (Table 25) which is *Chilli leaf curl virus* a begomovirus infecting to chilli from Raichur, Karnataka, India. This result along with SDT color code matrix (Fig. 7) indicated that PaLCuV-4 isolate is variant of ChiLCV and based on sampling location and host the PaLCuV-6 isolate has been given a descriptor as *Chilli leaf curl virus*-[India:Karnataka:Bagalkote:Papaya:2021] Hunugunda1 variant. It is designated as ChiLCV-[IN:Kar:Bgk:Pap:21].

The PaLCuV-7 isolate showed high nucleotide sequence identity range of 75.2-98.5 per cent with *Chilli leaf curl virus*, followed by *Papaya leaf curl virus* (74.4-96.0 %) and *Tomato leaf curl virus* (72.9-86.0 %). The lowest nucleotide sequence identity was found with *Mungbean yellow mosaic India* virus (62.5 %), followed by *Okra enation leaf curl virus* (72.5 %) (Table 24). Further, PaLCuV-7 isolate has recorded maximum per cent nucleotide sequence identity of 98.5 per cent with ChiLCV-[IN:Kar:Rai:Chi:17] (MK161454) (Table 25) which is *Chilli leaf curl virus* a begomovirus infecting to chilli from Raichur, Karnataka, India. This result

along with SDT color code matrix (Fig. 7) indicated that PaLCuV-4 isolate is variant of ChiLCV and based on sampling location and host the PaLCuV-7 isolate has been given a descriptor as *Chilli leaf curl virus*-[India:Karnataka:Bagalkote:Papaya:2021] Hunugunda2 variant . It is designated as ChiLCV-[IN:Kar:Bgk:Pap:21].

The PaLCuV-8 isolate showed high nucleotide sequence identity range of 74.9-98.0 per cent with *Chilli leaf curl virus,* followed by *Papaya leaf curl virus* (74.5-96.7 %) and *Tomato leaf curl virus* (72.8-85.7 %). The lowest nucleotide sequence identity was found with *Mungbean yellow mosaic India* virus (62.2 %), followed by *Okra enation leaf curl virus* (72.2 %) (Table 24). Further, PaLCuV-8 isolate has recorded maximum per cent nucleotide sequence identity of 98.0 per cent with ChiLCV-DU[IN:ND:DU:Pap:09] (HM140364) (Table 25) which is *Chilli leaf curl virus* a begomovirus infecting to chilli from New Delhi, India. This result along with SDT color code matrix (Fig. 7) indicated that PaLCuV-4 isolate is variant of ChiLCV and based on sampling location and host the PaLCuV-8 isolate has been given a descriptor as *Chilli leaf curl virus*-[India:Karnataka:Kalaburagi:Papaya:2021] Afjalpur variant. It is designated as ChiLCV-[IN:Kar:Kal:Pap:21].

The PaLCuV-9 isolate showed high nucleotide sequence identity range of 73.4-93.5 per cent with *Chilli leaf curl virus,* followed by *Papaya leaf curl virus* (73.6-92.0 %) and *Tomato leaf curl virus* (71.1-83.9 %). The lowest nucleotide sequence identity was found with *Mungbean yellow mosaic India* virus (61.8 %), followed by *Okra enation leaf curl virus* (71.1 %) (Table 24). Further, PaLCuV-9 isolate has recorded maximum per cent nucleotide sequence identity of 93.5 per cent with ChiLCV-DU[IN:ND:DU:Pap:09] (HM140364) (Table 25) which is *Chilli leaf curl virus* a begomovirus infecting to chilli from New Delhi, India. This result along with SDT color code matrix (Fig. 7) indicated that PaLCuV-4 isolate is strain of ChiLCV and based on sampling location and host the PaLCuV-9 isolate has been given a descriptor as *Chilli leaf curl virus*-[India:Karnataka:Kalaburagi:Papaya:2021] Afjalpur strain. It is designated as ChiLCV-[IN:Kar:Kal:Pap:21].

The PaLCuV-10 isolate showed high nucleotide sequence identity range of 72.9-92.4 per cent with *Papaya leaf curl virus,* followed by *Croton yellow vein mosaic virus* (84.8-89.3 %) and *Tomato leaf curl virus* (70.1-84.9 %). The lowest nucleotide sequence identity was found with *Mungbean yellow mosaic India* virus

(61.0 %), followed by *Okra enation leaf curl virus* (71.6 %) (Table 24). Further, PaLCuV-10 isolate has recorded maximum per cent nucleotide sequence identity of 92.4 per cent with PaLCuV-[IN:Kar:Madi:Cal:18] (MK087120) (Table 25) which *Papaya yellow leaf curl virus* a begomovirus infecting to calandula from Madikeri, Karnataka, India. This result along with SDT color code matrix (Fig. 7) indicated that PaLCuV-4 isolate is strain of PaYLCuV and based on sampling location and host the PaLCuV-10 isolate has been given a descriptor as *Papaya leaf curl virus*-[India:Karnataka:Kalaburagi:Papaya:2021] Kalburgi strain. It is designated as PaYLCuV-[IN:Kar:Kal:Pap:21].

The PaLCuV-11 isolate showed high nucleotide sequence identity range of 87.7-93.5 per cent with *Croton yellow vein mosaic virus,* followed by with *Papaya leaf curl virus* (69.3-91.9 %) and *Tomato leaf curl virus* (67.0-82.1 %). The lowest nucleotide sequence identity was found with *Mungbean yellow mosaic India* virus (58.4 %), followed by *Okra enation leaf curl virus* (68.3 %) (Table 24). Further, PaLCuV-11 isolate has recorded maximum per cent nucleotide sequence identity of 93.5 per cent with CYVMV-[IN:ND:Cro:08] (JN817516) (Table 25) which *Croton yellow vein mosaic virus* infecting to croton from New Delhi, India. This result along with SDT color code matrix (Fig. 7) indicated that PaLCuV-11 isolate is strain of CYVMV and based on sampling location and host the PaLCuV-10 isolate has been given a descriptor as *Croton yellow vein mosaic virus*-[India:Karnataka: Kalaburagi:Papaya:2021] Kalburgi strain. It is designated as CYVMV-[IN:Kar:Kal:Pap:21].

The PaLCuV-12 isolate showed high nucleotide sequence identity range of 79.0-93.0 per cent with *Papaya leaf curl virus,* followed by *Croton yellow vein mosaic virus* (84.4-88.8 %) and *Tomato leaf curl virus* (69.5-84.2 %). The lowest nucleotide sequence identity was found with *Mungbean yellow mosaic India* virus (59.9 %), followed by *Okra enation leaf curl virus* (70.7 %) (Table 24). Further, PaLCuV-12 isolate has recorded maximum per cent nucleotide sequence identity of 93.5 per cent with PaLCuV-[IN:Kar:Madi:Cal:18] (MK087120) (Table 25) which *Papaya yellow leaf curl virus* infecting to croton from Madikeri, Karnataka, India. This result along with SDT color code matrix (Fig. 7) indicated that PaLCuV-12 isolate is strain of PaYLCuV and based on sampling location and host the PaLCuV-10

isolate has been given a descriptor as *Papaya leaf curl virus*-[India:Karnataka:Vijayapura:Papaya:2021] Vijayapura strain. It is designated as PaYLCuV-[IN:Kar:Vij:Pap:21].

The PaLCuV-13 isolate showed high nucleotide sequence identity range of 69.7-87.2 per cent with *Tomato leaf curl virus*, followed by *Chilli leaf curl virus* (68.5-80.8 %) and *Papaya leaf curl virus* (67.9-79.9 %). Among the all begomovirus isolates infecting papaya, the lowest nucleotide sequence identity was found with *Mungbean yellow mosaic India* virus (59.6 %), followed by *Okra enation leaf curl virus* (68.7 %) (Table 24). Further, PaLCuV-13 isolate has recorded maximum per cent nucleotide sequence identity of 87.2 per cent with ToLCV-[In:Kar:Ben:Chr:17] (MG758145) (Table 25) which *Tomato leaf curl virus* infecting to chrysanthemum from Bengaluru, Karnataka, India. This result along with SDT color code matrix (Fig. 7) indicated that PaLCuV-13 isolate is different species of ToLCV and based on sampling location and host the PaLCuV-13 isolate has been given a descriptor *Papaya leaf curl Haveri virus*-[India:Karnataka:Haveri:Papaya:2021] Haveri. It is designated as PaLCuHV-[IN:Kar:Hav:Pap:21].

4.1.3.16 Pair wise distance nucleotide identity of DNA-A component between 13 begomovirus isolates

The pair wise distance nucleotide identity was analyzed between 13 isolated begomoviruses from surveyed samples (Table 26) to study genetic variability among the begomoviral isolates of Karnataka. The PaLCuV-1 isolate has recorded maximum per cent identity with PaLCuV-4 (91.3 %), while PaLCuV-2 isolate with PaLCuV-8 (96.5 %). The PaLCuV-3 and PaLCuV-4 isolates shared maximum per cent identity of 93.4 per cent with each other. While, PaLCuV-5 isolate recorded maximum per cent identity of 97.0 per cent with both PaLCuV-6 and PaLCuV-7 isolates. The PaLCuV-6 and PaLCuV-7 isolates shared maximum per cent identity of 99.3 per cent with each other. PaLCuV-8 isolate maximum per cent shred identity with PaLCuV-7 (96.9 %), PaLCuV-9 isolate with PaLCuV-8 (93.1%). PaLCuV-10 and PaLCuV-12 share 93.2 per cent identity with each other. Similarly, PaLCuV-11 with PaLCuV-10 (87.2 %) and PaLCuV-13 with PaLCuV-8 (80.7 %) share sequence identities.

Table 26: Pair wise distance nucleotide identity of DNA-A component between begomovirus isolates

Begomovirus isolates	Per cent nucleotide sequence identity												
	PaLCuV - 1	**PaLCuV -2**	**PaLCu V -3**	**PaLCu V -4**	**PaLCu V -5**	**PaLCu V -6**	**PaLCu V -7**	**PaLCu V -8**	**PaLCu V -9**	**PaLCuV -10**	**PaLCuV -11**	**PaLCuV -12**	**PaLCuV -13**
PaLCuV -1	100												
PaLCuV -2	77.0	100											
PaLCuV -3	90.1	77.1	100										
PaLCuV -4	91.3	79.2	93.4	100									
PaLCuV -5	76.6	93.4	76.7	79.0	100								
PaLCuV -6	77.9	95.0	78.0	80.4	97.0	100							
PaLCuV -7	78.0	95.2	78.2	80.5	97.0	99.3	100						
PaLCuV -8	77.7	96.5	77.8	80.3	94.9	96.8	96.9	100					
PaLCuV -9	81.5	92.6	81.3	83.7	90.2	91.9	92.0	93.1	100				
PaLCuV -10	88.3	76.9	89.3	92.5	76.9	78.3	78.3	78.0	81.7	100			
PaLCuV -11	82.0	73.5	82.5	82.6	73.9	75.1	75.2	75.0	78.5	87.2	100		
PaLCuV -12	87.04	75.1	87.1	88.9	75.1	76.3	76.5	76.3	79.8	93.2	87.4	100	
PaLCuV -13	71.1	79.9	71.2	72.8	78.7	80.4	80.3	80.7	77.0	70.7	67.4	69.7	100

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4.1.3.17 Phylogenetic analysis of full length genome of DNA-A component of isolated begomoviruses with other begomoviruses

The complete DNA-A genome sequence of 13 begomoviruses isolated from papaya was aligned with those of other selected begomoviruses available in the database (Table 12). The phylogenetic tree was generated from multiple alignment and pairwise alignment by using the CLUSTALW having 60.0 per cent cut-off value with 1,000 bootstrap replicates in MEGA.11 version. Phylogenetic tree based on alignment of complete DNA-A nucleotide sequences of other selected begomoviruses with 13 begomovrus isolates of present study given in fig. 8.

The phylogenetic analysis revealed that 13 begomoviruses isolates was clustered into two groups. PaLCuV-1 isolate formed single separate branch with ToLCKV-[IN:GJ:AHM:Tom:16] infecting tomato. Thus PaLCuV-1 isolate is a new distinct species of ToLCKV-[IN:GJ:AHM:Tom:16].

PaLCuV-2, PaLCuV-8 and PaLCuV-9 isolates formed the distinct branch along with ChiLCV-DU[IN:ND:DU:Pap:09] virus infecting papaya but differed with branching length,. Thus PaLCuV-2 and PaLCuV-8 isolates are new variants of ChiLCV-DU[IN:ND:DU:Pap:09], whereas PaLCuV-9 is a new species. Isolates PaLCuV-3, PaLCuV-10 and PaLCuV-12 formed distant branching with PaYLCuV-[IN:Kar:Madi:Cal:18], where as PaLCuV-10 and PaLCuV-12 isolates formed close branching. Thus PaLCuV-10 and PaLCuV-12 are new strain and PaLCuV-3 is a new different species of PaYLCuV-[IN:Kar:Madi:Cal:18].

The PaLCuV-4 isolate formed the distant branch with BYVBV-[IN:OD:Bhu:Okra:03], thus it is a new different species of this virus. The isolates PaLCuV-5, PaLCuV-6 and PaLCuV-7 isolates formed very close branching with ChiLCV-[IN:Kar:Rai:Chi:17], thus these are new variants of ChiLCV-[IN:Kar:Rai:Chi:17] virus.

PaLCuV-8 and PaLCuV-9 isolates have formed close branching with ChiLCV-DU[IN:ND:DU:Pap:09] but differed in branching length. Thus PaLCuV-8 is a new variant of ChiLCV-DU[IN:ND:DU:Pap:09], whereas, PaLCuV-9 is a new species of the same virus. PaLCuV-11 isolate formed a single separate branching with CYVMV-[IN:ND:Cro:08], thus it is a new strain of CYVMV-[IN:ND:Cro:08].

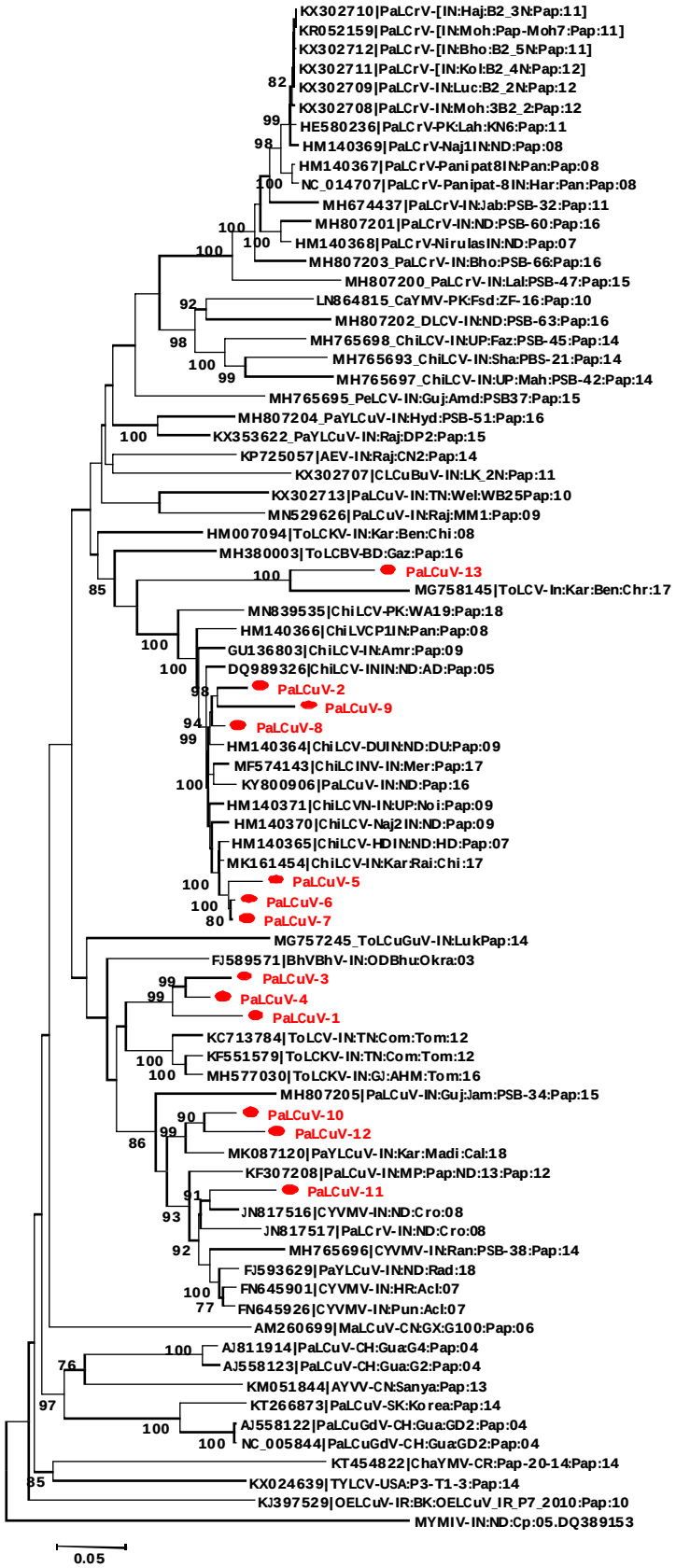

Fig. 8: Phylogenic tree showing the relationship of isolated begomoviruses with other selected begomoviruses based on their full length DNA-A genome nuclieotide sequence (The number of each node indicates 60 percentage bootstrap value with 1000 replicates)

PaLCuV-13 isolate formed a single separate branching with ToLCV-[In:Kar:Ben:Chr:17], thus it is a new species of ToLCV-[In:Kar:Ben:Chr:17]-MG758145. These results are also supported by DNA-A nt sequence identity.

4.1.3.18 Identification and comparison of intergenic region of isolated begomoviruses with other begomoviruses

The per cent nucleotide sequence identity of intergenic regions of 13 isolates with other begomoviruses is given in appendix IV-B and maximum per cent nucleotide sequence identity is given in table 27. Intergenic region of PaLCuV-1 isolate was found from position of 2612nd nt to 2763th nt and 1st to 144th nt position in which AC1 (Rep) gene and AV2 (Pre coat protein) gene coincides. This is the region which consists of total 296 nt in number with the stem-loop structure having nanonucleotide sequence “TAATATTAC” which is noncoding sequence lies in the region called common region.

In PaLCuV-2 isolate, intergenic region was found from position of 2609th nt to 2760 th nt and 1st to 146th nt with 298 nt. In PaLCuV-3 isolate, intergenic region was found from position of 2616 th nt to 2757 th nt and 1st to 144th nt with 286 nt. In PaLCuV-4 isolate, intergenic region was found from position of 2617 th nt to 2773 rd nt and 1st to 145th nt with 302 nt. In PaLCuV-5 isolate, intergenic region was found from position of 2612nd nt to 2763rd nt and 1st to 146th nt position which consists of total 293 nt. In PaLCuV-6 isolate, intergenic region was found from position of 2611st nt to 2762 nd nt and 1st to 145th nt with 297 nt. In PaLCuV-7 isolate. intergenic region was found from position of 2613rd nt to 2765th nt and 1st to 145th nt position which consists of total 297 nt. In PaLCuV-8 isolate, intergenic region was found from position of 2612nd nt to 2763rd nt and 1st to 146th nt with 298 nt. In PaLCuV-9 isolate, intergenic region was found from position of 2612nd nt to 2763rd nt and 1st to 146th nt with 298 nt. In PaLCuV-10 isolate, intergenic region was found from position of 2617th nt to 2773rd nt and 1st to 145th nt with 303 nt. In PaLCuV-11 isolate, intergenic region was found from position of 2615th nt to 2750th nt and 1st to 145th nt with 280 nt. In PaLCuV-12 isolate, intergenic region was found from position of 2614th nt to 2772 nd nt and 1st to 146th nt with 305 nt. In PaLCuV-13 isolate, intergenic region was found from position of 2585th nt to 2737th nt and 1st to 119th nt with 290 nt.

Table 27: Identification of intergenic region and maximum percentage nucleotide sequence identity of PaLCuV isolated from papaya with other selected isolates of begomoviruses

Begomovirus Isolate	Start-Stop codon (nt)	Predicted size of IR (nt)	Maximum Percentage nucleotide sequence (%)	Begomovirus
PaLCuV -1	2612-2763 and 1-144	296	82.3	ToLCKV-[IN:TN:Com:Tom:12]-KF551579
PaLCuV -2	2609-2760 and 1-146	298	97.9	ChiLCVN-[IN:UP:Noi:Pap:09]-HM140371
PaLCuV -3	2616-2757 and 1-144	286	81.2	PaYLCuV-[IN:ND:Rad:18]-FJ593629
PaLCuV -4	2617-2773 and 1-145	302	91.0	BYVBV-[IN:OD:Bhu:Okra:03]-FJ589571
PaLCuV -5	2612-2763 and 1-146	293	94.6	ChiLCV-HD[IN:ND:HD:Pap:07]-HM140365
PaLCuV -6	2611-2762 and 1-145	297	98.3	ChiLCVN-[IN:UP:Noi:Pap:09]-HM140371
PaLCuV -7	2613-2765 and 1-145	297	97.9	ChiLCVN-[IN:UP:Noi:Pap:09]-HM140371
PaLCuV -8	2612-2763 and 1-146	298	97.9	ChiLCVN-[IN:UP:Noi:Pap:09]-HM140371
PaLCuV -9	2612-2763 and 1-146	298	98.9	ChiLCVN-[IN:UP:Noi:Pap:09]-HM140371
PaLCuV -10	2617-2773 and 1-145	303	88.1	BYVBV-[IN:OD:Bhu:Okra:03]-FJ589571
PaLCuV -11	2615-2750 and 1-145	280	83.3	CYVMV-[IN:ND:Cro:08]-JN817516
PaLCuV -12	2614-2772 and 1-146	305	84.5	PaLCuV-[IN:Kar:Madi:Cal:18]-MK087120
PaLCuV -13	2585-2732 and 1-119	290	76.5	PaLCrV-Nirulas[IN:ND:Pap:07]-HM140368

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The maximum nucleotide sequence identity of intergenic region of 13 begomovirus isolate is given in table 27. PaLCuV-1 isolate has recorded maximum nucleotide sequence identity with ToLCKV-[IN:TN:Com:Tom:12]-KF551579 (82.3 %). The PaLCuV-2, PaLCuV-6, PaLCuV-7, PaLCuV-8 and PaLCuV-9 isolates recorded maximum nucleotide sequence identity with ChiLCVN-[IN:UP:Noi:Pap:09]-HM140371 (97.9 %). The maximum nucleotide identity of PaLCuV-3 isolate was with PaYLCuV-[IN:ND:Rad:18]-FJ593629 (81.2 %); PaLCuV-4 isolate was with BYVBV-[IN:OD:Bhu:Okra:03]-FJ589571(91.0 %); PaLCuV-5 isolate was with ChiLCV-HD[IN:ND:HD:Pap:07]-HM140365 (94.6 %); PaLCuV-10 isolate was with BYVBV-[IN:OD:Bhu:Okra:03]- FJ589571 (88.1 %); PaLCuV-11 isolate was with CYVMV-[IN:ND:Cro:08]-JN817516 (83.3 %); PaLCuV-12 isolate was with PaLCuV-[IN:Kar:Madi:Cal:18]-MK087120 (84.5 %) and PaLCuV-13 isolate was with PaLCrV-Nirulas[IN:ND:Pap:07]-HM140368 (76.5 %).

4.1.3.19 Recombination analysis of complete nucleotide sequence of DNA-A component of isolated begomoviruses with other begomoviruses

The recombination analysis provided evidence for the presence of past recombination events in all the isolated begomoviruses. Detailed recombination events with major and minor parent are given in table 28 and fig. 9.

PaLCuV-1 recorded the four recombination events which are of major parent and minor parent ToLCKV-[IN:Kar:Ben:Chi:08] (HM007094) and PaLCuV-10 (at 40^{th} nt to 2148^{th} nt position); CLCuBuV-[IN:LK_2N:Pap:11] (KX302707) and OELCuV-[IR:BK:OELCuV_IRP72010:Pap:10] (KJ397529) (at 2262^{nd} to 2440^{th}); PaLCuV-6 and PaLCrV-[IN:Haj:B2_3N:Pap:11] (KX302710) (at 282^{nd} nt to 1175^{th} nt position); CYVMV-[IN:HR:Acl:07] (FN645901) and PaLCuV-5 (at 1339^{th} nt to 2070^{th} nt position) respectively.

PaLCuV-2 recorded two recombinations at 1907^{th} nt to 2019^{th} nt and with major parent *i.e* ChiLCV-DU[IN:ND:DU:Pap:09] (HM140364) and minor parent AYVV-[CN:Sanya:Pap:13] (KM051844); 2250^{th} nt to 2433^{rd} nt with major parent

Table 28: Recombination analysis of breakpoint and its putative parental sequences of PaLCuV isolated from papaya

Begomovirus		Break point (nt)		Recombination parents		p-value					
	Events	Begin	End	Major parent	Minor parent	RDP	Geneconv	MaxChi	Chimera	SiScan	3Seq
PaLCuV-1	1	40	2148	ToLCKV-[IN:Kar:Ben:Chi:08].HM007094	PaLCuV-10	2.702 x 10^{-10}	NS	7.363x10^{-21}	1.682x10^{-11}	3.720x10^{-29}	8.168 x 10^{-57}
	2	2262	2440	CLCuBuV-[IN:LK_2N:Pap:11].KX302707	OELCuV-[IR:BK:IRP72010:Pap:10] KJ397529	NS *	1.534 x 10-^{02}	2.646 x 10^{-02}	9.615 x 10^{-03}	1.455 x 10-^{07}	NS
	3	282	1175	PaLCuV -6	PaLCrV-[IN:Haj:B23N:Pap:11] KX302710	5.543 x 10^{-03}	NS	1.510 x 10^{-06}	7.523 x 10^{-09}	8.680 x 10-^{06}	1.096 x 10^{-02}
	4	1339	2070	CYVMV-[IN:HR:Acl:07].FN645901	PaLCuV -5	9.624 x 10^{-04}	2.182 x 10^{-09}	9.177 x 10^{-04}	2.534 x 10^{-07}	1.291 x 10-^{11}	1.090 x 10^{-08}
PaLCuV-2	1	1907	2019	ChiLCV-DU[IN:ND:DU:Pap:09] HM140364	AYVV-[CN:Sanya:Pap:13] KM051844	7.740 x 10^{-13}	2.037 x 10^{-02}	2.936 x 10^{-04}	3.688 x 10^{-02}	NS	4.868 x 10^{-02}
	2	2250	2433	PaYLCuV-[IN:Raj:DP2:Pap:15] KX353622	PaLCrV-[PK:Lah:KN6:Pap:11] HE580236	1.893 x 10^{-03}	NS	4.571 x 10^{-05}	1.875 x 10^{-03}	5.966 x 10^{-07}	6.564 x 10^{-03}
PaLCuV-3	1	1272	2158	CYVMV-[IN: HR: Acl: 07]. FN645901	PaLCuV -5	9.624 x 10^{-04}	2.182 x 10^{-09}	9.177 x 10^{-04}	2.534 x 10^{-07}	1.291 x 10^{-11}	1.090 x 10^{-08}
	2	11	2185	PaLCuV-[CH:Gua:G2:Pap:04] AJ558123	CYVMV-[IN:Pun:Acl:07] FN645926	8.115 x 10^{-16}	8.946 x 10^{-27}	2.130 x 10^{-22}	1.040 x 10^{-27}	4.436 x 10^{-39}	9.474 x 10^{-37}
	3	2696	2785	BYVBV-[IN:OD;Bhu:Ok:03] FJ589571	PaLCrV-[IN:ND:Cro:08] JN817517	5.844 x 10^{-06}	4.486 x 10^{-05}	8.898 x 10^{-03}	NS	NS	NS
	4	294	1174	PaYLCuV-[IN:ND:Rad:18] FJ593629	PaLCrV-[IN:Haj:B23N:Pap:11] KX302710	5.543 x 10^{-03}	NS	1.510 x 10^{-06}	7.523 x 10^{-09}	8.680 x 10^{-06}	1.096 x 10^{-02}
	5	2408	2834	PaLCuV-3	ChiLCV-[IN:Kar:Rai:Chi:17] MK161454	6.983 x 10-^{03}	NS	3.204 x 10^{-05}	6.595 x 10^{-04}	3.089 x 10^{-04}	1.835 x 10^{-03}
	6	2508	2941	PaLCuV-1	BYVBV-[IN:OD;Bhu:Ok:03] FJ589571	6.310 x 10^{-06}	7.023 x 10^{-05}	4.530 x 10^{-09}	8.036 x 10^{-09}	3.340 x 10^{-10}	1.932 x 10^{-17}
PaLCuV-4	1	1273	2158	CYVMV-[IN:HR:Acl:07] FN645901	PaLCuV -5	9.624 x 10^{-04}	2.182 x 10^{-09}	9.177 x 10^{-04}	2.534 x 10^{-11}	1.291 x 10^{-11}	1.090 x 10^{-08}
	2	282	1174	PaLCuV -6	PaLCrV-[IN:Haj:B23N:Pap:11] KX302710	5.543 x 10^{-03}	NS	1.510 x 10^{-06}	7.523 x 10^{-09}	8.680 x 10^{-06}	1.096 x 10^{-02}
	3	2486	2941	PaLCuV -1	BYVBV-[IN:OD;Bhu:Ok:03] FJ589571	6.310 x 10^{-06}	7.023 x 10^{-05}	4.530 x 10^{-09}	8.036 x 10^{-09}	3.340 x 10^{-10}	1.932 x 10^{-17}

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Begomovirus		Break point (nt)		Recombination parents		p-value					
	Events	Begin	End	Major parent	Minor parent	RDP	Geneconv	MaxChi	Chimera	SiScan	3Seq
PaLCuV-5	1	2241	2433	PaLCuV-[IN:Raj:DP2:Pap:15] KX353622	PaLCrV-[PK:Lah:KN6:Pap:11] HE580236	1.893×10^{-03}	NS	4.517×10^{-05}	1.875×10^{-03}	5.966×10^{-07}	6.564×10^{-03}
PaLCuV-6	1	2251	2453	PaLCuV-[IN:Raj:DP2:Pap:15] KX353622	PaLCrV-[PK:Lah:KN6:Pap:11] HE580236	1.893×10^{-03}	NS	4.571×10^{-05}	1.875×10^{-03}	5.966×10^{-07}	6.564×10^{-03}
PaLCuV-7	1	2251	2453	PaLCuV-[IN:Raj:DP2:Pap:15] KX353622	PaLCrV-[PK:Lah:KN6:Pap:11] HE580236	1.893×10^{-03}	NS	4.571×10^{-05}	1.875×10^{-03}	5.966×10^{-07}	6.564×10^{-03}
PaLCuV-8	1	362	1363	ChiLCV-[IN:Kar:Rai:Chi:17] MK161454	PaLCuV -5	NS	NS	3.368×10^{-02}	1.898×10^{-03}	NS	NS
	2	2250	2433	PaYLCuV-[IN:Raj:DP2:Pap:15] KX353622	PaLCrV-[PK:Lah:KN6:Pap:11] HE580236	1.893×10^{-03}	NS	4.571×10^{-05}	1.875×10^{-03}	5.966×10^{-07}	6.564×10^{-03}
PaLCuV-9	1	550	1074	ChiLCVN-[IN:UP:Noi:Pap:09] HM140371	PaLCuV -11	4.860×10^{-63}	3.671×10^{-58}	1.179×10^{-20}	5.644×10^{-21}	3.778×10^{-16}	6.621×10^{-12}
	2	2250	2433	PaLCuV-[IN:Raj:DP2:Pap:15] KX353622	PaLCrV-[PK:Lah:KN6:Pap:11] HE580236	1.893×10^{-03}	NS	4.571×10^{-05}	1.875×10^{-03}	5.966×10^{-07}	6.564×10^{-03}
PaLCuV10	1	520	1246	ToLCKV-[IN:Kar:Ben:Chi:08] HM007094	CYVMV-[IN:Pun:Acl:07] FN645926	3.857×10^{-08}	1.194×10^{-14}	2.228×10^{-07}	2.651×10^{-05}	1.698×10^{-33}	2.521×10^{-26}
	2	2241	28	PaLCrV-Nirulas[IN:ND:Pap:07] HM140368	PaYLCuV-[IN:Raj:DP2:Pap:15] KX353622	4.539×10^{-03}	NS	3.218×10^{-05}	1.893×10^{-06}	1.291×10^{-07}	1.140×10^{-03}
	3	1268	2249	CYVMV-[IN:HR:Acl:07] FN645901	PaLCuV -5	9.624×10^{-4}	2.182×10^{-09}	9.177×10^{-04}	2.534×10^{-07}	1.291×10^{-11}	1.090×10^{-08}
	4	2702	2863	ChiLCV-[IN:Kar:Rai:Chi:17] MK161454	PaLCuV -1	6.983×10^{-03}	NS	3.204×10^{-05}	6.595×10^{-04}	3.089×10^{-04}	1.835×10^{-03}
	5	282	1178	PaLCuV -6	PaLCrV-[IN:Haj:B2_3N:Pap:11] KX302710	5.543×10^{-03}	NS	1.510×10^{-06}	7.523×10^{-09}	8.680×10^{-06}	1.096×10^{-02}
PaLCuV-11	1	284	1227	PaLCuV -6	PaLCrV-[IN:Haj:B2_3N:Pap:11] KX302710	5.543×10^{-03}	NS	1.510×10^{-06}	7.523×10^{-09}	8.680×10^{-06}	1.096×10^{-02}
	2	1916	2237	PaLCuV -7	PaLCuGdV-[CH:Gua:GD2:Pap:04] NC_005844	NS	NS	NS	2.474×10^{-01}	3.534×10^{-13}	NS
	3	2255	2894	PaLCuV-[IN:Raj:DP2:Pap:15] KX353622	PaLCrV- [IN:ND:Pap:07] HM140368	4.539×10^{-03}	NS	3.218×10^{-05}	1.893×10^{-06}	1.291×10^{-07}	1.140×10^{-03}

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Begomovirus		Break point (nt)		Recombination parents		p-value					
	Events	Begin	End	Major parent	Minor parent	RDP	Geneconv	MaxChi	Chimera	SiScan	3Seq
PaLCuV-11	4	2392	2884	PaLCrV-[IN:Bho:PSB-66:Pap:16] MH807203	ChiLCV- [IN:ND:DU:Pap:09] HM140364	9.495×10^{-08}	2.999×10^{-05}	2.996×10^{-03}	1.190×10^{-06}	1.704×10^{-12}	$4.864.10^{-05}$
	5	1079	1226	PaLCuV-[IN:Kar:Madi:Cal:18] MK087120	PaLCuV -3	NS	6.970×10^{-18}	1.050×10^{-10}	1.595×10^{-10}	7.464×10^{-14}	5.281×10^{-22}
PaLCuV-12	1	1268	2182	CYVMV-[IN:HR:Acl:07] FN645901	PaLCuV -5	9.624×10^{-04}	2.182×10^{-09}	9.177×10^{-04}	2.534×10^{-07}	1.291×10^{-11}	1.090×10^{-08}
	2	2836	2914	ToLCKV-[IN:GJ:AHM:Tom:16] MH5770301	PeLCV-[IN:Guj:Amd:PSB37:Pap:15] MH765695	NS	4.820×10^{-02}	1.992×10^{-02}	NS	1.298×10^{-06}	2.476×10^{-02}
	3	2240	2513	PaLCrV-Nirulas[IN:ND:Pap:07] HM140368	PaLCuV-[IN:Raj:DP2:Pap:15] KX353622	4.539×10^{-03}	NS	3.218×10^{-05}	1.893×10^{-06}	1.291×10^{-07}	1.140×10^{-03}
	4	11	2513	ToLCKV-[IN:Kar:Ben:Chi:08 HM007094	CYVMV-[IN:Pun:Acl:07] FN645926	3.857×10^{-08}	1.194×10^{-14}	2.338×10^{-07}	2.651×10^{-05}	1.698×10^{-33}	2.521×10^{-26}
	5	235	1178	PaLCuV -6	PaLCrV-[IN:Haj:B23N:Pap:11] KX302710	5.543×10^{-03}	NS	1.510×10^{-06}	7.523×10^{-09}	8.680×10^{-06}	1.096×10^{-02}
	6	2540	2613	PaLCuV-[IN:Guj:Jam:PSB-34:Pap:15] MH807205	ToLCKV-[IN:Kar:Ben:Chi:08] HM007094	NS	NS	2.164×10^{-02}	NS	2.884×10^{-07}	9.162×10^{-03}
PaLCuV-13	1	218	992	ToLCV-[In:Kar:Ben:Chr:17] MG758145	PaLCuV -2	5.828×10^{-33}	7.920×10^{-10}	3.819×10^{-23}	1.461×10^{-21}	1.329×10^{-20}	1.622×10^{-16}
	2	2178	2729	PaLCuV-[IN:TN:Wel:WB2&5:Pap:10] KX302713	PaLCuV -8	1.053×10^{-31}	3.869×10^{-38}	7.021×10^{-18}	1.121×10^{-23}	2.285×10^{-30}	6.621 x 10-$^{-12}$
	3	1190	2862	PaLCrV-[IN:Jab:PSB-32:Pap:11] MH674437	ChiLCV-HD[IN:ND:HD:Pap:07] HM140365	4.051×10^{-02}	NS	1.296×10^{-02}	4.714×10^{-04}	NS	2.030×10^{-04}
	4	1200	1166	PaLCrV-Panipat8[IN:Har:Pan:Pap:08] NC_014707	PaLCuV -11	NS	NS	1.620×10^{-02}	8.354×10^{-06}	8.499×10^{-06}	1.712×10^{-19}

*Non significant value

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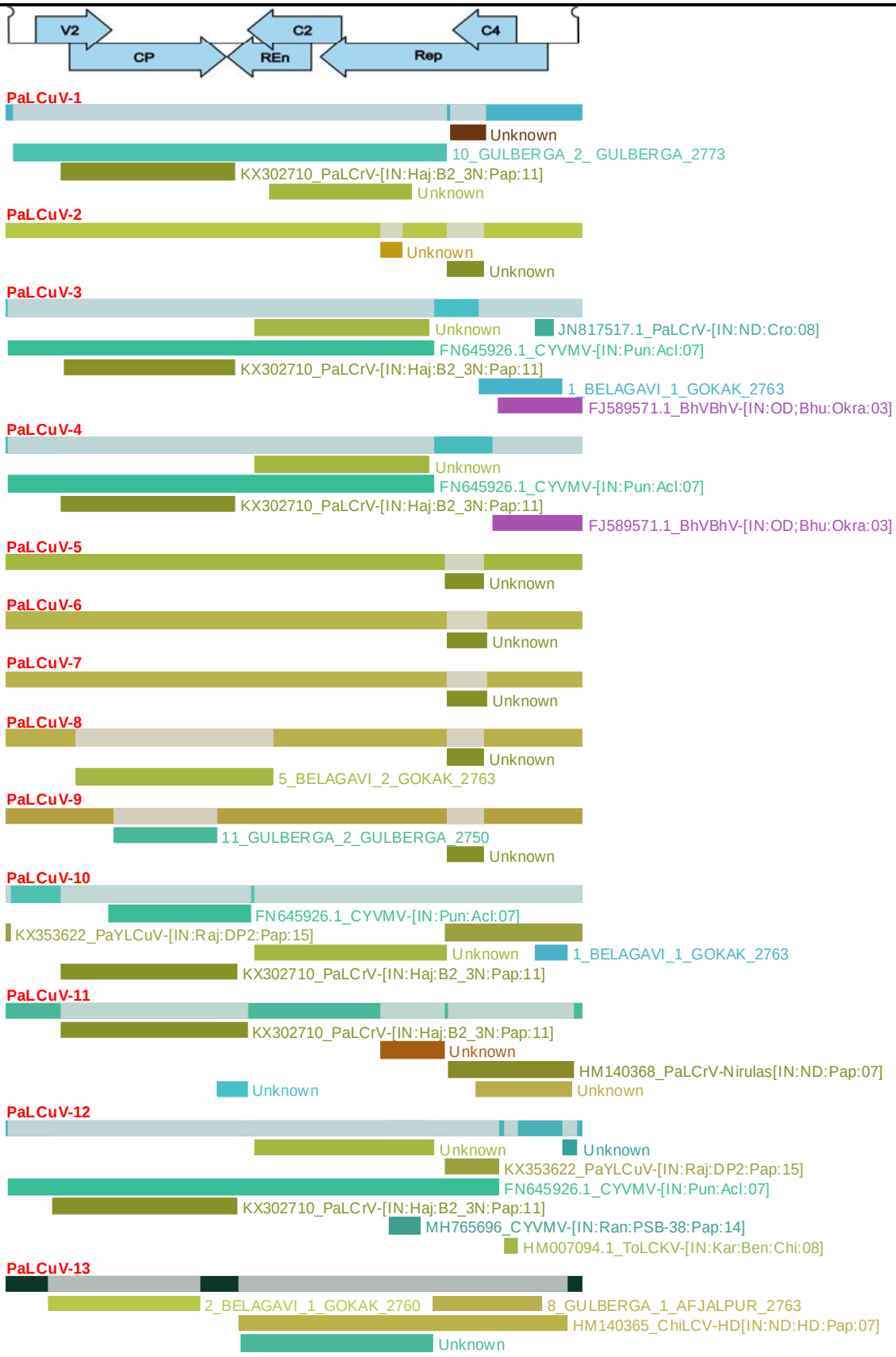

Fig. 9: Recombination analysis of breakpoints and its putative parental sequences of isolated PaLCuV

[For each of the PaLCuV isolates recombinant fragments are shown as shaded bars with the origin (parental virus species)]

PaYLCuV-[IN:Raj:DP2:Pap:15] (KX353622) and minor PaLCrV-[PK:Lah: KN6: Pap:11] (HE580236) respectively.

PaLCuV-3 recorded six recombination events *viz.,* CYVMV-[IN:HR:Acl:07] (FN645901) and PaLCuV-5 at 1272nd nt to 2158nd nt position; PaLCuV-[CH:Gua:G2:Pap:04] (AJ558123) and CYVMV-[IN:Pun:Acl:07] (FN645926) at 11st nt to 2185th nt postion; BYVBV-[IN:OD;Bhu:Okra:03] (FJ589571) and PaLCrV-[IN:ND:Cro:08] (JN817517) at 2696th nt to 2785th nt position; PaYLCuV-[IN:ND:Rad:18] and PaLCrV-[IN:Haj:B2_3N:Pap:11] (KX302710) at 294th nt to 1174th nt position; PaLCuV-3rd and ChiLCV-[IN:Kar:Rai:Chi:17] (MK161454) at 2408th nt to 2834th nt position; PaLCuV-1 and BYVBV-[IN:OD;Bhu:Okra:03] (FJ589571) at 2508th nt 2941st nt position, as major and minor parent respectively.

PaLCuV-4 recorded 3 different recombinations between major and minor parents *i.e* CYVMV-[IN:HR:Acl:07] (FN645901) and PaLCuV-5 (at 1273rd nt to 2158th nt position); PaLCuV-6 and PaLCrV-[IN:Haj:B2_3N:Pap:11] (KX302710) (at 282nd nt to 1174th nt position); PaLCuV-1 and BYVBV-[IN:OD;Bhu:Okra:03] (FJ589571) (2486th nt to 2941st nt position)

Whereas, PaLCuV-5, PaLCuV-6 and PaLCuV-7 recorded single recombination with same major and minor parent *i.e* PaYLCuV-[IN:Raj:DP2:Pap:15] (KX353622) and PaLCrV-[PK:Lah:KN6:Pap:11] (HE580236) at 2241st nt to 2433rd nt, 2251st nt to 2453rd nt and 2251st to 2453rd nt positions respectively

In PaLCuV-8 recorded recombination accorded at two different nucleotide position *i.e* 362nd nt to 1363rd nt and 2250th nt to 2433rd nt between ChiLCV-[IN:Kar:Rai:Chi:17] (MK161454) and PaLCuV-5; PaYLCuV-[IN:Raj:DP2:Pap:15] (KX353622) and PaLCrV-[PK:Lah:KN6:Pap:11] (HE580236) parents respectively

Similarly PaLCuV-9 recorded two recombination events at 550th nt to 1074th nt and 2250th to 2433rd nt position with major and minor parents *viz.*, ChiLCVN-[IN:UP:Noi:Pap:09] (HM140371) and PaLCuV-11; PaYLCuV-[IN:Raj:DP2:Pap:15] (KX353622) and PaLCrV-[PK:Lah:KN6:Pap:11] (HE580236) respectively.

PaLCuV-10 recorded showed high level of recombination at five different locations *i.e* 520th nt to 1246th nt position (ToLCKV-[IN:Kar:Ben:Chi:08]

(HM007094) and CYVMV-[IN:Pun:Acl:07] (FN645926)); at 2241st nt to 28th nt position (PaLCrV-Nirulas[IN:ND:Pap:07] (HM140368) and PaYLCuV-[IN:Raj:DP2:Pap:15] (KX353622)); at 1268th nt to 2249th nt position (CYVMV-[IN:HR:Acl:07] (FN645901) and PaLCuV-5); at 2702nd nt to 2863rd nt position (ChiLCV-[IN:Kar:Rai:Chi:17] (MK161454) and PaLCuV-1) and at 282nd nt to 1178th nt position (PaLCuV-6 and PaLCrV-[IN:Haj:B2_3N:Pap:11] (KX302710))

Similarly PaLCuV-11 isolate recorded five recombination *i.e* 284th nt to 1227th nt position (PaLCuV-6 and PaLCrV-[IN:Haj:B2_3N:Pap:11] (KX302710) parents); at 1916th nt to 2237th nt position (PaLCuV-7 and PaLCuGdV-[CH:Gua:GD2:Pap:04] (NC_005844) parents); at 2255th nt to 2894th nt position (PaYLCuV-[IN:Raj:DP2:Pap:15] (KX353622) and PaLCrV-Nirulas[IN:ND:Pap:07] (HM140368) patents); at 2392nd nt to 2884th nt position (PaLCrV-[IN:Bho:PSB-66:Pap:16] (MH807203) and ChiLCV-DU[IN:ND:DU:Pap:09] (HM140364) parents); at 1079th nt to 1226th nt position (PaLCuV-[IN:Kar:Madi:Cal:18] (MK087120) and PaLCuV-3 parents)

PaLCuV-12 recorded maximum of six recombination at different locations with different majour and minor parents *i.e* 1268th nt to 2182nd nt position between CYVMV-[IN:HR:Acl:07] (FN645901) and PaLCuV-5; at 2836 nt to 2914 nt position between ToLCKV-[IN:GJ:AHM:Tom:16] (MH5770301) and PeLCV-[IN:Guj:Amd:PSB37:Pap:15] (MH765695); at 2240th nt to 2513th nt position between PaLCrV-Nirulas[IN:ND:Pap:07] (HM140368) and PaYLCuV-[IN:Raj:DP2:Pap:15] (KX353622); at 11th nt to 2513rd nt position between ToLCKV-[IN:Kar:Ben:Chi:08 (HM007094) and CYVMV-[IN:Pun:Acl:07] (FN645926); at 235th nt to 1178th nt position between PaLCuV-6 and PaLCrV-[IN:Haj:B2_3N:Pap:11] (KX302710) and at 2540th nt to 2613rd nt position between PaLCuV-[IN:Guj:Jam:PSB-34:Pap:15] (MH807205) and ToLCKV-[IN:Kar:Ben:Chi:08] (HM007094).

Whereas, PaLCuV-13 recorded four recombinations at different nucleotide positions *viz.*, 218th nt to 992nd nt position (between ToLCV-[In:Kar:Ben:Chr:17] (MG758145) and PaLCuV-2); at 2178th nt to 2729th nt position (between PaLCuV-[IN:TN:Wel:WB2&5:Pap:10] (KX302713) and PaLCuV-8); at 1190th nt to 2862nd nt position (between PaLCrV-[IN:Jab:PSB-32:Pap:11] (MH674437) and ChiLCV-

HD[IN:ND:HD:Pap:07] (HM140365)) and at 1200th nt to 1166th nt position between PaLCrV-Panipat8[IN:Har:Pan:Pap:08] (NC_014707) and PaLCuV-11.

4.1.4 Virus indexing/identification of reservoir hosts through host range study.

Host indexing helps to detect and identify the type of viruses, based on the expression of typical symptoms like localized lesions and mosaic on indicator hosts. Indexing of PRSV virus was studied by inoculating the crude sap mechanically at a specific leaf stage on the 8 species of indicator host plants belonging to different families *viz.*, *Chenopodium quinoa* (Chenopodiaceae), *Cucumis sativus* (Cucurbitaceae), *Phaseolus vulgaris* (Fabaceae). *Datura stramonium, Datura metel, Capsicum annuum cv. California Wonder, Nicotiana tabacum* and *Nicotiana glutinosa* (all Solanaceae).

Table 29 revealed that *Chenopodium quinoa* expressed local lesions on leaves while *Cucumis sativus* expressesd mosaic symptoms due to PRSV infection. Both the indicator hosts took 10 days to produce symptoms after inoculation. Other indicator hosts did not produce any symptoms (Plate 16).

Further PCR analysis was carried out for the inoculated indicator host crops using a set of primers MB 11A/MB 11B (Bateson *et al.*, 1994) to detect the presence of PRSV. Results confirmed the presence of PRSV as there was positive amplification (~900 bp) in *Chenopodium quinoa* and *Cucumis sativus* (Table 29 and Plate 17) samples.

4.2 Epidemiological studies on the incidence of PRSV disease

4.2.1 Effect of different months of planting on the incidence of PRSV disease, growth and yield parameters of papaya at different stage of growth

The effect of planting papaya at different monthly intervals on the PRSV incidence at different growth stages was studied. In this study, planting has been done at monthly interval starting from the 1st week of June 2019 to 1st week of March 2020 and recorded the incidence of the disease along with growth and yield parameters. Data is presented in Table 30 to 38 and Plate 18A.

Table 29: Symptoms expressed by PRSV on different indicator hosts

Sl. No	Plant species	Common name	Family	Leaf stage for Inoculation	Symptoms	Days taken for expression	RT-PCR Detection
1.	*Chenopodium quinoa*	Chenopodium	Chenopodiaceae	4th	Local lesion symptoms	10	+ ve
2.	*Cucumis sativus*	Cucumber	Cucurbitaceae	2nd	Mosaic symptoms	10	+ ve
3.	*Phaseolus vulgaris*	Beans	Fabaceae	2nd	No symptoms	-	- ve
4.	*Datura stramonium*	Datura	Solanaceae	6th	No symptoms	-	- ve
5.	*Datura metel*	Datura	Solanaceae	6th	No symptoms	-	- ve
6.	*Capsicum annuum cv. California Wonder*	Chilli	Solanaceae	8th	No symptoms	-	- ve
7.	*Nicotiana tabacum*	Tobacco	Solanaceae	6th	No symptoms	-	- ve
8.	*Nicotiana glutinosa*	Tobacco	Solanaceae	6th	No symptoms	-	- ve

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(A)

(B)

Plate 16: Symptom produced by PRSV on (A) *Chenopodium quinona* (Local lesions) and (B) *Cucumis sativus* (Mosaic)

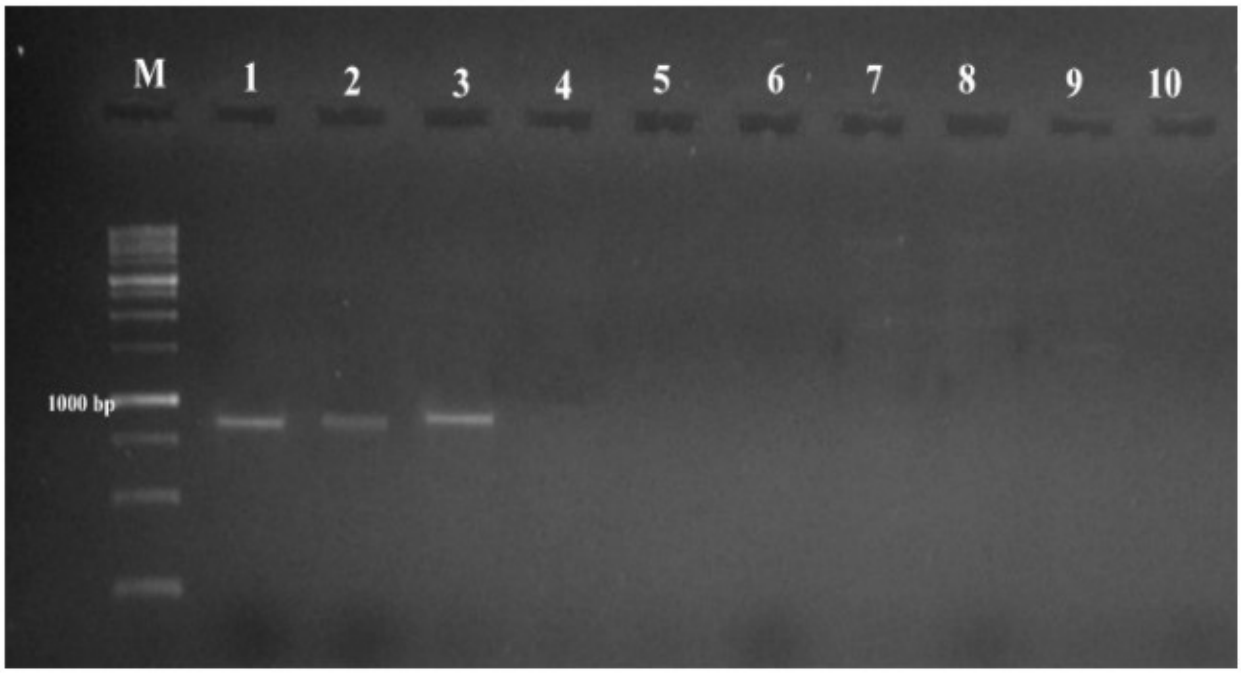

Plate 17: Agarose gel photograph showing amplification of coat protein fragment of PRSV

(Lane 1: +ve control), *Chenopodium quinoa* (Lane 2)and *Cucumis* sativus (Lane 3) by using a set of primer MB 11A/MB 11B (~905 bp). Lane L: 1 kb lader (StepUp™ 1 kb DNA Ladder)

4.2.1.1 PRSV disease incidence

Table 30 recorded the significant influence of different months of planting on disease incidence. The June planting recorded no disease incidence up to 90 DAT. At 120 DAT the incidence was 8.33 per cent which further increased to 19.44 per cent at 150 DAT, 38.89 per cent at 180 DAT, 66.67 per cent at 210 DAT and finally reached 100 per cent at 240 DAT.

In July planting the disease started at 90 DAT (11.11 %) which increased gradually to 22.22 per cent at 120 DAT, 44.44 per cent at 150 DAT, 77.78 per cent at 180 DAT and reached a maximum of 100 per cent at 210 DAT. September (47.22 %), October (52.78 %), November (58.33 %), December (77.78 %), January (55.56 %) and February (19.44 %) plantings recorded moderate amount of disease incidence at 30 DAT. December planting reached 100 per cent at 60 DAT, while October, November and January plantings at 90 DAT. February planting took 180 DAT to record 100 per cent incidence.

March planting recorded the least incidence of disease (5.56 %) at 60 DAT, which increased gradually to 13.89 per cent at 90 DAT, 16.67 per cent at 120 DAT, 19.44 per cent at 150 DAT, 30.56 per cent at 180 DAT, 72.22 per cent at 210 DAT, 97.22 per cent at 240 DAT and finally reached 100 per cent at 270 DAT. At all the crop growth stages except 270 DAT, March planting recorded significantly least incidence compared to all the treatments. At 270 DAT all the treatments recorded 100 per cent (Fig. 10).

4.2.1.2 Growth parameters

The plant height at different growth stages was recorded and presented in the table 31.The table 31 revealed that there was significant difference between the treatments at all the growth stages *viz.*, 30, 60, 90, 120, 150, 180, 210, 240 and 270 DAT. June planting recorded highest plant height of 38.10 cm at 30 DAT which increased to 71.11 cm at 60 DAT, 90.86 cm at 90 DAT, 120.86 cm at 120 DAT, 156.27 cm at 150 DAT, 181.36 cm at 180 DAT, 215. 97 cm at 210 DAT, 221.77 cm at 240 DAT and the maximum of 224.37 cm at 270 DAT. Similar trend was observed in all the plantings. March planting recorded significantly superior plant height compared

Table 30: Influence of different month of planting on PRSV disease incidence at different crop growth stages

Treatment	Disease incidence (%) at								
	30 DAT*	**60 DAT**	**90 DAT**	**120 DAT**	**150 DAT**	**180 DAT**	**210 DAT**	**240 DAT**	**270 DAT**
T_1-1st week of June	0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	8.33 (13.62)	19.44 (26.06)	38.89 (38.56)	66.67 (54.84)	100 (90.00)	100 (90.00)
T_2-1st week of July	0.00 (0.00)	0.00 (0.00)	11.11 (19.22)	22.22 (28.03)	44.44 (41.75)	77.78 (62.18)	100 (90.00)	100 (90.00)	100 (90.00)
T_3-1st week of August	0.00 (0.00)	25.93 (30.48)	58.33 (49.84)	61.04 (55.89)	83.33 (66.38)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T_4-1st week of September	47.22 (43.40)	69.44 (56.81)	88.89 (73.94)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T_5-1st week of October	52.78 (46.60)	94.44 (78.81)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T_6-1st week of November	58.33 (49.84)	94.44 (78.81)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T_7-1st week of December	77.78 (61.97)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T_8-1st week of January	55.56 (48.40)	97.22 (84.41)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T_9-1st week of February	19.44 (26.06)	44.44 (41.75)	63.89 (53.20)	83.33 (67.06)	97.22 (84.41)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T_{10}-1st week of March	0.00 (0.00)	5.56 (11.19)	13.89 (21.66)	16.67 (24.09)	19.44 (26.06)	30.56 (33.51)	72.22 (59.41)	97.22 84.41	100 (90.00)
S Em ±	2.30	4.24	3.24	5.80	2.66	1.43	2.69	1.77	0.00
C D @ 5 %	6.83	12.59	9.62	17.22	7.90	4.25	7.99	5.25	NS

*DAT-Days After Transplanting; ** Figures in parentheses are arc sine transformed values

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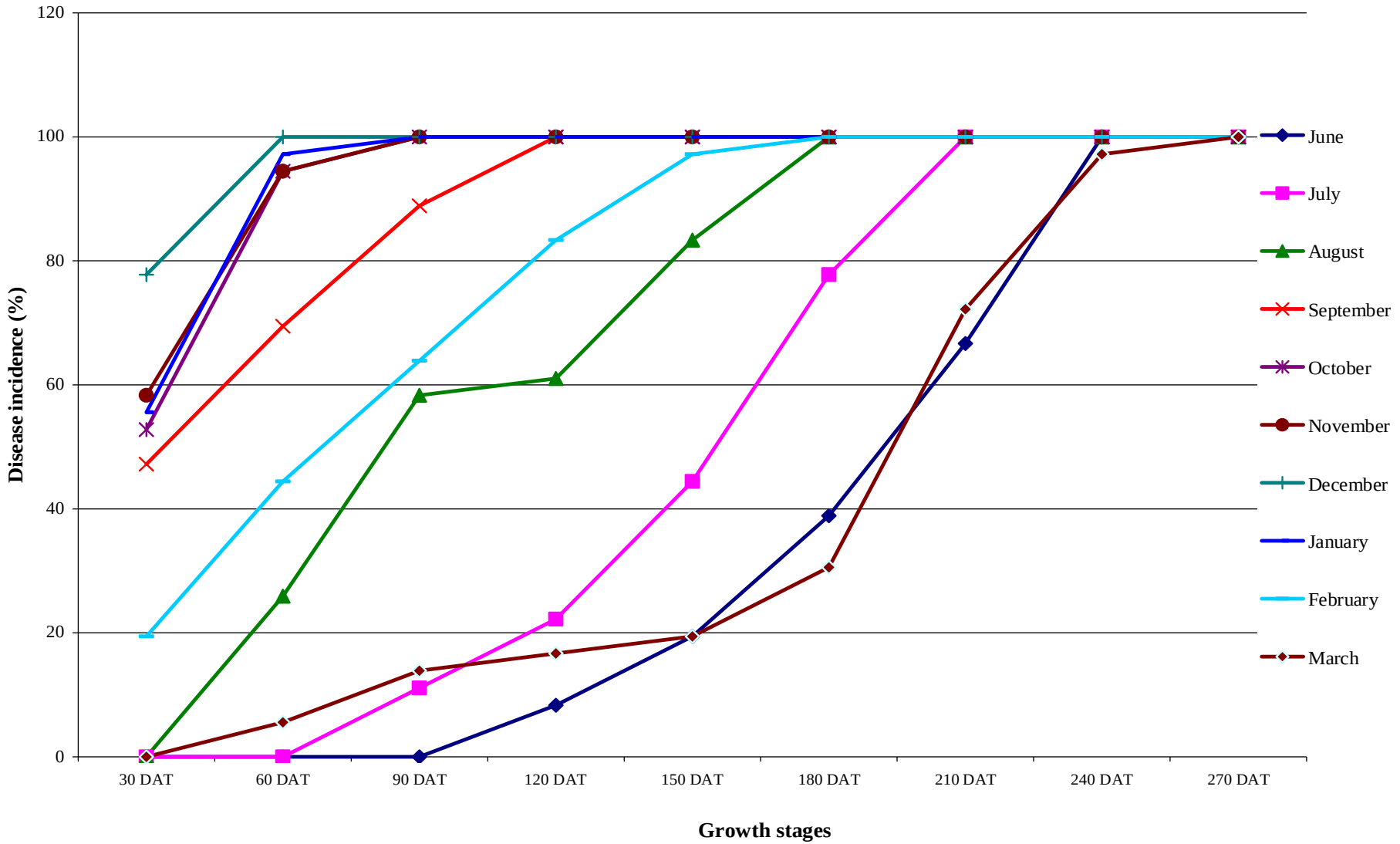

Fig. 10: Influence of different month of planting on disease incidence at different growth stages

Table 31: Influence of different month of planting on plant height at different crop growth stages

	Plant height (cm) at								
Treatment	**30 DAT***	**60 DAT**	**90 DAT**	**120 DAT**	**150 DAT**	**180 DAT**	**210 DAT**	**240 DAT**	**270 DAT**
T_1-1st week of June	38.13	71.11	90.86	120.86	156.27	181.36	215.97	221.77	224.37
T_2-1st week of July	37.39	69.94	89.46	118.83	154.91	179.30	214.36	216.50	218.91
T_3-1st week of August	37.52	69.80	89.41	118.94	154.08	178.33	187.50	196.41	206.25
T_4-1st week of September	38.27	69.93	89.91	118.41	141.00	164.47	175.55	186.11	194.91
T_5-1st week of October	38.75	58.97	78.61	97.97	119.20	138.00	143.52	156.58	171.11
T_6-1st week of November	38.66	58.86	77.30	96.77	118.69	137.86	143.00	154.02	162.77
T_7-1st week of December	34.73	40.33	57.56	78.22	86.72	94.38	119.30	125.36	132.91
T_8-1st week of January	33.39	38.90	55.73	77.36	85.72	93.38	114.69	123.72	132.13
T_9-1st week of February	37.80	61.77	80.77	99.72	125.76	144.77	165.12	176.12	180.36
T_{10}-1st week of March	40.50	74.75	94.66	125.77	161.94	186.83	221.91	226.37	227.19
S Em ±	0.37	0.69	0.53	0.98	0.91	1.01	17.50	0.99	1.15
C D @ 5 %	1.10	2.06	1.57	2.90	2.70	3.01	52.01	2.96	3.41

*DAT-Days After Transplanting

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to all other treatments at all growth stages. At 270 DAT March planting (227.19 cm) recorded significantly highest plant height than all the treatments but on par with June planting (224.37 cm) while least plant height was observed in January (132.13 cm) and December (132.91) planting.

Similarly, March planting at 30 DAT (5.52 cm) recorded highest internodal length than all other treatments. This treatment was significantly superior to all the treatments at all growth stages *viz.,* 60 DAT (6.33 cm), 90 DAT (7.38 cm), 120 DAT (10.35 cm), 150 DAT (10.30 cm), 180 DAT (9.14 cm), 210 DAT (6.91 cm) and 240 DAT (4.22 cm). The significantly least internodal length was recorded in January and December plantings at all the growth stages (Table 32).

In concern to plant girth, at 30 DAT in all the different months of plantings it ranged between 7.54 to 7.56 cm. As the growth stage of the plant increased the girth of the plant also increased in all the treatments and reached maximum. At 270 DAT, March planting (41.47 cm) recorded significantly highest plant girth followed by February (40.53 cm) and June (40.30 cm) plantings while January planting (36.19 cm) recorded the least plant girth (table 33).

The influence of different months of planting on number of leaves per plant was recorded at different time intervals. March planting has recorded significantly more number of leaves per plant at all the growth stages *i.e.* 6.80 at 30 DAT, 14.90 at 90 DAT, 20.12 at 120 DAT, 24.55 at 150 DAT, 25.52 at 180 DAT, 28.71 at 210 DAT, 28.94 at 270 DAT and 29.72 at 270 DAT (table 34).

4.2.1.3 Disease incidence on yield and yield parameters

Table 35 and 36 revealed that the treatments differed significantly with respect to yield per plant, yield per hectare, number of days taken for first flowering, number of days taken from flowering to first fruit set, number of days taken from flowering to first fruit set to harvest, number of fruits per plant, fruit diameter, fruit length, fruit breadth and fruit cavity diameter.

Number of days taken for first flowering recorded minimum in March planting (89.33) which was significantly superior to all other treatments. The next best treatments recorded are June (91.11) and July (91.56) plantings while it was maximum in

Table 32: Influence of different month of planting on plant internode length at different crop growth stages

	Internode length (cm) at								
Treatment	**30 DAT***	**60 DAT**	**90 DAT**	**120 DAT**	**150 DAT**	**180 DAT**	**210 DAT**	**240 DAT**	**270 DAT**
T_1-1st week of June	5.33	6.11	7.29	10.29	10.12	8.45	6.22	4.13	4.09
T_2-1st week of July	5.30	6.11	7.27	10.24	10.11	8.33	6.20	4.08	4.05
T_3-1st week of August	5.32	6.11	7.24	10.23	10.10	8.31	5.92	3.98	3.95
T_4-1st week of September	5.31	6.11	7.23	10.14	10.10	8.28	5.91	3.96	3.96
T_5-1st week of October	5.30	6.10	7.22	10.12	10.10	8.27	5.90	3.92	3.92
T_6-1st week of November	5.29	6.09	7.21	10.10	10.10	8.27	5.89	3.91	3.91
T_7-1st week of December	5.09	6.04	7.09	10.00	10.03	8.13	5.92	3.86	3.86
T_8-1st week of January	5.00	5.94	7.04	9.97	10.02	8.08	5.92	3.86	3.85
T_9-1st week of February	5.33	6.12	7.30	10.29	10.14	8.41	5.92	4.13	4.13
T_{10}-1st week of March	5.52	6.33	7.38	10.35	10.30	9.14	6.91	4.22	4.09
S Em ±	0.03	0.02	0.01	0.01	0.01	0.03	0.01	0.01	0.01
C D @ 5 %	0.10	0.05	0.04	0.04	0.04	0.10	0.03	0.04	0.04

*DAT-Days After Transplanting

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Table 33: Influence of different month of planting on plant girth at different crop growth stages

Plant girth (cm) at									
Treatment	**30 DAT***	**60 DAT**	**90 DAT**	**120 DAT**	**150 DAT**	**180 DAT**	**210 DAT**	**240 DAT**	**270 DAT**
T_1-1st week of June	7.55	15.33	25.56	35.58	37.85	39.22	40.30	40.30	40.30
T_2-1st week of July	7.56	15.30	25.44	35.39	37.21	38.58	39.66	39.66	39.66
T_3-1st week of August	7.55	15.28	25.27	35.35	37.11	38.49	39.56	39.56	39.56
T_4-1st week of September	7.56	15.25	25.17	34.95	36.76	38.13	39.21	39.21	39.21
T_5-1st week of October	7.55	15.11	24.91	34.06	36.37	37.75	38.79	38.79	38.79
T_6-1st week of November	7.54	15.08	23.61	33.96	36.27	37.65	38.73	38.73	38.73
T_7-1st week of December	7.55	15.06	23.34	33.09	34.47	35.38	36.19	36.19	36.19
T_8-1st week of January	7.55	14.83	22.15	32.50	33.71	34.42	34.57	34.57	34.57
T_9-1st week of February	7.57	15.33	25.74	35.76	38.07	39.45	40.53	40.53	40.53
T_{10}-1st week of March	7.56	15.35	26.59	36.61	39.02	40.39	41.47	41.47	41.47
S Em ±	0.01	0.02	0.10	0.21	0.19	0.21	0.20	0.20	0.20
C D @ 5 %	0.02	0.07	0.28	0.62	0.58	0.63	0.60	0.60	0.60

*DAT-Days After Transplanting

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Table 34: Influence of different month of planting on number of leaves per plant at different crop growth stages

Number of leaves per plant at									
Treatment	**30 DAT***	**60 DAT**	**90 DAT**	**120 DAT**	**150 DAT**	**180 DAT**	**210 DAT**	**240 DAT**	**270 DAT**
T_1-1st week of June	6.43	14.52	19.75	22.41	24.27	25.13	28.41	28.63	29.41
T_2-1st week of July	6.38	14.51	19.69	22.33	24.19	25.11	28.38	28.61	29.39
T_3-1st week of August	6.28	14.37	19.51	22.23	24.06	24.98	28.25	28.47	29.35
T_4-1st week of September	6.13	14.23	19.35	22.07	23.94	24.79	28.07	28.29	29.27
T_5-1st week of October	6.00	14.10	19.15	21.94	23.81	24.66	27.94	28.16	29.24
T_6-1st week of November	5.75	13.58	19.07	21.66	23.52	24.38	27.66	27.88	29.16
T_7-1st week of December	5.21	12.70	17.44	20.83	22.69	23.55	26.83	27.05	29.13
T_8-1st week of January	5.19	12.54	17.02	20.58	22.44	23.30	26.57	26.80	29.08
T_9-1st week of February	6.43	14.52	19.75	22.44	24.27	25.16	28.44	28.66	29.44
T_{10}-1st week of March	6.80	14.90	20.12	22.82	24.55	25.52	28.71	28.94	29.72
S Em ±	0.10	0.16	0.20	0.08	0.08	0.08	0.07	0.07	0.07
C D @ 5 %	0.30	0.46	0.60	0.23	0.23	0.23	0.22	0.22	0.22

*DAT-Days After Transplanting

(101.89) January planting (Table 35). Similarly, number of flowers per plant was significantly superior in March planting than all other plantings (Table 36)

Similarly, number of days taken from flowering to first fruit set was significantly least in March planting (91.97) followed by June (92.64) and July (94.14) planting while it was highest in January (99.39) followed by December (98.64) planting (Table 35).

Number of days taken for first fruit set to harvest recorded least in January (106.06) planting followed by December planting (108.72). The Maximum days taken for fruit set to harvest recorded in March planting (122.41) (Table 35).

With respect to number of fruits per plant, March (25.92) planting was significantly superior over other treatments. Further June (24.67) and July (23.33) plantings were the next best treatments, while least numbers of fruits were recorded in January (7.86) and December (8.42) plantings (Table 35).

Fruit diameter was significantly highest in March planting (12.31 cm) which was on par with June (12.27 cm) planting while January plantings (8.14 cm) recorded the least. Fruit length was significantly highest in March (29.92 cm) planting compared to all the other treatments except June (29.72 cm) planting which was on par. Least length of the fruit was recorded in January (19.75 cm) followed by December (20.47 cm) planting (Table 35).

Fruit breadth was highest in March (14.63 cm) planting which was on par with June (14.59 cm) planting while it was least in January (9.14 cm) followed by December (9.70 cm) planting. March (6.97 cm) planted fruits were having a bigger cavity diameter which were on par with June (6.90 cm) planting. Smaller cavity diameter was recorded in January (5.91 cm) planting followed by December (6.03 cm) planting (Table 35).

Yield per plant was recorded significantly highest in March (60.12 kg) planting compared to all the treatments. The planting at June (55.46 kg) and July (51.23 kg) months were next best while it was least in January (13.84 kg) planting followed by December (15.40 kg) plantings (Table 35).

Table 35: Influence of different month of planting on yield and yield parameters

Treatment	No. of days taken for first flowering	No. of days taken from flowering to first fruit set	No. of days taken for first fruit set to harvest	No. of fruits per plant	Fruit diameter (cm)	Fruit length (cm)	Fruit breadth (cm)	Fruit cavity diameter (cm)	Yield per plant (kg)	Yield per hectare (t)
T_1-1st week of June	91.11	92.64	121.14	24.67	12.27	29.72	14.59	6.90	55.46	171.15
T_2-1st week of July	91.56	93.97	120.64	23.33	11.89	27.78	14.12	6.56	51.23	158.09
T_3-1st week of August	92.75	94.14	120.72	20.83	11.28	26.97	12.62	6.44	48.20	148.75
T_4-1st week of September	93.56	95.81	117.89	17.28	11.16	24.50	12.53	6.43	41.20	127.16
T_5-1st week of October	94.39	96.86	112.03	12.78	10.33	23.00	10.75	6.32	25.30	78.08
T_6-1st week of November	94.72	97.03	110.64	12.47	10.44	22.33	10.19	6.77	23.82	73.51
T_7-1st week of December	98.58	98.64	108.72	8.42	8.52	20.47	9.70	6.03	15.40	47.53
T_8-1st week of January	101.89	99.39	106.06	7.86	8.14	19.75	9.14	5.91	13.84	42.70
T_9-1st week of February	92.06	94.58	120.64	19.47	11.23	24.97	12.63	6.38	43.76	135.04
T_{10}-1st week of March	89.33	91.97	122.41	25.92	12.31	29.92	14.63	6.97	60.12	185.54
S Em ±	0.11	0.14	0.11	0.12	0.10	0.29	0.07	0.11	0.24	0.74
C D @ 5 %	0.31	0.42	0.33	0.36	0.29	0.86	0.21	0.32	0.71	2.19

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Table 36: Influence of different month of planting on number of flowers per plant at different crop growth stages

	Number of flowers per plant at						
Treatment	**150 DAT***	**180 DAT**	**210 DAT**	**240 DAT**	**270 DAT**	**300 DAT**	**330 DAT**
T_1-1st week of June	4.41	19.39	84.25	72.44	48.86	20.86	18.86
T_2-1st week of July	4.39	18.86	83.91	72.22	47.55	19.55	17.63
T_3-1st week of August	4.30	17.08	83.08	71.54	46.63	18.66	16.91
T_4-1st week of September	4.27	16.55	80.94	70.75	44.00	15.97	14.22
T_5-1st week of October	4.16	15.94	78.66	70.08	42.36	14.38	12.47
T_6-1st week of November	4.05	14.02	77.38	65.47	41.66	13.69	11.86
T_7-1st week of December	3.89	13.94	76.77	53.38	39.64	11.64	9.89
T_8-1st week of January	3.11	11.11	74.91	47.02	33.94	6.94	5.19
T_9-1st week of February	4.44	19.55	81.36	72.64	48.94	20.91	19.16
T_{10}-1st week of March	4.89	20.02	86.38	74.11	51.66	24.13	22.13
S Em ±	0.08	0.19	0.79	0.47	0.50	0.50	0.47
C D @ 5 %	0.23	0.58	2.34	1.40	1.49	1.48	1.39

*DAT-Days After Transplanting

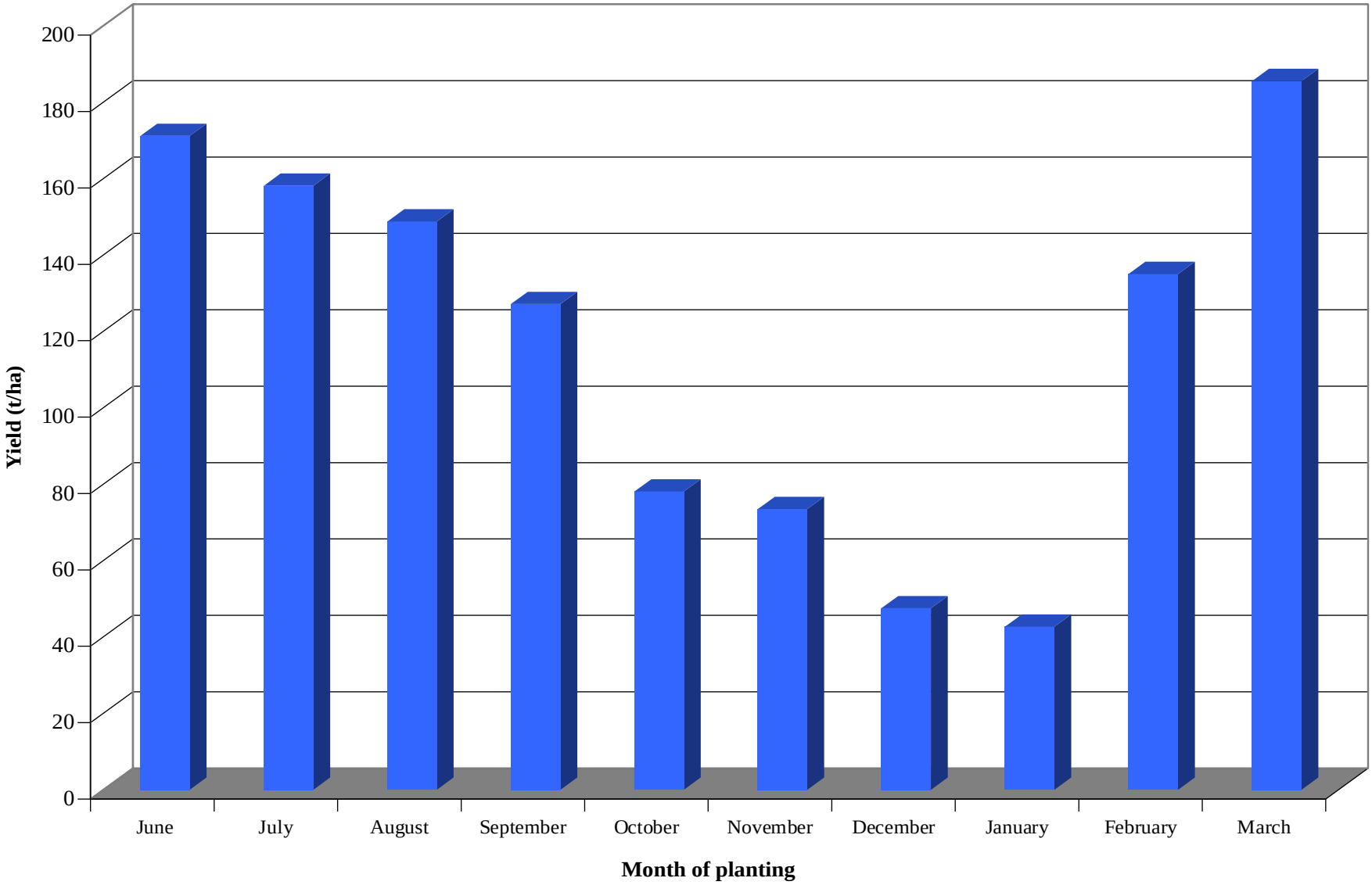

Fig. 11: Influence of different month of planting on yield of papaya

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Plate 18: Fruits on papaya plants planted at different months

Concerning yield per hectare, March planting (185.54 t) recorded the maximum yield and was significantly superior to all the treatments followed by June (171.15 t) and July (158.09 t) plantings, while minimum yield was recorded in January plantings (42.70 t) followed by December (47.53 t) planting (Table 35 and Fig. 11).

4.2.1.4 Aphid population during crop growth stage at monthly intervals

The number of aphids were counted weekly (appendix IV-C) and cumulative population was calculated at monthly interval (Table 37). The June, July and August plantings recorded zero infestation of aphid at 30 DAT. Whereas, planting did at September (30), October (94), November (111), December (192), January (344), February (329) and March (31) recorded moderate aphid population at 30 DAT. The June, July and August plantings recorded least aphid population up to 150 DAT, after which there was drastic increase. Whereas, September to February planting had high initial aphid activity, but after 150 DAT there was least aphid activity. March planting has recorded initial aphid load, but cumulative aphid population at each growth stage was least than all other month of plantings.

4.2.1.5 Correlation studies between disease incidence with aphid population, growth, yield and yield parameters at different crop growth stages

The correlation coefficient between PRSV disease incidence, aphid population, yield per plant, number of leaves per plant, plant girth, internodal length, plant height, days to first flowering, days from flowering to first fruit set, days from fruit set to harvest, fruits per plant, fruit diameter, fruit length, fruit breadth, fruit cavity diameter and number of flowers per plant were analyzed for different growth stages of the papaya *viz.*, 30, 60, 90, 120, 150, 180, 210 and 240 DAT. Data presented in Table 38.

Table 38 revealed that at 30 DAT disease incidence had a positive correlation with the aphid population (0.501) but was nonsignificant. Whereas remaining growth and yield attributes are negatively correlated *i.e.* yield per plant (-0.929), number of leaves per plant (-0.863), internodal length (-0.672), days to fruit set to harvest (-0.899), fruits per plant (-0.940), fruit length (-0.844), fruit diameter (-0.925), fruit breadth (-0.900), fruit cavity diameter (-0.634). While days to first flowering (0.786)

Table 37: Monthly aphid population (cumulative) at different crop growth stages

Treatment	Aphid population (cumulative) after									
	30 DAT*	**60 DAT**	**90 DAT**	**120 DAT**	**150 DAT**	**180 DAT**	**210 DAT**	**240 DAT**	**270 DAT**	**300 DAT**
T_1-1st week of June	0	0	0	30	124	235	427	771	1100	1131
T_2-1st week of July	0	0	30	124	235	427	771	1100	1131	1139
T_3-1st week of August	0	30	124	235	427	771	1100	1131	1139	1145
T_4-1st week of September	30	124	235	427	771	1100	1131	1139	1145	1148
T_5-1st week of October	94	205	397	741	1070	1101	1109	1115	1118	1119
T_6-1st week of November	111	303	647	976	1007	1015	1021	1024	1025	1027
T_7-1st week of December	192	536	865	896	904	910	913	914	916	930
T_8-1st week of January	344	673	704	712	718	721	722	724	738	833
T_9-1st week of February	329	360	368	374	377	378	380	394	489	617
T_{10}-1st week of March	31	39	45	48	49	51	65	160	288	490

Note: Cumulative aphid population is presented at different time period, *DAT-Days After Transplanting

Table 38: Correlation studies between disease incidence with aphid population, growth, yield and yield parameters at different crop growth stages

Disease incidence (%) at	Aphid population	Plant height (cm) at 270 DAT***	Internodal length (cm) at 270 DAT	Plant girth (cm) at 270 DAT	No. of leaves per plant at 270 DAT	No. of flowers at 270 DAT	Days to first flowering	Days from flowering to first fruit set	Days for fruit set to harvest	Fruits per plant	Fruit diameter (cm)	Fruit length (cm)	Fruit breadth (cm)	Fruit cavity diameter (cm)	Yield per plant (t)
30 DAT	0.501^{NS}	-0.489^{NS}	-0.672^{*}	-0.515^{NS}	-0.863^{**}	-	0.786^{**}	0.916^{**}	-0.899^{**}	-0.940^{**}	-0.844^{**}	-0.925^{**}	-0.900^{**}	-0.634^{*}	-0.929^{**}
60 DAT	0.786^{**}	-0.792^{**}	-0.610^{NS}	-0.819^{**}	-0.797^{**}	-	0.803^{**}	0.933^{**}	-0.907^{**}	-0.962^{**}	-0.856^{**}	-0.954^{**}	-0.953^{**}	-0.672^{*}	-0.949^{**}
90 DAT	0.838^{**}	-0.694^{*}	-0.708^{*}	-0.711^{*}	-0.664^{*}	-	0.745^{*}	0.886^{**}	-0.808^{**}	-0.911^{**}	-0.800^{**}	-0.923^{**}	-0.920^{**}	-0.695^{*}	-0.883^{**}
120 DAT	0.881^{**}	-0.743^{*}	-0.770^{**}	-0.731^{*}	-0.671^{*}	-	0.706^{*}	0.861^{**}	-0.755^{*}	-0.879^{**}	-0.760^{*}	-0.912^{**}	-0.879^{**}	-0.700^{*}	-0.849^{**}
150 DAT	0.851^{**}	-0.726^{*}	-0.640^{*}	-0.644^{*}	-0.607^{NS}	-0.604^{NS}	0.662^{*}	0.813^{**}	-0.665^{*}	-0.819^{**}	-0.711^{*}	-0.869^{**}	-0.833^{**}	-0.734^{*}	-0.788^{**}
180 DAT	0.811^{**}	-0.603^{NS}	-0.805^{**}	-0.586^{NS}	-0.557^{NS}	-0.624^{NS}	0.604^{NS}	0.748^{*}	-0.568^{NS}	-0.727^{*}	-0.635^{*}	-0.789^{**}	-0.747^{*}	-0.740^{*}	-0.703^{*}
210 DAT	0.718^{*}	-0.641^{*}	-0.756^{*}	-0.490^{NS}	-0.457^{NS}	-0.625^{NS}	0.519^{NS}	0.672^{*}	-0.485^{NS}	-0.629^{NS}	-0.556^{NS}	-0.707^{*}	-0.648^{*}	-0.700^{*}	-0.612^{NS}
240 DAT	0.712^{*}	-0.446^{NS}	-0.595^{NS}	-0.437^{NS}	-0.391^{NS}	-0.271^{NS}	0.439^{NS}	0.502^{NS}	-0.365^{NS}	-0.458^{NS}	-0.378^{NS}	-0.484^{NS}	-0.438^{NS}	-0.508^{NS}	-0.463^{NS}

* indicate significant at 5 per cent probability level, **indicate significant at 1 per cent probability level, ***DAT-Days After Transplanting, NS- Non Significant

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and days from flowering to first fruit set (0.916) recorded a strong positive correlation.

Disease incidence at 60 DAT had a significantly strong positive correlation with aphid population (0.786), days from flowering to first flowering (0.803) and flowering to first fruit set (0.933). While remaining attributes had a strong negative correlation with yield per plant (-0.949), number of leaves per plant (-0.797), plant girth (-819), plant height (-0.792), days from fruit set to harvest (-0.907), fruits per plant (-0.962), fruit length (-0.856), fruit diameter (-0.954), fruit breadth (-0.953) and fruit cavity diameter (-0.672).

At 90 DAT, disease incidence had significantly correlated with all the traits. The aphid population (0.838), days to first flowering (0.803) and flowering to first fruit set (0.933) had a strong positive correlation. Fruit yield (-0.883), number of leaves per plant (-0.664), plant girth (-711), internodal length (-0.708), plant height (-0.694), days to fruit set to harvest (-0.886), fruits per plant (-0.911), fruit length (-0.800), fruit diameter (-0.923), fruit breadth (-0.920) and fruit cavity diameter (-0.695) are significantly and negatively correlated to disease incidence.

At 120 DAT, disease incidence had a significantly strong positive correlation with aphid population (0.881) along with days to first flowering (0.745) and flowering to first fruit set (0.886) at the stage while fruit yield (-0.849), number of leaves per plant (-0.671), plant girth (-731), internodal length (-0.770), plant height (-0.743), days from fruit set to harvest (-0.755), fruits per plant (-0.879), fruit length (-0.760), fruit diameter (-0.912), fruit breadth (-0.879) and fruit cavity diameter (-0.700) had a significantly negative correlation.

Disease incidence at 150 DAT recorded the significant positive correlation with aphid population (0.851), days to first flowering (0.662) and flowering to first fruit set (0.813) while fruit yield (-0.788), plant girth (-644), internodal length (-0.640), plant height (-0.726), days from fruit set to harvest (-0.665), fruits per plant (-0.962), fruit length (-0.856), fruit diameter (-0.954), fruit breadth (-0.953) and fruit cavity diameter (-0.672) had a significantly negative correlation. The number of leaves per plant (0.607) and number of flowers per plant (0.604) though positive there were nonsignificant.

Disease incidence at 180 DAT recorded a significant and positive correlation with the aphid population (0.811) and number of fruits set (0.748). The fruit yield (-703), internodal length (-805), fruits per plant (-0.727), fruit length (-0.635), fruit diameter (-0.789), fruit breadth (-0.747), fruit cavity diameter (-0.740) are recorded negative correlation while other traits were nonsignificant to disease incidence.

Disease incidence still at the stage of 210 and 240 DAT had a significantly positive correlation with the aphid population *i.e.* 0.718 and 0.712 respectively. While most of the other attributes were nonsignificant with disease incidence at 210 and 240 DAT.

4.2.2 Identification of susceptible stage of papaya for PRSV infection

To find out susceptible growth stage of papaya proned to infection of PRSV, the plants were inoculated through aphids at different growth stages *viz.,* 30, 60, 90, 120, 150, 180, 210, 240 and 270 DAT and observations ware recorded as explained in the material and methods. The data on inoculation studies are presented in table 39 to 42, Plate 19 (B and C) and Plate 20.

4.2.2.1 Per cent transmission and days taken for symptom expression

Table 39 revealed that PRSV inoculation at 30 and 60 days after transplanting has recorded early symptomatic expression *i.e.* 15th day after inoculation with 100 per cent transmission. The plants inoculated at 60 DAT also expressed 100 per cent transmission, but taken 17 days for symptom expression. This is followed by inoculation at 120, 150 and 180 DAT which recorded a decrease in transmission rate (75.00, 58.00 and 25.00 % respectively) and there was delay in express symptoms (20th, 24th and 25th days after inoculation respectively). The plants inoculated at 210, 240 and 270 DAT recorded the least rate of transmission (8.33 %) and there was further delay in expression of symptoms *i.e.* 25th, 26th and 28th days after inoculation respectively.

Concerning to type of symptomatic expression, plants inoculated at 30, 60 and 90 DAT have recorded the most severe symptoms like vein clearing, mosaic, leaf reduction, leaf distortion, shoe string, stunted growth, mottling, chlorotic ring and chlorotic spots on fruits. Inoculation done at 120 and 150 DAT has recorded vein

Plate 19: Overview of the experimental plot on effect of different months planting (A) and Identification of susceptible stage of papaya for PRSV infection (B & C)

Table 39: Effect of challenge inoculation of PRSV at different crop growth stages of papaya on percent transmission and symptoms

Treatment (Stage of inoculation)	No. of Plant		Per cent transmission (%)	Days for symptom expression after inoculation	Symptoms
	Inoculated	Infected			
T_1-30 DAT**	12	12	100 (90.00)*	15	VC, MS, LR, LD, SS, SG, MO, CR, CS
T_2-60 DAT	12	12	100 (90.00)	15	VC, MS, LR, LD, SS, SG, MO, CR, CS
T_3-90 DAT	12	12	100 (90.00)	17	VC, MS, LR, LD, SS, SG, MO, CR, CS
T_4-120 DAT	12	9	75.00 (60.00)	20	VC, MS, LR, LD, SS, CR ,CS
T_5-150 DAT	12	7	58.33 (50.00)	24	VC, MS, LR, LD, SS, CR, CS
T_6-180 DAT	12	3	25.00 (30.00)	25	VC, MS, LR, LD,
T_7-210 DAT	12	1	8.33 (10.00)	25	VC, MS
T_8-240 DAT	12	1	8.33 (10.00)	26	VC, MS
T_9-270 DAT	12	1	8.33 (10.00)	28	VC
T_{10}-Un inoculated	0	0	0.00 0.00)	0	-
S Em ±			5.85		
C D @ 5 %			17.37		

* Figures in parentheses are arc sine transformed values**,** **DAT: Days After Transplanting, VC-Vein Clearing; MS-Mosaic Symptom; LR-Leaf Reduction, LD-Leaf Distortion; SS-Shoe String; SG-Stunted Growth; MO-Mottling; CR-Chlorotic Ring and CS-Chlorotic Spots on fruits.

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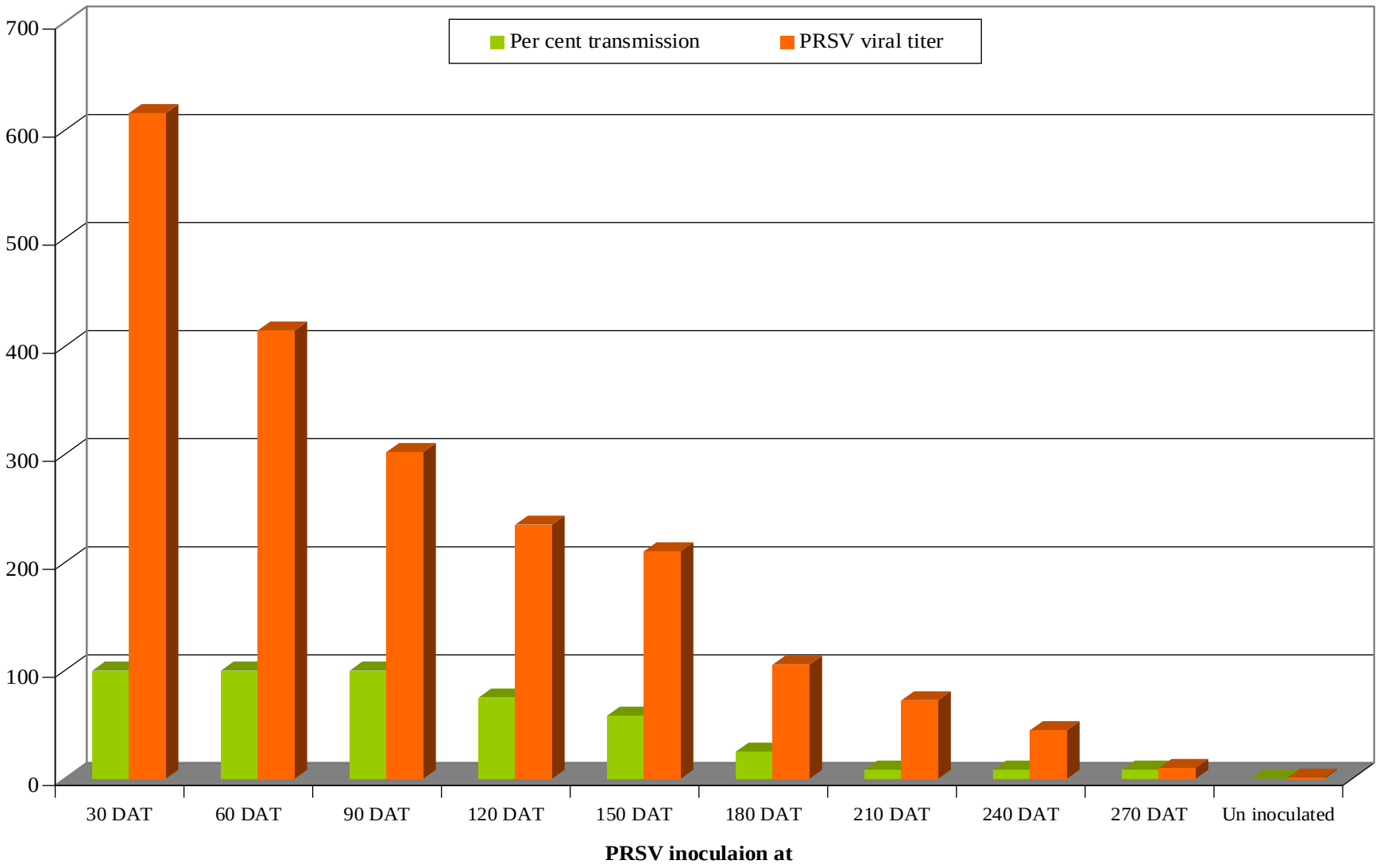

Fig. 12: Effect of artificial inoculation of PRSV at different growth stages of papaya on per cent transmission and viral titer

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Plate 20: Different symptoms expresses by papaya plants after artificial inoculation with PRSV

A). Vein Clearing, B). Mosaic Symptom, C). Leaf Reduction, D). Leaf Distortion, E). Shoe String, F). Stunted Growth, G). Mottling, H). Chlorotic Ring and I). Chlorotic Spot on fruits

clearing, mosaic, leaf reduction, leaf distortion, shoe string, stunted growth, mottling and chlorotic spots on fruits. The plants inoculated at 180 DAT recorded only vein clearing, mosaic, leaf reduction, leaf distortion. Delayed inoculation at 210 and 240 DAT recorded reduced rate of severity with expression of only vein clearing and mosaic symptoms on leaves, while inoculation at 270 DAT recorded only vein clearing (Plate 20 and Fig. 12).

4.2.2.2 Quantification of viral load in inoculated papaya plants by qRT-PCR

Challenge inoculation of PRSV at different crop growth stages *viz.*, 30, 60, 90, 120, 150, 180, 210, 240 and 270 DAT was made and quantification of viral titer present in plants was recorded after 15 days of inoculation using qRT-PCR assay. Data are presented in table 40 and melting curve and amplification plot of TATA-binding protein-2 (house keeping gene) and PRSV in qRT-PCR given in fig. 13, 14 and fig. 15.

Plants inoculated at early growth stage *i.e.* 30 DAT have recorded a high quantity of viral titer *i.e.* 615.97. Inoculation done at 60 DAT recorded 414.78 viral titer. Delay in the stage of inoculation recorded the decreased amount of viral titer in plants *i.e.* inoculation at 90, 120, 150 and 180 DAT recorded viral titer of 302.36, 235.09, 210.42 and 105.65 respectively. Further delay in inoculation recorded a low amount of viral titer *i.e.* 72.58 at 210 DAT, 44.89 at 240 DAT while least quantity of viral titer (9.86) was recorded in plants inoculated at 270 DAT.

4.2.2.3 Challenge inoculation of PRSV at different growth stages on growth and yield parameters of papaya

The effect of challenge inoculation of PRSV at different growth stages of papaya on growth, yield and yield parameters was recorded. Data presented in table 41 and fig. 16.

Table 41 revealed that the treatments differed significantly with respect to fruit yield per plant, fruit yield per hectare, plant height, plant internode length, plants girth, number of leaves per plant, number of days taken for first flowering, number of flowers per plant, number of days taken from flowering to first fruit set, number of

Table 40: Quantification of PRSV viral load in papaya plants challenge inoculated at different crop growth stages

Treatment (Stage of inoculation)	Average Ct mean ±S.E.		PRSV viral titer at 15 DAI**
	TATA-binding protein-2 (House keeping gene)	PRSV	
T_1-30 DAT*	17.91 ± 0.07	23.21 ± 0.14	615.97
T_2-60 DAT	17.94 ± 0.16	23.81 ± 0.20	414.78
T_3-90 DAT	17.73 ± 0.47	24.06 ± 0.14	302.36
T_4-120 DAT	17.89 ± 0.29	24.58 ± 0.38	235.09
T_5-150 DAT	18.26 ± 0.62	25.11 ± 0.21	210.42
T_6-180 DAT	18.03 ± 0.26	25.88 ± 0.33	105.65
T_7-210 DAT	17.85 ± 0.51	26.23 ± 0.29	72.58
T_8-240 DAT	18.16 ± 0.21	27.25 ± 0.38	44.89
T_9-270 DAT	18.05 ± 0.25	29.32 ± 0.41	9.86
T_{10}-Un inoculated	18.15 ± 0.27	32.72 ± 0.35	1.00

*DAT: Days After Transplanting, **DAI: Days After Inoculation, Ct =Cycle threshold

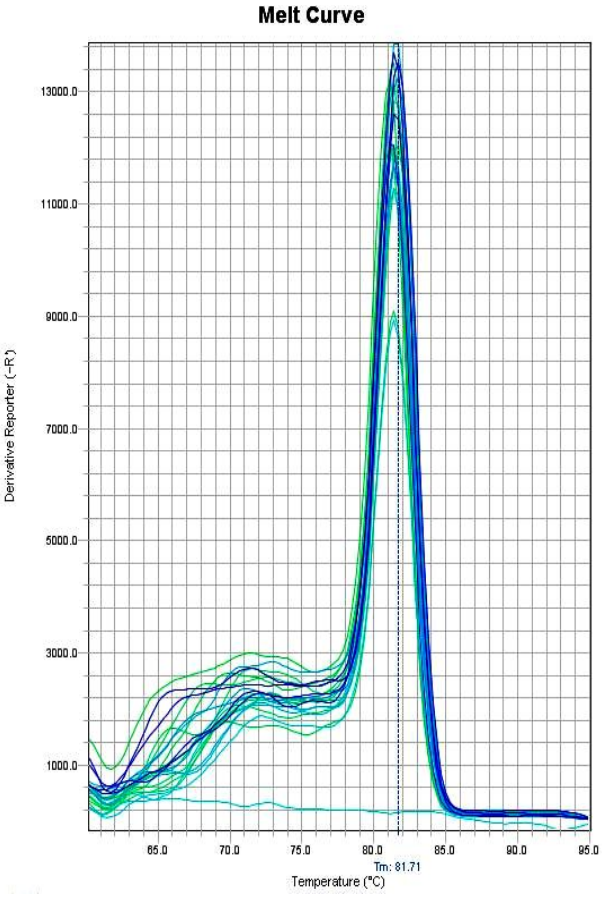

Fig. 13: Melt curve of (qRT-PCR) TATA-binding protein-2 (House keeping gene)

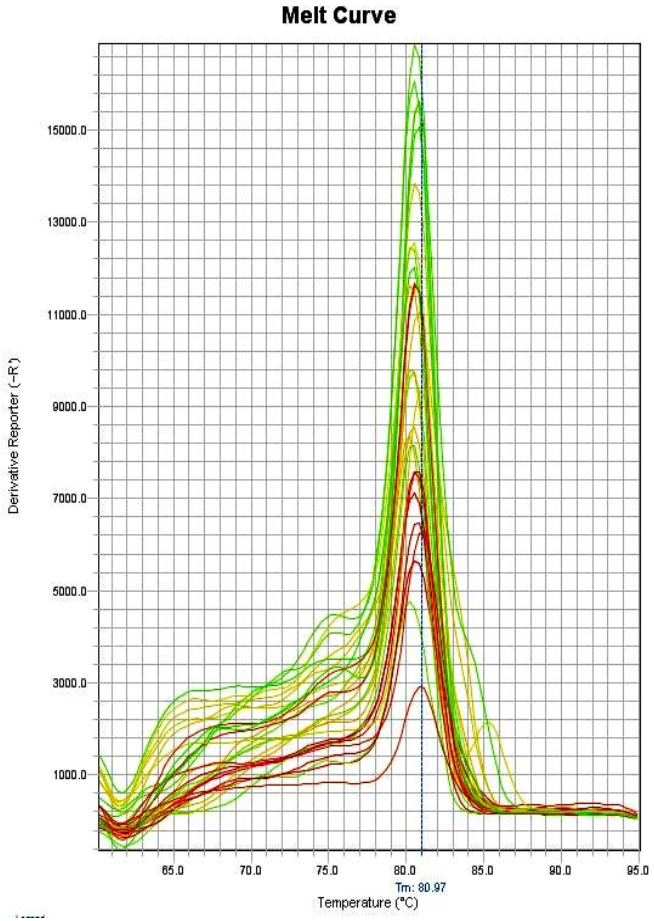

Fig. 14: Melt curve of PRSV viral titer (qRT-PCR) from papaya plants inoculated at months

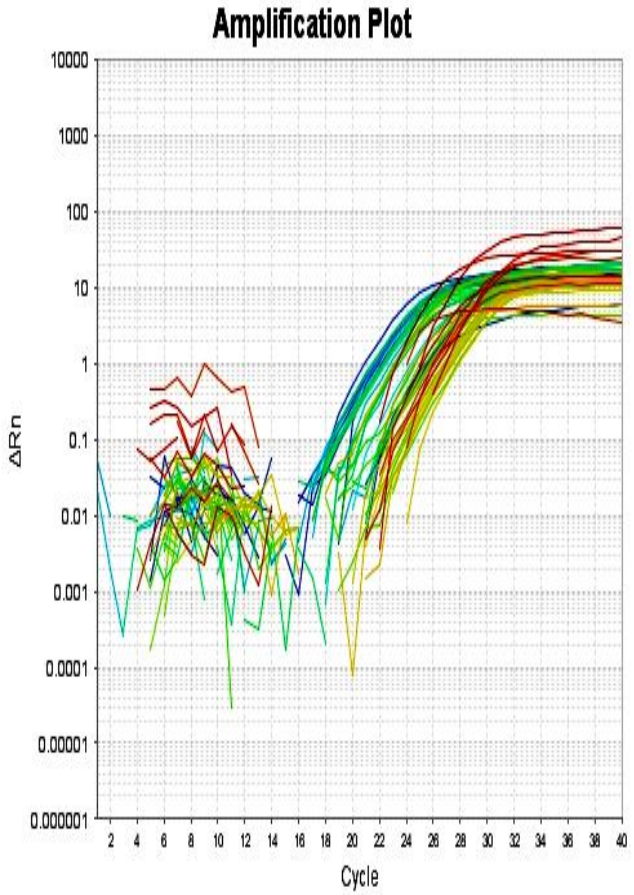

Fig. 15: Amplification plot (qRT-PCR) of TATA-binding protein-2 (House keeping gene) and PRSV viral titer from papaya plants inoculated at months

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Table 41: Effect of challenge inoculation of PRSV at different growth stages on growth, yield and yield parameters of papaya

Treatment (Stage of inoculation)	Plant height (cm) at 270 DAT*	Plant internode length (cm) at 270 DAT	Plants girth (cm) at 270 DAT	No. of leaves per plant at 270 DAT	No. of Days taken for First flowering	No. of flowers per plant at 270 DAT	No. of days taken from flowering to first fruit set	No. of days taken to first fruit set to harvest	No. of fruits per plant	Fruit diameter (cm)	Fruit length (cm)	Fruit breadth (cm)	Fruit cavity diameter (cm)	Yield per plant (kg)	Yield per hectare (t)
T_1-30 DAT*	145.69	4.03	33.74	28.70	105.04	9.21	91.56	102.86	1.75	8.95	21.21	9.73	6.11	1.92	5.94
T_2-60 DAT	168.58	4.04	35.70	28.83	101.71	13.91	90.72	105.64	1.92	9.33	21.93	10.30	6.41	2.22	6.84
T_3-90 DAT	193.25	4.09	36.90	29.86	97.88	15.96	89.11	107.56	3.58	11.13	23.79	10.79	6.51	5.06	15.62
T_4-120 DAT	194.91	4.10	40.96	29.61	97.57	16.65	88.94	108.94	10.86	11.24	24.46	11.35	6.63	19.43	59.96
T_5-150 DAT	219.52	4.14	42.51	30.64	96.74	18.24	87.89	114.81	21.72	12.08	25.96	13.13	6.64	29.12	89.86
T_6-180 DAT	230.44	4.13	42.73	31.39	95.93	20.93	86.89	117.64	33.58	12.69	28.43	13.22	6.76	46.66	144.00
T_7-210 DAT	236.25	4.13	42.70	31.75	95.18	21.82	86.67	117.72	35.33	12.93	29.24	14.71	7.10	54.68	168.75
T_8-240 DAT	239.79	4.22	42.47	32.11	94.63	23.13	86.06	119.31	37.03	13.03	31.18	15.16	7.20	57.52	177.51
T_9-270 DAT	241.72	4.26	42.70	32.14	94.24	23.18	85.75	119.17	37.67	13.07	31.57	15.28	7.25	58.64	180.95
T_{10}-Un inoculated	242.07	4.26	43.64	32.13	93.68	23.49	85.81	119.72	38.50	13.07	31.78	15.18	7.30	59.39	183.28
S Em ±	0.78	0.01	0.15	0.19	0.14	0.49	0.04	0.09	0.25	0.09	0.22	0.08	0.04	0.96	2.97
C D @ 5 %	2.32	0.04	0.45	0.56	0.43	1.46	0.12	0.28	0.76	0.27	0.65	0.23	0.12	2.86	8.83

*DAT-Days After Transplanting

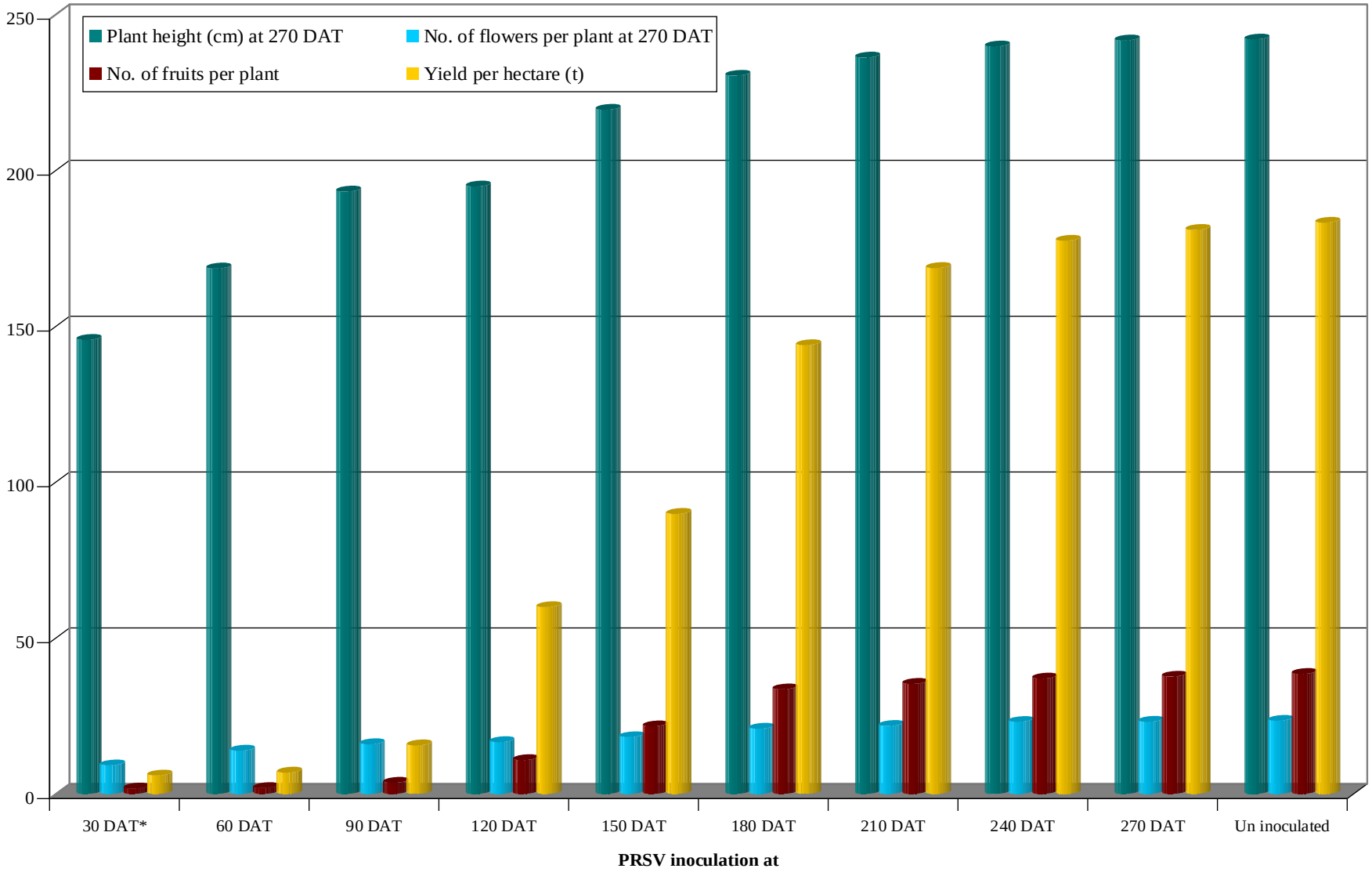

Fig. 16: Effect of artificial inoculation of PRSV at different growth stages of papaya on growth, yield and yield parameters

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days taken from first fruit set to harvest, number of fruits per plant, fruit diameter, fruit length, fruit breadth and fruit cavity diameter.

There was a linear increase in number of flowers per plant, number of days to first fruit set to harvest, number of fruits per plant, fruit diameter, fruit length, fruit breadth, fruit cavity diameter and yield when there is delay in inoculation. Whereas, linear decrease was observed in number of days to first flowering and flowering to first fruit set.

Plant height was recorded significantly highest in uninoculated control plants (242.07 cm) which was on par with plants inoculated 270 DAT (241.72 cm). Plants inoculated at 240 DAT recorded 239.79 cm followed by 210 DAT (236.25 cm). Lowest plant height was recorded in early inoculated plants *i.e.* 30 DAT (145.69 cm) followed by 60 DAT (168.58 cm).

Internodal length was recorded at 270 DAT. Plants inoculated at 30 DAT (4.03 cm) had shorter internodal length which was on par with 60 DAT (4.04 cm) followed by 90 DAT (4.09 cm). As there was delay in the stage of inoculation, longer internodal lengths was recorded. The highest internodal length was recorded in control plants (4.26 cm) and which was on par with 270 DAT (4.26 cm) and 240 DAT (4.22 cm).

Plant girth in control plants (43.64 cm) was significantly highest. This is followed by inoculation at 270 DAT (42.70 cm) and 240 DAT (42.47 cm). Least plant girth was recorded in plants inoculated at 30 DAT (33.74 cm) is followed by 60 DAT (35.70 cm).

Significantly highest numbers of leaves were recorded in plants inoculated at 270 DAT (32.14) which was on par with control plants (32.13) and 240 DAT (32.11). The least number of leaves were recorded in plants inoculated at 30 DAT (28.70) which were on par with 60 DAT (28.83).

Number of days taken for first flowering were significantly highest in early inoculated plants *i.e.* at 30 DAT (105.04) followed by 60 DAT (101.71). The number of days taken for first flowering was least in uninoculated control (93.68) which was followed by 270 DAT (94.24).

Number of flowers per plant at 270 days was significantly highest in uninoculated control plants (23.49) which were on par with plants inoculated at 270 DAT (23.18) and 210 DAT (23.13). Significantly fewer flowers were recorded in 30 DAT (9.21) followed by 60 DAT (13.91) and 90 DAT (15.96).

Number of days taken from flowering to first fruit set was significantly least in control plants (85.81) compared to all the other treatments except 270 DAT (85.75). While it was highest at 30 DAT (91.56).

Early inoculated plants *i.e.* 30 DAT (102.86) have taken the least number of days from first fruit set to harvest followed by 60 DAT (105.64) and 90 DAT (107.56). Significantly maximum number of days was recorded in uninoculated control (119.72) over other treatments. Maximum number of fruits per plant was recorded in uninoculated plants (38.50). The plants inoculated at 270 DAT (37.67) and 240 DAT (37.03) were the next best treatments. The number of fruits was least in 30 DAT (1.75) which is on par with 60 DAT (1.92).

Fruit diameter was superior in control plants (13.07 cm) which was on par with plants inoculated at 270 DAT (13.07 cm) and 240 DAT (13.03 cm). Least fruit diameter was recorded in plants inoculated at 30 DAT (8.95 cm) followed by 60 DAT (9.33 cm). Similarly, the length of the fruit was significantly maximum in the control plants (31.78 cm) compared to other treatments except inoculated at 270 DAT (31.57 cm) and 240 DAT (31.18 cm). The least fruit length was recorded in plants inoculated at 30 DAT (21.21 cm) followed by 60 DAT (21.93 cm).

Least fruit breadth was recorded in plants inoculated at 30 DAT (9.73 cm) followed by 60 DAT (10.30 cm). Maximum fruit breadth was recorded in 270 DAT (15.28 cm) which was on par with uninoculated control (15.18 cm). Similarly, fruit cavity diameter was highest in control plants (7.30 cm) which was on par with 270 DAT (7.25 cm) and 240 DAT (7.20 cm). The least cavity diameter was recorded in plants inoculated at 30 DAT (6.11 cm) followed by 60 DAT (6.41 cm).

The highest fruit yield per plant was recorded in uninoculated control (59.39 kg) which was on par with plants inoculated at 270 DAT (58.64 kg) and 240 DAT (57.52 kg). The next best treatments were 210 DAT (54.68 kg) followed by 180 DAT

(46.66 kg). The least yield per plant was recorded in plants inoculated at 30 DAT (1.92 kg) which was on par with 60 DAT (2.22 kg).

The uninoculated plants (183.28 t) recorded the highest fruit yield per hectare and were on par with plants inoculated at 270 DAT (180.95 t) and 240 DAT (177.51 t). The plants inoculated at 210 DAT (168.75 t) were the next best followed by 180 DAT (144.00 t). The least yield per hectare was recorded in plants inoculated at 30 DAT (5.94 t) and 60 DAT (6.84 t).

4.2.2.4 Correlation studies between papaya ringspot viral load in host with per cent transmission, growth and yield parameters of papaya

The correlation coefficient between papaya ringspot viral load in host with per cent transmission, fruit yield per hectare, number of leaves per plant, plant girth, plant internode length, plant height, number of days taken for first flowering, number of flowers per plant, number of days taken from flowering to first fruit set, number of days taken to first fruit set to harvest, number of fruits per plant, fruit diameter, fruit length, fruit breadth and fruit cavity diameter was analyzed and presented in table 42.

Table 42 revealed that the quantity of papaya ringspot viral load had a strong positive correlation to per cent transmission (0.917) with high significance. All the growth attributes like number of leaves per plant (-0.947 and -0.962), plants girth at 270 days (-0.943 and -0.900), plant internode length (-0.882 and -0.881) and plant height (-0.985 and -0.928) recorded highly significant negative correlation with viral load and per cent transmission respectively.

Similarly, all the flower and fruit parameters like number of flowers per plant (-0.994 and -0.934), number of days taken from first fruit set to harvest (-0.957 and -0.963), number of fruits per plant (-0.917 and -0.986), fruit diameter (-0.973 and -0.918), fruit length (-0.948 and -0.973), fruit breadth (-0.936 and -0.978) and fruit cavity diameter (-0.953 and -0.949) recorded highly significant negative correlation with viral load and per cent transmission respectively. While number of days taken for first flowering (0.988 and 0.866) and number of days taken from flowering to first fruit set (0.866 and 0.949) were positive and highly significant. Finally concerning fruit yield (-0.942 and -0.995), a highly significant negative correlation with viral load and per cent transmission was observed.

Table 42: Correlation studies between papaya ringspot viral load with percent transmission, growth, yield and yield parameters of papaya

	Viral titer	Per cent transmission (%)	Yield per plant (t)	No. of leaves per plant at 270 DAT*	Plant girth (cm) at 270 DAT	Internodal length (cm) 270 DAT	Plant height (cm) at 270 DAT	Days to first flowering	Flowers per Plant at 270 DAT	Days from flowering to first fruit set	Days to first fruit set to harvest	Fruits per Plant	Fruit diameter (cm)	Fruit length (cm)	Fruit breadth (cm)	Fruit cavity diameter (cm)
Viral titer	1															
Per cent transmission (%)	0.917**	1														
Yield per plant (t)	-0.924**	-0.995**	1													
No. of leaves per plant at 270 DAT	-0.947**	-0.962**	0.977**	1												
Plant girth (cm) at 270 DAT	-0.943**	-0.900**	0.896**	0.887**	1											
Internodal length (cm) 270 DAT	-0.882**	-0.881**	0.885**	0.909**	0.806**	1										
Plant height (cm) at 270 DAT	-0.985**	-0.928**	0.941**	0.972**	0.951**	0.876**	1									
Days to first flowering	0.988**	0.866**	-0.873**	-0.921**	-0.935**	-0.865**	-0.976**	1								
Flowers per Plant at 270 DAT	-0.994**	-0.934**	0.945**	0.968**	0.925**	0.894**	0.988**	-0.973**	1							
Days from flowering to first fruit set	0.982**	0.949**	-0.962**	-0.988**	-0.932**	-0.919**	-0.992**	0.966**	-0.988**	1						
Days to first fruit set to harvest	-0.957**	-0.963**	0.976**	0.984**	0.938**	0.886**	0.983**	-0.926**	0.973**	-0.984**	1					
Fruits per Plant	-0.917**	-0.986**	0.996**	0.977**	0.901**	0.869**	0.945**	-0.866**	0.939**	-0.960**	0.984**	1				
Fruit diameter (cm)	-0.973**	-0.918**	0.931**	0.966**	0.950**	0.861**	0.991**	-0.974**	0.969**	-0.986**	0.968**	0.933**	1			
Fruit length (cm)	-0.948**	-0.973**	0.982**	0.989**	0.878**	0.944**	0.954**	-0.911**	0.967**	-0.982**	0.972**	0.973**	0.942**	1		
Fruit breadth (cm)	-0.936**	-0.978**	0.982**	0.983**	0.895**	0.918**	0.957**	-0.900**	0.957**	-0.971**	0.978**	0.976**	0.940**	0.983**	1	
Fruit cavity diameter (cm)	-0.953**	-0.949**	0.945**	0.951**	0.856**	0.929**	0.934**	-0.921**	0.966**	-0.957**	0.931**	0.921**	0.913**	0.974**	0.966**	1

*DAT-Days After Transplanting, ** indicate significant at 1 per cent probability level

4.3 Integrated management of PRSV disease under field conditions

4.3.1 Effect of insecticides and bio rationals on PRSV incidence, growth and yield parameters

The effect of insecticides, bio rationals on PRSV disease incidence on papaya plants at different growth stages *viz.,* 30, 60, 90,120, 150, 180, 210, 240, 270, 300, 330 and 360 DAT was assessed under natural disease pressure for two seasons *viz.,* 2019-20 and 2020-21. The data is presented in the table 43 to 49, Plate 21 to 23.

4.3.1.1 2019-20

4.3.1.1.1 Disease incidence

Table 43 showed that at 30, 60, 90 DAT there was no incidence of disease in any of the treatments. At 120, 150, 180, 210, 240, 270, 300, 330 and 360 DAT treatments differed significantly with respect to PRSV incidence. At 360 DAT the treatments were non significant as all the treatments recorded 100 per cent incidence.

At 120 DAT, T_9 (63.89 %) recorded significantly highest disease incidence followed by T_8 (11.11 %), while all the other treatments recorded zero incidences. At 150 DAT, the same trend was followed wherein T_9 and T_8 recorded 94.44 per cent and 25.0 per cent respectively, while all other treatments being zero.

At 180 DAT, T_9 reached 100 per cent incidence which was significantly superior, followed by T_8 (50.0 %), T_6 (27.78 %) T_2 (16.67 %), T_3 (8.33 %) and T_7 (2.78 %). Still at this stage T_1, T_4 and T_5 recorded zero incidence.

At 210 DAT, T_4 (13.89 %) and T_5 (8.33 %) recorded disease incidence for the first time, while T_1 still continued with zero incidence. At 240 DAT, T_1 (2.78 %) recorded the first incidence of disease.

At 270 DAT, both T_9 and T_8 (100 %) were significantly highest followed by T_2 (77.78 %), T_3 (72.22 %) and T_6 (72.22 %) which were on par. T_7 recorded 50.0 per cent while T_1 (22.22 %) was significantly least than all other treatments. The same trend continued at 300 DAT.

Plate 21: Overview of the experimental plot of integrated diseases management
(A. 2019-20 & B. 2020-21) and evaluation of IDM module (C. 2020-21) for PRSV

Table 43: Effect of insecticides and bio rationals on PRSV disease incidence (during 2019-20)

	Disease incidence (%) at											
Treatment	**30 DAT***	**60 DAT**	**90 DAT**	**120 DAT**	**150 DAT**	**180 DAT**	**210 DAT**	**240 DAT**	**270 DAT**	**300 DAT**	**330 DAT**	**360 DAT**
T_1	0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.78 (5.59)	22.22 (28.03)	50.00 (45.05)	80.56 (68.57)	100 (90.00)
T_2	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	16.67 (23.62)	50.00 (45.05)	52.78 (46.80)	77.78 (66.49)	91.67 (76.38)	100 (90.00)	100 (90.00)
T_3	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.33 (13.62)	41.67 (39.63)	44.44 (41.60)	72.22 (63.40)	88.89 (73.94)	100 (90.00)	100 (90.00)
T_4	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	13.89 (18.03)	16.67 (23.62)	41.67 (39.79)	77.78 (62.65)	100 (90.00)	100 (90.00)
T_5	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.33 (13.62)	11.11 (19.22)	36.11 (35.59)	69.44 (56.81)	100 (90.00)	100 (90.00)
T_6	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	27.78 (31.75)	44.44 (41.80)	47.22 (43.40)	72.22 (58.46)	88.89 (74.41)	100 (90.00)	100 (90.00)
T_7	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.78 (5.59)	22.22 (27.03)	25.00 (29.46)	50.00 (45.37)	72.22 (58.57)	100 (90.00)	100 (90.00)
T_8	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	11.11 (16.06)	25.00 (29.79)	50.00 (45.16)	80.56 (68.25)	83.33 (70.21)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T_9	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	63.89 (53.25)	94.44 (78.81)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
S Em ±	NS	0.00	0.00	3.08	2.15	3.88	6.91	5.41	8.7	5.66	3.89	NS
C D @ 5 %	NS	0.00	0.00	9.23	6.44	11.64	20.72	16.20	26.2	16.97	11.67	NS

Treatments	**1st Spray at 30 DAT**	**2nd Spray at 60 DAT**	**3rd Spray at 90 DAT**	**4th Spray at 120 DAT**	**5th Spray at 150 DAT**	**6th Spray at 180 DAT**	**7th Spray at 210 DAT**	**8th Spray at 240 DAT**
T_1	Tolfenpyrad 15% EC 1 ml/l → micronutrients***	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Tolfenpyrad 15% EC 1 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients
T_2	1% Neem oil 10 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients	1% Neem oil 10 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients
T_3	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient
T_4	Tolfenpyrad 15% EC 1 ml/l → micronutrients	1% Neem oil 10 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Thiacloprid 21.7 SC 1ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Mineral oil 10 ml/l → micronutrients
T_5	Tolfenpyrad 15% EC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_6	1% Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Seaweed extract 1gm/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_7	Tolfenpyrad 15% EC 1 ml/l → micronutrients	1%Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_8	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients
T_9	Control							

*DAT-Days After Transplanting, ** Figures in parentheses are arc sine transformed values, *** Zn-3.0%, Fe-2.0%, Mn-1.0%, B-0.50%, Mg-2.0%, → Followed by

At 330 DAT, except T_1 (80.56 %) all other treatments recorded 100 per cent incidence. At 360 DAT all the treatments recorded 100 per cent incidence.

4.3.1.1.2 Growth, yield and yield parameters

The table 44 revealed that there was a significant difference between treatments with respect to all the parameters *viz.,* plant height, internodal length, plant girth, number of leaves per plants, number of days taken for first flowering, number of flowers, number of days taken from flowering to first fruit set, number of days taken from first fruit set to harvest, number of fruits per plant, fruit diameter, fruit length, fruit breadth, fruit cavity diameter, fruit yield per plant and fruit yield per hectare.

Concerning growth attributes *i.e.* plant height, internodal length, plant girth and number of leaves per plant, T_1 (225.66 cm, 4.09 cm, 40.37 cm and 29.41 respectively) was superior over all the treatments. This was followed by T_5 (216.44 cm, 4.09 cm, 39.66 cm and 28.57 respectively) and T_4 (213.91 cm, 4.09 cm, 39.55 cm and 28.25 respectively) which were on par. While control treatment, T_9 recorded significantly the least plant height (135.27 cm), internodal length (3.27 cm), plant girth (20.83 cm) and number of leaves per plant (19.27).

The yield traits *viz.,* number of days taken for first flowering, number of flowers, number of days taken from flowering to first fruit set and number of days taken from first fruit set to harvest in treatment T_1 (92.89, 72.77, 93.47 and 125.06 respectively) were significantly superior over all the treatments. Further T_5 (96.03, 59.61, 95.83 and 123.28 respectively) and T_4 (99.08, 56.41, 95.39 and 123.25 respectively) were the next best treatments. While control (T_9) recorded least for all the parameters (106.69, 14.86, 114.64 and 98.00 respectively).

Number of fruits per plant, fruit diameter, fruit length, fruit breadth and fruit cavity diameter were significantly superior in T_1 (25.97, 12.73 cm, 27.36 cm, 14.31 cm and 6.75 cm respectively) followed by T_5 (24.17, 12.39 cm, 24.67 cm, 12.49 and 6.64 cm respectively) and T_4 (22.00, 11.15 cm, 23.61 cm, 11.89 cm and 6.50 cm respectively) which were on par with each other. The least fruit parameters were recorded in T_9 (1.33, 8.13 cm, 14.64 cm 8.79 cm and 5.56 cm respectively).

Table 44: Effect of insecticides and bio rationals on growth, yield and yield parameters of papaya influenced by PRSV disease (during 2019-20)

Treatment	Plant height (cm) at 270 DAT*	Internodal length (cm) at 270 DAT	Plant girth (cm) at 270 DAT	No. of leaves per plants at 270 DAT	No. of Days taken for first flowering	No. of flowers at 270 DAT	No. of days taken from flowering to first fruit set	No. of days taken to first fruit set to harvest	No. of fruits per plant	Fruit diameter (cm)	Fruit length (cm)	Fruit breadth (cm)	Fruit cavity diameter (cm)	Fruit yield per plant (Kg)	Fruit yield per hectare (t)
T_1	225.66	4.09	40.37	29.41	92.89	72.77	93.47	125.06	25.97	12.73	27.36	14.31	6.75	57.92	178.74
T_2	204.72	3.60	37.36	23.11	105.44	31.77	103.36	109.86	16.06	9.49	19.83	9.81	6.01	26.49	81.75
T_3	206.16	3.72	38.22	27.14	100.69	57.97	100.53	118.03	23.11	11.31	23.83	11.23	6.45	48.53	149.77
T_4	213.91	4.09	39.55	28.25	99.08	56.41	95.39	123.25	22.00	11.15	23.61	11.89	6.50	42.48	131.09
T_5	216.44	4.09	39.66	28.57	96.03	59.61	95.83	123.28	24.17	12.39	24.67	12.49	6.64	51.73	159.62
T_6	205.52	3.48	37.69	26.27	101.75	54.11	100.31	113.83	21.64	10.48	22.33	11.35	6.33	40.38	124.60
T_7	214.57	3.71	39.05	27.23	100.03	56.38	100.89	112.72	21.25	11.10	23.36	11.48	6.35	41.33	127.55
T_8	160.69	3.39	33.97	20.46	105.36	20.44	110.61	97.75	5.67	8.48	17.36	9.34	5.88	6.38	19.67
T_9	135.27	3.27	20.83	19.72	106.69	14.86	114.64	98.00	1.33	8.13	14.64	8.79	5.56	0.83	2.57
S Em ±	3.05	0.07	0.29	0.33	0.21	1.10	0.72	0.34	0.86	0.13	0.25	0.14	0.08	1.04	3.21
C D @ 5 %	9.13	0.20	0.87	0.98	0.62	3.29	2.17	1.01	2.57	0.39	0.74	0.43	0.23	3.12	9.62

Treatments	1st Spray at 30 DAT	2nd Spray at 60 DAT	3rd Spray at 90 DAT	4th Spray at 120DAT	5th Spray at 150 DAT	6th Spray at 180 DAT	7th Spray at 210DAT	8th Spray at 240DAT
T_1	Tolfenpyrad 15% EC 1 ml/l → micronutrients**	Imidacloprid 17.8%SL 0.2ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Tolfenpyrad 15% EC 1ml/l → micronutrients	Imidacloprid 17.8% SL 0.2ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients
T_2	1% Neem oil 10 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients	1% Neem oil 10 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients
T_3	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient
T_4	Tolfenpyrad 15% EC 1 ml/l → micronutrients	1% Neem oil 10 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Mineral oil 10 ml/l → micronutrients
T_5	Tolfenpyrad 15% EC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_6	1% Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Seaweed extract 1gm/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_7	Tolfenpyrad 15% EC 1 ml/l → micronutrients	1%Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_8	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients
T_9	Control							

*DAT-Days After Transplanting, ** Zn-3.0%, Fe-2.0%, Mn-1.0%, B-0.50%, Mg-2.0%, → Followed by

Finally, with respect to fruit yield per plant and fruit yield per hectare, T_1 (57.92 kg and 178.74 t respectively) was significantly best followed by T_5 (51.73kg and 159.62 t respectively) and T_3 (48.53 kg and 149.77 t respectively). The least yield was recorded in T_9 (0.83 kg and 2.57 t respectively).

4.3.1.2 2020-21

4.3.1.2.1 Disease incidence

Table 45 showed that at 30 and 60 DAT there was no incidence of disease in any of the treatments. Further at 90,120, 150, 180, 210, 240, 270, 300, 330 and 360 DAT treatments differed significantly. At 360 DAT the treatments were non-significant as all the treatments recorded 100 per cent incidence.

At 90 DAT, only control treatment T_9 recorded the 1.14 per cent disease incidence while the remaining was disease free. At 120 DAT, T_9 (67.33 %) recorded significantly highest disease incidence followed by T_8 (10.14 %) and all the other treatments recorded zero incidence. At 150 DAT highest incidence was recorded in T_9 (86.31 %) followed by T_8 (26.11 %), while T_2 (8.00 %) and T_6 (7.31%) recorded the first incidence of the disease.

At 180 DAT, T_9 (control) reached 100 per cent incidence followed by T_8 (61.11 %), T_2 (22.64 %), T_6 (22.42 %), T_3 (8.33 %), T_4 (5.22 %) and least incidence recorded in T_7 (1.61 %). Still at this stage T_1 and T_5 recorded zero incidence.

At 210 DAT, T_1 (2.97 %) and T_5 (13.61 %) recorded disease incidence for the first time. At 240 DAT, T_9 (100 %) and T_8 (90.72 %) were having high incidence, while T_1 (7.61 %) recorded least which was on par with T_5 (16.39 %).

At 270 DAT, both T_9 and T_8 (100 %) were significantly highest followed by T_2 (81.00 %), T_6 (76.36 %) and T_3 (75.92 %) which were on par. T_7, T_4 and T_5 recorded 54.55 per cent, 50.89 per cent and 43.50 per cent respectively while T_1 (25.92 %) recorded significantly least than all other treatments. Similarly, at 300 DAT, T_1 (51.81 %) recorded significantly least disease incidence.

At 330 DAT, except T_1 (87.94 %) all other treatments recorded 100 per cent incidences. At 360 DAT all the treatments recorded 100 per cent incidence.

Table 45: Effect of insecticides and bio rationals on PRSV disease incidence (during 2020-21)

	Disease incidence (%) at											
Treatment	**30 DAT***	**60 DAT**	**90 DAT**	**120 DAT**	**150 DAT**	**180 DAT**	**210 DAT**	**240 DAT**	**270 DAT**	**300 DAT**	**330 DAT**	**360 DAT**
T_1	0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.97 (9.50)	7.61 (15.98)	25.92 30.59)	51.81 (46.13)	87.94 (73.22)	100 (90.00)
T_2	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.00 (16.42)	22.64 (28.35)	58.31 (49.83)	70.33 (57.06)	81.00 (68.49)	93.97 (81.61)	100 (90.00)	100 (90.00)
T_3	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.83 (18.84)	44.42 (41.60)	51.14 (45.66)	75.92 (65.59)	89.78 (74.63)	100 (90.00)	100 (90.00)
T_4	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	5.22 (10.83)	16.86 (23.80)	22.42 (28.18)	50.89 (45.88)	85.36 (67.71)	100 (90.00)	100 (90.00)
T_5	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	13.61 (21.49)	16.39 (23.88)	43.50 (41.15)	74.31 (59.86)	100 (90.00)	100 (90.00)
T_6	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	7.31 (15.66)	22.42 (28.23)	47.64 (43.64)	50.67 (45.38)	76.36 (61.12)	92.11 (80.30)	100 (90.00)	100 (90.00)
T_7	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.61 (5.49)	24.97 (29.16)	31.44 (33.95)	54.61 (48.25)	78.89 (62.73)	100 (90.00)	100 (90.00)
T_8	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.14 (18.32)	26.11 (30.57)	61.11 (51.60)	82.39 (69.44)	90.72 (79.39)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T_9	0.00 (0.00)	0.00 (0.00)	1.14 (4.98)	67.33 (55.14)	86.31 (68.71)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
S Em ±	0.00	0.00	0.84	0.78	1.61	2.76	4.88	3.68	7.35	5.19	2.80	0.00
C D @ 5 %	0.00	0.00	2.53	2.33	4.83	8.26	14.63	11.04	22.04	15.56	8.38	0.00

Treatments	**1st Spray at 30 DAT**	**2nd Spray at 60 DAT**	**3rd Spray at 90 DAT**	**4th Spray at 120 DAT**	**5th Spray at 150 DAT**	**6th Spray at 180 DAT**	**7th Spray at 210 DAT**	**8th Spray at 240 DAT**
T_1	Tolfenpyrad 15% EC 1 ml/l → icronutrients***	Imidacloprid 17.8%SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Tolfenpyrad 15% EC 1 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients
T_2	1% Neem oil 10 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients	1% Neem oil 10 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients
T_3	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient
T_4	Tolfenpyrad 15% EC 1 ml/l → micronutrients	1% Neem oil 10 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Mineral oil 10 ml/l → micronutrients
T_5	Tolfenpyrad 15% EC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_6	1% Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Seaweed extract 1gm/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_7	Tolfenpyrad 15% EC 1 ml/l → micronutrients	1%Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_8	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients
T_9	Control							

*DAT-Days After Transplanting, ** Figures in parentheses are arc sine transformed values, *** Zn-3.0%, Fe-2.0%, Mn-1.0%, B-0.50%, Mg-2.0%, → Followed by

4.3.1.2.2 Growth, yield and yield parameters

Table 46 revealed that growth attributes like plant height, internodal length, plant girth and number of leaves per plant were significantly superior in T_1 (226.16 cm, 4.13 cm, 40.53 cm and 29.58 respectively) over all the treatments. This was followed by T_5 (216.97 cm, 4.11 cm, 39.71 cm and 28.77 respectively) and T_4 (214.44 cm, 4.10 cm, 39.41 and 28.33 respectively) which were on par. While control treatment, T_9 recorded significantly the least plant height (135.79 cm), internodal length (3.73 cm), plant girth (21.07 cm) and number of leaves per plant (19.88).

With respect to number of days taken for first flowering, number of flowers, number of days taken from flowering to first fruit set and number of days taken to first fruit set to harvest, treatment T_1 (93.14, 73.35, 94.56 and 126.22 respectively) was significantly superior over all the treatments. Further T_5 (95.86, 60.16, 96.92 and 124.47 respectively) and T_4 (99.53, 56.99, 96.47 and 123.75 respectively) were the next best treatments. While control T_9 recorded least (106.92, 15.44, 115.72 and 99.17 respectively).

Number of fruits per plant, fruit diameter, fruit length, fruit breadth and fruit cavity diameter recorded significantly superior in T_1 (26.21, 13.53 cm, 28.19 cm, 14.56 cm and 6.88 cm respectively) followed by T_5 (24.26, 13.18 cm, 26.17 cm, 12.61 cm and 6.82 cm respectively) and T_4 (22.86, 12.95 cm, 25.78 cm, 12.47 cm and 6.74 cm respectively) which were on par with each other. The least fruit parameters were recorded in T_9 (1.05, 8.93 cm, 15.47 cm 9.03 cm and 5.69 cm respectively).

Finally, with respect to fruit yield per plant and fruit yield per hectare, T_1 (57.81 kg and 178.39 t respectively) was significantly best which was followed by T_5 (52.60 kg and 162.33 t respectively) and T_3 (43.13 kg and 133.11 t respectively), while least yield was recorded in T_9 (0.79 kg and 2.45 t respectively)

4.3.1.3 Pooled analysis of 2019-20 and 2020-21

The data on effect of insecticides, bio oils and seaweed extract on PRSV disease incidence, growth and yield parameters of two season's *i.e* 2019-20 and 2020-21 were pooled and analyzed. The pooled data is presented in table 47 to 48 and fig. 17 and 18.

Table 46: Effect of insecticides and bio rationals on growth, yield and yield parameters of papaya influenced by PRSV disease (during 2020-21)

Treatment	Plant height (cm) at 270 DAT*	Internodal length (cm) at 270 DAT	Plant girth (cm) at 270 DAT	No. of leaves per plant at 270 DAT	No. of days taken for first flowering	No. of flowers at 270 DAT	No. of days taken from flowering to first fruit set	No. of days taken to first fruit set to harvest	No. of fruits per plant	Fruit diameter (cm)	Fruit length (cm)	Fruit breadth (cm)	Fruit cavity diameter (cm)	Fruit yield per plant (Kg)	Fruit yield per hectare (t)
T_1	226.19	4.13	40.53	29.58	93.14	73.35	94.56	126.22	26.21	13.53	28.19	14.56	6.88	57.81	178.39
T_2	205.24	3.61	37.52	23.27	105.22	32.33	104.44	111.03	16.39	10.29	20.67	10.05	6.14	25.87	79.83
T_3	206.69	3.70	38.30	27.25	100.97	58.55	101.61	118.86	22.94	11.54	25.67	11.48	6.62	43.13	133.11
T_4	214.44	4.10	39.41	28.33	99.53	56.99	96.47	123.75	22.86	12.95	25.78	12.47	6.74	42.42	130.92
T_5	216.97	4.11	39.71	28.77	95.86	60.16	96.92	124.47	24.26	13.18	26.17	12.61	6.82	52.60	162.33
T_6	206.05	3.49	37.80	26.43	102.11	54.69	101.39	115.00	21.73	11.16	23.53	10.73	6.48	41.15	127.00
T_7	214.41	3.75	39.19	27.39	100.36	56.96	101.97	119.22	22.26	11.33	25.42	11.64	6.56	42.16	130.12
T_8	161.22	3.40	34.13	20.62	105.58	21.02	111.69	98.92	5.65	9.27	18.19	9.59	6.01	6.48	20.01
T_9	135.79	3.28	21.07	19.88	106.92	15.44	115.72	99.17	1.05	8.93	15.47	9.03	5.69	0.79	2.45
S Em ±	2.59	0.06	0.27	0.33	0.30	1.11	0.72	0.34	0.82	0.11	0.20	0.09	0.08	1.06	3.28
C D @ 5 %	7.77	0.18	0.80	0.99	0.90	3.33	2.17	1.03	2.46	0.32	0.60	0.27	0.24	3.19	9.84

Treatments	1st Spray at 30 DAT	2nd Spray at 60 DAT	3rd Spray at 90 DAT	4th Spray at 120 DAT	5th Spray at 150 DAT	6th Spray at 180 DAT	7th Spray at 210 DAT	8th Spray at 240 DAT
T_1	Tolfenpyrad 15% EC 1 ml/l → micronutrients **	Imidacloprid 17.8%SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Tolfenpyrad 15% EC 1 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients
T_2	1% Neem oil 10 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients	1% Neem oil 10 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients
T_3	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient
T_4	Tolfenpyrad 15% EC 1 ml/l → micronutrients	1% Neem oil 10 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Mineral oil 10 ml/l → micronutrients
T_5	Tolfenpyrad 15% EC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_6	1% Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Seaweed extract 1gm/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_7	Tolfenpyrad 15% EC 1 ml/l → micronutrients	1%Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_8	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients
T_9	Control							

*DAT-Days After Transplanting, ** Zn-3.0%, Fe-2.0%, Mn-1.0%, B-0.50%, Mg-2.0%, → Followed by

Table 47: Effect of insecticides and bio rationals on PRSV disease incidence (Pooled analysis of 2019-20 and 2020-21)

	Disease incidence (%) at											
Treatments	**30 DAT***	**60 DAT**	**90 DAT**	**120 DAT**	**150 DAT**	**180 DAT**	**210 DAT**	**240 DAT**	**270 DAT**	**300 DAT**	**330 DAT**	**360 DAT**
T_1	0.00 (0.00)**	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.49 (4.75)	5.19 (10.79)	24.07 (29.31)	50.90 (45.59)	84.25 (70.89)	100 (90.00)
T_2	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	4.00 (8.21)	19.65 (25.99)	54.15 (47.44)	61.56 (51.93)	79.39 (67.49)	92.82 (78.99)	100 (90.00)	100 (90.00)
T_3	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	9.58 (16.23)	43.04 (40.61)	47.79 (43.63)	74.07 (64.50)	89.33 (74.28)	100 (90.00)	100 (90.00)
T_4	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.61 (5.42)	15.38 (20.92)	19.54 (25.90)	46.28 (42.83)	81.57 (65.18)	100 (90.00)	100 (90.00)
T_5	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.97 (17.56)	13.75 (21.55)	39.81 (38.37)	71.88 (58.34)	100 (90.00)	100 (90.00)
T_6	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	3.65 (7.83)	25.10 (29.99)	46.04 (42.72)	48.94 (44.39)	74.29 (59.79)	90.50 (77.35)	100 (90.00)	100 (90.00)
T_7	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.19 (5.54)	23.60 (28.09)	28.22 (31.71)	52.31 (46.81)	75.56 (60.65)	100 (90.00)	100 (90.00)
T_8	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.63 (17.19)	25.56 (30.18)	55.56 (48.38)	81.47 (68.84)	87.03 (74.80)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T_9	0.00 (0.00)	0.00 (0.00)	0.57 (2.49)	65.61 (54.19)	90.38 (73.76)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
S Em ±	NS	NS	0.53	1.43	2.22	2.35	3.85	3.04	5.13	3.48	2.17	NS
C D @ 5 %	NS	NS	1.51	4.09	6.35	6.72	11.02	8.68	14.66	9.94	6.21	NS

Treatments	**1st Spray at 30 DAT**	**2nd Spray at 60 DAT**	**3rd Spray at 90 DAT**	**4th Spray at 120 DAT**	**5th Spray at 150 DAT**	**6th Spray at 180 DAT**	**7th Spray at 210 DAT**	**8th Spray at 240 DAT**
T_1	Tolfenpyrad 15% EC 1 ml/l → micronutrients***	Imidacloprid 17.8%SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Tolfenpyrad 15% EC 1 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients
T_2	1% Neem oil 10 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients	1% Neem oil 10 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients
T_3	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient
T_4	Tolfenpyrad 15% EC 1 ml/l → micronutrients	1% Neem oil 10 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Mineral oil 10 ml/l → micronutrients
T_5	Tolfenpyrad 15% EC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_6	1% Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Seaweed extract 1gm/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_7	Tolfenpyrad 15% EC 1 ml/l → micronutrients	1%Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_8	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients
T_9	Control							

*DAT-Days After Transplanting, ** Figures in parentheses are arc sine transformed values, *** Zn-3.0%, Fe-2.0%, Mn-1.0%, B-0.50%, Mg-2.0%, → Followed by

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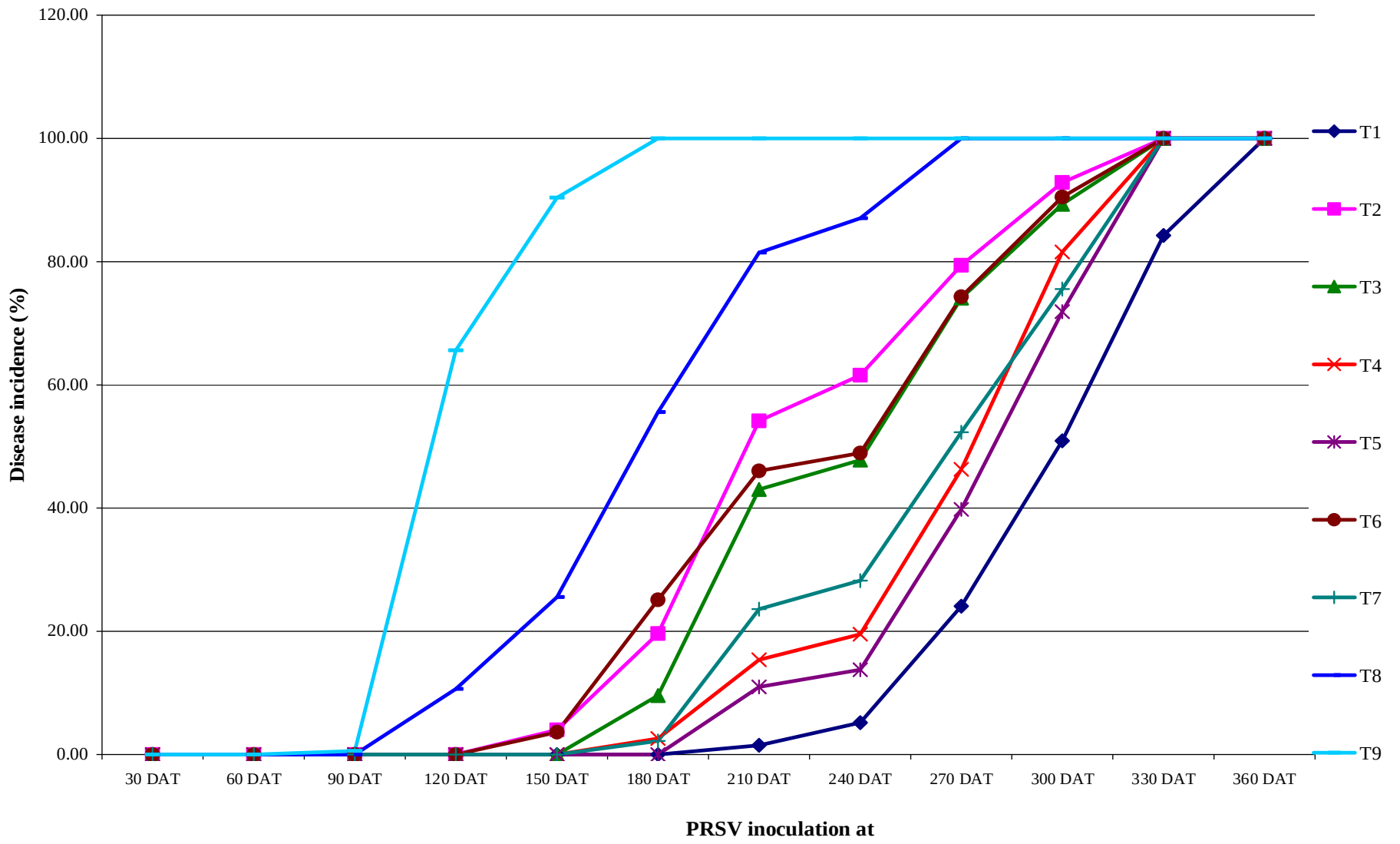

Fig. 17: Effect of insecticides and bio rationals on disease incidence

Plate 22: Effective treatments (T_1, T_3, T_5 and T_8) and control plot (T_9) view of experiment on integrated management of PRSV disease

Plate 23: Fruits on papaya plants in different treatments of experiment on integrated management of PRSV disease

4.3.1.3.1 Disease incidence

The pooled data showed that at 30 and 60 DAT there was no incidence of disease in any of the treatments. At 360 DAT the treatments were non-significant as the treatments recorded 100 per cent incidence. At 90 DAT, only T_9 (control) recorded disease incidence of 0.57 per cent, while the remaining were free from disease (Table 47).

At 120 DAT, T_9 (65.61 %) recorded significantly the highest disease incidence followed by T_8 (10.63 %) while zero incidence was recorded in all the other treatments. At 150 DAT highest incidence was recorded in T_9 (90.38 %) followed by T_8 (25.56 %) and the least incidence was recorded in T_6 (3.65 %) and T_2 (4.00%) while remaining treatments recorded zero incidence.

At 180 DAT, T_9 (control) reached 100 per cent incidence followed by T_8 (55.56 %), T_6 (25.10 %), T_2 (19.65 %), T_3 (9.58 %), T_4 (2.61 %) and T_7 (2.19 %). Still at this stage T_1 and T_5 recorded zero incidence. At 210 DAT, T_1 (1.49 %) and T_5 (10.97 %) recorded first disease incidence. Significantly highest incidence was recorded in T_9 (100 %) and T_8 (81.47 %). The same trend has followed in 240 DAT.

At 270 DAT treatments, T_9 and T_8 (100 %) were significantly highest followed by T_2 (79.39 %), T_6 (76.36 %) and T_3 (74.07 %) which were on par. T_7, T_4 and T_5 recorded 52.31 per cent, 46.28 per cent and 39.81 per cent respectively while T_1 (24.07 %) recorded significantly least than all other treatments.

Still at 300 DAT treatments, T_1 recorded only 50.90 per cent disease incidence and significantly least. At 330 DAT, except T_1 (84.25 %) all other treatments recorded 100 per cent incidence. At 360 DAT all the treatments recorded 100 per cent incidence.

4.3.1.3.2 Growth, yield and yield parameters

The plant height, internodal length, plant girth and number of leaves per plant in T_1 (225.93 cm, 4.11 cm, 40.45 cm and 29.50 respectively) were superior over all the treatments. This was followed by T_5 (216.71 cm, 4.10 cm, 39.68 cm and 28.67 respectively) and T_4 (214.18 cm, 4.10 cm, 39.48 cm and 28.29 respectively) which were on par. While control treatment, T_9 recorded significantly the least plant height

(135.53 cm), internodal length (3.28 cm), plant girth (20.95 cm) and number of leaves per plant (19.80) (Table 48).

Number of days taken for first flowering, number of flowers, number of days taken from flowering to first fruit set and number of days taken from first fruit set to harvest in T_1 (93.01, 73.06, 94.01 and 125.64 respectively) recorded significantly superior over all the treatments. Further T_5 (95.94, 59.88, 96.38 and 123.88 respectively) and T_4 (99.31, 56.70, 95.93 and 123.50 respectively) were the next best treatments. While control (T_9) was least performing treatment with respect to number of days taken for first flowering (106.81), number of flowers (15.15), number of days taken from flowering to first fruit set (115.18), number of days taken from first fruit set to harvest (98.58).

The fruit parameters *viz.*, number of fruits per plant, fruit diameter, fruit length, fruit breadth and fruit cavity diameter were significantly superior in T_1 (26.09, 13.13, 27.78, 14.43 and 6.81 cm respectively) followed by T_5 (24.21, 12.78, 25.42, 12.55 and 6.73 cm respectively) and T_4 (22.43, 12.05, 24.69, 12.18 and 6.62 cm respectively) which were on par with each other. Least fruit parameters were recorded in T_9 (1.19, 8.53, 15.06, 8.91 and 5.63 cm respectively).

Finally, with respect to fruit yield per plant and fruit yield per hectare, T_1 (57.86 kg and 178.56 t respectively) was significantly best which was followed by T_5 (52.16 kg and 160.97 t respectively) and T_3 (45.83 kg and 141.44 t respectively). The least yield was recorded in control T_9 (0.83 kg and 2.57 t respectively).

4.3.1.3.3 Economics of the experiment on management of PRSV disease on papaya using the insecticides and bio rationals.

The economics of the experiment on management of PRSV using insecticides and bio rationals was analyzed and presented in table 49.

Table 49 revealed that highest net returns were obtained in T_1 (Rs. 7,69,064/ha) followed by T_5 (Rs. 6,58,229/ha), T_3 (Rs. 5,36,754/ha) and T_4 (Rs. 4,83,757/ha). The B:C followed the same trend which recorded 3.54 in T_1, 3.14 in T_5, 2.72 in T_3 and 2.60 in T_4. The incremental cost benefit ratio (ICBR) was highest in T_1 (121), followed T_4 (88), T_5 (68) and T_6 (59)

Table 48: Effect of insecticides and bio rationals on growth, yield and yield parameters of papaya influenced by PRSV disease (Pooled analysis of 2019-20 and 2020-21)

Treatments	Plant height (cm) at 270 DAT*	Internodal length (cm) at 270 DAT	Plant girth (cm) at 270 DAT	No. of leaves per plants at 270 DAT	No. of days taken for first flowering	No. of flowers at 270 dat	No. of days taken from flowering to first fruit set	No. of days taken to first fruit set to harvest	No. of fruits per plant	Fruit diameter (cm)	Fruit length (cm)	Fruit breadth (cm)	Fruit cavity diameter (cm)	Fruit yield per plant (Kg)	Fruit yield per hectare (t)
T_1	225.93	4.11	40.45	29.50	93.01	73.06	94.01	125.64	26.09	13.13	27.78	14.43	6.81	57.86	178.56
T_2	204.98	3.60	37.44	23.19	105.33	32.05	103.90	110.44	16.22	9.89	20.25	9.93	6.07	26.18	80.79
T_3	206.43	3.71	38.26	27.19	100.83	58.26	101.07	118.44	23.03	11.43	24.75	11.36	6.53	45.83	141.44
T_4	214.18	4.10	39.48	28.29	99.31	56.70	95.93	123.50	22.43	12.05	24.69	12.18	6.62	42.45	131.01
T_5	216.71	4.10	39.68	28.67	95.94	59.88	96.38	123.88	24.21	12.78	25.42	12.55	6.73	52.16	160.97
T_6	205.79	3.49	37.75	26.35	101.93	54.40	100.85	114.42	21.69	10.82	22.93	11.04	6.40	40.76	125.80
T_7	214.49	3.73	39.12	27.31	100.19	56.67	101.43	115.97	21.75	11.21	24.39	11.56	6.45	41.75	128.83
T_8	160.95	3.39	34.05	20.54	105.47	20.73	111.15	98.33	5.66	8.88	17.78	9.46	5.94	6.43	19.84
T_9	135.53	3.28	20.95	19.80	106.81	15.15	115.18	98.58	1.19	8.53	15.06	8.91	5.63	0.81	2.51
S Em ±	1.79	0.04	0.18	0.21	0.17	0.70	0.46	0.46	0.54	0.13	0.19	0.10	0.05	0.79	2.45
C D @ 5 %	5.11	0.11	0.51	0.59	0.49	2.00	1.31	1.32	1.54	0.36	0.54	0.30	0.14	2.27	7.01

Treatments	1st Spray at 30 DAT	2nd Spray at 60 DAT	3rd Spray at 90 DAT	4th Spray at 120 DAT	5th Spray at 150 DAT	6th Spray at 180 DAT	7th Spray at 210 DAT	8th Spray at 240 DAT
T_1	Tolfenpyrad 15% EC 1 ml/l → micronutrients**	Imidacloprid 17.8%SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Tolfenpyrad 15% EC 1 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients
T_2	1% Neem oil 10 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients	1% Neem oil 10 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients
T_3	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient
T_4	Tolfenpyrad 15% EC 1 ml/l → micronutrients	1% Neem oil 10 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Mineral oil 10 ml/l → micronutrients
T_5	Tolfenpyrad 15% EC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_6	1% Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Seaweed extract 1gm/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_7	Tolfenpyrad 15% EC 1 ml/l → micronutrients	1%Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_8	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients
T_9	Control							

*DAT-Days After Transplanting, ** Zn-3.0%, Fe-2.0%, Mn-1.0%, B-0.50%, Mg-2.0%, → Followed by

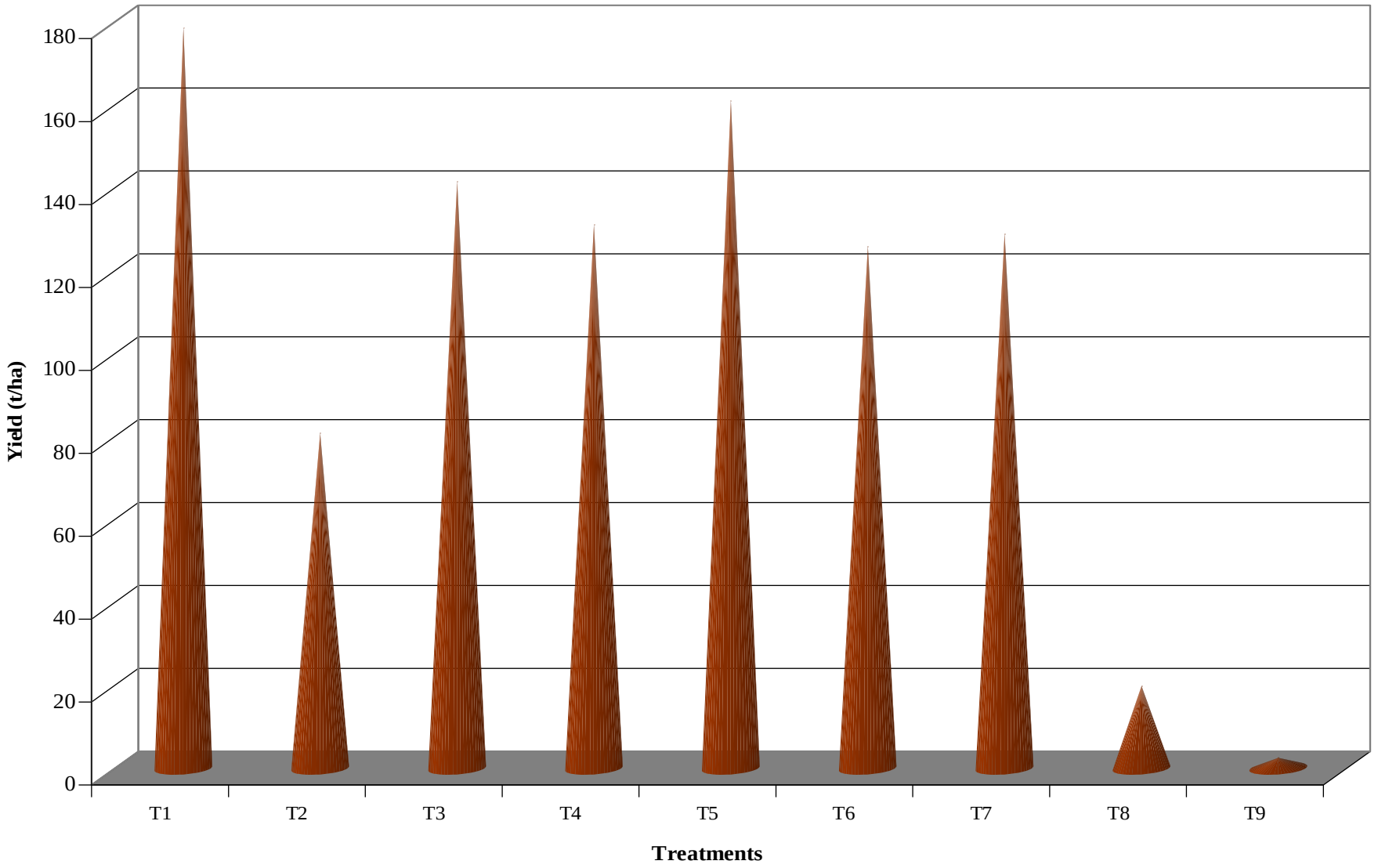

Fig. 18: Effect of insecticides and bio rationals on yield of papaya

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Table 49: Economics of the experiment on management of PRSV using insecticides and bio rationals

Treatments	Yield (t/ha)	Gross returns (Rs. /ha)*	Additional returns over control	Cost of cultivation (Rs. /ha)	Cost of treatment (Rs. /ha)	Total cost (Rs. /ha)	Additional cost over control	Net returns (Rs. /ha)	ICBR	B:C
T_1	178.56	10,71,360	10,56,300	2,93,536	8,760	3,02,296	8,760	7,69,064	121	3.54
T_2	80.79	4,84,740	4,69,680	2,93,536	8,038	3,01,574	8,038	1,83,166	58	1.61
T_3	141.44	8,48,640	8,33,580	2,93,536	18,350	3,11,886	18,350	5,36,754	45	2.72
T_4	131.01	7,86,060	7,71,000	2,93,536	8,767	3,02,303	8,767	4,83,757	88	2.60
T_5	160.97	9,65,820	9,50,760	2,93,536	14,055	3,07,591	14,055	6,58,229	68	3.14
T_6	125.8	7,54,800	7,39,740	2,93,536	12,598	3,06,134	12,598	4,48,666	59	2.47
T_7	128.83	7,72,980	7,57,920	2,93,536	13,835	3,07,371	13,835	4,65,609	55	2.51
T_8	19.84	1,19,040	1,03,980	2,93,536	2,350	2,95,886	2,350	-1,76,846	44	0.40
T_9	2.51	15,060	0	2,93,536	0	2,93,536	0	-2,78,476	0	0.05

Treatments	1st Spray at 30 DAT	2nd Spray at 60 DAT	3rd Spray at 90 DAT	4th Spray at 120 DAT	5th Spray at 150 DAT	6th Spray at 180 DAT	7th Spray at 210 DAT	8th Spray at 240 DAT
T_1	Tolfenpyrad 15% EC 1 ml/l → micronutrients**	Imidacloprid 17.8%SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Tolfenpyrad 15% EC 1 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients
T_2	1% Neem oil 10 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients	1% Neem oil 10 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients
T_3	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient	Seaweed extract 4ml/l → micronutrient
T_4	Tolfenpyrad 15% EC 1 ml/l → micronutrients	1% Neem oil 10 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l→micronutrients	Groundnut oil 10 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Mineral oil 10 ml/l → micronutrients
T_5	Tolfenpyrad 15% EC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_6	1% Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Pongamia oil 10 ml/l → micronutrients	Seaweed extract 1gm/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Mineral oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_7	Tolfenpyrad 15% EC 1 ml/l → micronutrients	1%Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
T_8	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients	Micronutrients
T_9	Control							

* Calculated using market value of papaya @ Rs.6/→, ** Zn-3.0%, Fe-2.0%, Mn-1.0%, B-0.50%, Mg-2.0%, → Followed by

4.3.3 Integrated disease management modules for management of PRSV disease on papaya

4.3.3.1 Disease incidence

Three integrated disease management modules were designed using results of experiments conducted on effect of insecticides and bio rationals for two seasons for the management of PRSV disease on papaya plants. The three modules were evaluated along with package of practices of UHS Bagalkot as check under natural disease pressure during 2020-21. The data is presented in table 50 to 51and Plate 21C.

Table 50 showed that at 30, 60, 90 and 120 DAT there was no incidence of disease in any of the modules. At 150 DAT, M_4 recorded 2.76 per cent disease incidence, while M_3 and M_2 recorded 1.93 and 1.14 per cent incidence respectively. M_1 recorded zero incidence.

At 180 DAT, M_1 (0.44 %) recorded the least incidence. M_2 (10.72 %) and M_3 (11.89 %) and M_4 (16.63 %) recorded moderate incidence.

The M_1 module recorded less disease (4.14 %, 20.11 %, and 56.44 %) at 210, 240 and 270 DAT respectively. At 300 DAT, except M_1 (80.64 %) all other treatments recorded 100 per cent incidence. At 330 DAT all four treatments recorded 100 per cent incidence.

4.3.3.2 Growth, yield and yield parameters

The effect of integrated disease management modules on growth and yield parameters was recorded and presented in table 51.

The plant height, internodal length, plant girth and number of leaves per plant in M_1 (227.02 cm, 4.24 cm, 40.46 cm and 29.19 respectively) were more than other modules. M_4 (recommended package) recorded the least plant height (216.11 cm), internodal length (4.08 cm), plant girth (39.36 cm) and number of leaves per plant (28.72).

Number of days taken for first flowering, number of flowers, number of days taken from flowering to first fruit set and number of days taken from first fruit set to

Table 50: Effect of IDM modules on PRSV diseased incidence at different crop growth stages

	Disease incidence (%) at										
Module	**30 DAT***	**60 DAT**	**90 DAT**	**120 DAT**	**150 DAT**	**180 DAT**	**210 DAT**	**240 DAT**	**270 DAT**	**300 DAT**	**330 DAT**
M_1	0.00	0.00	0.00	0.00	0.00	0.44	4.14	20.11	56.44	80.64	100
M_2	0.00	0.00	0.00	0.00	1.14	10.72	14.56	40.47	71.64	100	100
M_3	0.00	0.00	0.00	0.00	1.93	11.89	15.25	47.42	74.53	100	100
M_4 (UHS POP)	0.00	0.00	0.00	0.00	2.76	16.63	23.81	51.11	81.43	100	100

Module	1st Spray at 20 DAT	2nd Spray at 40 DAT	3rd Spray at 60 DAT	4th Spray at 80 DAT	5th Spray at 100 DAT	6th Spray at 120 DAT	7th Spray at 140 DAT	8th Spray at 160 DAT	9th Spray at 180 DAT	10th Spray at 200 DAT	11th Spray at 220 DAT	12th Spray at 240 DAT
M_1	Tolfenpyrad 15 EC 1 ml/l → micronutrients**	Imidacloprid 17.8% SL0.2ml/l → micronutrients	Thiacloprid 21.7 SC 1ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Tolfenpyrad 15EC 1 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Tolfenpyrad 15EC 1 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients
M_2	Tolfenpyrad 15EC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Tolfenpyrad 15EC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
M_3	Tolfenpyrad 15EC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Seaweed extract 4ml/l → micronutrients	1%Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
M_4 (UHS POP)	Dimethoate 30 % EC 1.7ml/l-1% Neem oil 10 ml/l	Neam Leaf Extract 1500 ppm 2 ml/l	Oxydemeton Methyl 25% EC 1.5ml/l-1% Groundnut oil 10 ml/l	Neam Leaf Extract 1500 ppm 2 ml/l	Imidacloprid 17.8% SL 0.25ml/l-1%Neem oil 10 ml/l	Neam Leaf Extract 1500 ppm 2 ml/l	Thiamethoxam 25% Wg 0.20 ml/l-1% Groundnut oil 10 ml/l	Neam Leaf Extract 1500 ppm 2 ml/l	Acephate 75 % SP 1.0 gm/l-1%Neem oil 10 ml/l	Neam Leaf Extract 1500 ppm 2 ml/l	Dimethoate 30 % EC 1.7ml/l-1% Groundnut oil 10 ml/l	Neam Leaf Extract 1500 ppm 2 ml/l

*DAT-Days After Transplanting, ** Zn-3.0%, Fe-2.0%, Mn-1.0%, B-0.50%, Mg-2.0%, → Followed by

Table 51: Effect of IDM modules on growth, yield and yield parameters of papaya influenced by PRSV disease

Module	Plant height (cm) at 270 DAT*	Internodal length (cm) at 270 DAT	Plant girth (cm) at 270 DAT	No. of leaves per plants at 270 DAT	No. of days taken for first flowering	No. of flowers at 270 DAT	No. of days taken from flowering to first fruit set	No. of days taken to first fruit set to harvest	No. of fruits per plant	Fruit diameter (cm)	Fruit length (cm)	Fruit breadth (cm)	Fruit cavity diameter (cm)	Fruit yield per plant (Kg)	Fruit yield per hectare (t)
M_1	227.02	4.24	40.46	29.19	92.78	73.38	94.63	125.61	28.31	14.23	28.00	14.63	7.14	62.40	192.56
M_2	218.64	4.17	39.63	28.78	94.35	60.41	95.71	125.34	25.33	13.19	26.03	12.49	6.78	54.82	169.19
M_3	216.30	4.12	39.59	28.66	94.47	60.30	95.89	125.38	25.24	13.07	25.83	12.37	6.67	54.55	168.34
M_4 (UHS POP)	216.11	4.08	39.36	28.25	98.00	56.95	96.10	125.43	25.17	12.95	25.81	12.25	6.58	54.54	168.32

Module	1st Spray at 20 DAT	2nd Spray at 40 DAT	3rd Spray at 60 DAT	4th Spray at 80 DAT	5th Spray at 100 DAT	6th Spray at 120 DAT	7th Spray at 140 DAT	8th Spray at 160 DAT	9th Spray at 180 DAT	10th Spray at 200 DAT	11th Spray at 220 DAT	12th Spray at 240 DAT
M_1	Tolfenpyrad 15 EC 1 ml/l → micronutrients**	Imidacloprid 17.8% SL0.2ml/l → micronutrients	Thiacloprid 21.7 SC 1ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Tolfenpyrad 15EC 1 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Tolfenpyrad 15EC 1 ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients
M_2	Tolfenpyrad 15EC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Tolfenpyrad 15EC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
M_3	Tolfenpyrad 15EC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Imidacloprid 17.8% SL 0.2 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Thiacloprid 21.7 SC 1 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Dinotefuran 20 % SG 0.5g/l → micronutrients	Seaweed extract 4ml/l → micronutrients	1%Neem oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients	Groundnut oil 10 ml/l → micronutrients	Seaweed extract 4ml/l → micronutrients
M_4 (UHS POP)	Dimethoate 30 % EC 1.7ml/l 1% Neem oil 10 ml/l	Neam Leaf Extract 1500 ppm 2 ml/l	Oxydemeton Methyl 25% EC 1.5ml/l 1% Groundnut oil 10 ml/l	Neam Leaf Extract 1500 ppm 2 ml/l	Imidacloprid 17.8% SL 0.25ml/l 1%Neem oil 10 ml/l	Neam Leaf Extract 1500 ppm 2 ml/l	Thiamethoxam 25% Wg 0.20 ml/l 1% Groundnut oil 10 ml/l	Neam Leaf Extract 1500 ppm 2 ml/l	Acephate 75 % SP 1.0 gm/l 1%Neem oil 10 ml/l	Neam Leaf Extract 1500 ppm 2 ml/l	Dimethoate 30 % EC 1.7ml/l 1% Groundnut oil 10 ml/l	Neam Leaf Extract 1500 ppm 2 ml/l

*DAT-Days After Transplanting, ** Zn-3.0%, Fe-2.0%, Mn-1.0%, B-0.50%, Mg-2.0%, → Followed by

harvest were more in M_1 (92.78, 73.38, 94.63 and 125.61 respectively). Further, M_2 (94.35, 60.41, 95.71 and 125.34 respectively) and M_3 (94.47, 60.30, 95.89, 125.38 respectively) are the next best. M_4 recorded least (98.00, 56.95, 96.10 and 125.43 respectively).

The fruit parameters *viz.*, number of fruits per plant, fruit diameter, fruit length, fruit breadth and fruit cavity diameter were more in M_1 (28.31, 14.23 cm, 28.00 cm, 14.63 cm and 7.14 cm respectively). The least fruit parameters were recorded in M_4 (25.17, 12.95 cm, 25.81 cm 12.25 cm and 6.58 cm respectively).

With respect to fruit yield per plant and fruit yield per hectare, M_1 (62.40 kg and 192.56 t respectively) recorded highest followed by M_2 (54.82 kg and 169.19 t respectively) and M_3 (54.55 kg and 168.34 t respectively). The least yield was recorded in check, M_4 (54.54 kg and 168.32 t respectively).

5. DISCUSSION

Carica papaya L. belongs to the family *Caricaceae*, commonly known as Papaya and it is one of the important fruit crops of Karnataka. It is a commercially cultivated and most economically important fruit crop of the tropical and subtropical regions of the world. Apart from consumption purposes, it is also used in pharmaceutical industries due to its anti cancerous, anti inflammatory, anti diabetic and anti oxidant properties (Aravind *et al.*, 2013 and Srivastava and Singh, 2016). Global papaya production has grown significantly increased over the last few years because of high yielding commercial cultivars, as a result of which increased area and production are recorded.

The papaya is vulnerable to many pests and diseases due to its extreme delicacy and succulence. Numerous arrays of fungi, bacteria and viruses frequently invade crops and cause sizeable economic losses. Papaya is affected by large numbers of viral diseases throughout the world. Among these viral diseases papaya ringspot caused by PRSV and leaf curl caused by PaLCuV are devastating and have dreadfully threatened papaya cultivation and productivity worldwide (Saxena *et al.*, 1998a; Sharma and Tripathi, 2014).

Considering the severity of PRSV and PaLCuV diseases and economic loss caused in papaya, the present investigation on studies on papaya viruses was carried out to know the prevalence and distribution of these viruses, molecular detection, characterization of the causal agent, host indexing, epidemiological studies and integrated disease management. The results of this experiment are discussed here.

5.1 Survey for the prevalence, distribution, collection and characterization of PRSV and PaLCuV diseases associated with papaya in Karnataka

5.1.1 Survey for the prevalence and distribution of PRSV and PaLCuV diseases in Karnataka

The survey was conducted in ten major papaya growing districts of Karnataka from November 2019 to April 2021 and recorded the prevalence of PRSV and PaLCuV disease. The incidence of PRSV varied with the location. The diseases recorded more severe in Koppal (100 %) and Yadgir (100 %), followed by Haveri

(91.47 %), Bagalkote (85.81 %), Ballari (70.26 %), Belagavi (76.12 %), Vijayapura (66.97 %), Chitradurga (61.93 %), Gadag (50.50 %) and Kalaburagi (53.53 %) districts, where in papaya was grown on a commercial scale. Apart from Koppal and Yadgiri many fields in Belagavi and Kalaburagi districts recorded 100 per cent disease incidence.

These findings indicated that the disease is widespread in different parts of Karnataka with varying levels of incidence. The average disease incidence across the districts noticed was 50.50 to 100 per cent. This may be due to increased demand with increased area under papaya cultivation and availability of crop around the year in these districts may help to build up and survive easily the viral infection on papaya. The differences in the incidence of disease were recorded in surveyed areas might be due to the variation in the source of inoculums, vector population, prevalent climatic conditions and stage of crop plant susceptible for infection by virus.

The PRSV disease has been previously reported from these surveyed districts by earlier workers. The average disease incidence in Bagalkote was recorded 30.0 to 100 per cent (Lakshminarayana Reddy, 2000; Ranebennur, 2005; Kunkalikar, 2003 and Mallikarjun, 2009), 15.0 to 100 per cent in Kalaburagi (Mallikarjun, 2009 and Kunkalikar *et al.*, 2007), 5.0 to 80.0 per cent in Koppal (Kunkalikar, 2003 and Mallikarjun, 2009) and 0 to 100 per cent incidence was recorded in Belagavi, Bellari, Chitradurga, Gadag, Haveri and Vijayapura (Shaikh, 1996; Hegde, 1998; Jahir Basha, 2002; Ranebennur, 2005; Kunkalikar, 2003; Mallikarjun, 2009, Vinayakumar Reddy *et al.*, 2011) by previous scientists.

The most commercially cultivated papaya varieties in Karnataka are Red Lady and Ice Burg, but both varieties recorded 100 per cent incidence in many of surveyed areas and were found susceptible to PRSV infection. Similar findings were also observed by Pushpa (2014), where Red Lady, Sunrise Solo and Arka Surya were found susceptible to PRSV in south Karnataka. According to Lokhande *et al*. (1992), the survey on different cultivars with respect to PRSV incidence in the Vidharbha region of Maharashtra showed up to 100 per cent incidence.

Further, during the current survey symptoms like a green mosaic, yellow mosaic, ringspot, stunted growth, puckering of leaves, leaf curling, mottling,

blistering, distortion of leaves, shoestring, oily streak, chloretic spots on fruits and distorted fruit were noticed in PRSV infected papaya plants. Viral infection can disrupt hormonal pathways, that manifests the simultaneous induction of synergistic or antagonistic hormones, the triggering of defense responses and reprogram the cellular environment (Zhao *et al.*, 2012). Such alterations often lead to the appearance of symptoms and are closely related to viral movement, replication, and systemic infection. Disruption of auxin signaling has been linked to developmental phenotypes with auxin biosynthesis and viral disease symptoms such as stunting, leaf curling and loss of apical dominance (Kazan and Manners, 2009). Viral component or proteins can cause the development of symptoms in cauliflower *i.e. Cauliflower mosaic virus* (CaMV) P6 protein (Rodriguez *et al.*, 2014). Furthermore, this multifunctional P6 viral protein plays a vital role in virus movement, replication, and RNAi suppression (Kakumani *et al.*, 2013; Angel *et al.*, 2013; Rodriguez *et al.*, 2014). P6 induces the symptoms like stunting, chlorosis, and banding of veins in leaves (Geri *et al.*, 2004). Expression of P6 results in interference with the ET pathway's response in Arabidopsis plants (Geri *et al.*, 2004). It has been observed that P6 can cause inference with ET signaling which produce symptoms (Wang *et al.*, 2006).

Green mosaic symptoms were commonly observed in PRSV infected surveyed fields and even in young plants which are less than 5 months old. Similar observation were made earlier by Lindner *et al.* (1945), Holmes *et al.* (1948) and Jensen (1949a). The mosaic is due to the activity of viral silencing suppressors which is linked to alterations in auxin signaling by HC-Pro and the development of symptoms. Specifically, transgenic Arabidopsis plants constitutively expressing the *Turnip mosaic virus* (TuMV) silencing suppressor HC-Pro, display leaf developmental abnormalities and produce mosaic symptoms (Collum and Culver, 2016). Under enhanced diseased conditions auxin biosynthesis was disrupted largely and express mottle and leaf distraction symptoms (Schaffer *et al.*, 1995; Manojkumar and Subbaiya, 2016) which are associated with severe crop damage. An experiment was conducted to know the effect of different symptoms associated with PRSV on growth and yield of the papaya *i.e.* mild mosaic, mosaic, severe mosaic, vein clearing, fern leaf, chlorotic leaf spot and leaf distortion. Among these symptoms, leaf distraction was found to cause severe damage to the growth and yield. The lowest plant height and number of leaves per plant were recorded in leaf distraction which was followed

by severe mosaic. However, in most of the cases, mild mosaic was found to cause minimum damage to plant height and the number of leaves per plant. Almost similar results were obtained in the case of fruits per plant and single fruit weight per plant and concluded that different symptoms are varied in their pathogenic effect on papaya (Akhter and Akanda, 2008).

During the survey in few districts of Karnataka *viz.,* Bagalkote, Belagavi, Haveri, Kalaburagi and Vijayapura, the infection of PaLCuV on papaya was recorded. The symptoms were severe leaf curl, downward leaf curling, vein thickening, vein clearing, rubbery, fragile, distorted petioles, deformation, yellowing, twisted petioles, vein enation, rolling, leathery leaf and the zigzag vein. Similar types of symptoms were observed on papaya plants infected by leaf curl virus by earlier scientists (Thomas and Krishnaswami, 1939; Sen *et al.,* 1946; Nariani, 1956; Govindu, 1964; Sureka *et al.,* 1977; Pandey and Marathe, 1986; Verma, 1991; Saxena *et al*., 1998a; Saxena *et al.*, 1998c; Singh, 2006; Patel, 2006; Raj *et al.*, 2008; Krishnareddy *et al.*, 2010; Singh-Pant *et al.*, 2012; Varun and Saxena, 2012; Sinha *et al.*, 2013; Usharani *et al.*, 2013; Dwivedi *et al.*, 2016; Dubey *et al.*, 2015; Sinha, 2015; Sinha *et al.*, 2016; Singh and Awasthi, 2017; Nehra *et al.*, 2019; Kumar *et al.*, 2021)

The severe leaf curl is the most common symptom associated with this disease and having a direct link with the auxin signaling hormone. A study showed that the *Tobacco mosaic virus* (TMV) replication protein has been shown to disrupt auxin signaling *via.* interaction with selected Aux/IAA family members (Padmanabhan *et al.,* 2006). These Aux/IAA proteins function as negative regulators of auxin responsive transcription factors (ARF) and control their ability to modulate genes involved in a range of plant processes (Wang *et al.,* 2014). Interaction with the TMV proteins disrupts the nuclear localization of interacting Aux/IAA proteins and correlates with the development of leaf curling and severe disease symptoms.

Previously there were no reports of PaLCuV on papaya in these surveyed areas. This might be due to the cultivation of papaya during the summer season, during the period warm climate helps in persistence of whiteflies resulting in infection of papaya with the leaf curl virus. During current study whitefly activity has not observed in infected plants. It might be due to disliking of papaya sap by whitefly

which didn't feed continuously on papaya plants. This infection is due to occasional visits to papaya (Guo *et al.,* 2015).

Similarly, the incidence of leaf curl disease of papaya has been reported during the survey in different countries of Asia and the North American continent by earlier scientists. Incidence of 6.0 to 64.0 per cent in Bangladesh (Hamim *et al.,* 2019), 42.0 to 100 per cent in Indonesia (Sutrawati *et al.*, 2021), 30.0 to 80.0 per cent in Oman (Khan *et al.,* 2012, Ammara *et al.,* 2015 and Haq *et al.,* 2018) and 40.0 to 100 per cent incidence in USA (Alabi *et al.*, 2016) has also been reported.

Further in India, an extensive survey has been conducted at Uttar Pradesh and recorded 5.0 to 35.0 per cent incidence of leaf curl virus by many scientists (Saxena *et al.*, 1998a; Saxena *et al.*, 1998c; Krishnareddy *et al.*, 2010; Dubey *et al.*, 2015; Singh and Awasthi, 2017). While, 0 to 30.0 per cent incidence was reported from Gujarat (Patel, 2006)

5.1.2 Collection, detection and characterization of virus isolates associated with papaya collected during survey

5.1.2.1 Collection and molecular detection

A total of 107 papaya surveyed leaf samples were subjected for PCR based detection for PRSV using set of specific primer designed by Bateson *et al.* (1994) and recorded positive amplification in 75 samples which are showed typical PRSV symptoms in field. Similar PRSV coat protein specific primers have been used to report the PRSV-P incidence in papaya from Philippines by Cruz *et al.* (2009) and from Thailand by Temaja and Darmiati (2015).

Further, to identify the any other RNA viruses associated with 75 PRSV infection confirmed samples, set of specific primers for *Papaya milk vetch dwarf virus, Papaya leaf distortion virus, Papaya mosaic virus* and *Zucchini yellow mosaic virus* were subjected for PCR based detection. But none of the reaction showed positive amplification for any of these viruses. This confirmed that there is no association of these viruses along with PRSV in surveyed region. However, Villamor *et al.* (2003) Tuo *et al.* (2014) and Lal *et al.* (2020) used similar primers to detect the infection of these viruses in papaya.

Meanwhile, some samples showing papaya bunchy top symptoms are collected during survey are subjected for PCR based detection using set of phytoplasma specific primers (Deng and Hiruki, 1991; Schneider, 1995). But there was no positive amplification. This confirmed that plants expressesing symptoms of papaya bunchy top are not because of phytoplasma infection, it may be due to any other biotic or abiotic factor. Pallavi *et al.* (2012) has successfully used similar primers for detection and molecular characterization of phytoplasma associated with chickpea phyllody disease in south India.

The 32 papaya leaf samples collected from different locations of Karnataka which having typical leaf curl symptoms showed positive amplification for begomovirus degenerative primers designed by Rojas *et al.* (1993) and Wyatt and Brown (1996) thus confirmed the presence of begomoviral association in these samples. Further, these samples were subjected for detection of both DNA-A and DNA-B components, all these samples only showed positive amplification for DNA-A specific degenerative primers component but not for DNA-B specific degenerative primers designed by Rojas *et al.* (1993). This confirmed that all 32 begomoviral infected papaya samples contain only DNA-A which means that collected begomoviral samples are monopartite in nature. Bananej *et al.* (2016) successfully detected the begomoviral infection in papaya using same primers and reported *Okra enation leaf curl virus* from Iran causing leaf curl in papaya. Whereas Varun and Saxena (2012) reported the association of *Tomato leaf curl Gujarat virus* on papaya showing typical leaf curl symptoms in North India and confirmed the absence of bipartite nature. Shen *et al.* (2014) reported *Ageratum yellow vein virus* association with papaya leaf curl for the first time in China.

Further, these 32 samples were subjected for PCR based detection of beta and alpha satellite virus association. No alpha satellite was detected, but interestingly beta satellite amplicon of 0.7 kb was obtained which is a naturally defective satellite, an evidence for recombination between alpha satellite and beta satellite (Huang *et al.*, 2013**).** Similar to present findings defective beta satellite DNA of 0.6 kb was reported in *Chilli leaf curl virus* and *Tomato leaf curl virus* isolates of begomovirus infecting papaya from New Delhi, India (Kumar *et al.*, 2021). Some scientists reported 1.3 kb length beta satellite virus in association with papaya *viz.*, *Papaya leaf curl China*

betasatellite virus from China (Shen *et al.*, 2014) and *Tomato leaf curl Bangladesh betasatellite* from north India (Varun and Saxena, 2012) and *Papaya yellow leaf curl* betasatellite from the Indian subcontinent (Nehra *et al.*, 2019).

5.1.2.2 Molecular characterization

5.1.2.2.1 PRSV

Among 75 PRSV infection confirmed samples, a representative sample collected from Bagalkote, Karnataka, India was subjected for molecular characterization of complete full length genome using nine sets of overlapping PRSV primers reported by Ortiz-Rozas and Chaves-Bedoya (2017) which were specifically designed for amplifying the full length of PRSV.

The PRSV-BGK isolate (Bagalkote, Karnataka) was cloned and sequenced. The full length genome consisting of 10,341 nt in length and having a single major polyprotine with ten mature peptides located at different location in the genome having variable length of nucleotides *viz.*, P1 (1,638 nt), HC-Pro (1,371 nt), P3 (1,035 nt), 6K1 (156 nt), CI (1,905 nt), 6K2 (171 nt), VPg (567 nt), NIa-Pro (714 nt), NIb (1,611 nt) and CP (861 nt) which are all located in +ve sense stand and runs in a clockwise direction along with 5' and 3' untranslated regions at terminal end. Further, recorded the nine cleavage sites around polyproteins *viz.*, MEQY/N (P1/HC-Pro); HYIVG/G (HC-Pro/P3); VIHQ/A (P3/6K1); VYHQ/S (6K1/ CI); VYHQ/G (CI/6K2); VFHQ/G (6K2/VPg); VHHE/G (VPg/NIa-Pro); VFEQ/S (NIa-Pro/NIb) and VYHQ/S (NIb/CP). Well characterized motifs were also been identified in PRSV-BGK genome *viz.*, KITC in the HC-Pro region for aphid transmission, AVGSGKST in the CI region for helicase function, GDD in the NIb region for replicase activity, and DAG in the CP region for aphid transmission.

This result confirms with earlier study in which complete genome of the PRSV-DEL isolate from north India consisted of 10,317 nucleotides with a single large ORF of 10,023 nt and shared maximum sequence identity at the amino acid level (92 %) with isolates from the America (Parameswari *et al.*, 2007). Similarly, PRSV-Pune isolate reported from India (PRSV-Pune) contained 10,326 nt and having close clustering with Indian PRSV isolates (Gorane *et al.*, 2019)

Detailed analysis of complete nucleotide sequence of PRSV-BGK isolate showed a high nucleotide sequence identity of 95.84 per cent with PRSV-[IN:TS:Hyd:HYD:Pap:15] (KP743981) from Telangana. Further it is found by phylogenetic analysis, that PRSV-BGK isolate formed the close cluster with PRSV Indian isolates and formed distinct branching with PRSV-[IN:TS:Hyd:HYD:Pap:15] which indicated emergence of new PRSV variant in Karnataka. Hence name of new variant is proposed as *Papaya ringspot Virus*-[India:Karnataka:Bagalkote: Papaya:2021] as per ICTV guidelines.

Recombination provides a means by which viruses invade new hosts or develop greater pathogenicity and/or virulence (Kehoe *et al.,* 2014 and Maina *et al.,* 2017). Using the RDP 4.7 recombination analysis package, 6 recombination events were recorded in PRSV-BGK isolate and indicated that there is a differential variability and recombination across the PRSV genome.

The diversity and genetic variation of PRSV with regions to regions is due to occurrence of spatial recombination and temporal evolution within the province of occurrence (Saleem *et al.*, 2020). PRSV diversity appears to be changing at different rates; presumably driven by introductions, movement of plant materials, geographical isolation and disease management practices (Chin *et al.*, 2007). Recombination is an important factor to promote virus evolution, which can increase genomic biodiversity (Dietrich *et al*., 2007) and enhance the virulence of the virus and expand its host range (Escriu *et al.*, 2007). The recombination hotspot of PRSV is P1, P3, CI, and HcPro genes suggesting that the 5’ end of the genome has played a vital role in the genome dynamics (Mangrauthia *et al.,* 2008). Chaves-Bedoya and Ortiz-Rojas (2015) reported the emergence of PRSV genetic variability at the local level in Colombia due to recombination. The P1 gene was the most variable while the NIb and CP was the most conserved making it a good target for the control of PRSV through RNA-interference technology (Mishra and Patil, 2018 and Mishra *et al.,* 2019). In addition, Maina *et al.* (2017) detected recombination breakpoints not only within the P1 region, but also in PRSV’s CI, NIa-Pro, NIb, 6K2, and 5′ UTR regions, which support the recombination results of current study. Hence, indicating that P1 is an ideal region for variability studies which may reveal the population differentiation and eventually the incipient speciation (Zhao *et al.,* 2016 and Mishra and Patil, 2018).

5.1.2.2.2 PaLCuV

Out of 32 samples collected during survey, 13 representative samples containing DNA-A component were subjected for cloning and sequencing. The full length genome of all these isolates analyzed and recorded the variation in their length of nucleotide, intergenic region and ORFs. These have a common stem-loop structure of nanonucleotide sequence "TAATATTAC" which is non coding sequence. All these 13 begomovirus isolates were similar to that of new world monopartite begomoviruses causing leaf curl diseases worldwide.

Among 13 isolates diversification of begomovirus populations is observed. Based on ICTV species demarcation criteria (Anon., 2021) four new distinct species, four new strain and five new variants were identified.

Maximum nucleotide sequence identity of PaLCuV-1 was 88.6 per cent with ToLCKV-[IN:GJ:AHM:Tom:16] (MH5770301); PaLCuV-3 was 85.4 per cent with PaLCuV-[IN:Kar:Madi:Cal:18] (MK087120); PaLCuV-4 was 88.6 per cent with BYVBV-[IN:OD:Bhu:Okra:03] (FJ589571) and PaLCuV- 13 was 87.2 per cent with ToLCV-[In:Kar:Ben:Chr:17] (MG758145). All these four isolates (PaLCuV-1, PaLCuV-3, PaLCuV-4 and PaLCuV-13) were demarcated as new species and proposed the name as *Papaya leaf curl Bagalkote virus*, PaLCuBKV-[IN:Kar:Bel: Pap:21] Gokak; *Papaya leaf curl Bagalkote virus*, PaLCuBKV-[IN:Kar:Bgk:Pap:21] Bagalkote; *Papaya leaf curl Bagalkote virus*, PaLCuBKV-[IN:Kar:Bgk:Pap:21] Bilgi and *Papaya leaf curl Haveri virus*, PaLCuHV-[IN:Kar:Hav:Pap:21] Haveri respectively based as ICTV guidelines.

Maximum nucleotide sequence identity of PaLCuV-9 was 93.5 per cent with ChiLCV-DU[IN:ND:DU:Pap:09] (HM140364); PaLCuV-10 was 92.4 per cent with PaLCuV-[IN:Kar:Madi:Cal:18] (MK087120); PaLCuV-11 was 93.5 per cent with CYVMV-[IN:ND:Cro:08] (JN817516); PaLCuV-12 was 93.0 per cent with PaLCuV-[IN:Kar:Madi:Cal:18] (MK087120). All these four isolates (PaLCuV-9, PaLCuV-10, PaLCuV-11 and PaLCuV-12) were demarcated as new strain and proposed the name as *Chilli leaf curl virus*, ChiLCV-[IN:Kar:Kal:Pap:21] Afjalpur strain; *Papaya leaf curl virus*, PaLCuV-**[**IN:Kar:Kal:Pap:21] Kalaburagi strain; *Croton yellow vein mosaic virus*, CYVMV-**[**IN:Kar:Kal:Pap:21], Kalaburagi strain; *Papaya leaf curl*

virus and PaLCuV-[IN:Kar:Vij:Pap:21] Vijayapura strain respectively based on ICTV guidelines.

Maximum nucleotide sequence identity of PaLCuV-2 was 96.3 per cent with ChiLCV-DU[IN:ND:DU:Pap:09] (HM140364), PaLCuV-5 was 96.5 per cent with with ChiLCV-[IN:Kar:Rai:Chi:17] (MK161454); PaLCuV-6 was 98.5 per cent with ChiLCV-[IN:Kar:Rai:Chi:17] (MK161454), PaLCuV-7 was 98.5 per cent with ChiLCV-[IN:Kar:Rai:Chi:17] (MK161454) and PaLCuV-8 was 98.0 per cent with ChiLCV-DU[IN:ND:DU:Pap:09] (HM140364). All these five (PaLCuV-2, PaLCuV-5, PaLCuV-6, PaLCuV-7 and PaLCuV-8) isolates were demarcated as new strain and proposed the name as *Chilli leaf curl virus*, ChiLCV-[IN:Kar:Bel:Pap:21] Gokak variant; *Chilli leaf curl virus*, ChiLCV-[IN:Kar:Bel:Pap:21] Ramadurga variant; *Chilli leaf curl virus*, ChiLCV-[IN:Kar:Bgk:Pap:21] Hunugunda1 variant; *Chilli leaf curl virus*, ChiLCV-[IN:Kar:Bgk:Pap:21] Hunugunda2 variant and *Chilli leaf curl virus*, ChiLCV-[IN:Kar:Kal:Pap:21] Afjalpur variant respectively. These results are supported by SDT and phylogenetic analysis.

Pair wise distance nucleotide identity of DNA-A component between the 13 begomoviruses isolated was analyzed and recorded that PaLCuV-1 (91.3 %), PaLCuV-3 (93.4 %) and PaLCuV-4 (93.4 %) isolates are strains of each other. PaLCuV-5 (97.0 %), PaLCuV-6 (99.3 %), PaLCuV-7 (99.3 %) and PaLCuV-8 (93.1 %) are variant of each other. Whereas, PaLCuV-9 (93.1 %) is strain of PaLCuV-8 but PaLCuV-2 (96.5 %) is variant of PaLCuV-8. Although PaLCuV-10 (93.2 %), PaLCuV-12 (93.2 %) and PaLCuV-11 (87.2 %) are in same cluster but PaLCuV-10 and PaLCuV-12 are strains of each other; whereas PaLCuV-11 is different species emerged out from this cluster. PaLCuV-13 (80.78 %) shared maximum identity and emerged as distinct species from PaLCuV-8. These results are supported by phylogenetic analysis which indicates high level of genetic variability existing in these 13 begomoviral population.

Similar results have been published by several scientists with respect to association new begomoviruses infecting papaya from Indian subcontinent *viz.*, *Tomato leaf curl New Delhi virus* from Uttar Pradesh (Raj *et al.*, 2008), *Ageratum enation virus* (AEV), *Croton yellow vein mosaic virus* (CrYVMV), *Papaya leaf curl virus* (PaLCuV) and *Tomato leaf curl New Delhi virus* (ToLCNDV) from different

parts of India (Krishnareddy *et al.*, 2010), *Chilli leaf curl virus* (ChiLCuV), *Tomato leaf curl New Delhi virus* (ToLCuNDV) and *Papaya leaf crumple virus* (PaLCrV) from different parts of India (Singh-Pant *et al.*, 2012). Varun and Saxena (2012) conducted molecular characterization of begomoviruses infecting papaya showing severe leaf curl symptoms and revealed highest similarity percentage of 91.0 per cent with *Tomato leaf curl Gujarat virus* (ToLCuGuV) and reported the emergence new strain in regions of Lucknow. Similarly, Hamim *et al.* (2019) who characterized the 29 surveyed severe leaf curl symptomatic papaya samples and reported *Tomato leaf curl Bangladesh virus* (ToLCBV) infection in papaya for the first time from Bangladesh.

The high genetic variability of begomovirus populations has been primarily attributed their high rates of nucleotide substitution, similar to those of RNA viruses (Duffy and Holmes, 2008 and Duffy and Holmes, 2009) and frequent occurrence of recombination, which may significantly accelerate their evolution by maximizing the combinations of pre-existing nucleotide polymorphisms created by mutation (Padidam *et al.*, 1999 and Pita *et al.*, 2001). Thus, mutation and recombination are often referred as the major contributors to the genetic variability of begomovirus populations. Over the years, various studies have shown that recombination occurs at high frequencies in begomovirus populations (Padidam *et al.*, 1999; Pita *et al.*, 2001 and Martin *et al.*, 2011). A nonrandom location of recombination breakpoints is a conserved feature amongst ssDNA viruses which use a rolling circle mechanism for replicating their genomes (Lefeuvre *et al.*, 2007; Prasanna and Rai, 2007 and Martin *et al.*, 2011). Recently, Lima *et al.* (2017) detected the begomovirus recombination events hotspot between origin of replication and in the 50-terminal/ central regions of the rep gene and showed that recombinants that exchange whole domains have less disrupted intragenome interaction networks and are favored by selection (Martin *et al.* 2011).

All the 13 isolates showed past recombination events, PaLCuV-1 recorded the four recombination events at different nucleotide positions *i.e* 40^{th} nt to 2148^{th} nt, 2262^{nd} to 2440^{th}, 282^{nd} nt to 1175^{th} nt and 1339^{th} nt to 2070^{th} nt position respectively. PaLCuV-2 recorded two recombinations at 1907^{th} nt to 2019^{th} nt and 2250^{th} nt to 2433^{rd} nt. PaLCuV-3 recorded six recombination events at 1272^{nd} nt to

2158nd nt , 11st nt to 2185th nt, 2696th nt to 2785th nt, 294th nt to 1174th nt, 2408th nt to 2834th nt and 2508th nt 2941st nt position. PaLCuV-4 recorded 3 different recombinations at 1273rd nt to 2158th nt, 282nd nt to 1174th, 2486th nt to 2941st nt position. Whereas, PaLCuV-5, PaLCuV-6 and PaLCuV-7 recorded single recombination at 2241 nt to 2433 nt, 2251st nt to 2453rd nt and 2251 to 2453 nt position respectively. In PaLCuV-8 recombination accorded at two different nucleotide position *i.e* 362nd nt to 1363rd nt and 2250th nt to 2433rd nt. Similarly, PaLCuV-9 recorded two recombination events at 550th nt to 1074th nt and 2250th to 2433rd nt position. PaLCuV-10 recorded showed high level of recombination at five different locations *i.e* 520th nt to 1246th nt, 2241st nt to 28th nt, 1268th nt to 2249th nt, 2702nd nt to 2863rd nt and 282nd nt to 1178th nt position. Similarly, PaLCuV-11 isolate recorded five recombination *i.e* 284th nt to 1227th nt, 1916th nt to 2237th nt, 2255th nt to 2894th nt, 2392nd nt to 2884th nt and 1079th nt to 1226th nt. PaLCuV-12 recorded maximum of six recombination at different locations with different major and minor parent *i.e* 1268th nt to 2182nd nt, at 2836th nt to 2914th nt, 2240th nt to 2513th nt, 11th nt to 2513rd nt, 235th nt to 1178th nt and 2540th nt to 2613rd nt. Whereas, PaLCuV-13 recorded four recombination at different nucleotide positions at 218th nt to 992nd nt, 2178th nt to 2729th nt, 1190th nt to 2862nd nt and1200th nt to 1166th nt.

These results showed the homologous recombination events occured at intraspecific level which is much more often in DNA viruses with genetic crossing over (Bujarski, 2008). Similar intraspecific recombination was reported by Venkataravanappa *et al.* (2012), Chandrakant (2014) and Premchand (2015) in different begomoviruses. Recombination is one of the major forces in increasing plant virus variability and adaptation to new hosts, often leading to emergence of new variants and resistance breaking virus strains. Recombination can also increase the fitness of plant RNA viruses by repairing defective viral genomes or efficiently removing deleterious mutations that result from error prone replication. The frequency of recombination is affected by several factors, including the viral replication proteins and various features of the viral RNA templates involved. Host genes also affect viral RNA recombination, suggesting complex interaction between a given virus and its host during viral adaptation and evolution (Nagy, 2008).

5.1.3 Virus indexing/identification of reservoir hosts through host range study.

Concerning host indexing, efforts were made to ascertain the role of collateral hosts belonging to family other than *Caricaceae*. The results of the host indexing study revealed that PRSV can infect host belonging to families Chenopodiaceae and Cucurbitaceae. In the case of *Chenopodium quinoa* belonging to *Chenopodiaceae,* only local lesions were observed on the inoculated leaves at 10 days after inoculation. However, on *Cucumis sativas* belong to family Cucurbitaceae mosaic symptoms were noticed.

This host range is based on the genetic diversity among the strains of PRSV. These strains were grouped into two, PRSV-P and PRSV-W type. Virus which grouped into the PRSV-P (infecting type papaya) affects both papaya and cucurbits and the PRSV-W (cucurbit infecting type) affect only cucurbits but not papaya.

It also has been reported earlier that, PRSV-P is mostly restricted to Chenopodiaceae and Cucurbitaceae families other than Caricaceae (Conover, 1962; Purcifull *et al.,* 1984b; Yeh and Gonsalves., 1984; Thomas and Dodman., 1993; Dahal *et al.*, 1997; Perera *et al.*, 1998; Parmar, 2000; Lakshminarayana Reddy, 2000; Kelaniyangoda and Madhubashini, 2010; Kunkalikar, 2003; Tripathi *et al.,* 2008; Limkar, 2017; Singh *et al.*, 2017; Navanath *et al.*, 2017; Harish, 2018; Kumar *et al.*, 2014).

5.2 Epidemiological studies on the incidence of PRSV disease

5.2.1 Effect of different months of planting on the incidence of PRSV disease, growth and yield parameters of papaya at different stage of growth

The field study on the effect of planting dates on PRSV disease was conducted at COH Bagalkot to know the best month of papaya planting to manage the PRSV disease incidence. June planting recorded zero disease incidence up to 90 DAT while in July planting up to 60 DAT. The incidence gradually increased and finally reached 100 per cent at 240 DAT in June planting, while at 210 DAT in July planting. December planting reached 100 per cent incidence at 60 DAT, while October, November and January plantings reached at 90 DAT. February plantings took 180 DAT to record 100 per cent incidence. March planting recorded mild incidence at

early stage (5.56 % at 60 DAT), which further increased gradually and took 270 DAT to reach 100 per cent.

Crop planted in June and July coincided with rainfall during the early phase of development which might have resulted in less aphid population and zero incidence of disease up to 90 DAT. The planting during September to January is coincided with winter, during which period aphid population was high. The aphid activity reached its peak in January month. Hence in the later stage of growth the buildup of aphid population lead to more incidence.

March planting although recorded mild disease incidence at early stage it took long duration of 210 days to reach 100 per cent. Apart from that, March panting was superior in all growth and yield traits compared to other months of plantings. During March planting although some aphid activity was recorded, it decreased gradually which lead to reduction in the further spread of the disease in the field.

In the present study winter season planting had high disease severity due to the increased aphid's population. Planting during the lean period of aphid population (spring season) lead to delayed incidence till monsoon. By the time aphid population start building up plants cross the flowering and fruit bearing stage. These results are in line with Sharma *et al*. (2010). So best time for papaya planting is found to be March followed by June, while September to January month of plantings need to be avoided for minimizing PRSV damage.

This fact is substantiated by the research of Mora-Aguilera *et al.* (1992) who illustrated that aphid populations had a bimodal distribution with the highest population peak in December-February and a second peak in August to September, He demonstrated the change in PRSV disease incidence on papaya was influence of aphid population echelon of the previous month.

A study was conducted to record the aphid population and its impact on the incidence of PRSV by planting papaya during different months of the year by Chandrashekar *et al*. (2015). The aphid populations reached a peak in January and recorded low populations from March onwards till September. Papaya planted from February to April showed significantly less incidence of PRSV compared to those planted from September to January. Singh and Shukla (2011) conducted studies on

PRSV disease severity and yield of the papaya at different months. Inoculations were done in pre-*kharif* season (March to April) and *kharif* season (July to August). The symptoms were very severe in plants inoculated in *kharif* and the yield was recorded only 63.9 per cent. However, plants of the pre-*kharif* season showed mild mottling and only a slight reduction. This is because symptoms were masked at high temperatures resulting in a mild incidence of the disease. Whereas in low temperatures severe expression of symptoms and also a heavy yield loss and concluded that, disease severity in the plants inoculated in the *kharif* season was high due to the increased population of aphids. This is confirmed by the study where planting during winter season resulted in more incidence of disease than spring season.

At the same time reports contradictory to present study were recorded by Thiribhuvanamala *et al*. (2016) who reported August to December month papaya planting has minimum PRSV incidence (10 to 20 %) compared to February and March planted crop. This due to influence of weather parameters on population dynamics of different aphid species (Pushpa *et al.*, 2019).

The planting did in March month recorded superior growth parameters *viz.*, plant height (227.19 cm), internodal length (4.22 cm), plant girth (41.47 cm) and number of leaves (29.31) which is followed by June planting. Whereas least growth parameters were recorded from September to January planting at all the growth stages due to early PRSV infection.

The reduced plant height and internodal length are reaction to changes in genomic regions associated with replication, virus movement and the processes that control the level of virus accumulation in the infected plants (Lazarowitz, 1992; Timmermans *et al.*, 1994 and Brown *et al.*, 1997). Auxin act as a key factor in regulating plant growth and development (Benjamins and Scheres, 2008), which is disrupted directly by increased viral particles and movement in the plant (Jin *et al.*, 2016) which lead to severe developmental abnormalities, such as stunting. During viral replication the viral outer capsid protein P2 interacts with *ent*-kaurene oxidases, which are crucial to the biosynthesis of GAs. This result in an inhibition of *ent*-kaurene oxidase activity and a subsequent decline of GA concentrations, leading to stunting and other associated symptoms (Zhu *et al.*, 2005).

Reduced number of leaves in severally infected plants are due to early leaf senescence. Ethylene is senescence promoting hormone in plants. Viral infection at an early stage affects the hormonal imbalance by regulating ethylene in the plants that lead to early senescence of leaves (Gan, 2003). Similarly, senescence of leaves is related to some auxin and abscisic acid responsive genes which are expressed at higher levels in virus infected leaves (Espinoza *et al.*, 2007). Similar studies conducted in okra against *Bhendi yellow vein mosaic virus* (BYVMV) by Ndunguru and Rajabu (2004) recorded the reduction in the growth and yield components incurred by virus infection in okra. Plant height was reduced by 19.50 per cent, number of fruits by 34.70 per cent and petiole length by 32.10 per cent. Sheikh *et al.* (2013) recorded the reduced plant height (24.0 %), number of fruits (32.0 %), fruit length (31.0 %), stem girth (16.0 %), root length (50.0 %) and fruit malformation in diseased plants.

Concerning to flowering and fruiting traits, March planting was found superior over other months of planting with respect to number of days taken from first flowering (89.33), number of days taken from flowering to first fruit set (91.97), number of days taken to first fruit set to harvest (122.41), number of fruits per plant (25.92), fruit diameter (12.31 cm), fruit length (29.93 cm), fruit breadth (14.63 cm), fruit cavity diameter (6.97 cm), yield per plant (60.12 kg) and yield per hectare (185.54 t) followed by June planting. The planting done in January month recorded least flowering and fruiting traits. Further, disease incidence at early to middle crop growth stages (*i.e.* 30, 60, 90, 120, 150 and 180 DAT) had a significant and negative correlation with yield. Whereas, disease incidence at later crop growth stage (*i.e.* 210, 240 and 270 DAT) had no significance with yield. Similarly, remaining all growth, flowering and fruiting traits are recorded significant and negatively correlated and contributed to the reduction of yield.

The severely infected plants recorded delay in flowering traits due to viral multiplication which alters the ethylene production by affecting the activity of S-adenosyl-L-methionine synthetase (SAMS), a key component of the ethylene synthesis pathway, resulting in elevated susceptibility to viral infection and leading to late flowering (Li *et al.*, 2011). Shalit-Kaneh *et al.* (2019) reported the single flower truss (SFT) gene, an ortholog of FT sft induces late flowering, suppresses sympodial

growth and forms single flower truss inflorescences, which is supported by a study where PRSV infected plant has delayed the flowering (81.50 days) in comparison to healthy plant which recorded normal flowering (64.67 days) in papaya cultivar Pusa nanha (Prakash *et al.*, 2015).

Fruiting traits are linked with growth, flowering and photosynthetic activity which is affected by viral infection. As we know viral infection caused an increase in the content of malondialdehyde, alterations in the activities of peroxidase enzymes and quantitative and qualitative changes in their molecular isoforms. A comparison of thylakoid membrane polypeptides from virus infected leaves indicated a decrease in the content of the thylakoid membrane polypeptides. PSII efficiency and the content of chlorophylls (a and b) were significantly lower in the virus infected leaves which should be key in inducing yield losses of infected crops (Huseynova *et al.*, 2018). Irizarry (2016) reported yield loss and seed quality deterioration due to infection of *Soybean vein necrosis virus*, Nancarrow *et al.* (2021) reported yield losses in wheat and barley by *Barley yellow dwarf virus* infection, by Seda-Martinez *et al.* (2021) noticed yield reduction (35-80 %) in cucurbits due to PRSV.

Altering traditional planting times may enable a crop to avoid virus vectors. With viruses that have airborne vectors, the choice of planting date may influence the time and amount of infection. The best planting time will depend on the time of migration of the vectors. While making a multivariant comparison of *Papaya ringspot virus* epidemics, Mora-Aguilera *et al.* (1992) reported the usefulness of transplanting dates in controlling the *Papaya ringspot virus* in Central Veracruz, Mexico. However, for any particular crop, the effectivity of changed planting dates in minimizing virus infection has to be considered in relation to other economic factors (Broadbent, 1952; Cadman and Chambers, 1960).

5.2.2 Identification of susceptible stage of papaya for PRSV infection

The studies on the effect of challenge inoculation of PRSV on papaya at different growth stages (30, 60, 90, 120, 150,180, 210, 240 and 270 DAT) were carried out to know the susceptible stage for infection. The plants inoculated at an early stage *i.e.* 30 and 60 DAT have taken 15 days while those inoculated at 90 DAT took 17 days for symptom expression with a 100 per cent transmission rate. Also,

these plants recorded severe symptoms *viz.*, vein clearing, mosaic symptom, leaf reduction, leaf distortion, shoestring, stunted growth, mottling, chlorotic ring and chlorotic spots on fruits. The inoculation at 120, 150 and 180 DAT delayed symptom expression (20, 24, 25 dpi respectively) with reduced per cent transmission (75.00, 58.33 and 25.00 % respectively) and recorded all the above mentioned symptoms but lacking in mottling symptom. Further delay in inoculation *i.e.* 210, 240 and 270 DAT recorded more delay in (25, 26 and 28 dpi respectively) symptom expression, least per cent transmission (8.33 %) and with only vein clearing symptom.

The severity and frequency of foliar symptoms on PRSV inoculated papaya plants were drastically faster in plants inoculated at early growth stage than the plants inoculated at a later growth stage. Early viral infection generally resulted in severe local and systemic symptoms, whereas later inoculation had little or no effect and caused milder symptoms. Further, PRSV viral titer as detected by qRT-PCR was very high (615.96) in plants inoculated at an early stage (30 DAT). Plants inoculated at later growth stage *i.e.* at 60, 90, 120, 150 and 180 DAT recorded the viral titer of 414.78, 302.36, 235.09, 210.42 and 105.65 respectively. The plants inoculated at the mature stage *i.e.* at 210, 240 and 270 DAT recorded the very least viral titer of 72.58, 44.89 and 9.86 respectively. There was a higher significant and positive correlation between viral titer and per cent transmission rate (0.917).

Early viral infection has a high replication rate and more cell to cell movement of viral particles which is an important step for initiation and spreading of virus infection in plants. This process occurs through the intercellular connections, termed plasmodesmata, and is usually mediated by one or more virus encoded movement proteins which interact with multiple cellular factors (Hong and Ju, 2017). Tran *et al.*, 2021 reported that BAM_1 is a multiple cellular factor required for the efficient cell to cell movement of TMV MP (movement protein), suggesting that BAM_1 interacts with TMV MP to support the early movement of the virus. Which in turn enhances the number and spread of the viral particles. Further, these virus particles hijacking the auxin signaling pathways resulted in morphogenic alterations in the cell with enhanced viral symptoms (Jin *et al.*, 2016).

Host plants may acquire or increase their resistance with growth and development (Develey-Riviere *et al.*, 2007). Wang *et al.* (2018) reported that wheat

lines infected by *Barley yellow dwarf virus* (BYDV) at the adult stage, had up-regulated certain defense responses *viz.,* ROS scavenging, GA and JA biosynthesis genes. This lead to the suppression of BYDV replication, movement and accumulation. Whereas in Arabidopsis and turnip, long distance transport of the CaMV occurs in the phloem. During the course of host development, sink source relationships change and the region of plants that CaMV can invade is progressively reduced, leading to resistance which results into to restricted migration and reduced viral load (Leisner *et al.*, 1992).

Delayed viral inoculation recorded superior growth parameters. Inoculation did at 270 DAT has recorded plant height (241.72 cm), plant internode length (4.26 cm), plants girth (42.70 cm) and the number of leaves per plant (32.14), which were on par with uninoculated control plants. Early inoculated plants recorded least with respect to all these growth parameters and all these growth traits were highly significant and negatively correlated with viral titer in the plant (-0.985, -0.882, -0.943 and -0.947 respectively). The increased viral titer in the plant lead to decrease in the growth attributes. Similarly, delayed inoculation (at 270 DAT) also recorded superior flowering traits *viz.,* the number of days taken for first flowering (94.24) and the number of flowers per plant at 270 days (23.49) compared to early inoculated plants. The correlation between the viral titer and the number of days taken for the first flowering (0.988) was recorded as positive and highly significant. The number of flowers per plant at 270 days (-0.994) was highly negatively significant, which indicated that the viral titer has a negative effect on flowering traits.

Delayed inoculation (at 270 DAT) has recorded superior fruiting traits *viz.,* number of days taken from flowering to first fruit set (85.75), number of days taken from first fruit set to harvest (119.17), number of fruits per plant (37.67), fruit diameter (13.07 cm), fruit length (31.57 cm), fruit breadth (15.28 cm) and fruit cavity diameter (7.25 cm) which was almost similar to uninoculated control plant and viral titer has recorded the strong negatively significant correlation co-efficient with all the fruiting traits. Finally, the severity of foliar symptoms, number of small and malformed fruits decreased as the inoculation was delayed.

Viral inoculation at 270 DAT recorded superior yield per plant (58.64 kg) and yield per hectare (180.95 t) over early inoculated plants and were on par with

uninoculated plants. Inoculation at 30, 60, 90, 120, 150, 180, 210, 240 and 270 DAT has recorded yield reduction of 96.76 per cent, 96.27 per cent, 91.48 per cent, 67.29 per cent, 50.97 per cent, 21.43 per cent, 7.93 per cent, 3.15 per cent and 1.27 per cent respectively over uninoculated plants. As viral inoculation delayed, plants reported less viral load and minimum yield loss which are evidenced by the negative and highly significant correlation between yield and viral titer (-0.924).

Zhu *et al*. (2010) showed that *Sugarcane yellow leaf virus* (SCYLV) infected sugarcane plants recorded high virus titer and severe symptoms like yellow leaf symptoms and yielded only 54.0-60.0 per cent of cane and sugar tonnage compared to plants of low virus titer. Agrios *et al*. (1985) experimented by mechanically inoculating the pepper with *Cucumber mosaic virus* (CMV) at successive weekly intervals and noticed that early inoculations had severe symptoms whereas later inoculations had mild or no symptoms. Further CMV titer was detected greater in early inoculated plants than subsequent inoculation when detected through ELISA. Niblett and Claflin *et al*. (1978) and Uyemoto *et al*. (1981) demonstrated that M*aize dwarf mosaic virus* (MDMV) in maize (*Zea mays*) resulted up to 91.0 per cent yield loss and death of many plants especially when infection occurred early. Similarly, Levy and Lapidot, (2008) determined the effects of plant age on the expression of genetic resistance against *Tomato yellow leaf curl virus* (TYLCV) by inoculating TYLCV at different days after sowing and recorded maximum yield loss in plants inoculated at early stage *i.e.* 14 DAS followed by 28 DAS. Further, he found that delayed inoculation recorded less yield loss (45 DAS) and confirmed the occurrence of age related (or mature plant) resistance in tomato plants against TYLCV.

5.3 Integrated management of PRSV disease under field conditions

5.3.1 Effect of insecticides and bio rationals on PRSV incidence, growth and yield parameters

The effect of insecticides and bio rationals was evaluated to manage PRSV disease on papaya under field conditions. The pooled analysis result of two seasons showed that T_1 (8 sprays of four different insecticide and micronutrients at 30 days interval) is the significantly best treatment in managing the PRSV which recorded zero disease incidence up to 180 DAT. It has taken 360 DAT to reach 100 per cent.

This treatment also recorded superior growth and flowering traits with higher yield than other treatments *viz.*, plant height (225.93 cm), internodal length (4.11 cm), plant girth (40.45 cm), number of leaves per plant (29.50), number of days taken for first flowering (93.01), number of flowers at 270 days (73.06), number of days taken from flowering to first fruit set (94.01), number of days taken from first fruit set to harvest (125.64), number of fruits per plant (26.09), fruit diameter (13.13 cm), fruit length (27.78 cm), fruit breadth (14.43 cm), fruit cavity diameter (6.81 cm), fruit yield per plant (57.86 kg) and fruit yield per hectare (178.56 t). Further, highest cost-benefit ratio of 1:3.54 and highest net returns of 7,69,064 Rs./ha were recorded in T_1.

The T_1 treatment contains micronutrients with four different insecticides which belong to two classes namely pyrazole (tolfenpyrad) and neonicotinoid (imidacloprid, thiacloprid and dinotefuran). These two classes of insecticides are very effective in controlling sucking pests like aphids. All these compounds have different mode of action on insects. Pyrazole acts mainly through the inhibition of the mitochondrial electron transport system (Das, 2013), whereas neonicotinoids target insects' nervous systems *via* nicotinic acetylcholine receptors (nAChRs), exhibiting high selective toxicity (Ihara and Matsuda, 2018).

Neonicotinoids are the most widely used insecticides in the world. They are systemic in action, traveling through plant tissues and protect the crop from sucking pests and safer to natural enemies compared to conventional insecticides. Ahmed *et al*. (2001) reported that two applications at four rates of confidor, an imidacloprid insecticide (47.6, 71.4, 95.2, and 119 g a.i./ha) indirectly controlled *Tomato yellow leaf curl virus* (TYLCV) in field plantings of tomato. Similarly, Csinos *et al*. (2001) reported that imidacloprid (Admire 2F) at 67.2 g a.i./7,000 plants is significantly effective in reducing the *tomato spotted wilt virus* (TSWV) disease on Tobacco

Further, a study conducted by Patel *et al*. (2018) reported that dinotefuron @ 0.2 per cent was significantly effective in controlling the insect vector population (94 %) and decreasing the rice tungro disease infection to 75.0 per cent under glasshouse condition. Mahalakshmi *et al*. (2015) recorded the significant reduction in whiteflies population and incidence of *Yellow mosaic virus* in blackgram when treated with neonicotiniods such as imidacloprid 200 SL @ 0.3 ml/l, thiamethoxam 25 WG @ 0.2 g/land thiacloprid 21.7 SC @ 1.25 ml/l.

The second best treatment is T_5 (8 sprays, combination of four different insecticide and seaweed extract with micronutrients at 30 days interval) in controlling disease incidence along with good growth, flowering and yield attributes. This treatment gave net returns of 6,58,229 Rs./ha with a high cost-benefit ratio of 1:314. However, T_5 is the combination of micronutrients with insecticide and seaweed extract where seaweed extract is sprayed at two month intervals to avoid residual toxicity due to continuous incecticidal sprays. Also seaweed extract is known to activate plant's immune response (Bolles and Chatfield, 2009; Macho and Zipfel, 2014). Specific recognition of these elicitors and their subsequent transduction may trigger defense responses leading to downstream effects such as; thickening of plant cell walls, increased activity of defense enzymes and the production of phytoalexin like defense compounds *etc.*, (Keen and Yoshikawa, 1983 and Bonhoff and Grisebach, 1988). More recently, the red seaweed *Schyzimenia binderi* derived oligo sulphated galactan, poly-Ga, has been shown to induce long term protection against TMV in tobacco plants (Vera *et al.*, 2012).

During the process of cellular damage or infection induced necrosis, plant cells are known to produce few molecules that potentially activate plant's immune response termed as DAMPs (Damage Associated Molecular Patterns) immunity (Tang *et al.*, 2012; Choi *et al.*, 2016). It is pertinent to note here that oligogalacturonides (OGs), the fragments of pectic polysaccharide, are well known DAMPs elicitors. The oligogalacturonide, a linear polymer of 1, 4-linked α-D galacturonic acid, was shown to bind to leucine rich repeat containing TLR (TLR2 and TLR4) receptors to induce immune responses such as MAPK activation, callose deposition, production of reactive oxygen species (ROS), elevated cytosolic Ca^{2+} and defense gene activation (Chandra and Low, 1997; Denoux *et al.*, 2008; Brutus *et al.*, 2010). It is possible that the sulphated oligosugars or any unknown chemical compounds in *K. alvarezii* extract may mimic as DAMPs elicitors leading to activation of plant's immune response.

The third best treatment is T_3 which contains eight sprays of only seaweed extract along with micronutrients. Although T_3 recorded early disease incidence (9.58 % at 180 DAT) the effect of disease incidence was not reflected too much on yield (141.44 t/ha). Seaweeds have been reported to produce diverse secondary metabolites

including antimicrobial, antifungal and antiviral compounds (Madhusudhan *et al.*, 2011; Abu-Ghannam and Rajauria, 2013) due to which, T_3 was free from PRSV incidence up to 150 DAT.

In a similar study conducted by Pushpa *et al.* (2018) foliar application of aqueous sea weed extract (*Kappaphycus alvarezii*) at 4 ml/l on papaya against PRSV disease at every 15 days interval recorded the delay and relatively lesser per cent disease incidence with less symptoms and well formed fruits than the control plants. Moreover, plants that were treated sea weed extract were relatively taller in height with a dense foliar canopy compared to those in the untreated. The average number of fruits in case of the treated plants was higher (30/ plant) than those in the untreated group (15/ plant). However, despite of growing evidence on the functions of diverse defense molecule present in seaweed extract their complex mode of action remain elusive (Arioli *et al.*, 2015). Further studies in this direction are essential for detailed understanding of their molecular mechanisms.

Though the good yield was obtained in T_3 treatment with seaweed extract, it is not encouraging to go for its recommendation at field level as cost benefit ratio is very less (1:2.60) due to its higher cost. Seaweed extract may be incorporated judicially in IDM module as one of the component.

It may be also noted that complete dependence on the bio rationals is not advisable as the treatment T_2 (eight spray of bio oils with micronutrients) recorded higher disease incidence and lesser yield (80.79 t). However, these bio rationals in combination with insecticide (T_4) performed better having less disease incidence and 62.16 per cent increase in yield. The bio rationals can be incorporated in between the insecticidal sprays to reduce the toxicity. Chemicals and bio rationals significantly delayed the incidence of PRSV infection. This finding was encouraging, as escape from early infection is reported to avoid severe reduction in yield (Gonsalves *et al.*, 1998). However, different combination of insecticides and oils differed in their effectiveness in delaying PRSV infection. A combination of neem oil 1 % + dimethoate 1.05 % recorded the least disease incidence of 6.66 per cent and 41.66 per cent respectively at 60 and 150 DAP (Singh *et al.*, 2008b). Similarly, combination of reflective row cover, mineral oil and imidacloprid spray was the most effective

treatment in delaying the PRSV incidence (83.33 % at 18 months after planting) while only mineral oil or neem oil application did not protect the crop.

5.3.2 Integrated disease management modules for management of PRSV disease

Looking into the results of two seasons, three integrated disease management modules were designed and evaluated in farmer field having recommended package of practice (POP) as a check.

The results of the IDM module showed that Module I (12 sprays of four different insecticides and micronutrients at every 20 days interval) is the significantly best module as this module recorded less disease incidence, thereby enhancing the plant growth, flowering and fruiting attributes which ultimately maximized the yield (192.56 t/ha) over check. The next best treatment is M_2 which is followed by M_3 and M_4 (POP).

6. SUMMARY AND CONCLUSIONS

An investigation was conducted on characterization of *Papaya ringspot virus* (PRSV) and *Papaya leaf curl virus* (PaLCuV) infecting papaya in Karnataka and epidemiology and management of PRSV disease at the Department of Plant Pathology, College of Horticulture Bagalkot, UHS, Bagalkot during 2019-20 and 2021. The salient features of the present investigations were summarized hereunder.

The roving survey carried out during January 2019 to March 2021 in major papaya growing districts of Karnataka *viz.*, Bagalkote, Belagavi, Ballari, Chitradurga, Gadag, Haveri, Kalaburagi, Koppal, Vijayapura and Yadgiri revealed that average disease incidence ranged from 50.5-100 per cent. Many fields in Belagavi and Kalaburagi districts recorded 100 per cent incidence.

During survey 107 samples were collected from 75 locations and subjected for PCR based detection using specific primers. Among them 75 samples were tested positive for PRSV infection and the remaining 32 leaf curl samples were detected for begomovirus. In the 32 leaf samples, only DNA-A components of begomovirus was detected indicating that all samples were associated with monopartite begomovirus. Further, no alpha satellite was detected but interestingly defective betasatellite DNA of 0.7 kb was detected in association with all 32 begomovirus infected samples. All the samples were subjected for detection of other RNA viruses *viz., Papaya milk vetch dwarf virus*, *Papaya leaf distortion virus*, *Papaya mosaic virus* and *Zucchini yellow mosaic virus* and phytoplasma. However, no such viruses association was detected.

Complete genome characterization of PRSV-BGK isolate revealed that this isolate contains length of 10,341 nt and consists of a single major polyprotine with ten mature peptides (P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb and CP) located at different locations in +ve sense stand, runs in a clockwise direction along with 5’ and 3’ untranslated regions at terminal end and it contain two conserved regions 5’-AAATAAAACATCT and -CTCTTAGAATGAG-3’. Further, nine cleavage sites around polyproteins and well characterized motifs were identified in PRSV genome. The PRSV-BGK isolate showed high nucleotide sequence identity of 95.84 per cent with PRSV-[IN:TS:Hyd:HYD:Pap:15] (KP743981) from Telangana followed by 95.54 with PRSV-[IN:MH:Pun:VC:Pap:15] (MF405299) from Maharashtra and

formed close cluster with Indian isolates. The present finding is first report on complete genome sequence of an isolate of PRSV (designated as PRSV-BGK) from South India. Further, recombination analysis revealed the past 6 recombination events in PRSV-BGK isolate indicating that there is a differential variability and recombination across the viral genome.

Among 32 begomoviruses infected samples 13 representative isolates from different surveyed locations were subjected for molecular characterization by sequencing the complete genome. The complete genome sequence analysis revealed that among 13 isolates, 4 isolates (PaLCuV-1, PaLCuV-3, PaLCuV-4 and PaLCuV-13) were demarcated as new distinct species in begomovirus and proposed the name as *Papaya leaf curl Bagalkote virus*, PaLCuBKV-[IN:Kar:Bel:Pap:21] Gokak; *Papaya leaf curl Bagalkote virus*, PaLCuBKV-[IN:Kar:Bgk:Pap:21] Bagalkote; *Papaya leaf curl Bagalkote virus*, PaLCuBKV-[IN:Kar:Bgk:Pap:21] Bilgi and *Papaya leaf curl Haveri virus*, PaLCuHV-[IN:Kar:Hav:Pap:21] Haveri respectively.

Further, PaLCuV-9, PaLCuV-10, PaLCuV-11 and PaLCuV-12 isolates were demarcated as new strains and proposed the name as *Chilli leaf curl virus*, ChiLCV-[IN:Kar:Kal:Pap:21] Afjalpur strain; *Papaya leaf curl virus*, PaLCuV-[IN:Kar:Kal:Pap:21] Kalaburagi strain; *Croton yellow vein mosaic virus*, CYVMV-[IN:Kar:Kal:Pap:21], Kalaburagi strain and *Papaya leaf curl virus*, PaLCuV-[IN:Kar:Vij:Pap:21] Vijayapura strain respectively.

Whereas, five isolates (PaLCuV-2, PaLCuV-5, PaLCuV-6, PaLCuV-7 and PaLCuV-8) were demarcated as new variant and suggested the name as *Chilli leaf curl virus*, ChiLCV-[IN:Kar:Bel:Pap:21] Gokak variant; *Chilli leaf curl virus*, ChiLCV-[IN:Kar:Bel:Pap:21] Ramadurga variant; *Chilli leaf curl virus*, ChiLCV-[IN:Kar:Bgk:Pap:21] Hunugunda1 variant; *Chilli leaf curl virus*, ChiLCV-[IN:Kar:Bgk:Pap:21] Hunugunda2 variant and *Chilli leaf curl virus*, ChiLCV-[IN:Kar:Kal:Pap:21] Afjalpur variant respectively these results are supported by SDT and phylogenetic analysis.

Recombination analysis revealed the evolution of all 13 begomoviral isolates by past intraspecific recombinations with different major and minor parents at different nucleotide position.

Host indexing study was conducted using eight host species out of which only two host species *i.e. Chenopodium quinoa* and *Cucumis sativus* identified as indicator hosts for PRSV which produced local lesion and mosaic symptoms respectively. Presence of PRSV in transmitted host plant was confirmed through PCR.

Studies on the different months of planting under field conditions revealed that planting during March is effective for the management of PRSV as it recorded least disease incidence (5.56 % at 60 DAT and took 270 DAT to reach 100 %) and maximum yield (185.54 t) along with good growth and yield parameters. This was followed by planting during June. Whereas planting during winter months (September to January) recorded highest diseases incidence. Especially December planting recorded maximum incidence of disease (77.78 %) at 60 DAT. Correlation studies revealed papaya growth stage up to 180 DAT is the very critical period for the incidence of disease as plants infected up to 180 DAT recorded higher yield loss.

The effect of inoculation of PRSV at different growth stages of papaya revealed that per cent transmission and severity of symptoms on PRSV inoculated papaya plants were drastically greater in early inoculated plants than in plants inoculated at later stages. Transmission was 100 per cent when inoculation at 30, 60 and 90 DAT, 75.0 per cent at 120 DAT, 58.33 per cent at 150 DAT, 25.0 per cent at 180 DAT and 8.33 per cent transmission at 210, 240 and 270 DAT. Similarly, the PRSV titer as detected by qRT-PCR was also greater in early inoculated plants than in subsequent inoculations (615.97 at 30 DAT, 414.78 at 60 DAT, 302.36 at 90 DAT, 235.09 at 120 DAT, 210.42 at 150 DAT, 105.65 at 180 DAT, 72.58 at 210 DAT, 44.89 at 240, 9.86 at 270 DAT). Further, early inoculated plants recorded the least values for growth and yield attributes. As the inoculation delayed there was increase in growth and yield parameters (1.92 t/ha at 30 DAT, 2.22 t/ha at 60 DAT, 5.06 t/ha at 90 DAT, 19.43 t/ha at 120 DAT, 29.12 t/ha at 150 DAT, 46.66 t/ha at 180 DAT, 54.68 t/ha at 210 DAT, 57.52 t/ha at 240 DAT, 58.64 t/ha at 270 DAT). Moreover, there was a strong significant positive correlation between per cent transmission. Whereas negative correlation between plant growth, flowering and yield traits with higher viral titer.

The studies on the management of PRSV under field condition for two seasons (2019-20 and 2020-21) using insecticides and bio rationales revealed that T_1 (8 sprays

of four different insecticide *i.e* tolfenpyrad 15 % EC @1 ml/l, imidacloprid 17.8 % SL @ 0.2 ml/l, thiacloprid 21.7 SC @ 1 ml/l and dinotefuran 20 % SG @ 0.5g/l alternatively and micronutrients at every 30 days intervals) proved as the best treatment in managing PRSV under field conditions. It recorded least diseases incidence (1.49 % at 210 DAT and took 360 DAT to reach 100%) and maximum yield (178.56 t/ha) along with high cost-benefit ratio (1:3.54) was seen in this treatment. Treatment, T_5 (8 sprays, combination of four different insecticide *i.e* tolfenpyrad 15 % EC @1 ml/l, imidacloprid 17.8 % SL @ 0.2 ml/l, thiacloprid 21.7 SC @ 1 ml/l and dinotefuran 20 % SG @ 0.5g/l and seaweed extract alternatively with micronutrients at 30 days interval) was next best treatment.

Looking into results of two seasons of PRSV management experiment three integrated diseases management module were designed and evaluated along with POP as a check and found that M_1 (contains 12 sprays insecticides *i.e* tolfenpyrad 15% EC @1 ml/l, imidacloprid 17.8%SL @ 0.2 ml/l, thiacloprid 21.7 SC @ 1 ml/l and dinotefuran 20 % SG @ 0.5g/l alternatively 3 times and micronutrients at 20 days interval) is the best module with respect to managing diseases (0.44 % diseases incidence at 180 DAT and took 330 DAT to reach 100%) and getting higher yield (192.56 t/ha) and good net returns (Rs. 84,8691/-).

Conclusion

- PRSV is widely distributed throughout the major papaya growing areas of Karnataka.
- The complete genome sequencing done for PRSV Bagalkote isolate from Karnataka is a first full length genome reported from south India. This helps in detailed understanding the diversity of south Indian isolate with other PRSV isolates of India and world.
- The samples showing leaf curl symptoms were detected for the presence of begomoviruses, which is the first report from Karnataka.
- Planting in the month of March is found non-chemical, ecofriendly and effective for the management of PRSV under field condition. Farmers need to avoid planting during September to January months to avoid disease and higher losses.

- Early crop growth stage (up to 180 DAT) is very critical period for PRSV infection, so need to take effective disease management measures up to 180 DAT for managing disease and getting higher yield

- T1 (8 sprays of four different insecticide *i.e* tolfenpyrad 15% EC @1 ml/l, imidacloprid 17.8%SL @ 0.2 ml/l, thiacloprid 21.7 SC @ 1 ml/l and dinotefuran 20 % SG @ 0.5g/l alternatively and micronutrients at every 30 days intervals) proved as the best treatment in managing PRSV under field conditions. It recorded least diseases incidence and maximum yield along with high cost-benefit ratio was seen in this treatment.
- The module (M1) which contains 12 sprays insecticides *i.e* tolfenpyrad 15% EC @1 ml/l, imidacloprid 17.8%SL @ 0.2 ml/l, thiacloprid 21.7 SC @ 1 ml/l and dinotefuran 20 % SG @ 0.5g/l alternatively 3 times and micronutrients at 20 days interval is found best integrated diseases module for managing the PRSV disease.

Future line of work

- Extensive survey and proper documentation of prevalence, distribution, incidence and molecular diversity of PRSV and PaLCuV in all part of Karnataka.

- Development of diagnostic tool such as multiplex PCR which is helpful in early and multiple virus detection in a single reaction for planning of disease management practices.

- Production of polyclonal antibody and development of serological diagnostic technique for PaLCuV detection.

- Development of infectious clones for proving Koch’s postulates through molecular approach.

- Development of disease forecasting models

- Management of PRSV can be still be fine tuned using sea weed extract to get higher yield even after infection.

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Appendix I-A

DNA extraction buffer:

2 per cent CTAB (w/v), 1.4 M NaCl, 20 mM EDTA of pH 8.0, 100 mM Tris-HCl of pH 8.0, 10.0 per cent polyvinyl pyrolidone and 0.2 per cent mercaptoethanol (added *in situ* just before DNA extraction)

Appendix I-B

Reagents for preparation of agarose gel for electrophoresis

i.10X TAE (Tris Acetic acid EDTA buffer, 1L):

108 g Tris base, 25 ml acetic acid 0.5 M EDTA (pH 8.0); volume was made up to 1 liter with distilled water pH was adjusted to 8.0 and stored at room temperature.

ii.6X Loading dye:

10 mM Tris HCl (pH 7.6), 0.03 % bromophenol blue, 0.03 % xylene cyanol, 60 % glycerol, 60 mM EDTA.

iii.0.1 % ethidium bromide stock solution (10 ml) :

100 mg ethidium bromide was dissolved in 10 ml distilled water; Stored in dark bottle at 4^0 C

iv.1kb DNA ladder (StepUp™ 1 kb DNA Ladder, 50 µg, GeNei SKU: MBD20):

It containg 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA. Two micro liters of loading mixture was required for an agarose gel lane.

Appendix I-C

Reagents for preparation of Luria-Bertani (LB) agar and broth:

i.Luria-Bertani (LB)

Composition of LB medium:		**Composition of LB broth:**	
Agar-agar	20 gm	-	-
Bactotryptone	10 gm	Bactotryptone	10 gm
Yeast extract agar	10 gm	Yeast extract agar	10 gm
Sodium chloride	05 gm	Sodium chloride	05 gm
Distilled water	01 liter	Distilled water	01 liter

ii.Calcium chloride ($CaCl_2$) : 0.1 M: 1.4 gm $CaCl_2$ in 100 ml double distilled water.

iii.Ampicillin: 100 mg volume of Ampicillin (Himedia) in 1 ml double distilled water.

iv.X-Gal: 20 mg/ml stock solution of X-Gal (GeNei, SKU: FC5L) in N, N-dimethylformamide (DMF), or dimethylsulfoxide (DMSO).

v.IPTG (Isopropyl-beta-thio galactopyranoside): 2.383 g of IPTG (GeNei, SKU: FC1L) in 10 ml double distilled water.

Appendix I-D

Preparation of plasmid DNA by alkaline lysis with sodium dodecyl sulfate (SDS)

Mini preparation of plasmid DNA by alkaline lysis method is as follows.

Reagents and media required

Solution I: 50mM glucose, 25 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0). 100 ml of solution I was prepared from standard stocks autoclaved for 15 min at 15 PSI on liquid cycle and stored at 4^0 C

Solution II: 0.2 N NaOH**,** 1 % SDS (w/v) and SDW (Prepared freshly).

Solution III: 7.5 M Ammonium acetate: 60 ml, Glacial acetic acid: 11.5 ml**,** SDW: 28.5 ml, Ethanol (70 %), RNase, TE buffer: 10 mM Tris-HCl and 1 mM EDTA pH 8.

Appendix II-A

Extraction of total DNA using cetyl trimethyl ammonium bromide (CTAB) method from papaya leaf samples

The genomic DNA was extracted from the healthy and virus-infected surveyed samples were done based on the method of Lodhi *et al.* (1994). One hundred milligrams of fresh leaf tissues were ground with 1000 ml of pre-warmed (60^0 C) DNA extraction buffer using sterile mortar and pestle. The whole crude sap was transferred into a fresh 1.5 ml eppendorf tube and incubated for 30 min at 60^0 C in a water bath with occasional mixing. The supernatant (750 ml) was transferred into a fresh 1.5 ml eppendorf tube and mixed with an equal amount (750 ml) of chloroform: isoamyl alcohol (24:1) by vortexing. The samples were then centrifuged at 13000 rpm for 10 min using a microcentrifuge. The aqueous supernatant was collected into a fresh 1.5 ml eppendorf tube. The DNA was precipitated by mixing with 300 ml of chilled isopropanol by inversion and incubated at -20^0 C for at least one hour. The tubes were centrifuged at 13000 rpm for 10 min. The resulted pellet was washed with 70.0 per cent ethanol, dried in a vacuum drier for 10 min and re-suspended with 50 ml of $T_{10}E_1$ buffer (10 mM Tris-HCl of pH 8.0 and 0.1 mM EDTA of pH 8.0). DNA was assessed by agarose gel electrophoresis.

Appendix II-B

Phenol-chloroform extraction of DNA purification

DNA isolation from papaya leaves was usually compromised by excessive contamination of RNA, secondary metabolites, polysaccharides and polyphenols which impede the extraction of high quality intact genomic nucleic acids (Sambrook and Russell, 2006).

Purification of nucleic acids by extraction with phenol: chloroform was done based on Sambrook and Russell (2006). DNA samples were treated with 1 µl of 10µg/ml RNase A (GeNei, SKU: FC34S), and incubate at 37^0C for 30 mins for the removal of RNA from genomic DNA samples. After RNase A treatment, to the DNA samples, 200ml of $T_{10}E_1$ buffer and 200ml of phenol was added and mixed well by shaking for 1min and mixed with an equal amount (400 ml) of chloroform: isoamyl alcohol (24:1) by vortexing for 1 min. The samples were then centrifuged at 12000 rpm for 10 min. The aqueous supernatant was collected into a fresh 1.5 ml eppendorf tube and mixed with 400 ml of chloroform: isoamyl alcohol (24:1) by vortexing for 1 min. The samples were then centrifuged at 12000 rpm for 10 min. The aqueous supernatant was collected into a fresh 1.5 ml eppendorf tube. The $1/10^{th}$ volume of 7.5 M of ammonium acetate added to the supernatant. The DNA was precipitated by mixing with an equal volume of chilled isopropanol by inversion and incubated at -20^0 C overnight. The tubes were centrifuged at 12000 rpm for 15 min. The resulted pellet was washed with 70.0 per cent ethanol, dried in a vacuum drier for 10 min and re-suspended with 50 ml of $T_{10}E_1$ buffer (10 mM Tris-HCl of pH 8.0 and 0.1 mM EDTA of pH 8.0).

Appendix II-C

Extraction of total RNA from virus infected papaya plant leaves

Isolation of total RNA from the leaf of healthy Papaya, virus infected surveyed samples and crop plant used in epidemiology study was done by using Spectrum™ Plant Total RNA Kit from Sigma-Aldrich (Sigma-Aldrich, Catalog No. STRN50).

To avoid the RNases contamination, all the glassware and plastic wares like tubes and pipette tips were washed thoroughly, dried and treated with 0.1 per cent DEPC (diethylene pyrocarbonate) overnight at room temperature and then autoclaved. During the RNA extraction period the working areas, glassware, plastic surfaces, reaction vessels, countertops, pipettors and gloves were applied with RNaseZAP™ (Sigma-Aldrich, Catalog No. R2020) rigorously to eliminate RNase contamination to maintain RNase-free condition.

The healthy Papaya, virus infected surveyed samples and PRSV infected crop plants used in the epidemiology study were brought under ice-cold conditions and plant tissue were submerge in liquid nitrogen as soon as possible to prevent RNA degradation. Grinded the tissue to a fine powder in liquid nitrogen using a dried pre-chilled mortar and pestle. After liquid nitrogen had evaporated from the frozen tissue powdered, quickly weighed approximately 100 mg of the tissue powder in a 2 ml microcentrifuge tube, pre chilled on dry ice. Keep the weighed sample on dry ice or at –70 °C before the lysis solution was added. Pipetted 500 µl of the Lysis Solution/2 ME Mixture to 100 mg of tissue powder and vortexed immediately and vigorously for at least 30 seconds. Incubated the sample at 56° C for 3-5 minutes.

Centrifuged the sample at maximum speed (14,000–16,000 × *g*) for 3 minutes to pellet cellular debris. Pipetted the lysate supernatant into a filtration column seated in a 2 ml collection tube by positioning the pipette tip at the bottom of the tube but away from the pellet. If there was a layer of floating particulates, position the pipette tip below the floating layer and away from the pellet before pipetting the supernatant. Closed the cap and centrifuged at maximum speed (14,000–16,000 × *g*) for 1 minute to remove residual debris. Save the clarified flow-through lysate.

Pipetted 500 µl of binding solution into the clarified lysate and mix immediately and thoroughly by pipetting at least 5 times or vortex briefly.

Pipetted 700 µl of the mixture into a binding column seated in a 2 ml collection Tube. Closed the cap and centrifuged at maximum speed (14,000–16,000 × *g*) for 1 minute to bind RNA. Decanted the flow-through liquid and taped the collection tube (upside down) briefly on a clean absorbent paper to drain the residual liquid. Returned the column to the collection tube and pipetted the remaining mixture to the column and repeated the centrifugation and decanting steps.

Pipetted 500 µl of wash solution 1 into the column. Closed the cap and centrifuged at maximum speed (14,000–16,000 × *g*) for 1 minute. Decanted the flow-

through liquid and taped the collection tube (upside down) briefly on a clean absorbent paper to drain the residual liquid. Returned the column to the collection tube.

Then pipetted 500 µl of the diluted wash solution 2 into the column. Closed the cap and centrifuged at maximum speed (14,000–16,000 × *g*) for 30 seconds. Discarded the flow-through liquid and taped the collection tube (upside down) briefly on a clean absorbent paper to drained the residual liquid. Returned the column to the collection tube.

Pipetted another 500 µl of the diluted wash solution 2 into the column, closed the cap and centrifuged at maximum speed (14,000–16,000 × *g*) for 30 seconds. Discarded the flow-through liquid and taped the collection tube (upside down) briefly on a clean absorbent paper to drained the residual liquid. Returned the column to the collection tube.

Centrifuged the column at maximum speed (14,000–16,000 × *g*) for 1 minute to dry. Carefully removed the column-tube assembly from the centrifuge to avoid splashing the residue flow-through liquid on the dried column.

Transfered the column to a new, clean 2 ml collection tube. Pipetted 50 µl of elution solution directly onto the center of the binding matrix inside the column. Closed the cap and let the tube sit for 1 minute. Centrifuged at maximum speed for 1 minute to elute. Purified RNA was now in the flow-through elute and ready for immediate use or storage at –20° C (short term) or –70° C (long term).

Appendix II-D

Reverse transcription (cDNA Synthesis) of RNA

Extracted total RNA from healthy, virus infected surveyed samples and crop plants used in epidemiology study infected by PRSV samples were quantified and dilute the RNA to 1000ng/µl. The diluted RNA was taken for reverse transcription for synthesizing cDNA using PrimeScript™ 1st strand cDNA Synthesis Kit (Takara Bio, Catalog No. #6110A).

RT mixture was prepared by adding the following ingredients into the PCR tube. 1 µl Oligo dT Primer (50 µM) or 1µl Random 6 mers (50 µM), 1µl dNTP Mixture (10 mM each), 3 µl template total RNA (1 µg) and 7 µl RNase Free dH_2O a total of 10 µl reaction mixture was incubated it for 5 min at 65^0 C then cooled immediately on ice. Finally prepared the reaction mixture in a total volume of 20 µl by adding 10 µl Template RNA Primer mixture, 4µl 5X PrimeScript buffer, 0.5µl RNase inhibitor (40 U/µl), 1 µl PrimeScript RTase (200 U/µl) and 4.5 µl RNase free dH_2O and Mixed gently. Then incubated the reaction mixture using the following conditions; 30^0 C for 10 min (required when using Random 6 mers), 42^0 C for 60 min and inactivated the enzyme by incubating at 95^0 C for 5 min then cooled on ice immediately. The cDNA thus obtained was used for performing PCR.

Appendix II-E

Polymerase chain reaction and reverse transcription-PCR (RT-PCR) mediated amplification of virally infected samples of papaya

The DNA or cDNA synthesized by reverse transcription was subjected to PCR or RT-PCR respectively. Specific primers were used to amplify the DNA and RNA viruses were mentioned in table 12. The primers were got synthesized by eurofinsTM, Eurofins Genomics India Private Limited, Bangalore.

a. PCR reaction mixture used for detection of the virus

PCR reaction was carried using KAPA Taq PCR (KK1016-500 U) Reagents,

KAPA^{TM} Taq DNA Polymerase (5 U/µL)

KAPA^{TM} Taq Buffer (10X) containing 15 mM $MgCl_2$ (1.5 mM at 1X)

KAPA^{TM} dNTP Mix (10 mM each)

b . Procedure

i. 0.2 ml PCR tubes were taken, labeled and kept on ice crystals.

ii. Samples were taken for PCR along with positive control and negative control (distilled water).

iii. 25 µl PCR mixture was prepared by adding the following ingredients into the eppendorf tube.

Sterile distilled water	18.9 µl
10X KAPA^{TM} Taq Buffer with 15 mM $MgCl_2$ (1.5 mM at 1X)	2.5 µL
KAPA^{TM} dNTP Mix (10 mM each)	0.5 µL
Primer Forward (10 mM)	1.0 µl
Primer Reverse (10 mM)	1.0 µl
5 U/µL KAPA^{TM} *Taq** DNA Polymerase	0.1 µl
Template DNA	1.0 µl
Total reaction mixture	**25.0 µl**

**Thermus aquaticus* DNA polymerase

Appendix II-F

Analysis of PCR and RT-PCR products by agarose gel electrophoresis

Reagents for preparation of agarose gel for electrophoresis

The gel casting tray was cleaned by washing and subsequent wiping with distilled water. The required volume of 1% agarose was prepared by melting agarose in 1X TAE buffer in a microwave oven. Once agarose solution was cooled up to 45-50^0 C, ethidium bromide was added at the rate of 0.5 mg/ml of agarose. The gel casting tray was prepared with the desired number of wells and taping the ends. After the comb was placed in the boat, molten agarose was poured into the boat without forming air bubbles and allowed 30 min for proper solidification (polymerization). Five microliters of PCR or RT-PCR products were separately mixed with loading dye 3 ml on a clean polythene strip. After removing the tapes from the ends of the casting tray, the tray was placed in the electrophoresis tank filled with 1X TAE buffer maintaining the buffer level 2 mm above the gel slab. The comb was removed carefully. Amplified products were analyzed through gel electrophoresis under 90 volts for 1 hour and then visualized using ethidium bromide stain under an alpha imager gel documentation system. DNA ladder set (StepUp™ 1 kb DNA Ladder) was included as a sized molecular marker. DNA from healthy plants and double distilled water were used as experimental controls.

Appendix II-G

Elution of amplified PCR fragments from agarose gel using NucleoSpin® Gel and PCR Clean-up kit

The DNA from agarose gel in TAE buffer was extracted and purified using NucleoSpin® Gel and PCR Clean-up kit (Takara Bio, Catalog No. # 740986.50) according to the instructions given by the manufacturer. PCR amplified product (about 50-100ml) containing the desired fragment was loaded into 2.0 % low melting agarose fresh gel in fresh buffer. The desired DNA fragment was excised from the agarose gel with a clean sharp scalpel while observing through a UV transilluminator. Minimize UV exposure time to avoid damaging the DNA. The gel slice was weighed in a 1.5 ml eppendorf tube. Determine the weight of the gel slice and transfer it to a clean tube. For each 100 mg of agarose gel add 200 µL Buffer NTI. Incubate the sample for 5–10 min at 50° C. Vortex the sample briefly every 2–3 min until the gel slice is completely dissolved.

Placed a PCR clean-up column into a collection tube (2 mL) and loaded up to 700 µL sample. Centrifuged for 30 s at 11,000 x *g*. Discarded the flow-through and placed the column back into the collection tube. Loaded the remaining sample if necessary and repeated the centrifugation step. Added 700 µL Buffer NT3 to the PCR Clean-up Column. Centrifuged for 30 s at 11,000 x *g*. Discarded the flow-through and placed the column back into the collection tube. Centrifuged for 1 min at 11,000 x *g* to remove Buffer NT3 completely. Make sure the spin column does not come in contact with the flow-through while removing it from the centrifugde and the collection tube. Placed the PCR clean-up column into a new 1.5 mL microcentrifuge tube. Added 15–30 µL buffer NE and incubated at room temperature (18-25° C) for 1 min. Centrifuged for 1 min at 11,000 x *g*.

Kit contents:

Contents	**50 preparation**
Binding Buffer NTI	40 ml
Wash Buffer NT3	25 ml
Elution Buffer NE	13 ml
NucleoSpin® Gel and PCR Clean-up Columns	50 ml
Collection Tubes	50 ml

Appendix II-H

Cloning and ligation

The PCR amplified DNA fragments were cloned into the plasmid vector pMD20-T vector (Takara Bio Mighty TA-cloning Kit, Catalog No. #6028) (Fig. 1). PCR-amplified products obtained using a Taq-based DNA polymerase had a single base, deoxyriboadenosine (dA), added at the 3' end. The PCR products with 3' A overhangs utilize a T vector with a single deoxyribothymidine (dT) addition at the 3' end. This cloning mechanism took advantage of the complementarity between the single dT overhang of the vector and the single dA overhanged at the end of the PCR product. The vector contained AmpR in the pMD20-T vector as a selectable marker.

Reagents for ligation

Ligation of PCR product was carried out using TA cloning method followed by using the plasmid vector pMD20-T. For the final volume of 10 ml for each PCR fragment.

Plasmid vector pMD20-T of 50 ng/µl, Ligation Mighty Mix and sterile purified water. The following components were mixed in a 0.5 ml eppendorf tube.

PCR product	1 µl
pMD20-T vector	1 µl
Sterile purified water	3 µl
Ligation Mighty Mix	5 µl

Procedure:

1. Added 1 µl of the PCR product above to a new microtube.
2. Added 1 µl of pMD20-T vector and 3 µl of sterile purified water to the tube, and mixed. Adjusted accordingly so that the PCR product and sterile purified water were 4 µl of the total volume.
3. Add 5 µl of Ligation Mighty Mix and gently mix.
4. The ligation reaction mixture was incubated at 16^0 C minutes followed by 4^0 C overnight and used for transformation.

Appendix II-I

Preparation of competent cells

Competent cells of *Escherichia coli* strain DH5α were prepared by the calcium chloride method as described by Sambrook and Russel (2001).

Procedure:

The Luria-Bertani (LB) agar and broth were conical flasks for the preparation of respective media which were plugged with a sterilized cotton plug. The media and broth were autoclaved at 121^0 C at 15 Lbs pressure for 15 minutes, after the pressure decreased the flasks containing the media were taken out of the autoclave and kept at room temperature.

The LB agar medium was melted gently, taken to the laminar airflow chamber (LAFC) and 100 ml of LB agar medium was poured into the autoclaved Petri dishes where *E.coli* was used for inoculation on solidified LB agar medium and it was streaked on medium with the help of autoclaved toothpicks. The inoculated plates were incubated at 37^0C overnight by keeping the Petri dishes invert. Then the growth of the bacterium *E. coli* was observed in the Petri dishes. The single colony was picked and streaked with the help of toothpicks; the streaked cells were inoculated into the 50 ml of LB broth medium and grown for 2 hours at 37^0 C at 130 rpm until the turbidity was observed in the media. Then this conical flask containing the turbid growth was incubated for one hour at ice by covering the flask up to its neck. After one hour incubation period this conical flask was taken to LAFC and turbid growth was transferred to the chilled oak ridge tube. And this tube was centrifuged at 5200 rpm for 10 minutes at 4^0 C. After 10 minutes of centrifugation, the bacterial pellet was observed under the oak ridge tube, so the supernatant was discarded without disturbing that bacterial pellet. Then about 25 ml of 0.1M $CaCl_2$ solution was added to this oak ridge tube and the pellet was mixed properly into calcium chloride solution. This mixture was kept on ice for one-hour incubation by mixing the mixture. After one hour this tube was centrifuged at 5000 rpm for 10 minutes at 4^0 C. Then again, the bacterial pellet was observed in the tube, again discarded the supernatant without disturbing the pellet and 2-3 ml of 0.1M $CaCl_2$ solution was added to this tube and mixed the pellet into that solution and kept on ice for ten minutes.

Transformation

The 100 ml of competent cells were aliquoted into a sterilized microcentrifuge tube. The ligated products were transferred to the respective microcentrifuge tubes which were containing the competent cells with $CaCl_2$ solution, then tubes mixture was mixed properly by inversion and kept on ice for 1 hour in-between tapping, then after one-hour heat shock was given to these tubes at 42^0 C for exactly two minutes to make cloned plasmid vector into the host cell. Exactly after two minutes, these tubes were kept on ice for five minutes. Then the 800 ml of LB broth medium was added to all the respective tubes, mixed properly and kept for growing in incubation cum shaker at 130 rpm at 37^0 C. After one hour, the tubes were taken out and centrifuged at 12,000 rpm for 30 seconds to get the bacterial pellet. Then the supernatant was decanted by retaining about 100 ml of the suspension including pellets inside the tube. All the respective pellets formed in the tubes were resuspended into the LB medium and spread on the plates of LB agar medium containing ampicillin (per 100 ml of media containing 100 ml of ampicillin), X-Gal and IPTG. The inoculated plates were then kept under an incubator at 37^0 C overnight to get colony development.

Appendix II-J

Preparation of plasmid DNA by alkaline lysis with Sodium dodecyl sulfate (SDS)

The colonies were selected from each fragment for the isolation of plasmid DNA. A single white colony was selected from each plate and inoculated into 10 ml of LB broth medium containing ampicillin (10 ml of LB broth medium contains 10 ml of ampicillin) in the 50 ml size test tube and grown by incubating at 37^0 C at 130 rpm for 16 hours. After 16 hours of incubating cum shaking the growth suspension was taken to LAFC and prepared glycerol stocks by mixing 200 ml of 50% glycerol and 800 ml of suspension in a 1.5 ml size eppendorf tube.

1. The grown bacterial culture was poured into a microcentrifuge tube and centrifuged the tube for 30 seconds at 12000 rpm.
2. Removed the LB broth from the tubes leaving the bacterial pellets dry as possible.
3. Resuspended the bacterial pellet into 200 ml of solution I by proper vortexing and kept at room temperature for 5 minutes then again centrifuged the tube to remove the solution I.
4. Again resuspended the bacterial pellet into Solution I, mixed properly by vortexing and kept at RT for 5 minutes.
5. Then 400 ml of freshly prepared solution II was added to the tubes containing suspension then mixed 3-4 times by inversion and incubated the tubes on ice for 10 minutes.
6. Then 250 ml of Solution III was added to these tubes and mixed 3-4 times by inversion.
7. Then lysed cells were separated by centrifuging the tubes at 12,000 rpm for 10 minutes and the supernatant was transferred to the newly labeled respective tubes.
8. This supernatant was mixed with 600 ml of isopropanol by inverting the tubes and incubated at -20^0 C for one hour.
9. After incubation, the tubes were centrifuged at 13000 rpm for 10 minutes, the supernatant was decanted and the pellet was washed with 500 ml of 70% ethanol.
10. Then pellets were dried at 37^0 C for 30 minutes.
11. Dissolved the pellets in 100 ml of TE, then tapped the tubes gently and kept at RT for 20 minutes.
12. 50 ml of solution III was added to these tubes, mixed properly and kept on ice for 30 minutes.
13. After 30 minutes, centrifuged the tubes for 10 minutes at 12,000 rpm and the supernatant was transferred to new respective tubes.
14. Then 150 ml of isopropanol was added, mixed by inverting two to three times and kept for incubation at -20^0 C for about 1 hour.
15. Then the incubated tubes were centrifuged at 12,000 rpm for 10 minutes.

16. After decanting the supernatant, the pellet was washed with 500 ml of 70% ethanol and dried the pellet under 37^0 C.
17. Then pellets were resuspended and dissolved in 100 ml of TE and 1 ml of RNase was added to this suspension.
18. These tubes were briefly spun, tapped, again brief spun for 30 seconds and tubes were incubated at 37^0 C for 30 minutes.
19. Tubes were taken out, added 50 ml of solution III and mixed properly.
20. Then added 150 ml of isopropanol to these tubes, mixed by inverting and kept for incubation at -20^0 C for 1 hour.
21. Then after incubation, the tubes were centrifuged at 12,000 rpm for 10 minutes.
22. The pellet was washed with 500 ml of 70% ethanol and dried at 37^0 C for 30 minutes.
23. Then finally the dried pellets were stored at -20^0 C by adding 40 ml of TE.

Appendix II-K

Preparation of 0.005 M phosphate buffer

Reagents

0.05 M phosphate buffer, pH7.0 (to prepare 1 liter)

KH_2PO_4: 2.4 g

K_2HPO_4: 5.4 g

Thioglycerol: 0.75 ml

Dissolved in 1lt of distilled water, by adjusting pH 7.0.

0.02 M 2-mercaptoethanol (1.56 ml/l)

Selection of PRSV infected tissue

Young infected tissue showing primary symptoms were used to those from older plants because of high infective virus concentration and fewer inhibitory compounds. The leaf stage used for inoculation given in table 22**.**

Preparation of infected leaf extracts

The PRSV infected leaf tissue was triturated in a sterilized pre-cold mortar and pestle in chilled 0.05 M Phosphate buffer. Grind the tissues in 1:9 dilutions *i.e* 1 gm tissue in 9 ml buffer till a fine homogenate was obtained. Keep the inoculums chilled till it was used for inoculation. The homogenate was sieved through a muslin cloth. Before inoculation, celite was added to the inoculum (0.025 gm/ml) to serve as an abrasive. Mechanical inoculation was carried out by swabbing with a small piece of sterilized absorbent cotton wool soaked in the inoculum on the upper and lower surface of the young leaves of the host crop. Inoculated plants will be observed daily for the development of phenotypic symptoms. Different kinds of symptoms developed on individual plant species will be recorded separately.

Appendix II-L

Designing of qRT-PCR Primers for PRSV

Primer pairs were designed using the NCBI Primer-Blast online tool based on sequence data obtained after amplification of the HC-Pro gene of PRSV Bagalkote, Isolate. Primer-BLAST helped to design target-specific primers in one step as well as to check the specificity of pre-existing primers.

Procedure:

1. Primer Designed in Primer Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi) online tool
2. Paste the FASTA sequence of HC-Pro gene of PRSV Bagalkote Isolate, in PCR template box.
3. Got the primers by keeping the default settings in the tool, but changing the few parameters like,

   PCR product size: 100-150bp (for qRT-PCR assay)

   Primer Pair Specificity Checking Parameters: Database - nR

   Organism- PRSV (taxid:12205 and 12206)

4. Primer-Blast results gave the best of 10 primer pairs, for the input FASTA sequence.
5. Selected the best primer pair based on the values of,

   GC content: Must have less than 3.0 per cent difference

   Tm Value: Near to 60^0 C

   Self complementarity: Near to 3

   Product length: In-between 100-140 bp

6. Selected primers were subjected to Secondary Structure analysis using OligoevaluatorTM (http://www.oligoevaluator.com/LoginServlet) online tool. The secondary structure value must be none or week. The complementarity primer sequence obtained by Sequence "Massager" (http://biomodel.uah.es/en/lab/cybertory/analysis/massager.htm) online tool using antiparallel complementary 5-3 function key.
7. Selected primer's specificity was confirmed by using NCBI Nucleotide BLAST: Blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch) tool by pasting the FASTA Forward and reveres primer sequence separated by adding 5n (nnnnn) in-between two sequences. During the blast, it must hit other PRSV sequences in the DATA base.

Appendix III-A

Complete genome sequence of PRSV-BGK

>PRSV-BGK

AAATAAAACATCTCAACACAACACAATTCGAAGCAACCAAACAAATTTAAGCACTTTCTACTTACAAATCTCATTATCTGA
AATCATGTCTTCGTTATATCAATTGCAACCAATTGCACTGAAAGACCGTCTGTTAGGTCATAAGGAAGGAAGCGGAGGGAT
CGAGCATAAGCTCGAGAGAAAGGGAGATAGAGGAAATACTCGCCATGTTGGTGAGTTTGTTATAAGTGAAGGAGCTAAAAT
CCTTCAGCTTATTCAAATTGGCGATGCTGAAATTGGAAGGACCTTCCTGGGAGGTGATCGGCGAGTCCGCGCCGATGTTTT
TGAAATCGTTAAGAAGACGATGGTTGGCTGCCTGGGCTACAACTTTGAAAGTGAATTATGGTTTTGCCATTCATGCGATAA
GACTTCTGACAAGTATTTTAAGAAATGTGACTGCGGAGATAAGTATTACTACTCCGAGAGGAATTTGATGAAAACGCTACA
AGATTTGATGTATCAATTTGACATGACCCCATCTGAAATCGACGCAGTTGGATATGATTATCTAGCTGACGCGGTTGATTT
TGCTGAACGATCTGTCAAGGAACCTCAAGTCGTGAAATCTGTGGAGCCTGAGGTGTCGAAACTTGTGAAATCTGATGAAGG
AACCCTGGCGACGCCTAAGCCTGGATCACCATTAATCACCACGAAACAGGAAGATGCTTGGACAATACAAATTGGAGAAAT
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Contd…..

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Appendix III-B

Complete genome sequence of PaLCuV-1

>PaLCuV-1

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CGAAACCGTACATGGGTTTAGATGTATGTTAGCAGTTAAATATCAGCAGTTAGTAGAGAAAAGTTATGTTCTTGACACATTGGGGC
GCGATTTAATTAGGGAGTTAATTTCAGTAATTAGGGCTAGAAATTATGTCGAAGTGTCCAGCAGAAATAATCATTTACACGTCCGC
TTCGAAGGTACGCCGTCGTCTCAACTTTGTCATCCCATATTTGAACCGTGCTGCTGCCCCCATTGTCCGGGTCGCCAAAGCAAAGG
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TCCTATATATATGGAGACTCCAATGGCATAAATGTAATTAATGAACTTTAATTCAAAATTCTCAATCGCTCCAAAAAGCGGCCATC
CGTATAATATT

Appendix III-C

Complete genome sequence of PaLCuV-2

>PcLCuV-2

ACCGGATGGCCGCGATTTTTTTTACCGTGGGCCCCACCACGCACGTGCTGACAAAGACATGTGCACCAATTAAAATCGTTCCTCAT
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TCCTGAAACCGTTCACGGTCTTAGGTGTATGTTAGCAGTTAAATATCTGCAGCTAATAGAAAATACATATTCTCCAGACACTCTGG
GGTACGATTTAATCAGGGAGTTGATCTCCGTTATTAGGGCTAAGAATTATGTCCAAGCGACCAGCAGATATAATCATTTCCACGCC
CGCCTCGAAGGTACGCCGCCTTCTCAACTTCGCCAACCCTTATTCCACACGTGCTGCTGCCCCCACTGTCCGCGTCACAAAGCCCG
CCGCACGGGTGAACAGGCCCAAGAACAGGAAGCCCCGGACGTCCAGGGTGTACAGAAGCCCAGATGTTCCTAGGGGGTGTGAAGGC
CCATGTAAGGTCCAGTCTTTGAGTCTAGACTCGATGTAGTTCATATAGGGAAGGTTAAGTGTATTAGTGATATTACCCGTGGTAC
TGGGTTAACTCATAGAGTAGGTAAGCGTTTCTGTGTTAAGTCTGTGTACGTATTAGGGAAGATATGGAAGGATGAGAATATCAAGA
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AACAAGTTTGACAACGAGCCTAGCACTGCCACTGTGAAGAATAAGCATAGAGATCGTTATCAGGTTTTGAGGAAATGGCACGCAAC
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AATATATAGTGTGAGTACCAAATGGCATGTTCGTAATTTTGAAAGCAAATTCAAAATTCAAATTCCGACAAAAGCGGCCATCCGCA
CTAATATT

Appendix III-D

Complete genome sequence of PaLCuV-3

>PaLCuV-3

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CGCGGGTTAATTAGGGAGTTTATTTCAGTAATTAGGGCTAGAAATTATGTCGAAGTGTCCAGCAGATATAATCATTTACACGTCCG
CTTCGAAGGTACGCCGTCGTATCAACTTGGTCATCCCATATGTGAACCGTGCTGCTGCCCCCATTGTCCGGGTCACCAAAGCAAAG
GCATGGGCCAACAGACCCCTGTCCCCGAAGCCCAGGAGGTGCAGGATGTACAGAAGCCCAGATGTCCATAAAGGATGTGTAGTCCC
ATGTAGAGTGCGGTCTTTTGATGCGAAGAATGATATTGGTCACATGGGTAAGGTTATTTGTCTCTCTGATGTTACTAGGGGTATTG
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TCACAGGTGGTCAATATGCTGCTAAGGAACAAGCTATAATTAGGAAATTCTATCGGGTTAACAATTATGTGGGGTATAATCCCCAG
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ATATT

Appendix III-E

Complete genome sequence of PaLCuV-4

>PaLCuV-4

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GCGCGATTTAATTAGGGAGTTAATTTCAGTAATTAGGGCTAGAAATTATGTCGAAGCGTCCAGCAGATATAATCATTTCCACGCCC
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GGCATGGACCAACAGACCCATGTCCCGGAAGCCCAGGATGTGCAGGATGTACAGAAGCCCAGATGTCCCTAAGGGATGTGAAGGCC
CATGTAAGGTGCAGTCTTTTGATGCGAAGAATGATATTGGTCACATGGGTAAGGTTATTTGTCTTTCTGATGTTACTAGGGGTATT
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GTCACAGGTGGTCAATATGCTGCTAAGGAACAAGCTATAATTAGGAAATTCTATCGTGTTAACAATTATGTGGTGTATAATCACCA
GGAAGCTGGGAAGTATGAAAATCATACTGAGAATGCTTTGTTGTTGTATATGGCATGTACTCATGCCTCTAACCCTGTGTATGCTA
CTTTGAAAGTTAGGAGTTACTTCTACGATTCTGTAACAAATTAACATTAATAAAGATTGAATTTTATTGAATATGATTGGTCTACA
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Appendix III-F

Complete genome sequence of PaLCuV-5

>PaLCuV-5

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Appendix III-G

Complete genome sequence of PaLCuV-6

>PaLCuV-6

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CCTGAAACCGTTCTCGGTTTTAGGTGTATGTTAGCAGTTAAATATCTGCAGCTACTAGAAAATACATATGCTCCAGACACTCTGGG
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AGCATGGGTGAACAGGCCCATGAACAGGAAGCCCAGGATGTACAGGATGTACAGAAGCCCAGATGTTCCTAGGGGTTGTGAAGGCC
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TCAATATATAGTGTGAGTACCAAATGGCATGTTCGTAATTTTGAAGGCAAATTCAAAATTCAAATTCCGACAAAAGCGGCCATCCG
CACTAATATT

Appendix III-H

Complete genome sequence of PaLCuV-7

>PaLCuV-7

ACCGGATGGCCGCGATTTTTTGACCGTGGGCCCCACCACGCACGTGCTGACAAAGACATGTGCACCAATTAAAATCGTCCCTCATA
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CCTGAAACCGTTCACGGTTTTAGGTGTATGTTAGCAGTTAAATATCTGCAGCTACTAGAAAATACATATGCTCCAGACACGCTGGG
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GCCTCGAAGGTACGCCGCTTGCTCAACTTCGACAGCCCTTATTCCACCCGTGCTGCTGCCCCCACTGTCCGCGTCACAAAGGCCAG
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GAGCTTTCTCCCTTAATATATTGAGGGCCGCAGCCTTGGACCCTGAATTGATTGCCTCGGCATATGCGTCGTTGGCAGACTGCTGA
CCTCCTCTAGCTGATCGTCCATCGACTTGGAAAACTCCAAAATCAATGAAGTCTCCGTTTTTTTCCACATAGGCCTTGACATCTGA
CGAGCTTTTAGCTCCCTGAATGTTCGGATGGAAATGTGCTGGCCTGGTTGGGGAAACGAGGTCGAAGAATCTGTTGTTTTGGCATT
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TACTCAATATATAGTGTGAGTACCAAATGGCATGTTCGTAATTTTGAAGGCAAATTCAAAATTCAAATTCCGACAAAAGCGGCCAT
CCGCACTAATATT

Appendix III-I

Complete genome sequence of PaLCuV-8

>PaLCuV-8

ACCGGATGGCCGCGATTTTTGTTACCGTGGGCCCCATCACGCACGTGCTGACAAAGACATGTGCACCAATTAAAATCGTCCCTCAT
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TCCTGAAACCGTTCACGGTTTTAGGTGTATGTTAGCAGTTAAATATTTGCAGCTAATAGAAAATACATATTCGCCAGACACTCTGG
GGTACGATTTAATCAGGGAGTTGATATCCGTTATTAGGTGTAAGAATTATGTCCAAGCGACCAGCAGATATAATCATTTCCACGCA
CGCCTCGAAGGTACGCCGCCTTCTCAACTTCGACAGCCCTTATTCCACCCGTGCTGCTGCCCCCGCTGTCCGCGTCACAAAGGCCA
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TGGGTTAACCCATAGAGTAGGTAAGCGTTTCTGTGTTAAGTCTGTGTACGTATTAGGGAAGATATGGATGGATGAGAATATCAAGA
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AGCTTTTAGCTCCCTGAATGTTCGGATGGAAATGTGCTGGCCTGTTTGGGGAAACGAGGTCGAAGAATCTGTTGTTTTGGCATTTG
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GCACTAATATT

Appendix III-J

Complete genome sequence of PaLCuV-9

>PaLCuV-9

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TCCTGAAACCGTTCACGGTTTTAGGGGGATGTTAGCAGTTAAATATCTGCAGCTACTAGAAATAACATATTCTCCAGACACTCTGG
GGTACGATTTAATCAGGGATTTGATCTCCGTTATTAGGGCTAAGAATTATGTCCAAGCGACCAGCAGATATAATCATTTCCACGCC
CGCCTCGAAGGTACGCCGCCTTCTCAACTTCGCCAACCCTTATTCCACACGTGCTGCTGCCCCCACTGTCCGCGTCACAAAGCCCG
CCGCACGGGTGAACAGGCCCCTGAACAGGAAGTCCAGGATGTACAGGATGTACAGAAGCCCAGATGTCCCTAAGGGTTGTGAAGGC
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AGTCACAGGTGGTCAATATGCTGCTAAGGAACAAGCTATAATTAGGAAATTCTATCGTGTTAACAATTATGTGGTGTATAACCAGC
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GCACTAATATT

Appendix III-K

Complete genome sequence of PaLCuV-10

>PaLCuV-10

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GTCACAGGTGGTCAATATGCTGCTAAGGAACAAGCTATAATTAGGAAATTCTATCGTGTTAACAATTATGTGGTGTATAATCACCA
GGAAGCTGGGAAGTATGAAAATCACACTGAGAATGCTTTGTTGTTGTATATGGCATGTACTCATGCCTCTAACCCTGTGTATGCTA
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Appendix III-L

Complete genome sequence of PaLCuV-11

>PaLCuV-11

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CAGAGGTCTTTAGGAGCCACCTCCACGTGTCTGTGACACAATAACCTTAAAATTGGATGAATTTTATGTTTAAAAATTGATCTACA
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Appendix III-M

Complete genome sequence of PaLCuV-12

>PaLCuV-12

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GAGTAATTGTTTTGTGGTCCCTTATTTAAAGTTCGCCACCAAGTAGTGCATTGCGCTTTATGCGGGATGCATTAGTAAATGAGCTT
TCCGAAATCGTTTATGGGCTTAGAAGTATGTTAGCAGTTAAATGTCCGCAGTTAGTAGAGATGAATTATGCTCCTGACACAATGGG
GCGCGGGTTAATTGGGGAGTTAATTTCAGTCAGTAGGGGTCGAAATTATGTCGAAGCGTCCAGCAGATATAATCTTTTACACCTAC
GCTTGGAAGGTACGCCGTCGTCTCAACTTTGTCGTCCCAAATTTGACCCGTGCTGCTGCCCCCCTCGTCCGGGTCGCCAAAGCCAA
GGCATGGGCCAACAGACCCCTGTCCCCGAAACCCAGGAAGTACAGGATCTCCACAAGCCCAGATGTCCCTAAGGGATGTGAAGGCC
CATGTAAGGTGCAGTCTTTTGATGCGAAGAATGATATTGGTCACAAGGGTAAGGTCATTTGTCTTTCTGATGTTACTAGGGGTATT
GGGCTGACCCATCGAGTAGGGAAACGTTTCTGTGTGAAGTCGTTGTATTTTGTTGGCAAAATATGGAAGGACGAGAACATCAAGAC
TAAGAACCATACGAATACTGTTAAGTTTTGGATCGTTAGAGATAGGCGTCCTTCAGGAACCCCAAATGATTTCCAGCAAGTGTTCA
ATGTTTATGATAATGAGCCCTCTACGGCTACTGTGAAGAACGACCAGCGTGATCGTTTTCAGGTGTTGAGGAGGTTTCAAGCAACA
GTCACAGGTGGTCAATATGCTGCTAAGGAACAAGCTATAATTAGGAAATTCTATCGTGTTAACAATTATGTGGTGTATAATCACCA
GGAAGCTGGAAAGTATGAAAATCACACTGAGAATGCTTTGTTGTTGTATATGGCATGTACTCATGCCTCTAACCCTGTGTATGCTA
CTTTGAAAGTTAGGAGTTACTTCTACGATTCTGTAACAAATTAACATTAATAAATATTGAATTTTATTGAATATGATTGGTCTACA
TATACAACGTGATGTAATACATCCCATAATACATGATCAACCGATTTAATTACAGTGTTAATACTGATAACCCCTAAATTATTTAA
GTATTTAAGAACTTGAGTCTTAAATACCCTTAAGAAACGACCAGTCTGAGGCTGTGAAGTCATCCAGATTCGGAAGTTCAGAAAAC
ATTTGTGTATCCCCAACGCTTTCCTCAGGTTGTGATTGAACTGTATTCGTATTGTCATTATGTCTTCTGTCATGTTGAATGGACTG
TTGTGGTGCTCCAATATCTTGAAATAGAGGGGATTTCGAATCTCCCAGATAAACACGCCATTCTGTGCTTGAGCTGCAGTGATGGG
TTCCCCTGTGCGTGAATCCATGGTTTCTGCAGTTGATACTCAGGTAGTAAGAACAGCCACAGTCAAGATCCACTCGTCGACGCCTG
ATCCCCTTCTTGGCTAGCCTGTGTTGCACTTTGATTGGTACCTGAGTAGAGTGGGCCTTCGAGGGTGATGAAGGTCGCATTCTTTA
AAGCCCAAGTCTTGAGTGCGTTATTTTTCTCTTCATCCAAGAACTCTTTATAGCTGGAATTGGGTCCTGGATTGCAGAGGAAGATA
GTGGGAATTCCCCCTTTAATTTGAACTGGCTTCCCGTACTTTGTGTTGGACTGCCAGTCCTTTTGGGCCCCCATGAACTCCTTGAA
GTGCTTTAGGTAGTGGGGATCTACGTCATCAATGACGTTATACCAGGCATCATTATTGTAGACCTTTGGGCTAAGGTCTAGATGTC
CACACAAATAGTTATGTGGTCCGAGTGATCTGGCCCACATGGTCTTGCCCGTTCTACTATCACCCTCTATCACTATACTTATGGGC
CTCAAAGGCCGCGCAGCGGCACTGACAACGTTCTCGGCAGCCCATTCCTCAAGTTCTTCCGGAACTTGGTCAAAAGAAGAAGAAGA
AAAAGGAGAAACATAAACCTCCACTGGAGGTGTAAAAATCCTATCTAAATTACATTTTATATTATGATATTGAAAAATACAATCTT
TAGCGAGTTTTTCCCTAATTATTGCTAAAGCTGCGTCAGCTGAACCTGCGTTTAGCGCCTCTGCTGCTGCATCATTAGCTGTCTGT
TGACCTCCTCTAGCAGATCTTCCATCGATCTGAAACTGACCCCAGTCGATGTAATCACCGTCCTTCTCGATGTAGGACTTGACATC
GGAACTGGACTTAGCTCCCTGGAAGTTTGGGTGGAATTGGGTGGAGTTATTAGGGTGAGTGACATCGAAATGTCTGGGGTTTCTGA
ACTGGGATTTTCCTTCGAATTGGATGATAACATGCAAGTGAGGAGTCCCATCTTCGTGAAGCTCTCTGCAGATTCTAATGAATTTT
TTAGAAGTTGGGGTTTGGAGATTTAAGAATTGGGAAAGTGCTTCTTCTTTAGTAAGAGAGCACTTGGGATAAGTGAGAAAATAATT
TTGGCATATATTGATATCGTTTGGGTGAGGCATGTTGACTAAGTCAATCGGTGTCTCTCAAACTTGTCTATGTATTGGTGTACTGG
AGTCCTATATATAGGTGAGTACTCTAAATGGCATGATTGTAATTTGGTAAAGTGTGATTTGAAATTCAAAACTCTCACGCTCCAAA
GCGGCCATCCGTATAATATT

Appendix III-N
Complete genome sequence of PaLCuV-13

>PaLCuV-13

ACCGAATGGCCGCGAAATTTTTGTGGTGGGCCCCGAACCAATAGAATTGCAGCTACATGGCTTATTTAGTGCGTGGGGGCCAATAA
ATAGACTTGCTCACCAAGTTTGGATCCACAAACATGTGGGATCCATTAGTACACGAGCTTCTTGAAAACGTTCTCGGTCTTAGGTG
TATGTTAGCAGTTCAATATCTGCAGCAGATAGAAAATACATATTCTCCAGTCGCTCTGGGGTACGATCTAATCAGGGGGGTGATTT
CCGTCATTAGGGCTAAGAATTATGTCCAAGCGACCAGCAGATATAATCATTTCAACGCCCGCATCGAAGGTACGCCGCCGTCTCAA
CTTCGCCAACCCCTATGCCACACGCGCTGCTGCCCCCGCGGTCCGCGTCTCAAAGGCCGGGGCATGGCcGAACAGGCCCATGGGCA
GCAAGCCCCGAATGTACAGGATGTATAGAAGCCCTGATGTTCCTAGGGGGTGTGAAGGCCCATGTAAGGTCCAGTCTTTTGAATCT
AGACACGATGTCTTTCATATAGGGAAGGTTATGTGTATTAGTGATGTTACTCGTGGTACTGGGTTAACCCATAGAGTAGGTAAGCG
TTTCTGTGTTAAGTCTGTGTATGTATTAGGGAAGATATGGATGGATGAGAATATCAAGACTAAGAAACACACGAATAGTGTGATGT
TTTTCCTTGTCCGTGATCGTCGTCCTAGTAGATTACCACAAGATTTTGGAGAGGTGTTCAACATGTTTGACAACGAGCCGAGCACT
GCCACTGTGAAGAACATGCATAGAGATCGTTATCAGGTTTTGAGGAAATGGCACGCAACCGTTACTGGTGGGTCATACGCGTCGAA
GGAACAGGCTTTAGTTAAGAAGTTCGTTAGGGTTAATAATTATGTTGTTTACAATCAACAAGAGGCCGGCAAGTATGAGAATCATA
CTGAAAACGCATTAATGTTGTATATGGCCTGTACTCAAGCATCAAATCCGGTATACGCTACTTTGAAGATCCGGATCTATTTCTAT
GATTCGGTCACAAATTAATAAATATTGCTCTTTATATCATACGTTGCCAATACATCAACTGTTTTATGCAATACATTGTCTAAAAC
ATGATAAACAGCTCGTATTACATTATAAATGCTAACTACACCAAGCATATCTAGGTACTTAAGGACCTGCGTTCTAAAAACCCTCA
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TGGTTGAACTGTATTTGCACTTTGATTATGTCGTGATTGGTCAGGAACGGCCTGCTGTCGTGTTTCAATATTTTGAAATACAGGGG
ATTTGGAACGGTCCAGATAAACACGCCACTCTCTGCTCGATCCGCAGTGATGTACTCCCCTGTGCGTGAATCCGTGATCATGGCAG
TTGATCGATATGTAGTACGAACAGCCACACTGAAGATCAACTCGCCTCCTGCGAATTATTTTCTTCTTCTTCTGGGGGAGCGATGT
TTTCGCGACCGGAATAGAGTGGTTCTTCGAGTGTGATGAAGACTGCATTCTTGATTGCCCATTGCTTCAATGCTGCATTTTTTTCT
TCGTCCAGATATTCCTTATACCTGCCGTTTGGACCTTTATAGCAGAGGAAGATAGTGGGAAATCCCCCCTTAATCATGACAGGCTT
GCCGTACTTAGTCTTGCTTTGCCAGTCTCGCTGGGCCCCCCCGCCTTCTTTAAAGAACTTTAGATAGTGGGGATCACCGTCATCAG
AGACGTTGCACCAGGCACCATCGCCATACACCGTTGGGCTCAGGCCTAGATGCCCGCACAAGTAATGGTGTGGGCCTAAGGAACGA
GCCCACATTGTTTTTCCTGTTCTACTATCACCCTCTATTACTATGCTTATGGGCCTAATAGGCCGCGCAGCGGAACACACAACATT
GGAACTGACCCAATCGACGAGATCTGGAGGAACTCTGTCGAAAGAAGAAAACGAAAAAGGATAAACATAAACCTCCAAGGGAGGTG
TAAAAATCCTATCTAAATTAGCATTTAAATTATGAAATTGTAAAACATAATCTTTGGGAGCTTTCTCCCTTAATATATTGAGGGCC
GCAGCTTTGGACCCTGAATTGATTGCCTCGGCATATGCGTCGTTGGCAGACTGCTGACCTCCTCTAGCTGATCTTCCATCGACTTG
GAAAACTCCAAAATCTAGAACGTCTCCGTTTTTTTCCACATAGGCCTTGACATCTGACGAGCTTTTAGCTCCCTGAATGTTCGGAT
GGAAATGTGCTGGCCTGGTTGGGGAAACAAGGTCGAAGAATCTGTTGTTTTGGCATTTGAATTTACCTTCGAATTGTATGAGGACG
TGGAGATGAGGTTCCCCATTTTCATGTAATTCGCGACAAACCCTGATGAATTTATTCGAAGTGGGGGTGCTTAGGTTTTGAAGTTG
GGAAAGTGCTTCTTCTTTAGTGAGGGAACACTGTGGATATGTTAAGAAATAATTTTTAGCATATATTTGAAAACGTTTTGGAGGAG
CCATGTTACTTATCGTTTCGAATCGGACTCACTCAACTTGGCTATGCAATCGGTGAATGGACTCACATATATAGGTAGTACTCTAA
ATGGCATGTTCGTAATTTTGAAAAGAAAATTACTTTAATTCAAACCCTCATAGCGGCCATTCGTTAATATT

Appendix IV-A. Per cent nucleotide sequence identity of DNA-A component of begomovirus isolated from papaya with other selected isolates of begomoviruses

Begomovirus species	Accession number	Percentage nucleotide sequence identity												
		PaLCuV-1	PaLCuV-2	PaLCuV-3	PaLCuV-4	PaLCuV-5	PaLCuV-6	PaLCuV-7	PaLCuV-8	PaLCuV-9	PaLCuV-10	PaLCuV-11	PaLCuV-12	PaLCuV-13
Ageratum enation virus-[India:Rajasthan:CN2:Papaya:2014]	KP725057	78.6	79.6	78.1	81.0	79.4	80.9	80.9	80.7	79.2	81.0	76.8	78.4	72.8
Ageratum yellow vein virus-[China: Sanya: Papaya:2013]	KM051844	72.6	73.6	72.3	74.1	73.8	75.0	75.2	75.0	74.2	73.7	69.9	72.1	70.1
Bhendi yellow vein Bhubhaneswar virus-[India: Orissa:Okra:03]	FJ589571	84.2	78.5	85.0	88.6	78.5	80.0	80.1	79.6	81.7	87.9	81.9	84.3	72.2
Catharanthus yellow mosaic virus-[Pakistan:Faisalabad:ZF-16:Papaya:2009]	LN864815	72.1	74.9	72.7	73.4	74.9	76.3	76.5	76.3	74.1	76.4	78.5	76.1	69.2
Chayote yellow mosaic virus-[Cameroon:Papaya-20-14:Papaya:2014]	KT454822	66.4	68.1	66.1	68.5	68.2	69.3	69.5	69.6	69.3	69.1	65.9	67.5	66.2
Chilli leaf curl India virus-[India: Meerut:Papaya:2017]	MF574143	77.5	95.7	77.5	79.9	94.7	96.6	96.6	97.2	92.4	77.8	74.6	75.8	80.1
Chilli leaf curl virus-[Indi:Shahahanpur:PSB-21:Papaya:2014]	MH765693	72.1	74.1	72.6	73.3	73.8	75.2	75.4	75.5	73.5	75.6	77.3	74.6	68.9
Chilli leaf curl virus-[India:Amritsar:Papaya:2009]	GU136803	78.3	94.7	78.4	80.5	93.7	95.6	95.6	96.3	91.4	78.6	75.6	76.3	80.0
Chilli leaf curl virus-[India:Karnataka:Raichuru:chilli:2017]	MK161454	78.4	95.8	78.5	80.9	96.5	98.5	98.5	97.6	92.6	78.7	75.4	76.7	80.8
Chilli leaf curl virus-[India:Uttar Pradesh: Faizabad: PSB-45:Papaya:2014]	MH765698	73.0	75.1	73.6	74.5	75.0	76.5	76.6	76.6	74.4	77.1	79.7	76.6	68.9
Chilli leaf curl virus-[India:Uttar Pradesh:Mahoba:PSB-42:Papaya:2014]	MH765697	72.2	73.8	73.0	73.9	73.7	75.1	75.2	74.9	73.4	76.4	76.8	75.1	68.5
Chilli leaf curl virus-[Pakistan:WA19:Papaya:2018]	MN839535	78.2	89.9	78.3	79.4	89.3	91.2	91.3	91.5	87.0	77.2	75.2	76.0	77.6
Chilli leaf curl virus-DU [India:New Delhi:DU:Papaya:2009]	HM140364	78.1	96.3	78.2	80.5	94.9	96.8	96.8	98.0	93.5	78.4	75.3	76.4	80.7
Chilli leaf curl virus-HD [India:New Delhi:HD:Papaya:2007]	HM140365	78.5	95.4	78.6	80.9	96.2	98.1	98.2	97.2	92.3	78.8	75.6	76.8	80.8
Chilli leaf curl Virus-India [India: New Delhi: AD: Papaya:2005]	DQ989326	78.2	95.5	78.1	80.4	94.8	96.7	96.8	97.2	92.3	78.3	75.3	76.3	80.2
Chilli leaf curl virus-Najafgarh2 [India:New Delhi:Papaya:2009]	HM140370	78.0	95.5	78.0	80.4	95.1	97.0	97.1	97.1	92.4	78.3	75.1	76.3	80.4

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Begomovirus species	Accession number	Percentage nucleotide sequence identity												
		PaLCuV-1	PaLCuV-2	PaLCuV-3	PaLCuV-4	PaLCuV-5	PaLCuV-6	PaLCuV-7	PaLCuV-8	PaLCuV-9	PaLCuV-10	PaLCuV-11	PaLCuV-12	PaLCuV-13
Chilli leaf curl virus-Noida [India:Uttar Pradesh:Noida:Papaya:2009]	HM140371	78.1	96.1	78.1	80.5	95.2	97.0	97.1	97.7	92.8	78.5	75.3	76.5	80.7
Chilli leaf curl virus-Panipat1 [India:Panipat:Papaya:2008]	HM140366	78.4	93.8	78.2	80.6	92.4	94.3	94.3	95.3	91.2	78.4	75.5	76.7	79.5
Cotton leaf curl Burewala virus-[India: LK_2N:Papaya:2011]	KX302707	74.7	75.6	74.6	76.8	75.7	77.2	77.2	77.1	74.9	75.8	72.2	74.0	71.9
Croton yellow vein mosaic virus-[India:Hariyana:Acalypha:07]	FN645901	83.6	75.9	84.8	85.0	76.4	77.6	77.7	77.4	80.9	89.1	92.3	88.6	70.0
Croton yellow vein mosaic virus-[India:New Delhi:croton:2007]	JN817516	83.5	75.8	83.9	84.6	76.3	77.5	77.6	77.2	80.8	88.7	93.5	88.3	69.9
Croton yellow vein mosaic virus-[India:Panjab:Acalypha:2007]	FN645926	83.7	75.8	84.8	85.2	76.0	77.3	77.4	77.1	80.6	89.3	91.8	88.8	69.9
Croton yellow vein mosaic virus-[India:Ranchi:PSB-38:Papaya:2014]	MH765696	80.5	73.4	81.2	81.1	73.5	74.7	74.9	74.9	77.7	84.8	87.7	84.4	67.5
Duranta leaf curl virus-[India: New Delhi:PSB-63:Papaya:2016]	MH807202	71.9	73.5	71.9	72.7	73.5	74.6	74.6	74.9	72.9	75.0	75.3	73.7	69.8
Malvastrum leaf curl virus-[China:Guangxi:G100:Papaya:2006]	AM260699	74.6	73.5	74.9	76.2	73.6	74.9	75.0	74.8	76.5	76.0	73.3	74.3	70.7
Mungbean yellow mosaic India virus-[India:New Delhi: Cowpea :2005]	DQ389153	62.2	61.6	62.2	63.3	61.7	62.4	62.5	62.2	61.8	61.0	58.4	59.9	59.6
Okra enation leaf curl virus-[Iran:Bahu Kalat: OELCuV_IR_P7_2010:Papaya:2010]	KJ397529	70.7	70.8	70.2	72.3	70.9	72.3	72.5	72.2	71.1	71.6	68.3	70.7	68.7
Papaya leaf crumple virus-[India: Mohali:New Delhi:croton:2008]	JN817517	82.6	74.8	84.5	83.9	74.8	75.9	76.0	76.0	79.3	87.6	90.3	86.9	68.7
Papaya leaf crumple virus-[India: Mohali:Pap-Moh7:Papaya:2011]	KR052159	72.4	75.4	72.4	74.8	75.2	76.7	76.7	76.8	74.9	74.5	69.6	72.3	74.3
Papaya leaf crumple virus-[India:Bhopal:B2_5N:Papaya:2011]	KX302712	72.3	75.3	72.4	74.7	75.1	76.6	76.6	76.7	74.8	74.4	69.5	72.2	74.2
Papaya leaf crumple virus-[India:Bhopal:PSB-66:Papaya:2016]	MH807203	72.2	75.4	72.1	74.4	75.0	76.5	76.5	76.5	74.7	74.2	69.3	71.6	73.8
Papaya leaf crumple virus-[India:Hajipur:B2_3N:Papaya:2011]	KX302710	72.3	75.4	72.4	74.8	75.2	76.6	76.7	76.7	74.9	74.5	69.6	72.3	74.3
Papaya leaf crumple virus-[India:Jabalpur:PSB-32:Papaya:2016]	MH674437	71.5	74.3	71.5	73.7	74.3	75.6	75.8	75.7	73.8	73.6	68.7	71.5	73.2
Papaya leaf crumple virus-[India:Kolkata:B2_4N:Papaya:2012]	KX302711	72.4	75.4	72.5	74.8	75.2	76.7	76.8	76.8	74.9	74.6	69.6	72.3	74.4
Papaya leaf crumple virus-[India:Lalitpur:PSB-47:Papaya: 2015]	MH807200	70.0	72.8	70.0	72.6	72.6	74.0	74.2	74.2	72.5	72.4	68.2	70.8	72.3

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Begomovirus species	Accession number	Percentage nucleotide sequence identity												
		PaLCuV-1	PaLCuV-2	PaLCuV-3	PaLCuV-4	PaLCuV-5	PaLCuV-6	PaLCuV-7	PaLCuV-8	PaLCuV-9	PaLCuV-10	PaLCuV-11	PaLCuV-12	PaLCuV-13
Papaya leaf crumple virus-[India:Lucknow:B2_2N:Papaya:2012]	KX302709	72.4	75.4	72.5	74.8	75.2	76.7	76.8	76.8	74.9	74.6	69.6	72.3	74.4
Papaya leaf crumple virus-[India:Mohali:3B2_2:Papaya:2012]	KX302708	72.3	75.3	72.3	74.7	75.1	76.6	76.6	76.7	74.8	74.4	69.5	72.2	74.2
Papaya leaf crumple virus-[India:New Delhi:PSB-60:Papaya:2016]	MH807201	71.8	74.4	71.5	73.9	74.1	75.6	75.6	75.8	73.9	73.7	68.8	71.9	73.5
Papaya leaf crumple virus-[Pakistan:Lahore:KN6:Papaya:2011]	HE580236	72.3	75.5	72.5	74.8	75.4	76.8	76.9	76.9	74.9	74.5	69.6	72.3	74.5
Papaya leaf crumple virus-Najafgarh1[India:New Delhi:Papaya:2008]	HM140369	72.2	75.5	72.3	74.6	75.2	76.6	76.7	76.8	74.9	74.3	69.5	72.1	74.3
Papaya leaf crumple virus-Nirulas[India:New Delhi:Papaya:2007]	HM140368	72.7	75.5	72.4	74.6	75.3	76.7	76.8	76.9	74.9	74.5	69.9	72.7	74.2
Papaya leaf crumple virus-Panipat 8 [India:Panipat:Papaya:2008]	HM140367	72.2	75.5	72.2	74.5	75.4	76.8	76.9	76.9	75.0	74.3	70.1	72.2	74.3
Papaya leaf crumple virus-Panipat8[India:Haryana:Panipat:Papaya:2008]	NC_014707	72.2	75.5	72.2	74.5	75.4	76.8	76.9	76.9	75.0	74.3	70.1	72.2	74.3
Papaya leaf curl China virus-[China:Guangxi:G2:Papaya:2004]	AJ558123	74.9	76.0	74.6	76.5	76.0	77.3	77.4	77.1	76.6	73.5	69.8	72.1	72.2
Papaya leaf curl China virus-[China:Guangxi:G4:Papaya:2004]	AJ811914	75.1	76.4	75.2	76.9	76.3	77.8	77.8	77.5	76.9	74.1	70.3	72.5	72.3
Papaya leaf curl Guandong virus-[China:Guangxi:GD2:Papaya: 2004]	AJ558122	71.5	73.5	70.7	72.6	73.7	75.0	75.1	75.0	74.2	72.9	69.3	70.9	71.5
Papaya leaf curl Guandong virus-[China:Guangxi:GD2:Papaya:2004]	NC_005844	71.5	73.5	70.7	72.6	73.7	75.0	75.1	75.0	74.2	72.9	69.3	70.9	71.5
Papaya leaf curl virus-[India: Tamil Nadu:Wellington:WB2&5:Papaya:2010]	KX302713	74.6	77.0	76.0	76.3	77.1	78.3	78.5	78.5	76.2	79.1	81.8	78.4	69.4
Papaya leaf curl virus-[India::Rajasthan:MM1:Papaya:2019]	MN529626	76.1	77.7	77.0	77.1	78.1	79.4	79.4	79.2	76.0	80.4	82.4	79.7	70.7
Papaya leaf curl virus-[India:Gujarat:Jamnagar:PSB-34:Papaya:2015]	MH807205	80.0	73.8	80.8	80.9	73.8	75.1	75.2	75.1	78.3	83.7	84.9	82.8	67.9
Papaya leaf curl virus-[India:Karnataka:Madikeri:candela:2017]	MK087120	87.1	77.2	85.4	87.9	77.6	78.8	78.9	78.8	82.0	92.4	90.8	93.0	71.4
Papaya leaf curl virus-[India:Madhya Pradesh:Pap:ND:13:Papaya:2012]	KF307208	81.8	75.6	81.8	82.5	75.9	77.2	77.3	77.2	80.5	86.1	90.4	85.8	69.8

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Begomovirus species	Accession number	Percentage nucleotide sequence identity												
		PaLCuV-1	PaLCuV-2	PaLCuV-3	PaLCuV-4	PaLCuV-5	PaLCuV-6	PaLCuV-7	PaLCuV-8	PaLCuV-9	PaLCuV-10	PaLCuV-11	PaLCuV-12	PaLCuV-13
Papaya leaf curl virus-[India:New Delhi:Papaya:2016]	KY800906	77.1	95.3	77.2	79.6	94.1	95.9	96.0	96.7	92.0	77.2	74.0	75.2	79.9
Papaya leaf curl virus-[South Korea: Korea:Papaya:2014]	KT266873	72.1	72.9	71.1	73.0	73.2	74.5	74.7	74.5	73.6	73.4	69.9	72.2	71.4
Papaya yellow leaf curl virus-[India: Hyderabad:PSB-51:Papaya:2016]	MH807204	77.4	79.4	76.5	78.6	79.3	80.8	80.9	80.7	78.5	78.3	74.9	76.4	72.7
Papaya yellow leaf curl virus-[India: Rajasthan:DP2:Papaya:2015]	KX353622	79.2	81.2	77.4	79.6	81.4	82.7	82.9	82.5	80.5	79.3	76.5	79.0	72.9
Papaya yellow leaf curl virus-[India:New Delhi:Radish:2018]	FJ593629	83.6	75.9	84.7	84.6	76.2	77.6	77.7	77.6	80.9	88.6	91.9	88.1	69.8
Pedilanthus leaf curl virus-[India: Gujarat:Ahmedabad:PSB37:Papaya:2015]	MH765695	75.3	77.9	75.2	76.5	77.7	79.4	79.4	79.3	77.3	76.7	74.0	74.5	71.2
Tomato leaf curl Bangladesh virus-[Bangladesh:Gazipur:Papaya:2016]	MH380003	79.7	84.2	79.7	81.9	84.1	86.0	86.0	85.7	83.7	79.6	76.5	77.5	75.8
Tomato leaf curl Gujarat virus-[India:Lucknow:Papaya:2014]	MG757245	81.8	76.0	78.2	80.2	75.8	77.1	77.2	77.2	78.7	78.7	75.7	78.4	70.6
Tomato leaf curl Karnataka virus-India [India:Gujarat:Tomato:2008	MH5770301	88.6	78.9	84.7	86.6	79.4	80.5	80.5	80.4	83.9	84.9	82.1	84.2	72.7
Tomato leaf curl Karnataka virus-India [India:Karnataka:Banglore:Chilli:2008]	HM007094	83.6	82.6	80.4	82.5	82.5	84.0	84.0	84.0	80.9	80.2	77.5	79.6	75.5
Tomato leaf curl Karnataka virus-India [India:TamilnaduTomato:2012]	KF551579	88.2	79.3	84.2	86.1	79.8	80.9	80.9	80.7	83.3	84.3	81.6	83.7	73.1
Tomato leaf curl virus-[India:Karnataka:Bengaluru:Chrysanthamum:2017]	MG758145	69.6	71.6	69.0	70.9	71.3	72.7	72.9	72.8	71.1	70.1	67.0	69.5	87.2
Tomato leaf curl virus-[India:Tamilnadu:Tomato:2012]	KC713784	87.9	79.0	84.2	86.6	79.1	80.4	80.4	80.3	83.2	84.5	82.0	83.8	73.3
Tomato yellow leaf curl viru Israel -[USA:P3-T1-3:Papaya:2014]	KX024639	71.2	72.9	72.0	73.4	73.0	74.1	74.3	74.2	73.2	71.5	68.6	70.2	69.7

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Appendix IV-B. Per cent nucleotide sequence identity of intergenic region of begomovirus isolated from papaya with other selected isolates of begomoviruses

Begomovirus species	Accession number	Percentage nucleotide sequence identity												
		PaLCuV-1	PaLCuV-2	PaLCuV-3	PaLCuV-4	PaLCuV-5	PaLCuV-6	PaLCuV-7	PaLCuV-8	PaLCuV-9	PaLCuV-10	PaLCuV-11	PaLCuV-12	PaLCuV-13
Ageratum enation virus-[India:Rajasthan:CN2:Papaya:2014]	KP725057	78.6	79.6	78.1	81.0	79.4	80.9	80.9	80.7	79.2	81.0	76.8	78.4	72.8
Ageratum yellow vein virus-[China: Sanya: Papaya:2013]	KM051844	72.6	73.6	72.3	74.1	73.8	75.0	75.2	75.0	74.2	73.7	69.9	72.1	70.1
Bhendi yellow vein Bhubhaneswar virus-[India: Orissa:Okra:03]	FJ589571	84.2	78.5	85.0	88.6	78.5	80.0	80.1	79.6	81.7	87.9	81.9	84.3	72.2
Catharanthus yellow mosaic virus-[Pakistan:Faisalabad:ZF-16:Papaya:2009]	LN864815	72.1	74.9	72.7	73.4	74.9	76.3	76.5	76.3	74.1	76.4	78.5	76.1	69.2
Chayote yellow mosaic virus-[Cameroon:Papaya-20-14:Papaya:2014]	KT454822	66.4	68.1	66.1	68.5	68.2	69.3	69.5	69.6	69.3	69.1	65.9	67.5	66.2
Chilli leaf curl India virus-[India: Meerut:Papaya:2017]	MF574143	77.5	95.7	77.5	79.9	94.7	96.6	96.6	97.2	92.4	77.8	74.6	75.8	80.1
Chilli leaf curl virus-[Indi:Shahahanpur:PSB-21:Papaya:2014]	MH765693	72.1	74.1	72.6	73.3	73.8	75.2	75.4	75.5	73.5	75.6	77.3	74.6	68.9
Chilli leaf curl virus-[India:Amritsar:Papaya:2009]	GU136803	78.3	94.7	78.4	80.5	93.7	95.6	95.6	96.3	91.4	78.6	75.6	76.3	80.0
Chilli leaf curl virus-[India:Karnataka:Raichuru:chilli:2017]	MK161454	78.4	95.8	78.5	80.9	96.5	98.5	98.5	97.6	92.6	78.7	75.4	76.7	80.8
Chilli leaf curl virus-[India:Uttar Pradesh: Faizabad: PSB-45:Papaya:2014]	MH765698	73.0	75.1	73.6	74.5	75.0	76.5	76.6	76.6	74.4	77.1	79.7	76.6	68.9
Chilli leaf curl virus-[India:Uttar Pradesh:Mahoba:PSB-42:Papaya:2014]	MH765697	72.2	73.8	73.0	73.9	73.7	75.1	75.2	74.9	73.4	76.4	76.8	75.1	68.5
Chilli leaf curl virus-[Pakistan:WA19:Papaya:2018]	MN839535	78.2	89.9	78.3	79.4	89.3	91.2	91.3	91.5	87.0	77.2	75.2	76.0	77.6
Chilli leaf curl virus-DU [India:New Delhi:DU:Papaya:2009]	HM140364	78.1	96.3	78.2	80.5	94.9	96.8	96.8	98.0	93.5	78.4	75.3	76.4	80.7
Chilli leaf curl virus-HD [India:New Delhi:HD:Papaya:2007]	HM140365	78.5	95.4	78.6	80.9	96.2	98.1	98.2	97.2	92.3	78.8	75.6	76.8	80.8
Chilli leaf curl Virus-India [India: New Delhi: AD: Papaya:2005]	DQ989326	78.2	95.5	78.1	80.4	94.8	96.7	96.8	97.2	92.3	78.3	75.3	76.3	80.2
Chilli leaf curl virus-Najafgarh2 [India:New Delhi:Papaya:2009]	HM140370	78.0	95.5	78.0	80.4	95.1	97.0	97.1	97.1	92.4	78.3	75.1	76.3	80.4
Chilli leaf curl virus-Noida [India:Uttar Pradesh:Noida:Papaya:2009]	HM140371	78.1	96.1	78.1	80.5	95.2	97.0	97.1	97.7	92.8	78.5	75.3	76.5	80.7

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Begomovirus species	Accession number	Percentage nucleotide sequence identity												
		PaLCuV -1	PaLCuV -2	PaLCuV -3	PaLCuV -4	PaLCuV -5	PaLCuV -6	PaLCuV -7	PaLCuV -8	PaLCuV -9	PaLCuV-10	PaLCuV-11	PaLCuV-12	PaLCuV-13
Chilli leaf curl virus-Panipat1 [India:Panipat:Papaya:2008]	HM140366	78.4	93.8	78.2	80.6	92.4	94.3	94.3	95.3	91.2	78.4	75.5	76.7	79.5
Cotton leaf curl Burewala virus-[India: LK_2N:Papaya:2011]	KX302707	74.7	75.6	74.6	76.8	75.7	77.2	77.2	77.1	74.9	75.8	72.2	74.0	71.9
Croton yellow vein mosaic virus-[India:Hariyana:Acalypha:07]	FN645901	83.6	75.9	84.8	85.0	76.4	77.6	77.7	77.4	80.9	89.1	92.3	88.6	70.0
Croton yellow vein mosaic virus-[India:New Delhi:croton:2007]	JN817516	83.5	75.8	83.9	84.6	76.3	77.5	77.6	77.2	80.8	88.7	93.5	88.3	69.9
Croton yellow vein mosaic virus-[India:Panjab:Acalypha:2007]	FN645926	83.7	75.8	84.8	85.2	76.0	77.3	77.4	77.1	80.6	89.3	91.8	88.8	69.9
Croton yellow vein mosaic virus-[India:Ranchi:PSB-38:Papaya:2014]	MH765696	80.5	73.4	81.2	81.1	73.5	74.7	74.9	74.9	77.7	84.8	87.7	84.4	67.5
Duranta leaf curl virus-[India: New Delhi:PSB-63:Papaya:2016]	MH807202	71.9	73.5	71.9	72.7	73.5	74.6	74.6	74.9	72.9	75.0	75.3	73.7	69.8
Malvastrum leaf curl virus-[China:Guangxi:G100:Papaya:2006]	AM260699	74.6	73.5	74.9	76.2	73.6	74.9	75.0	74.8	76.5	76.0	73.3	74.3	70.7
Mungbean yellow mosaic India virus-[India:New Delhi:Cowpea:2005]	DQ389153	62.2	61.6	62.2	63.3	61.7	62.4	62.5	62.2	61.8	61.0	58.4	59.9	59.6
Okra enation leaf curl virus-[Iran:Bahu Kalat: OELCuV_IR_P7_2010:Papaya:2010]	KJ397529	70.7	70.8	70.2	72.3	70.9	72.3	72.5	72.2	71.1	71.6	68.3	70.7	68.7
Papaya leaf crumple virus-[India: Mohali:New Delhi:croton:2008]	JN817517	82.6	74.8	84.5	83.9	74.8	75.9	76.0	76.0	79.3	87.6	90.3	86.9	68.7
Papaya leaf crumple virus-[India: Mohali:Pap-Moh7:Papaya:2011]	KR052159	72.4	75.4	72.4	74.8	75.2	76.7	76.7	76.8	74.9	74.5	69.6	72.3	74.3
Papaya leaf crumple virus-[India:Bhopal:B2_5N:Papaya:2011]	KX302712	72.3	75.3	72.4	74.7	75.1	76.6	76.6	76.7	74.8	74.4	69.5	72.2	74.2
Papaya leaf crumple virus-[India:Bhopal:PSB-66:Papaya:2016]	MH807203	72.2	75.4	72.1	74.4	75.0	76.5	76.5	76.5	74.7	74.2	69.3	71.6	73.8
Papaya leaf crumple virus-[India:Hajipur:B2_3N:Papaya:2011]	KX302710	72.3	75.4	72.4	74.8	75.2	76.6	76.7	76.7	74.9	74.5	69.6	72.3	74.3
Papaya leaf crumple virus-[India:Jabalpur:PSB-32:Papaya:2016]	MH674437	71.5	74.3	71.5	73.7	74.3	75.6	75.8	75.7	73.8	73.6	68.7	71.5	73.2
Papaya leaf crumple virus-[India:Kolkata:B2_4N:Papaya:2012]	KX302711	72.4	75.4	72.5	74.8	75.2	76.7	76.8	76.8	74.9	74.6	69.6	72.3	74.4
Papaya leaf crumple virus-[India:Lalitpur:PSB-47:Papaya: 2015]	MH807200	70.0	72.8	70.0	72.6	72.6	74.0	74.2	74.2	72.5	72.4	68.2	70.8	72.3

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Begomovirus species	Accession number	Percentage nucleotide sequence identity												
		PaLCuV -1	PaLCuV -2	PaLCuV -3	PaLCuV -4	PaLCuV -5	PaLCuV -6	PaLCuV -7	PaLCuV -8	PaLCuV -9	PaLCuV-10	PaLCuV-11	PaLCuV-12	PaLCuV-13
Papaya leaf crumple virus-[India:Lucknow:B2_2N:Papaya:2012]	KX302709	72.4	75.4	72.5	74.8	75.2	76.7	76.8	76.8	74.9	74.6	69.6	72.3	74.4
Papaya leaf crumple virus-[India:Mohali:3B2_2:Papaya:2012]	KX302708	72.3	75.3	72.3	74.7	75.1	76.6	76.6	76.7	74.8	74.4	69.5	72.2	74.2
Papaya leaf crumple virus-[India:New Delhi:PSB-60:Papaya:2016]	MH807201	71.8	74.4	71.5	73.9	74.1	75.6	75.6	75.8	73.9	73.7	68.8	71.9	73.5
Papaya leaf crumple virus-[Pakistan:Lahore:KN6:Papaya:2011]	HE580236	72.3	75.5	72.5	74.8	75.4	76.8	76.9	76.9	74.9	74.5	69.6	72.3	74.5
Papaya leaf crumple virus-Najafgarh1[India:New Delhi:Papaya:2008]	HM140369	72.2	75.5	72.3	74.6	75.2	76.6	76.7	76.8	74.9	74.3	69.5	72.1	74.3
Papaya leaf crumple virus-Nirulas[India:New Delhi:Papaya:2007]	HM140368	72.7	75.5	72.4	74.6	75.3	76.7	76.8	76.9	74.9	74.5	69.9	72.7	74.2
Papaya leaf crumple virus-Panipat 8 [India:Panipat:Papaya:2008].	HM140367	72.2	75.5	72.2	74.5	75.4	76.8	76.9	76.9	75.0	74.3	70.1	72.2	74.3
Papaya leaf crumple virus-Panipat8[India:Haryana:Panipat:Papaya:2008]	NC_014707	72.2	75.5	72.2	74.5	75.4	76.8	76.9	76.9	75.0	74.3	70.1	72.2	74.3
Papaya leaf curl China virus-[China:Guangxi:G2:Papaya:2004]	AJ558123	74.9	76.0	74.6	76.5	76.0	77.3	77.4	77.1	76.6	73.5	69.8	72.1	72.2
Papaya leaf curl China virus-[China:Guangxi:G4:Papaya:2004]	AJ811914	75.1	76.4	75.2	76.9	76.3	77.8	77.8	77.5	76.9	74.1	70.3	72.5	72.3
Papaya leaf curl Guandong virus-[China:Guangxi:GD2:Papaya: 2004]	AJ558122	71.5	73.5	70.7	72.6	73.7	75.0	75.1	75.0	74.2	72.9	69.3	70.9	71.5
Papaya leaf curl Guandong virus-[China:Guangxi:GD2:Papaya:2004]	NC_005844	71.5	73.5	70.7	72.6	73.7	75.0	75.1	75.0	74.2	72.9	69.3	70.9	71.5
Papaya leaf curl virus-[India: Tamil Nadu:Wellington:WB2&5:Papaya:2010]	KX302713	74.6	77.0	76.0	76.3	77.1	78.3	78.5	78.5	76.2	79.1	81.8	78.4	69.4
Papaya leaf curl virus-[India::Rajasthan:MM1:Papaya:2019]	MN529626	76.1	77.7	77.0	77.1	78.1	79.4	79.4	79.2	76.0	80.4	82.4	79.7	70.7
Papaya leaf curl virus-[India:Gujarat:Jamnagar:PSB-34:Papaya:2015]	MH807205	80.0	73.8	80.8	80.9	73.8	75.1	75.2	75.1	78.3	83.7	84.9	82.8	67.9
Papaya leaf curl virus-[India:Karnataka:Madikeri:candela:2017]	MK087120	87.1	77.2	85.4	87.9	77.6	78.8	78.9	78.8	82.0	92.4	90.8	93.0	71.4
Papaya leaf curl virus-[India:Madhya Pradesh:Pap:ND:13:Papaya:2012]	KF307208	81.8	75,6	81.8	82.5	75.9	77.2	77.3	77.2	80.5	86.1	90.4	85.8	69.8

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Begomovirus species	Accession number	Percentage nucleotide sequence identity												
		PaLCuV-1	PaLCuV-2	PaLCuV-3	PaLCuV-4	PaLCuV-5	PaLCuV-6	PaLCuV-7	PaLCuV-8	PaLCuV-9	PaLCuV-10	PaLCuV-11	PaLCuV-12	PaLCuV-13
Papaya leaf curl virus-[India:New Delhi:Papaya:2016]	KY800906	77.1	95.3	77.2	79.6	94.1	95.9	96.0	96.7	92.0	77.2	74.0	75.2	79.9
Papaya leaf curl virus-[South Korea: Korea:Papaya:2014]	KT266873	72.1	72.9	71.1	73.0	73.2	74.5	74.7	74.5	73.6	73.4	69.9	72.2	71.4
Papaya yellow leaf curl virus-[India: Hyderabad:PSB-51:Papaya:2016]	MH807204	77.4	79.4	76.5	78.6	79.3	80.8	80.9	80.7	78.5	78.3	74.9	76.4	72.7
Papaya yellow leaf curl virus-[India: Rajasthan:DP2:Papaya:2015]	KX353622	79.2	81.2	77.4	79.6	81.4	82.7	82.9	82.5	80.5	79.3	76.5	79.0	72.9
Papaya yellow leaf curl virus-[India:New Delhi:Radish:2018]	FJ593629	83.6	75.9	84.7	84.6	76.2	77.6	77.7	77.6	80.9	88.6	91.9	88.1	69.8
Pedilanthus leaf curl virus-[India: Gujarat:Ahmedabad:PSB37:Papaya:2015]	MH765695	75.3	77.9	75.2	76.5	77.7	79.4	79.4	79.3	77.3	76.7	74.0	74.5	71.2
Tomato leaf curl Bangladesh virus-[Bangladesh:Gazipur:Papaya:2016]	MH380003	79.7	84.2	79.7	81.9	84.1	86.0	86.0	85.7	83.7	79.6	76.5	77.5	75.8
Tomato leaf curl Gujarat virus-[India:Lucknow:Papaya:2014]	MG757245	81.8	76.0	78.2	80.2	75.8	77.1	77.2	77.2	78.7	78.7	75.7	78.4	70.6
Tomato leaf curl Karnataka virus-India [India:Gujarat:Tomato:2008	MH5770301	88.6	78.9	84.7	86.6	79.4	80.5	80.5	80.4	83.9	84.9	82.1	84.2	72.7
Tomato leaf curl Karnataka virus-India [India:Karnataka:Banglore:Chilli:2008]	HM007094	83.6	82.6	80.4	82.5	82.5	84.0	84.0	84.0	80.9	80.2	77.5	79.6	75.5
Tomato leaf curl Karnataka virus-India [India:TamilnaduTomato:2012]	KF551579	88.2	79.3	84.2	86.1	79.8	80.9	80.9	80.7	83.3	84.3	81.6	83.7	73.1
Tomato leaf curl virus-[India:Karnataka:Bengaluru:Chrysanthamum:2017]	MG758145	69.6	71.6	69.0	70.9	71.3	72.7	72.9	72.8	71.1	70.1	67.0	69.5	87.2
Tomato leaf curl virus-[India:Tamilnadu:Tomato:2012]	KC713784	87.9	79.0	84.2	86.6	79.1	80.4	80.4	80.3	83.2	84.5	82.0	83.8	73.3
Tomato yellow leaf curl viru Israel -[USA:P3-T1-3:Papaya:2014]	KX024639	71.2	72.9	72.0	73.4	73.0	74.1	74.3	74.2	73.2	71.5	68.6	70.2	69.7

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Appendix IV-C: Aphid population at monthly intervals

Aphid population at monthly intervals (No./Trap)						
Month	**1st Week**	**2nd Week**	**3rd Week**	**4th Week**	**Total**	**Average**
Jun-19	0	0	0	0	0	0
Jul-19	0	0	0	0	0	0
Aug-19	0	0	0	0	0	0
Sep-19	0	7	10	13	30	7.5
Oct-19	18	24	25	27	94	23.5
Nov-19	26	28	32	25	111	27.75
Dec-19	49	43	48	52	192	48
Jan-20	76	87	86	95	344	86
Feb-20	84	80	82	83	329	82.25
Mar-20	14	6	4	7	31	7.75
Apr-20	3	0	2	3	8	2
May-20	0	4	2	0	6	1.5
Jun-20	0	0	3	0	3	0.75
Jul-20	0	1	0	0	1	0.25
Aug-20	0	0	0	2	2	0.5
Sep-20	2	4	5	3	14	3.5
Oct-20	23	19	25	28	95	23.75
Nov-20	35	30	25	38	128	32
Dec-20	53	44	51	54	202	50.5
Jan-21	84	93	83	85	345	86.25
Feb-21	81	68	58	59	266	66.5
Mar-21	21	7	8	6	42	10.5

* Two yellow sticky traps used at 50 mt distance

CHARACTERIZATION OF PAPAYA RING SPOT (PRSV) AND PAPAYA LEAF CURL (PaLCuV) VIRUSES INFECTING PAPAYA, EPIDEMIOLOGY AND MANAGEMENT OF PRSV DISEASE

PREMCHAND U. **2021** **Dr. RAGHAVENDRA K. MESTA**
Major Advisor

ABSTRACT

The present investigation was conducted at the Department of Plant Pathology, C.O.H Bagalkot during 2019-2021. The roving survey carried during January 2019 to March 2021 in major papaya growing districts of Karnataka revealed that PRSV disease incidence ranged from 50.5-100 %. The107 samples collected from 75 locations during survey were subjected to PCR based detection. Among them, 75 samples were tested positive for PRSV infection and the remaining 32 for PaLCuV (monopartite begomovirus).

Complete genome characterization of a representative PRSV-BGK (Collected from Bagalkote) isolate revealed that this isolate contains 10,341 nt with ten mature polyproteins and is demarcated as a variant. It is given a descriptor as PRSV-[IN:Kar:Bgk:Pap:21]. Among 32 begomoviral samples 13 representative isolates were subjected for characterization, out of which four were found new distinct species of begomovirus and proposed the name as PaLCuBKV-[IN:Kar:Bel:Pap:21] Gokak; PaLCuBKV-[IN:Kar:Bgk:Pap:21] Bagalkote; PaLCuBKV-[IN:Kar:Bgk:Pap:21] Bilgi and PaLCuHV-[IN:Kar:Hav: Pap:21] Haveri. Another four were found as new strains and proposed the name as ChiLCV-[IN:Kar:Kal:Pap:21] Afjalpur; PaLCuV-[IN:Kar:Kal:Pap:21] Kalaburagi; CYVMV-[IN:Kar:Kal:Pap:21] Kalaburagi and PaLCuV-[IN:Kar:Vij:Pap:21] Vijayapura. Five isolates were demarcated as new variants and suggested the name as ChiLCV-[IN:Kar:Bel:Pap:21] Gokak; ChiLCV-[IN:Kar:Bel:Pap:21] Ramadurga; ChiLCV-[IN:Kar:Bgk:Pap:21] Hunugunda1; ChiLCV-[IN:Kar:Bgk:Pap:21] Hunugunda2 and ChiLCV-[IN:Kar:Kal:Pap:21] Afjalpur.

Studies on the different months of planting under field conditions revealed that planting during March is effective for the management of PRSV as it recorded the least disease incidence (5.56 % at 60 DAT and took 270 DAT to reach 100 %) and maximum yield (185.54 t/ha) along with good growth and yield parameters. The effect of inoculation of PRSV at different growth stages of papaya revealed that percent transmission and severity of symptoms on PRSV inoculated papaya plants were drastically greater in early inoculated plants than in plants inoculated at later stages. As the inoculation was delayed there was reduction in diseases incidence and an increase in growth and yield parameters.

The studies on the management of PRSV under field conditions for two seasons (2019-20 and 2020-21) using insecticides and bio rationales revealed that T1 (8 sprays of four different insecticide *i.e* tolfenpyrad 15% EC @1 ml/l, imidacloprid 17.8%SL @ 0.2 ml/l, thiacloprid 21.7 SC @ 1 ml/l and dinotefuran 20 % SG @ 0.5g/l alternatively and micronutrients at every 30 days intervals) proved as the best treatment. It recorded the least diseases incidence (1.49 % at 210 DAT and took 360 DAT to reach 100%) and maximum yield (178.56 t/ha) along with a high cost-benefit ratio (1: 3.54). Looking into these results, three integrated diseases management modules were designed and evaluated along with recommended POP as a check and found that M1 (12 sprays insecticides *i.e* tolfenpyrad 15% EC @1 ml/l, imidacloprid 17.8%SL @ 0.2 ml/l, thiacloprid 21.7 SC @ 1 ml/l and dinotefuran 20 % SG @ 0.5g/l alternatively 3 times and micronutrients at 20 days interval) is the best module for managing PRSV (0.44 % incidence at 180 DAT and took 330 DAT to reach 100%).

¥¥ÁAiÄ jAUï¸Ámï (¦DgïJ¸ï«) ªÄvÄ ¥¥ÁAiÄ °Ã¥ïP®ð (¦JJ¯ï¹AiÄÄ«) £AeÁtÄ«£ UÄuÁgÆÃ¥u ¸ÁAPÁ«ÄP gÆÃU±Á¸¦ ªÄvÄ ¦DgïJ¸ï« gÆÃU ¤ªðºu

¥ÃªiïZAzï AiÄÄ. 2021 qÁ. gÁWªÃAz P. ªÄ¸Á ªÄÄR¦ ¸®ºUÁggÄ

¸ÁgÁA±

¥¸Äv CzÁAiÄ£ª£Ä¦ ¸¸gÆÃU ±Á¸¦ «¨ÁU, vÆÃlUÁjP ªÄºÁ«zÁ®AiÄ, ¨ÁU®PÆÃmAiÄ° 2019 jAz 2021g ªgU P£UÆ¼¯Á¬ÄvÄ. P£ÁðlPz ¥¥Á¬Ä ¨¼AiÄÄª ¥ªÄÄR f¯U¼° d£ªj 2019 jAz ªiÁZïð 2021g ªgU £q¸¯Áz ¸«ÄÃPAiÄ° ¦DgïJ¸ï« £AeÁtÄ gÆÃUz wÃª£vAiÄ ¥ªiÁtª¦ ±Ã. 50.50 jAz 100g ªgU EgÄª¦zÄ PAqÄ§A¢z. ¸«ÄÃP £q¹¢ 75 ¸¼U½Az MlÄ 107 gÆÃU¦Ãrv J¯ ªiÁzjU¼£Ä¸AU»¹ DzÁjv «±ÃµuU N¼¥r¹zÁU, 75 ªiÁzjU¼° ¦DgïJ¸ï« ¸ÆÃAPÄ ¥vAiiÁzg G½z 32 J¯ ªÄÄzÄrgÄª ªiÁzjU¼° ¦JJ¯ï¹AiÄÄ« (ªÆ£Æ¥Ám£ðmï ¨UÆªÆ) £AeÁtÄ ¥vAiiÁ¬ÄvÄ.

75 ¦DgïJ¸ï« ªiÁzjU¼° ¦DgïJ¸ï«-©fP AiÄ£Ä¦ (¨ÁU®PÆÃmAiÄ° ¸AU»¹z ¥w¤¢AiiÁV) G¥AiÆÃV¹ ¸A¥Ætð Cu£P CzÁAiÄ£ ªiÁq¯Á¬ÄvÄ. F L¸ÆÃ¯Ãmï ªiÁzjAiÄÄ 10,341 £ÆQAiÆÃm£qï C£Ä¦ ºÆA¢gÄª gÆ¥ÁAvjAiiÁVzÄ, EzP PRSV-[IN:Kar:Bgk:Pap:21] JA§ º¸g£Ä¦ ¥¸Á¦¸¯ÁVz. CzÃ jÃw 32 ¨UÆªÆ ª£g¯ï ªiÁzjU¼° 13 ¥Áw¤¢P L¸ÆÃ¯ÃmïU¼£Ä¦ C£ÄªA²P Cu£P CzÁAiÄ£ ªiÁq¯Á¬ÄvÄ. Eª¦U¼° 4 «©£¦Áz ºÆ¸ ¨UÆªÆ ª£g¸ï ¥¨ÃzP ¸ÃjgÄª¦zÄ PAqÄ§A¢z ªÄvÄ Eª¦U½U PaLCuBKV-[IN:Kar:Bel:Pap:21] UÆÃPÁPï; PaLCuBKV-[IN:Kar:Bgk:Pap:21] ¨ÁU®PÆÃm; PaLCuBKV-[IN:Kar:Bgk:Pap:21] ©Ã¼V ªÄvÄ PaLCuHV-[IN:Kar:Hav:Pap:21] ºÁªÃj JA§ º¸g£Ä¦ ¥¸Á¦¸¯ÁVz. CzÃ jÃw 4 ºÆ¸ v½U½zÄ, Eª¦U½U ChiLCV-[IN:Kar:Kal:Pap:21] C¥d¯ï¥Ág; PaLCuV-[IN:Kar:Kal:Pap:21] P®§AgV; CYVMY-[IN:Kar:Kal:Pap:21] P®§AgV ªÄvÄ PaLCuV-[IN:Kar:Vij:Pap:21] «dAiÄ¥Ág JA§ º¸gÄU¼£Ä¦ ¥¸Á¦¸¯ÁVz. E£Ä½z 5 L¸ÆÃ¯ÃmïU¼£Ä¦ ºÆ¸ gÆ¥ÁAvjU¼AzÄ UÄgÄw¸¯ÁVz. Cª¦U¼£Ä¦ ChiLCV-[IN:Kar:Bel:Pap:21] UÆÃPÁPï; ChiLCV-[IN:Kar:Bel:Pap:21] gÁªÄzÄUð; ChiLCV-[IN:Kar:Bgk:Pap:21] º£UÄAz1; ChiLCV-[IN:Kar:Bgk:Pap:21] º£UÄAz2 ªÄvÄ ChiLCV-[IN:Kar:Kal:Pap:21] C¥d¯ï¥Ág JAzÄ º¸j¸¯ÁVz.

ªiÁZïð wAU½£° ¥¥ÁAiÄ £Án ªiÁqÄª¦zjAz ¦DgïJ¸ï« gÆÃUª£Ä¦ CvÁAv ¥juÁªÄPÁjAiiÁV ¤ªðºu ªiÁq§ºÄzAzÄ w½zÄ§A¢z KPAzg D ¸ªÄAiÄz° £Án ªiÁrz ¨¼AiÄ° gÆÃUª¦ PrªÄ ¥ªiÁtz°zÄ (5.56% 60 ¢£U¼ £Avg ªÄvÄ 100% v®Ä¥®Ä 270 ¢£U¼Ä) ªÄvÄ Ujµ E¼Äªj (185.54 l£ï/º) zÁR¯Á¬ÄvÄ.

¥¥ÁAiÄ ¨¼ªtUAiÄ ««z ºAvU¼° ¦DgïJ¸ï« £AeÁtÄª£Ä¦ E£ÁPÄ¦¯Ã±£ï ªiÁrzÁU, ¨¼ªtUAiÄ ¥Ëq ºAvz° E£ÁPÄ¦¯Ã±£ï ªiÁrz ¥¥Á¬ÄU ºÆÃ°¹z° DgA©P ¨¼ªtUAiÄ ºAvz° E£ÁP¦¯Ã±£ï ªiÁrz ¥¥Á¬Ä VqU½U ±ÃPqÁªÁgÄ gÆÃU ºgqÄ«P ªÄvÄ gÆÃU ®PtªÄ CvÁAv wÃª£ vg¢Az PÆrgÄª¦zÄ PAqÄ§A¢z.

QÃlÉÁ±PU¼ÄÄ ªÄvÄ e£«P vPðU¼£Ä¦ §¼¹PÆAqÄ JgqÄ lÄvÄU¼° (2019-20 ªÄvÄ 2020-21) £q¸¯Áz ¦DgïJ¸ï« gÆÃU ¤ªðºu CzÁAiÄ£z° n1 (4 «©£¦ QÃlÉÁ±P CAzg mÆ¯ï¥£ï¥£gÁq 15% E¹ @ 1 «Ä°Ã/°Ã, E«ÄqÁPÆÃ¦qï 17.8% J¸ïJ¯ï @ 0.2 «Ä°Ã/°Ã, yAiiÁPÆÃ¦qï 21.7 J¸ï¹ @ 1 «Ä°Ã/°Ã ªÄvÄ q£ÆÃn¥Æ£gÁ£ï 20% J¸ïf @ 0.5 UÁA/°Ã ªÄvÄ ¸ÆPï ¥ÆÃµPÁA±U¼ 8 ¹A¥guU¼Ä, ¥w 30 ¢£U¼ ªÄzÁAvgz°) CvÄvªÄ aQvAiiÁV ¸Á©ÁvÁVzÄ, Prª Ä gÆÃUz ¨ÁzAiÄ£Ä¦ (1.49% 210 ¢£U¼° ªÄvÄ 100% v®Ä¥®Ä 360 ¢£U¼£Ä¦ vUzÄPÆArvÄ) ºÁUÆ Ujµ E¼ÄªjAiÄ£Ä¦ (178.56 l£ï/º) zÁR°¹vÄ. F ¥°vÁA±z DzÁgz ªÄÄ¯, 3 ¸ªÄU gÆÃUU¼ ¤ªðºuÁ ªiÁzjU¼£Ä¦ «£Á¸UÆ½¹ ²¥Ájv ¥zwAiÄ eÆvU ¥jÃQ¹zÁU, Jªii1 ªiÁzj (4 «©£¦ QÃlÉÁ±PU¼ÄÄ ªÄvÄ ¸ÆPï ¥ÆÃµPÁA±U¼ ¹A¥gu 20 ¢£U¼ ªÄzÁAvgz°) gÆÃU ¨ÁzAiÄ£Ä¦ (0.44% 180 ¢£U¼ £Avg ªÄvÄ 100% v®Ä¥®Ä 330 ¢£U¼) ¤ªð»¸Äª° CvÄvªÄªÁVz JAzÄ PAqÄ§A¢z.