MOLECULAR VARIABILITY AND INTEGRATED MANAGEMENT OF PAPAYA RINGSPOT VIRUS (PRSV)

ANIL PAPPACHAN

PALB 3070

DEPARTMENT OF PLANT PATHOLOGY

UNIVERSITY OF AGRICULTURAL SCIENCES-BENGALURU

BENGALURU 560 065

2017

MOLECULAR VARIABILITY AND INTEGRATED MANAGEMENT OF PAPAYA RINGSPOT VIRUS (PRSV)

ANIL PAPPACHAN

PALB 3070

Thesis submitted to the

UNIVERSITY OF AGRICULTURAL SCIENCES-BENGALURU

in partial fulfillment of the requirements for the award of the degree of

Doctor of Philosophy

in

PLANT PATHOLOGY

Bengaluru **September, 2017**

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
GKVK, BENGALURU- 560 065

CERTIFICATE

This is to certify that the thesis entitled **"MOLECULAR VARIABILITY AND INTEGRATED MANAGEMENT OF PAPAYA RINGSPOT VIRUS (PRSV)"** submitted by **Mr. ANIL PAPPACHAN, ID. No. PALB 3070** in partial fulfillment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY** in **PLANT PATHOLOGY** to the University of Agricultural Sciences, Bengaluru, is a record of *bona fide* research work done by him during the period of his study in this University 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 any other similar titles.

N. Nagaf 27/06/17

Bengaluru
September, 2017

N. NAGARAJU
Major Advisor

APPROVED BY:

Chairperson: N. Nagaf 25/09/17
(N. NAGARAJU)

Members:

1. K.T Rangaswamy
(K. T. RANGASWAMY)

2. Krishna Reddy
(M. KRISHNA REDDY)

Saradahni

External Examiner 25/09/2017
(R. SARADA JAYALAKSHMI DEVI)
Professor (Plant Pathology) & Univ. Librarian
A.N.G.R Agricultural University
Guntur, Andhra Pradesh -522 509

3. _____
(N. NATARAJA KARABA)

4. M. Chandre Gowda
(M. CHANDRE GOWDA)

ACKNOWLEDGEMENT

No creation in this world is a solo effort, neither is this thesis. I would like to take this opportunity to acknowledge all, who supported me directly or indirectly, bringing in this thesis to a good shape.

First, I thank the Almighty God for his love and blessings, without which I would not have been able to complete my studies hitherto and present this piece of work.

By the grace of Almighty I could put all efforts to fulfil the task assigned to me by learned members of the Advisory Committee. Fervently, I extol the genuine and constant encouragement, immaculate guidance and every suggestive help offered to me by the Chairperson of my advisory committee ***Dr. N. Nagaraju,*** *Professor, Department of Plant Pathology, UAS, GKVK, Bangalore for his wise counsel, concrete suggestions, inspiring, meticulous and affectionate guidance, constant help and persistent encouragement during the course of my study and research work. It was indeed a great opportunity and honour to work under his guidance.*

I express sincere thanks to my advisory committee member ***Dr. K. T. Rangaswamy,*** *Professor, Department of Plant Pathology, UAS, GKVK, Bangalore for his excellent guidance, support and for providing lab facilities.*

I deem it my privilege in expressing my deep sense of reverence, gratitude and indebtedness to my advisory committee members ***Dr. Krishna reddy,*** *Indian Institute of Horticultural Sciences, Bangalore;* ***Dr. Nataraja Karaba,*** *Professor, Department of Crop Physiology, UAS, GKVK, Bangalore and M Chandregowda, Professor, Department of Horticulture, UAS, GKVK, Bangalore, for their talented guidance, valuable suggestions, careful and reasoned criticism, meticulous attention to the details and also for thier constant encouragement which has led to the present investigation to the final shape.*

I thank ***Dr. M. K. Prasanna Kumar****, Assistant Professor, Department of Plant Pathology, UAS, GKVK, Bangalore for his moral support and encouragement during the research. I extend my sincere thanks to all teaching and non-teaching staff members of the Department of Plant pathology for their help rendered in many ways.*

I am very much thankful to and feel it a great privilege to place on my record with sincere regards and thanks to Indian Institute of Horticultural sciences, Bangalore forr providing seed material required for my investigation.

I am dearth of words to express my love to my beloved parents ***Smt. Mary*** *and* ***Sri. Pappachan,*** *to my dear teachers* ***Smt. Sonia M Paul*** *and* ***Dr. S. K. Pattanshetty,*** *for their dedicated efforts to educate me to this level and whose unparallel affection and persistent encouragement in keeping my career go along way throughout my life. I owe thanks from depth of my heart to Dr. K.B. Palanna and Dr. Sreeshail Sonyal for the affection, guidance and assistance they showered on me from the day first.*

With immense pleasure I thank my seniors ***Pushpa R.N, Basavaraj K*** *and* ***Manjunatha N;*** *my batchmates Bommalinga,* ***Kedarnath, Shanthamma, Jyothi and Pavithra****; and my juniors Gurudevi, Puneeth, Mahesh, Kavyashree, Manjunath, Bhaskar and Venkatesh for their affection and help during my college life.*

I offer my profuse regards and thanks to Mr. Bharat and Mr. Veere Gowda Progressive farmers for their support, keen interest and patience. I also thank ***Mr. Padmaraj****, Field assistant, MRS, Hebbal, Bangalore for his help throughout my research work.*

I greatly acknowledge the ***University Grant Commission, for providing MAULANA AZAD NATIONAL FELLOWSHIP*** *for my doctorate degree without which this task would have been impossible.*

Finally, yet importantly, I wish to express my indebtedness to all those helped me directly or indirectly during the period of my stay in GKVK, UAS campus. I frankly admit it is not possible to remember all the faces that stood behind the facade at this juncture and omission of any name does not mean lack of gratitude.

Sincerely

Bengaluru

September, 2017 (***Anil Pappachan***)

MOLECULAR VARIABILITY AND INTEGRATED MANAGEMENT OF PAPAYA RINGSPOT VIRUS (PRSV)

ANIL PAPPACHAN

Thesis abstract

Papaya Ringspot Virus (PRSV) is posing a major threat to papaya cultivation throughout India by rendering orchards economically unproductive. Survey conducted in 2014-15 revealed that 100 per cent PRSV incidence was observed at some locations of Bangalore rural and Bangalore urban districts of Karnataka, while highest average incidence was recorded at Ramanagara (82 %). In Andhra Pradesh, Kadapa district recorded highest average disease incidence of 70 per cent, while in Telangana, 24 per cent incidence of PRSV was observed in Hyderabad. In Kerala PRSV incidence was highest in Kottayam (77 %) and in Tamil Nadu, highest average incidence of 70 per cent was observed at Coimbatore. Comparison of P1 proteinase gene of PRSV deposited in NCBI GenBank revealed that nucleotide identity of South Indian PRSV isolates ranged from 87 to 72 per cent. None of the fifteen cultivated papaya varieties was found to possess resistance to PRSV both under glasshouse and field conditions. Extracts of *Acorus calamus* (5 %), *Boerhavia diffusa* (5 %), *Kappaphycus alvarezii* (KH-1 %), *Eucheuma spinosum* (SH-1 %) and Silicic acid (1 %) showed significant inhibitory effect on PRSV. Silver nanoparticles (60 to 100 nm) were prepared by green synthesis process using neem (*Azadirachta indica*) leaf extract. The extract of *B. diffusa* (5 %), colloidal Silver nanoparticles (100 ppm), Silver nanoparticles (50 ppm) prepared by green synthesis process and *K. alvarezii* (LBS3-1 %) showed significant inhibitory effect on PRSV multiplication both under field and glasshouse conditions. Adoption of integrated disease management module (III) a combination of maize (South African tall) as barrier crop, use of silver reflective mulch row cover and spraying with extract of *A. calamus* (5 %), *K. alvarezii* extract (KH-1 %) and insecticide imidacloprid (0.05 %) at monthly interval resulted in 142.39 per cent increase in yield over control with highest return per rupee invested (1.91).

Department of Plant Pathology
UAS, GKVK, Bangalore-560 065

(N. Nagaraju)
Major Advisor

ಪರಂಗಿಯ ಉಂಗುರ ಚುಕ್ಕೆ ನಂಜಾಣು (ಪಿ.ಆರ್.ಎಸ್.ವಿ.) ರೋಗದ ಅಣ್ವೀಕಶಾಸ್ತ್ರ, ವೈವಿಧ್ಯತೆ ಮತ್ತು ಸಮಗ್ರ ನಿರ್ವಹಣೆ

ಅನಿಲ್ ಪಪ್ಪಚ್ಚನ್

ಪ್ರಬಂಧದ ಸಾರಾಂಶ

ಭಾರತದಲ್ಲಿ ಪರಂಗಿ ಉಂಗುರ ಚುಕ್ಕೆ ನಂಜಾಣು ಪರಂಗಿ ಕೃಷಿಗೆ ಪ್ರಮುಖ ಅಪಾಯಕಾರಿಯಾಗಿದ್ದು ಹಣ್ಣು ತೋಟಗಳಲ್ಲಿ ಆರ್ಥಿಕವಾಗಿ ಅನುತ್ಪಾದನೆಯನ್ನು ಉಂಟು ಮಾಡುತ್ತಿದೆ. ೨೦೧೪–೧೫ ರಲ್ಲಿ ನಡೆಸಲಾದ ಸಮೀಕ್ಷೆಯ ಪ್ರಕಾರ ಪಿ.ಆರ್.ಎಸ್.ವಿ. ಯು ಕರ್ನಾಟಕದ ಬೆಂಗಳೂರು ಗ್ರಾಮಾಂತರ ಮತ್ತು ಬೆಂಗಳೂರು ನಗರ ಜಿಲ್ಲೆಗಳ ಕೆಲವು ಸ್ಥಳಗಳಲ್ಲಿ ಶೇ. ೧೦೦ ರಷ್ಟು ಕಂಡುಬಂದಿದ್ದು ಹಾಗೂ ರಾಮನಗರದಲ್ಲಿ ಗರಿಷ್ಠ ಸರಾಸರಿ ವ್ಯಾಪ್ತಿಯು (ಶೇ. ೮೪) ಕಂಡುಬಂದಿದೆ. ಆಂದ್ರಪ್ರದೇಶದ ಕಡಪ ಜಿಲ್ಲೆಯಲ್ಲಿ ಪಿ.ಆರ್.ಎಸ್.ವಿ. ವ್ಯಾಪ್ತಿಯು ಶೇ. ೭೦ ರಷ್ಟು ಹಾಗೂ ತೆಲಂಗಾಣದ ಹೈದರಾಬಾದ್‌ನಲ್ಲಿ ಶೇ. ೨೪ ರಷ್ಟು ಕಂಡುಬಂದಿದೆ. ಪಿ.ಆರ್.ಎಸ್.ವಿ. ಯ ವ್ಯಾಪ್ತಿಯು ಕೇರಳದ ಕೊಟ್ಟಾಯಂನಲ್ಲಿ ಗರಿಷ್ಠವಾಗಿದ್ದು (ಶೇ. ೭೦ ರಷ್ಟು) ಮತ್ತು ತಮಿಳುನಾಡಿನ ಕೊಯಮತ್ತೂರ್‌ನಲ್ಲಿ ಸರಾಸರಿ ವ್ಯಾಪ್ತಿಯು ಶೇ. ೭೦ ರಷ್ಟು ಕಂಡುಬಂದಿದೆ. ಎನ್.ಸಿ.ಬಿ.ಐ ಜೀನ್ ಬ್ಯಾಂಕ್‌ನಲ್ಲಿರುವ ಬೇರೆ ಪಿ.ಆರ್.ಎಸ್.ವಿ. ಪ್ರತ್ಯೇಕದ ಜೊತೆಗೆ ಹೋಲಿಕೆ ಮಾಡಿದಾಗ, ಪಿ.ಆರ್.ಎಸ್.ವಿ. ಯ ಪಿ೧ ಪ್ರೊಟೀನ್‌ನ ಅಣುವನ್ನು ಶೇ. ೮೭ ರಿಂದ ೭೫ ರಷ್ಟು ನ್ಯೂಕ್ಲಿಯೋಟೈಡ್‌ನ ಗುರುತನ್ನು ದಕ್ಷಿಣ ಭಾರತದ ಪ್ರತ್ಯೇಕಗಳ ಜೊತೆಗೆ ಹೊಂದಿದೆ ಎಂದು ದೃಢಪಡಿಸಲಾಯಿತು. ಗಾಜಿನ ಮನೆ ಮತ್ತು ಕ್ಷೇತ್ರಗಳಲ್ಲಿ, ಪರಂಗಿಯ ೧೫ ಪ್ರಭೇದಗಳು ಪಿ.ಆರ್.ಎಸ್.ವಿ. ರೋಗಕ್ಕೆ ಪ್ರತಿರೋಧ ವ್ಯಕ್ತಪಡಿಸುವುದರಲ್ಲಿ ವಿಫಲವಾಗಿರುವುದು ಕಂಡುಬಂದಿದೆ. *ಅಕೋರಸ್ ಕಲಾಮಸ್* (ಶೇ. ೫೦ ರಷ್ಟು) *ಬೋರ್ಹೆವಿಯ ಡಿಫ್ಯೂಸಾ* (ಶೇ. ೫ ರಷ್ಟು), *ಕಪ್ಪಾಫೈಕಸ್ ಅಲ್ವಿರೈಜಿ* (ಕೆ.ಹೆಚ್–೧, ಶೇ. ೧ ರಷ್ಟು), *ಯ್ಯೂಕೆಯುಮಾ ಸ್ಪೈನೋಸಮ್* (ಎಸ್.ಹೆಚ್–೧, ಶೇ. ೧ ರಷ್ಟು) ಮತ್ತು ಸಿಲಿಸಿಕ್ ಆಮ್ಲ (ಶೇ. ೧ ರಷ್ಟು) ಪಿ.ಆರ್.ಎಸ್.ವಿ. ಯ ಮೇಲೆ ಗಮನಾರ್ಹ ಪ್ರತಿಬಂಧಕ ಪರಿಣಾಮವನ್ನು ತೋರಿಸಿದೆ. ಹಸಿರು ಸಂಶ್ಲೇಷಣೆಯ ಮೂಲಕ, ಬೇವಿನ ಎಲೆಯ ರಸಸಾರದಿಂದ ಬೆಳ್ಳಿಯ ನ್ಯಾನೊಕಣಗಳನ್ನು (೬೦ ರಿಂದ ೧೦೦ ನ್ಯಾನೊಮೀಟರ್) ತಯಾರಿಸಲಾಯಿತು. ರಸಸಾರಗಳಾದ *ಬೋರ್ಹೆವಿಯ ಡಿಫ್ಯೂಸಾ* (ಶೇ. ೫ ರಷ್ಟು), ಹಸಿರು ಸಂಶ್ಲೇಷಣೆಯಿಂದ ತಯಾರಿಸಿದ ಬೆಳ್ಳಿಯ ನ್ಯಾನೊಕಣಗಳು (೧೦೦ ಪಿಪಿಎಮ್) ಮತ್ತು ಬೆಳ್ಳಿ ನ್ಯಾನೊಕಣಗಳು (೫೦ ಪಿಪಿಎಮ್) ಹಾಗೂ *ಕಪ್ಪಾಫೈಕಸ್ ಅಲ್ವಿರೈಜಿ* (ಎಲ್.ಬಿ.ಎಸ್–೩, ಶೇ. ೧ ರಷ್ಟು) ಗಾಜಿನ ಮನೆ ಮತ್ತು ಕ್ಷೇತ್ರದಲ್ಲಿ ಗಮನಾರ್ಹ ಪ್ರತಿಬಂಧಕತೆಯನ್ನು ಪಿ.ಆರ್.ಎಸ್.ವಿ ಗೆ ತೋರಿಸಿರುವುದು ಕಂಡುಬಂದಿದೆ. ಸಮಗ್ರ ರೋಗ ನಿರ್ವಹಣೆ ಪದ್ಧತಿ (ಖಖಖ) ಪ್ರಕಾರ, ಮೆಕ್ಕೆಜೋಳ (ಆಫ್ರಿಕನ್ ಟಾಲ್) ವನ್ನು ತಡೆಗೋಡೆ ಬೆಳೆಯಾಗಿ, ಬೆಳ್ಳಿಯ ಪ್ರತಿಫಲದ ಹಾಳೆಯನ್ನು ಸಾಲುಹೊದಿಕೆಯಾಗಿ ಮತ್ತು *ಅಕೋಲಸ್ ಕಲಮಸ್* (ಶೇ. ೫ ರಷ್ಟು), *ಕಪ್ಪಾಫೈಕಸ್ ಅಲ್ವಿರೈಜಿ* (ಕೆ.ಹೆಚ್–೧, ಶೇ. ೧ ರಷ್ಟು) ಗಳ ರಸಸಾರ ಮತ್ತು ಕೀಟನಾಶಕವಾದ ಇಮಿಡಾಕ್ಲೋಪ್ರಿಡ್ (ಶೇ. ೦.೦೫ ರಷ್ಟು) ನ್ನು ಮಾಸಿಕ ಮಧ್ಯಂತರದಲ್ಲಿ ಸಿಂಪರಣೆ ಮಾಡಿದಾಗ ಪ್ರತಿ ರೂಪಾಯಿ ಹೂಡಿಕೆಗೆ ಪ್ರತಿಫಲವಾಗಿ ೧.೯೧ ರಷ್ಟು ಆದಾಯ ಹೆಚ್ಚಾಗಿದ್ದು ಮತ್ತು ಶೇ. ೧೪೨.೩೯ ರಷ್ಟು ಹೆಚ್ಚಾಗಿರುವುದು ಕಂಡುಬಂದಿದೆ.

ಸಸ್ಯ ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ
ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ, ಜಿ.ಕೆ.ವಿ.ಕೆ.,
ಬೆಂಗಳೂರು–೫೬೦ ೦೬೫

(ಎನ್. ನಾಗರಾಜು)
ಪ್ರಧಾನ ಮಾರ್ಗದರ್ಶಕರು

C O N T E N T S

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF PLATES

LIST OF PLATES

LIST OF APPENDICES

I INTRODUCTION

Papaya (*Carica papaya* L.) is an important fruit crop of the tropical and subtropical regions of the globe. Carica is the largest among the four genera comprising 48 species, of which papaya is most important and cultivated species all over the world (Badillo, 1971). Papaya is the native of tropical America (Singh, 1990). Among the fruits, papaya occupies a special place because of its availability throughout the year, produces more income per unit area. Papaya fruits are easy to digest, with high nutritive and medicinal value.

Papaya plant is a large, quick growing, soft stemmed plant. The fruits can be harvested within a year (Chauhan and Chatterjee, 2005). Papaya fruit ranks second only to mango as a source of beta carotene. It is a good source of natural sugars, vitamin C and also contains fair amount of calcium and phosphorus (Saravanan *et al.,* 2004) but is low in calories and has got high medicinal value. It has been used as a laxative since ancient times. It used for the treatment of various digestive disorders, diabetes mellitus and is also effective in lowering blood cholesterol level (Cherian and Cheriyan, 2003).

Papaya is cultivated widely for consumption as a fresh fruit, for use in drinks, jams, candies and as dried and crystallised fruit (Villegas, 1997). Green fruit, leaves and flowers may also be used as cooked vegetable (Watson, 1997). Papain prepared from dried latex of raw fruits is used in meat tendering, preparation of chewing gum, cosmetics, for degumming silk and to give shrink resistance to wool. In addition, it is also used in pharmaceutical industries, textiles, garments, cleaning paper, adhesive manufacturing, sewage disposal and for many other purposes (Chadha, 2002). Chymopapain and antioxidant nutrients found in papaya have been found helpful in lowering inflammation and healing burns (Aravind *et al*., 2013).

Papaya is one of the fruit crop intensively grown in India. It is grown throughout the country and fruits are produced throughout the year. If the crop is managed properly very high yields and profits can be achieved. Papaya is a tropical fruit, but it can also be grown in the mild sub tropical regions of the country up to 1,000 m above the sea level. India is the largest producer of papaya, having increased its share in world production

upto 41.57 per cent in 2012-13. India is followed by Brazil and Indonesia which rank second and third in production (Anon., 2013). In India papaya occupies 0.133 million ha with a production of 5.69 million MT and average productivity of 42.8 MT/ha (Anon., 2015).

In India papaya is grown for table purpose, papin and pectin extraction in the states of Orissa, West Bengal, Karnataka, Assam, Kerala and Gujarat (Singhal, 1990). Among different states, Gujarat covers highest area of 19,l00 ha under papaya cultivation with a production of 1.17 million MT. Tamil Nadu stands first in productivity with an average of 236.4 MT/ ha. Other leading states in papaya production are Andhra Pradesh, Maharashtra, Karnataka, Madhya Pradesh and West Bengal. Karnataka is the fourth largest producer of papaya in India with an estimated production of 0.528 million MT from 7600 ha with an average productivity of 69.9 MT/ha (Anon., 2014).

Although the total area under cultivation has recorded a regular increase in the recent past, but fruit production has not shown proportional increase. Low productivity of papaya is mainly due to the losses caused by various diseases incited by fungal, bacterial, phytoplasmal and viral pathogens. In India, papaya ringspot and papaya leaf curl diseases are the major viral diseases causing considerable losses both in yield and quality of fruits (Singh, 2003). The ringspot virus disease is posing a major threat to papaya cultivation throughout India by rendering orchards economically unproductive. PRSV infection can result in crop losses up to 85-90 per cent (Lokhande *et al*., 1992; Hussain and Verma, 1994). PRSV is found to cause loss up to 100 per cent in some cases (Tennant *et al*., 2007). The PRSV disease drastically reduces fruit yield, fruit size quality and in some cases it results in total loss of production. There is a great scope to increase the productivity by at least 50 per cent if the PRSV disease is managed timely (Shikhamany, 2004).

Papaya ringspot virus spreads rapidly in the field by many species of aphids in a non-persistent manner and it is also readily transmitted mechanically through sap (Suzuki *et al*., 2007). It is not transmitted through seeds. The major symptoms on leaves are vein clearing, vein banding, mottling, distortion and yellow spots, puckering, blistering and in

severe cases shoe strings appear after two to three weeks of infection. Affected plants produce flowers meagrely and drastic reduction in the size of fruits occurs leading to the production of malformed fruits with less sugar content, reduced shelf life and market value (Gonsalves, 1994). Infected Papaya plants shows yellowing, leaf distortion and severe mosaic. Oily or water-soaked spots and streaks appear on the trunk and petioles. As the disease progresses plants appear bushy, tapering and finally leading to death of plants. The fruits exhibit bumps and classic ringspot symptoms. Severe isolates are known to cause tissue necrosis (Gonsalves *et al.,* 2010).

Papaya ringspot virus which belongs to the family *Potyviridae* causes one of the most important diseases in papaya and in members of Chenopodiaceae and Cucurbitaceae (Purcifull *et al.,* 1984). PRSV may have arisen in Asia in the region of the Indian subcontinent (Bateson *et al.,* 2002). PRSV strains are divided into two biotypes: the papaya-infecting type (PRSV-P), which affects both papaya and cucurbits, and the cucurbit-infecting type (PRSV-W) which affects cucurbits but not papaya (Gonsalves *et al.,* 2010). The two biotypes cannot be distinguished in serological tests (Bateson *et al.*, 2002).

Papaya ringspot virus is thought to have originated in the Indian subcontinent and was dispersed from there on several occasions to the rest of the world. PRSV-W probably reflects its ancestral state from which PRSV-P subsequently arose (Bateson *et al.,* 2002; Gibbs *et al.,* 2008; Castillo *et al.,* 2011). Although originally classified as different potyviruses (PRSV-W was originally *Watermelon mosaic virus* 1), amino acid similarity of approximately 98 per cent of the Nib, as well as the CP and 3'UTR of isolates of PRSV-P and PRSV-W, confirms they are strains of the same virus (Quemada *et al.,* 1990).

Papaya ringspot virus is morphologically characterized as flexuous filamentous rods measuring 750×12 nm (Kumar *et al.,* 2014). PRSV has a monopartite linear single-stranded positive sense RNA genome and is about 10,326 nucleotides long, excluding a poly-A-tract found at its 3' end. Genome encodes a single large protein which is subsequently cleaved into smaller proteins *viz.,* P1, HC-Pro, P3, CI, 6K, NIa-Pro, NIb

and CP. The P1 protein is one of three proteinases encoded by the potyvirus genome which autocatalytically cleaves itself from the polyprotein. P1 encodes for a 63 KD protein which is a proteinase and also possibly involved in cell-to-cell movement (Gonsalves *et al.,* 2010).

Sequence variability of viruses has important implications for the use of genes to develop transgenic plants by pathogen derived resistance (Savenkove and Valkonen, 2001). The selection of the transgene would be vital step to develop long-lasting virus resistant transgenic papaya. P1 protein gene of PRSV is reported to be highly variable (Gulsiri *et al.,* 2003). With PRSV, most studies have been focused on examining sequence variation in the CP gene. Identification of gene sequences in PRSV will provide valuable information as to the sequence of events that lead to infection and will lead to a better understanding of the significance of changing hosts in the molecular evolution of PRSV, an essential requirement for the development of suitable management strategies against PRSV (Srinivasulu and Saigopal, 2011).

The best way to manage a disease is to identify resistant germplasm and incorporation of the resistance into adapted varieties resulting in decreased production costs, improving product quality and reducing the detrimental effects of chemicals on ecosystem. Genetic resistance is considered to be the most economical and eco-friendly approach to virus control (Shukla *et al.,* 1994).

Plant breeding aims to evolve genotypes suitable for a given agro-climatic situation to optimize the level of genetic potentiality. Use of these resistant varieties is nonhazardous, eco-friendly and easily adoptable approach by the farmers to manage disease. The success of any crop improvement programme mainly depends on the strength and high yielding potential of the germplasm. It is important to screen the genotypes against the papaya ringspot disease prior to incorporating genotypes in hybridization programme.

At present, management of viral diseases of papaya is attempted through insecticides to prevent the movement of insect vectors. But all these chemicals and insecticides are causing environmental pollution, health hazards and phytotoxicity

besides their high cost. Many higher plants are known to contain endogenous proteins that act as virus inhibitors (Hansen, 1989; Chessin *et al.,* 1995; Parveen *et al.,* 2001; Choudhary *et al.,* 2008 and Srivastava *et al.,* 2015). Antiviral compounds present in plants may be easily absorbed into the leaves on which it is sprayed and translocated into the whole of the plant to induce the production or synthesis of some proteins that are actually antiviral and defend the plant against infection.

Because of the biological origin, anti microbial compounds from plants have many advantages over chemicals, as they are easily biodegradable, do not leave any residue, eco-friendly, non-phytotoxic, easily absorbed by the plant and cheap (Singh *et al*., 2011). Plants like *Boerhavia diffusa* and *Datura metal* grow abundantly as weeds posing a big problem for farmers; if they could be used as an antiviral agent, the problem of disease management as well as weed control may be solved simultaneously with minimum financial input.

The word nanotechnology is generally used when referring to material size of 0.1 to 100 nm (Morones *et al.,* 2005). Within this size range, chemical, physical and biological properties change in fundamental ways of both individual atoms/molecules and their corresponding bulk. Nanotechnology tenders prospects to explore the biological properties of already known antimicrobial compounds by manipulating their size to alter the effect. Based on enhanced effectiveness, the new age drugs are nanoparticles of polymers, metals or ceramics, which have several biological applications. Different types of nanomaterials like copper, zinc, titanium, magnesium, gold, alginate and silver have been developed, but silver nanoparticles have been proved to be most effective as they exhibit potent antimicrobial efficacy against bacteria, viruses and eukaryotic microorganisms (Guo *et al.,* 2003).

Nanoparticles could be used as an alternative to chemical pesticides for the management of plant pathogens even though there are some parameters to be evaluated for practical use. These may involve phytotoxicity, antimicrobial effects in hosts, and development of delivery systems of nanoparticles into host tissues colonized by

pathogens (Min *et al.,* 2009). There is an urgent need to address these unanswered questions.

A number of chemical, physical and biological approaches are available for the synthesis of silver nanoparticles (Goia and Matijevic 1998; Kowshik *et al.,* 2003). However, the chemical and physical methods are hazardous and harmful in one or the other way as the chemicals used are toxic, inflammable and do not get easily disposed off in the environment (Song and Kim, 2009). Chemical synthesis methods also lead to adsorbance of some toxic chemicals on the surface of nanoparticles that may have adverse effect in the plants.

Great deal of efforts has been made for the search of methods utilizing the biological system for nano material synthesis. The use of environmentally benign materials like plant leaf extract, fruit extract, bacteria, fungi, yeast and enzymes for the synthesis of silver nanoparticles have been used (Jain *et al.,* 2009). Advancement in green syntheses of nanoparticles is progressing as a key branch of nanotechnology. Use of biological entities like microorganisms, plant extract or plant biomass for the production of nanoparticles could be an alternative to chemical and physical methods in an eco-friendly manner (Reddy *et al.,* 2012). There is a need for development of economic, commercially viable and environment friendly synthesis process.

Papaya ringspot virus is difficult, if not impossible, to manage by conventional methods (Gonsalves, 1998). Management strategies of PRSV consist of earlier crop planting, using colored or reflective mulches and using insecticides to eliminate the vectors (Conway *et al.,* 1989). These control strategies are generally unsuccessful due to the short period of time it takes for the vector to transmit the virus from an infected plant to an uninfected plant, and the difficulty in removing all alternate hosts from crop production areas. The solution to minimize yield losses due to PRSV is to adopt an integrated disease management (IDM) approach. Economical and sustainable disease management could be achieved through the establishment of an integrated disease management system. As a rule, an integrated management system must always be eco-friendly.

An integrated approach towards PRSV management could be the best option for successful cultivation of papaya under PRSV infected regimes till PRSV resistant varieties are available. Major components of integrated disease management include host-plant resistance, cultural practices, biological and chemical components that strongly interact complementary to each other and environment. It is essential to break away from relying on a single-technology and to adopt a more ecological approach built around fundamental understanding of population biology of the vector at the local farm level and to rely on the integration of components which are readily available to the resource-poor farmers (Thomas, 1999). The solution to PRSV lies in the strategies integration of several methods (chemical, cultural, mechanical and biological) to manage both virus and its vectors.

In view of the above, present investigation has been undertaken with the following objectives,

1. Survey for the collection of Papaya Ringspot Virus (PRSV) infected samples of papaya from Southern India.
2. P1 protein gene sequencing of PRSV isolates to study their molecular variability.
3. Screening of papaya hybrids and varieties against PRSV under glasshouse and field conditions.
4. Evaluation of bioextracts and nanoparticles for the management of PRSV under glasshouse conditions.
5. Integrated management of papaya ringspot virus.

II REVIEW OF LITERATURE

Papaya ringspot virus (PRSV) has emerged as a major threat to papaya cultivation resulting in heavy reduction of area under this crop. It severely limits production, has become a major bottleneck to the growers and also a challenge to researchers across the globe. The virus has infringed beyond the conventional methods of management and paralyzed the papaya cultivation and industries dependent on it since the quick spread of the disease in the field leave little scope for adopting effective measures. The review presented here include, general properties of the virus, variability in symptoms, P1 protein sequence and coat protein gene sequences, reaction of papaya varieties against PRSV, evaluation of defence inducing molecules against PRSV and integrated management of PRSV.

2.1 Papaya ringspot virus

Papaya ringspot virus, a member of the aphid-transmitted genus Potyvirus, is the cause of a destructive disease and a major limiting factor for papaya and cucurbit cultivation worldwide (Purcifull *et al.,* 1984). PRSV belongs to the family *Potyviridae*, which forms the largest family of plant viruses, with 193 species (Shukla *et al.,* 1994).

2.1.1 General characters of PRSV

Papaya ringspot virus is a member of Potyviridae family, genus Potyvirus which typically have flexuous, filamentous particles, 760-800 nm long and 12 nm in diameter (Herold and Weibel, 1962; Purcifull *et al.,* 1984; Murphy *et al.,* 1995).

Papaya ringspot virus isolates are divided into two strains, type P and W. Webb and Scott (1965) were first to describe PRSV-W in cucurbits. PRSV-P and W were serologically and morphologically identical and could only be differentiated on the basis of their host range (Gonsalves and Ishii, 1980).

PRSV-P isolates infect both cucurbits and papaya, while PRSV-W isolates naturally infect only cucurbits. The experimental host range of PRSV-P includes 15 species in three families (Cahcaceae, Chenopodiaceae and Cucurbitaceae) while

PRSV-W infects 38 species of 11 genera in two families *viz., Cucurbitaceae* and *Chenopodiaceae* (Edwardson and Christie, 1986). Amino acid similarity of approximately 98 per cent of the Nib, as well as the CP and 3'UTR of US isolates of PRSV-P and W confirmed that they were strains of the same virus (Quemada *et al.,* 1990).

The virion appeares as flexious rod measuring 760-800nm x 12nm, with a positive sense single stranded RNA, producing often pinwheel inclusion bodies in the cytoplasm of the infected host tissue (Wu *et al*., 1983; Yeh and Gonsalves, 1985; Fauquet *et al.,* 2005). The buoyant density of the virus in CsCl is 1.32 g cm^{-3}. The cryptogram of PRSV is R/1, X/5.5, E/E, S/Ap (Fauquet *et al.,* 2005). PRSV got inactived at 54 - 56°C, which is the thermal inactivation point with a dilution end point of 10^{-3} and longevity *in vitro* of 3h (Conover, 1962).

According to Kumar *et al.* (2014) PRSV particles appeared as flexuous rod shape of 750×12 nm under electron microscope. Dilution end point of the virus was 10^{-4}. Thermal inactivation point and Longevity *in vitro* of the virus was found to be 55 ˚C and 30 h respectively.

Based on bio-assay, the virus isolates from papaya and sponge gourd were classified as P and W pathotypes respectively. Pathotype P could infect members of the family Caricaceae and Cucurbitaceae, while W pathotype could infect only cucurbitaceous hosts. Both localized and systemic infection were observed on cucurbitaceous hosts for pathotype W, while only localized infection was observed in few cucurbits for pathotype P (Parameswari and Jain, 2016)

2.1.2 Symptoms of PRSV infection

For the first time mosaic pattern on leaves and yellow rings on papaya fruits were observed by Linder *et al.* (1945). Holmes *et al*. (1948) reported different types of symptoms *viz*., puckering of leaf tissues between veins and veinlet of young leaves and mosaic pattern on expanded leaves, while swelling of leaf tissues between veins resulting in upward curling of leaves followed by chlorotic mottling, blistering of leaf tissue was

observed by Jensen (1949). Chlorotic ringspots, shoestring on leaves, blistering and distortion of leaves in severe cases, lobed and semi apocarpus conditions of fruits and overcrowding of leaves were also observed (Bhandari, 1952).

According to Gonslaves (1998) symptoms on the fruit consisted of dark green concentric rings or spots or C-shaped markings, often slightly sunken in the fruit were diagnostic. Fruits often showed uneven bumps, especially those fruits that develop after a tree was infected. The number of rings on fruits varied and the rings became less distinct as the fruit matures.

Initial symptoms on papaya became visible as yellowing and vein-clearing of the young leaves, followed by conspicuous yellow mottling and sometimes severe blistering and leaf distortion (Heu *et al.*, 2002). As the disease progress, leaf canopy became smaller due to the development of smaller leaves and stunting of the plant. Leaves often developed a shoestring appearance due to extreme reduction of leaf laminae. Singh *et al*. (2003) also recorded downward curling of leaf margins, elongation and distortion of leaves with scorched appearance. Development of dark green water-soaked oily streaks and rings on petiole, leaf stalks and upper part of the trunk or stems were also observed by Tripathi *et al*. (2008) and Talukdar *et al.* (2013).

Bare plants with no flowers, without any fruit set, stunted growth, beheaded appearance, tapered canopy and death of the plants were recorded by several workers in the early infected and abandoned orchards (Lokhande and Moghe, 1992; Thomas and Dodman, 1993; Hussain and Verma, 1994; Shaikh, 1996; Dahal *et al*., 1997).

Depending upon the age of the plant when infection starts, vigour of the trees and fruit set get usually reduced, fruit quality particularly flavor is adversely affected. Varying degrees of deformation and abnormalities on fruits and stunted growth and collapsing nature of plants in advanced stages of infection were also reported (Reddy, 2000; Kunkalikar, 2003; Hemavati, 2005; Verma *et al*., 2007; Mallikarjun, 2009 and Reddy *et al.,* 2011a).

Papaya plants showed yellowing, leaf distortion and severe mosaic. Oily or water-soaked spots and streaks appeared on the trunk and petioles. The fruit would exhibit bumps and the classic "ringspot". A severe isolate of PRSV has also been shown to cause tissue necrosis (Gonsalves *et al.,* 2010).

Seedlings showed prominent vein clearing and mottling of the young leaves in about 1-2 weeks after inoculation. After 2-3 weeks, the leaves become blistering and distorted, the lobes being markedly reduced in size resulting in filiformy. Only mosaic and mild mottling symptoms were produced on cucurbits (Parameswari and Jain, 2016).

Cucurbits produced similar symptoms to papaya including blisters, mosaic, yellowing, and leaf distortions (Dahal *et al.,* 1997). Mechanically inoculated seedlings of zucchini expressed symptoms like mosaic, leaf blistering and dark green spots after 7-10 days of inoculation. Mild mosaic, mosaic, puckering, mottling, vein clearing, vein banding, blistering, distortion and shoe strings in severe case appeared on leaves after two to three weeks of infection. Oily streaks on petioles, ringspots on leaves were also observed. In advanced case infected plants appeared bushy, back headed, tapering and finally death was noticed (Navanath *et al*., 2017).

2.1.3 Distribution of the PRSV

PRSV-P was first confirmed in Hawaii in 1949 (Jensen, 1949), USA, South America, Africa (Purcifull *et al.,* 1984), Mexico (Alvizo and Rojkind, 1987), South-East Queensland (Australia) in 1991 (Thomas and Dodman, 1993), Thailand, Taiwan, China and the Philippines (Gonsalves, 1994), Japan (Maoka *et al*., 1995), in Saipan, Northern Mariana Islands and Guam in 1994 (Kiritani and Su, 1999), in French Polynesia and the Cook islands (Davis *et al*., 2005), in Cote d'Ivoire, UK in 2006 (Diallo *et al.*, 2007) resulting in declined production. Zhu *et al*. (2016) found that the PRSV incidence was more than 35 per cent in most fields (>85 %) in selected vegetable greenhouses in Sichuan Province of Southwest China.

From India, PRSV was first reported by Capoor and Verma (1948) as papaya mosaic, later it was reported in Bihar by Mishra and Jha (1955) as mosaic, from Madhya

Pradesh by Garga (1963), from Uttar Pradesh by Khurana and Bhargava (1970). Subsequently PRSV was reported in Udaipur of Rajasthan (Surekha *et al*., 1977). Severe incidence of PRSV in Marathwada region of Maharastra was reported by Yemewar and Mali (1980) while Cheema and Reddy (1985) from Punjab reported it as papaya mosaic virus and Susan (1985) from Andhra Pradesh as papaya mosaic. Rao (1988) from Maharashtra stated that the virus causing mosaic, leaf distortion, shoestring and rings on fruits of papaya was due to PRSV- P, a member of potyvirus group.

PRSV disease has proved catastrophic to papaya gardens throughout India by rendering orchards unproductive and uneconomical. Irrespective of the agroclimatic conditions the virus was reported to occur in every region of the Indian subcontinent where papaya was grown (Lokhande *et al*., 1992; Hussain and Verma, 1994). Byadgi *et al*. (1995) first reported PRSV from Dharwad, Karnataka. Since then, the disease has been reported throughout south India causing severe yield loss. PRSV-W infecting snake gourd was confirmed in Tamil Nadu by Kumar *et al*. (2014) based on biological, physical and morphological properties, serology and molecular characterization

2.1.4 Crop loss due to PRSV incidence

Papaya ringspot virus was found to be a deadly disease of papaya inflicting heavy yield losses. In Southern Florida, incidence of the PRSV was found up to 100 per cent (Wan and Conover, 1983). In Malaysia out of 74 ha plantations surveyed, Wahab (1991) observed occurrence of PRSV in 26 ha. Among different viruses infecting papaya, PRSV has been reported to be most destructive disease in many tropical and subtropical region of the world (Prowidentii, 1996).

PRSV crippled the production of papaya in many countries of South East Asia (Philippines, Taiwan, Thailand, and Vietnam), India, Africa, South America (Brazil, Venezuela), the Caribbean islands, Mexico and the USA (Kiritani and Su, 1999). However, the disease had restricted distribution in Queensland, Australia and was not recorded from South Africa and several island nations of the South Pacific. PRSV is found to cause loss up to 100 per cent in some parts of the globe (Tennant *et al*., 2007).

PRSV infection is reported to occur in every region of the Indian subcontinent where papaya is grown irrespective of the agroclimatic conditions resulting in crop loss up to 85-90 per cent (Lokhande *et al*., 1992; Hussain and Verma, 1994). Singh *et al.* (2005) recorded 70 per cent yield loss due to PRSV infection in Eastern Uttar Pradesh.

2.2 Survey for the collection of PRSV infected samples of papaya from Southern India

2.2.1 Incidence of PRSV in India

In Vidharbha region of Maharashtra up to 75 to100 per cent PRSV incidence was recorded (Lokhande *et al.,* 1992). PRSV incidence ranged from 48 to 100 per cent in all the districts of Eastern Uttar Pradesh was reported by Singh *et al*. (2003). A survey conducted in papaya orchards of Eastern Uttar Pradesh by Singh *et al.* (2005) recorded 95 per cent disease incidence with 70 per cent yield loss.

PRSV disease has been recorded from almost all the states of India *viz*., Bihar (100 %), Maharashtra (3 to 100 %), Uttar Pradesh (74 to 90 %), Karnataka (60 %), Kerala (35 to 66 %) and West Bengal (40 %) by Verma *et al*., 2007.

In Uttar Pradesh Incidence of PRSV disease ranged from 45 to 85 per cent during 2004-06 and 2005-06. Minimum ringspot disease incidence was recorded in Varanasi district followed by Faizabad, Lucknow and Etawah during both years. Most of the papaya plants surveyed showed characteristics symptoms of PRSV (Singh and Awasthi, 2007)

Mallikarjun (2009) found severe incidence of PRSV in papaya growing locations of Maharashtra. 100 per cent disease incidence was found in Akola, Aurangabad¸ Beed, Hingoli, Jalna, Latur, Nagpur, Nanded, Parbhani, Pune, Sangali, Solapur, Usmanabad and Yawatmal districts and lowest disease incidence of 10 per cent was found in Satara and Ahmadnagar districts. The observations revealed that average incidence of each district in Maharashtra were higher compared to Karnataka.

Highest PRSV incidence of 74 per cent was noticed in Goalpara district of Assam followed by 70 per cent in Kokrajhar district and the lowest in Nagaon district (28 %) when 15 districts of Assam were surveyed (Talukdar *et al*., 2013).

2.2.2 Incidence of PRSV in Karnataka

First time incidence of PRSV from northern parts of Karnataka state was reported by Byadgi *et al.* (1995). PRSV incidence up to 100 per cent in different parts of Dharwad and Belgum districts of North Karnataka was reported by Shaikh (1996). 100 per cent PRSV incidence in different districts of Karnataka was reported by Hegde (1998). Similarly, Reddy (2000) and Basha (2002) observed 75 to100 per cent disease incidence in Bangalore district.

Complete absence of the PRSV during 2002-03 in three districts of coastal Karnataka *viz*., Udupi, Hassan and Kodagu was revealed from roving surveys of Kunkalikar *et al.* (2007); and Kalleshwaraswamy and Kumar (2008).

Highest PRSV incidence in north Karnataka as compared to south except Bangalore urban (70 %) was recorded by Mallikarjun, 2009. Bidar district showed highest per cent incidence (92 %) followed by Gulbarga (86 %), Bijapur (81 %), Bellary (78 %), Bagalkot (75 %) and Dharwad (72 %). Lowest average incidence was observed in Udupi and Mandya (1 %) followed by Mangalore (8 %), Hassan (11 %), Shimogga (13 %) and Tumkur (24 %) district.

A survey conducted by Reddy *et al.* (2011a) on commercial orchards and kitchen gardens in different districts of Southern Karnataka revealed that the maximum disease incidence of 94 % was observed in Bangalore followed by Kolar (76 %), Tumkur (57 %), Chitradurga (52 %), Chamarajanagar (35 %), Dakshin Kannada (34 %), Chickmaglur (19 %), Mandya (18 %) districts and minimum (14 %) in Shimoga.

Surveys conducted in major papaya growing areas of southern Karnataka during 2012-14 revealed maximum PRSV incidence in Bangalore Rural (78 %) followed by

Bangalore Urban (69 %), Kolar (65 %) and Chikkaballapura (52 %) districts (Pushpa, 2014).

2.3 Molecular variability of PRSV

Knowledge of sequence diversity among isolates of a virus and their distribution has the potential to deepen our understanding of viral origins, development, dispersal and disease etiology. This information would be useful in developing effective virus disease management programmes. Identification of gene sequences in PRSV will provide valuable information as to the sequence of events that lead to infection and will lead to a better understanding of the significance of changing hosts in the molecular evolution of PRSV, an essential requirement for the development of long-term sustainable control strategies against PRSV. With PRSV, most studies have focused on examining sequence variation in the CP gene.

Gulsiri *et al.,* 2003 determined the complete nucleotide sequence of PRSV type P, Thai isolate (PRSVthP). The viral genome was 10323 nucleotides long and contained an open reading frame encoding a polyprotein of 3343 amino acids, flanked with 5' and 3' non coding regions of 85 and 206 nucleotides, respectively. Sequence similarity among the type P and type W isolates suggested that the P type arose from type W. No significant difference between types P and W was discovered that would account for the host specificity.

Parameswari *et al*. (2007) did complete sequencing of New Delhi Indian isolate of PRSV (PRSV-DEL). Comparative sequence analyses revealed that the PRSV-DEL shared 83-89 per cent and 90-92 per cent overall sequence identity at the nucleotide and amino acid levels respectively, with other PRSV isolates. Maximum sequence identity at the amino acid level (92 %) was observed with isolates from the America forming one cluster, followed by 90 to 91 per cent identity with Asian isolates, forming a distinct cluster.

The genetic variation of PRSV in Venezuela was estimated by single strand conformation and nucleotide sequence analyses of two genomic regions of twenty-six

isolates. Phylogenetic analysis indicated that Venezuelan isolates were within a clade composed of isolates from the Americas and Australia (Rodriguez *et al.,* 2008)

A nucleotide BLAST search using the complete genome of isolate PRSV-W-TUL15 showed 83 to 92 % nucleotide sequence similarities with the published PRSV isolates (Ali, 2017).

2.3.1 Molecular variability of PRSV based on P1 protein gene sequence

P1 protein appeared to be the most variable potyviral protein and showed a wide variation in size (from 29K-63K) among reported potyviruses (Yeh, 1994). Between the P1 proteins of Taiwanese and Hawaiian PRSV isolates there was only 70.9 per cent nucleotide identity and 66.7 per cent amino acid identity (Wang and Yeh, 1997). This high level of variability was also seen within isolates from a particular country. Henderson (1999) reported 81.4 per cent nucleotide and 76.9 per cent amino acid similarity between the P1 proteins of PRSV-P and W from Thailand. The Australian PRSV-P and W isolates were found to be 96.89 per cent and 95.43 per cent similar at the nucleotide and amino acid levels, respectively (Henderson, 1999).

Out of the ten putative proteins P1was found most variable (73.9 per cent similarity) as compared to the PRSV type P (PRSV-P) sequences, while the CI protein was found to be most conserved (99.1 per cent similarity) among nucleotide sequences of a Thai isolate of PRSV-W (Attasart *et al.,* 2002).

Gulsiri *et al.* (2003) reported that among ten putative proteins of Thai PRSV isolate type P (PRSVthP), P1 was the most variable (73.9 per cent similarity) when compared to the other full PRSV sequences, while CI protein was the most conserved protein (99.1 per cent similarity). Sequence similarity among the type P and type W isolates also suggested that the P type arose locally from type W.

The comparative analysis of a severe PRSV isolate of Mexico (Mex-VrPO) and five others reported before showed that P1 was most variable with 13-33 per cent divergence while CP showed 5-9 per cent divergence only (Carrazana *et al*., 2007).

According to Urcuqui-lnchima *et al*. (2001) P1 protein appeared to be the least conserved protein among potyviruses. High variability in the P1 has been reported for other potyviruses including PVY (72.8 %-100 % amino acid sequence identity between 12 isolates) by Tordo *et al*. (1995) and Zucchini yellow mosaic virus (53.3 %-57 % amino acid sequence identity) by Wisler *et al*. (1995). Average intergroup nucleotide sequence identity identity between Yam mosaic virus (YMV) isolates was only 65 per cent in the P1 protein compared to about 80 per cent in the HC-Pro, P3 and Nib proteins (Verdaguer *et al*., 1997). Lin *et al*., 2001 also reported that in Zucchini yellow mosaic virus (isolate TW-TN3) P1 protein was most variable, with amino acid identities of 59.0-93.2 per cent.

2.3.2 Molecular variability of PRSV based on coat protein gene sequence

Bateson *et al.* (1994) sequenced the coat protein gene of six Australian and three Asian PRSV isolates and compared these with four previously reported sequences of PRSV. Up to 12 per cent sequence variation between isolates at the nucleotide level was observed and there was no significant difference between the sequences obtained from Australian isolates irrespective of whether they were PRSV type P or PRSV type W.

Comparative sequence analyses showed the CP genes of the P and W isolates from India were similar, with 87 per cent nucleotide identity and 93 per cent amino acid identity. The amino acid differences between the CP genes were mostly confined to the amino terminus (Jain *et al.,* 1998).

The sequences of the CP gene of Twelve PRSV isolates from Brazil were compared among themselves and an average of 97.3 per cent degree of homology at the nucleotide sequence was found. When compared to 27 isolates from outside Brazil in a homology tree, the Brazilian isolates were clustered with Australian, Hawaiian, and Central and North American isolates, with an average degree of homology of 90.7 per cent among them (Roberto *et al.,* 2002).

Bateson *et al*. (2002) studied evolution and molecular epidemiology of PRSV-P by sequencing CP genes of both PRSV P and W type isolates from Vietnam, Thailand,

India and Philippines and were compared with published sequences of 28 isolates. Phylogenetic analyses of PRSV showed its closest known relative, Moroccan watermelon mosaic virus, which indicated that PRSV might have originated in Asia, particularly in the Indian subcontinent.

The CP gene from twelve Brazilian isolates shared an average homology of 97.3 per cent at the nucleotide level among Brazilian isolates. When compared to 27 isolates from outside the Brazil, these isolates showed clustering with Australian, Hawaiian, Central and North American isolates, with an average degree of homology of 90.7 per cent among them (Lima *et al.,* 2002).

Marilia *et al.,* 2003 compared three mild and three severe strains of PRSV-W, based on nucleotide and amino acid sequences of the CP gene. The CP nucleotide sequences of the mild strains shared 98 per cent to 100 per cent identity.

CP gene sequence of south Indian PRSV strain (INP-UAS) with other reported sequences was compared by Hema and Prasad (2004). Coat protein gene of INP-UAS strain was relatively divergent from those of other PRSV-P isolates as it formed a separate and distinct group. This strain had deletion of 24 nucleotides that corresponded to eight amino acids in the N-terminal region of the CP.

Jain *et al.* (2004) compared the CP sequences of eleven PRSV isolates originating from different locations in India with other isolates of PRSV. The virus isolates from India showed considerable heterogeneity in the CP sequences. The CP-coding region varied in size from 840-858 nucleotides, encoding protein of 280-286 amino acids. Comparative sequence analysis revealed that the PRSV isolates originated from India were divergent up to 11 per cent. Though the PRSV isolates were differentiated into two clusters, the sequence variation could not be correlated with the geographical origin of the isolates.

Bag *et al*. (2007) studied the sequence diversity in the coat proteins of 28 PRSV isolates from India. There was heterogeneity in CP gene length (275-289 amino acids) among the isolates from central, eastern, northern, southern and western India (up to 23

%). Isolates, KA4, INU-01 and AP2 from southern India were found unique. Maximum heterogeneity was observed in southern isolates (up to 23 %), followed by central (up to 11 %), eastern and northern (up to 10 %) and western (up to 7 %) isolates. Lack of relationship between variability and geographical origin of the isolates was observed.

The CP gene nucleotide and deduced amino acid sequences of seven isolates were compared with each other and with sequences of 22 other PRSV isolates from Indian subcontinent by Srinivasulu and Saigopal (2011). The sequence comparisons revealed greater sequence divergence (Up to 18.4 per cent and 15.0 per cent at nucleotide and amino acid levels, respectively) within Indian PRSV populations. All south Indian isolates were clearly separated from isolates of other geographical regions and formed a major group in phylogenetic trees and the clustering pattern of isolates did not correlate well with their geographical origins.

Among 64 PRSV-W isolates collected from watermelon in commercial fields of Oklahoma nucleotide and amino acid sequence identities ranged from 95.2-100 per cent and 97.1-100 per cent, respectively. PRSW-W isolates clustered according to the locations where they were collected within Oklahoma, and each cluster contained two subgroups. All subgroups of Oklahoman PRSV-W isolates were on separate branches, when compared to 35 known isolates originating from other parts of the world, including the one reported previously from the USA (Osama and Ali, 2012).

The CP genes of 21 PRSV isolates from Brazil and 7 isolates from Cuba were sequenced and analyzed by Martinez *et al.* (2014). The American and Indian isolates were grouped together in the in phylogenetic tree. Cuban isolates from the eastern region were closer to the American isolates than to those from the central-west region. The variability of the coat protein genes confirmed that virus control using cross-protection and transgenic plants requires the selection of region-specific virus isolates in each country.

Zhu *et al*. (2016) reported that nucleotide BLAST analysis of the CP sequence of PRSV infecting bitter gourd in China, showed highest identity of 99 per cent with four isolates from Taiwan. The isolate clustered with the PRSV type P isolates available in

gene bank. Partial characterization of PRSV isolate BUH-1 by CP gene showed highest homology of 98 per cent with South Indian isolates and 87-92 per cent with Asian isolates (Pushpa, 2014)

Singh *et al*. (2017) cloned and sequenced coat protein (CP) genes of four PRSV isolates originating from different locations in India. The maximum per cent similarity of PRSV-FZD isolate of Uttar Pradesh was observed with the Lucknow isolate (AY458620)-97.7 per cent followed by Haryana isolate (DQ088670) with 91.7 per cent similarity. Also, the maximum divergences of 14.5 per cent and 14.2 per cent were found with Karnataka (AY458618) and Tamil Nadu (DQ077175) isolates, respectively.

2.4 Screening of papaya varieties against PRSV for identification of natural resistance

Although the total area under papaya cultivation has recorded a regular increase in the recent past, fruit production has not shown corresponding increase. Low productivity of papaya is mainly due to the losses caused by PRSV since managing the disease is very difficult. There is no known source of resistance to PRSV. Attempts were made to identify new sources of resistance from the time of devastating effects of PRSV were known (Roff, 2007). In the absence of a durable PRSV resistant papaya variety or hybrid, farmers often resort to indiscriminate application of insecticides to reduce yield loss (Kalleshwaraswamy and Kumar, 2008).

Capoor and Verma (1961) reported *Carica cauliflora* to be immune to PRSV. Wang (1982) reported that F1-TT-5 variety was tolerant to PRSV. Zee (1985) reported that a line 356-3 selected from Florida accession was most tolerant. Further tolerance was readily transferred from line 356-3 into papaya hybrid ‘Solo’ in a quantitative manner. Genetic analysis of tolerance to PRSV disease in line 356-3 had indicated multiple QTLs affecting various components of resistance. Introgression of resistance genes from wild species into commercial papaya varieties had been attempted (Chen *et al*., 1991).

Efforts to overcome PRSV in Florida by Conover *et al*. (1988) resulted in the development of ‘Cariflora’, a PRSV-tolerant variety. ‘Cariflora’ served as a source of

PRSV resistance in different papaya breeding programs around the world, resulting in currently popular PRSV-tolerant varieties such as 'Red Lady' (Davis *et al*., 2003).

Ram (1993) made two crosses involving *Carica papaya* x *C. cauliflora* (resistant to PRSV) at Indian Institute of Horticultural Research (IIHR), Bangalore but the hybrids were found to be susceptible to PRSV later. The varieties Cariflora, Thapra, Red Lady and Known You No.1 were found tolerant to the virus. However tolerant selections became susceptible to virus, but remained symptomless or showed mild symptom expression and produced economically useful yields (Gonsalves, 1994). Tolerance to PRSV was evident in some of the non-transgenic varieties *viz*., Red Lady, Cariflora, Tainung No. 5 and Washington No.5 in Florida (Crane *et al*., 1995).

Magdalita *et al*. (1997) screened *Carica papaya*, *C. cauliflora* and interspecific hybrids of these species for resistance to two Australian isolates of PRSV-P. All interspecific hybrids and *C. cauliflora* plants were manually inoculated in the glasshouse and planted in the field, failed to become infected and showed resistance to the Australian PRSV-P isolates. However, *C. papa*ya plants were infected by PRSV-P.

Among 26 varieties of papaya screened for resistance against PRSV disease under natural conditions, no variety was found to be resistant. 'Farm Selection-1' was the most promising variety, recording higher fruit yield per plant. Varieties Co.2 and M.F-1 showed high yield potential under diseased conditions. Minimum PRSV disease incidence was recorded in 'Pusa majesty' but it was a poor yielder (Kudada and Prasad, 2000).

C. cauliflora when inarched with *C. papaya* delayed PRSV symptom expression but did not confer resistance to the disease. Symptom expression of PRSV started when the inarched *C. cauliflora* deteriorated and delayed symptom expression could be attributed to a transmissible, water-soluble, low molecular weight protein factor that got transported from *C. cauliflora* to *C. papaya* (Valencia *et al.,* 2001).

In a breeding programme for development of resistance to PRSV initiated in Malaysia, using Tainung No. 5 and *Cauliflora* as tolerant parents, F1 hybri variety

'Eksotika' showed very good tolerance (Chan and Ong 1996). Seedling screening for PRSV tolerance by artificial inoculation at the F5 resulted in selection of 11 lines. When these lines were tested, four lines *viz.,* L41, L90, L248 and L13 were found most tolerant (Chan and Ong, 2003).

Fourteen varieties of papaya were tested to identify sources of resistance. However, none of the tested varieties was resistant to PRSV but variety Harichaap showed only 10 per cent incidence with an average yield loss of 10.38 per cent suggesting its better performance over others (Singh *et al.,* 2005). Out of 31 papaya varieties screened for resistance to PRSV-P under glasshouse conditions, none of them showed complete resistance to the disease while variety 'Cariflora' was found to be tolerant to PRSV-P (Roff, 2007).

Among 16 varieties screened against PRSV, minimum per cent disease incidence was recorded in variety Harichaap. Four varieties *viz.,* Co2, Co6, M.F-1 and Pusa Majesty were found moderately resistant, eight varieties showed susceptible reaction while two were highly susceptible to the disease (Awasthi and Singh, 2009a). Screening of 84 germplasm accessions for identifying the field tolerant lines of papaya showed that accession number CP-50, a cross between *Carica* (Wild) × Co6 (Cultivated variety) was found to have field tolerance. None of the *Carica papaya* was found to have natural-resistance to PRSV-P (Balamohan *et al*., 2010).

PRSV incidence was noticed in seedlings of eight papaya varieties *viz*., Red Lady, Madhubala, Pusa dwarf, Pusa nanha, Surya, P-04, P-06 and CO2 till 100 per cent flowering stage. variety 'Madhubala' showed lowest disease incidence (13.2 %) at 100 per cent flowering stage followed by varieties 'CO2' (39.8 %) and 'Pusa nanha' (44.8 %) while widely grown 'Red Lady' variety recorded incidence of 86.0 per cent (Chavan *et al*., 2010).

The resistance of three PRSV tolerant varieties *viz*., Sinta, Cariflora and Red Lady were characterized and development of infection was compared with that of the susceptible varieties Davao solo and Cavite Special. The disease incidence in Cariflora was significantly lower than Sinta, Cavite special and Davao solo. Red Lady had disease

onset and symptom severity similar to Davao solo, but Sinta had lower virus level than Red Lady, Davao solo and Cavite special. However, at later stages of infection, no difference in incidence among the varieties was observed (Alviar, 2011).

Commercially available papaya genotypes *viz*., Sunrise solo, Solo-109, Red Lady, Surya, Coorg honey dew, Co 2, Pusa nanha, Pusa dwarf, Pusa gaint and Pant papaya-1 were screened by mechanical inoculation to identify source of natural resistance. None of them was resistat to PRSV but variety Red Lady showed minimum disease incidence of 8.33 per cent with maximum days (26) for PRSV symptom expression (Reddy *et al*., 2011b).

Fifteen varieties of papaya were tested to identify sources of resistance but, none of the varieties was free from papaya ringspot and leaf curl disease. Variety Harichaap showed < 25 per cent incidence, which was found better than others (Singh and Singh, 2013). Three varieties *viz.,* Arka surya, Red Lady and Sunrise solo were found susceptible to PRSV upon mechanical inoculation (Pushpa, 2014).

Intergeneric hybrids were developed between *Carica papaya* (Var. Pusa nanha, CP 50 and Co7) with *Vasconcellea cauliflora* to incorporate the PRSV resistant gene from *V. cauliflora* into the varieties of papaya. Based on overall evaluation of F3 population, 7 progenies from the cross Pusa nanha /*V. cauliflora* and 4 progenies from the cross CP 50 /*V. cauliflora* were found to be good for forwarding to F4 generation for further evaluation (Sudha *et al*., 2015).

2.5 Induction of resistance against PRSV

The viral diseases of plants are difficult to manage but can be minimized by some preventive measures like induced systemic resistance through antiviral agents of plant origin or from some other sources.

2.5.1 Induction of resistance using bioextracts

Many higher plants contain endogenous proteins that act as virus inhibitors (Hansen, 1989; Chessin *et al.,* 1995; Parveen *et al.,* 2001 and; Choudhary *et al.,* 2008).

These substances were non-chemical, non-hazardous, easily biodegradable and found eco-friendly besides their very low cost and no residual effect.

Khurana and Bhargava (1970) reported prevention and spread of ringspot infection in papaya through plant extracts. Symptoms were delayed by use of leaf extracts from *Argemone mexicana*, *Carum capsicum* and seed extract of *Argemone mexicana*, *Datura fastuosa*, *Physachosia asnoris* and *Raphanus sativus*.

Glycoprotein from *Boerhavia diffusa* could prevent 60-90 per cent of the virus infection of tomatoes, potatoes, pea and French bean (Awasthi and Mukerjee, 1980). Verma and Singh (1994) stated that inhibitory effect of *B. diffusa* and *Clerodendrum aculeatum* may be due to the resistance inducer, which induced strong systemic resistance against tobacco mosaic virus (TMV) in tobacco, cucumber mosaic and TMV in tomato, cucumber green mottle mosaic virus in melon, sunn hemp rosette virus in *Crotalaria juncea,* Gomphrena mosaic virus in *G. globosa* and yellow mosaic virus in mung bean.

A commercial viricide product Virex-H containing water extract of *Acorus calamus*, *Datura metal*, *Boerhavia diffusa*, *Capsicum frutoscens* and *Chenopodium album* helped in the reduction of Radish mosaic virus in radish. However, there were no reports on effect of Virex-H on PRSV (Sharma *et al.*, 2005).

Systemic resistance inducing protein (CA-SRIP) was identified from *Clerodendrum aculeatum* against PRSV infection in papaya by Srivastava *et al.* (2006). No virus symptoms were observed up to 6 months after inoculation, following challenge inoculation with PRSV, to the CA-SRIP treated papaya plants. There was also a significant difference in the vegetative growth of the treated plants as compared to control set of plants.

Ten sprays of *Boerhavia diffusa* root extract at 5 per cent along with milk protein (1.0 %) was found significantly superior in preventing Papaya ringspot disease of papaya as reported by Awasthi and Singh (2009b).

The antiviral agents namely *Boerhavia diffusa* root extract and *Clerodendron aculeatum* leaf extract were found to be significantly effective in inducing systematic resistance against PRSV in papaya. *B. diffusa* root extract was better when compared to *C. aculeatum* leaf extract. Maximum reduction in disease incidence of 72 per cent and 74 per cent, were observed with the application of *B. diffusa* root extract as seed treatment + nursery treatment + field treatment during 2005-2006 and 2006-2007 respectively (Singh *et al*., 2011).

A protein (CAP-34) from *Clerodendrum aculeatum* was evaluated for management of PRSV infection in papaya. In the treated plants, mild mosaic symptoms appeared only in 10 per cent plants, while 95 per cent of control plants exhibited symptoms ranging from mosaic to filiformy. (Srivastava *et al*., 2009). Systemic antiviral resistance induced in papaya by CAP -34 was found to be associated with a proteinaceous virus inhibitory Agent CP-VIA-34 (Srivastava *et al*., 2015).

2.5.2 Induction of resistance using seaweed extracts

Liquid extracts obtained from seaweeds gained much interest as soil and foliar spray for inducing shoot growth and yield in orchards and horticultural plants. Seaweed extracts were found superior than chemicals because of the presence of high level of organic matter, micro nutrients, vitamins and fatty acids and also growth regulators such as auxins, cytokinin and gibberellins (Crouch and Staden, 1994).

Marine macroalgae and seaweeds contain a variety of unique polysaccharides (Kloareg and Quatrano, 1988), and some of these were shown to be potential sources of oligosaccharide elicitors of plant defence (Kobayashi *et al.,* 1993; Patier *et al.*, 1993; Klarzynski *et al.,* 2000 and Mercier *et al.,* 2001).

Laporte *et al.* (2007) reported that tobacco plants treated with structurally unrelated oligosaccharides obtained from Chilean marine macroalgae. Treated plants showed an increase in defense against tobacco mosaic virus (TMV) with corresponding decrease in the number of necrotic lesions. Stimulation of defense against TMV was

correlated with the activation of the defense enzyme phenylalanine ammonia-lyase (PAL).

Vera *et al.* (2011) recorded a progressive increase in PAL activity and accumulation of free and conjugated phenylpropanoid compounds (PPCs), after spraying marine alga oligo-sulphated-galactan Poly-Ga. Increase in PAL activity showed a linear correlation with the decrease in necrotic lesions and the decrease in TMV-CP transcript level. Induced systemic and long-term protection against TMV in tobacco plants could be attributed to a sustained activation of PAL and the accumulation of PPCs with potential antiviral activity.

Jimenez *et al.* (2011) reported that aqueous and ethanolic extracts from the brown-alga *Durvillaea antarctica* were able to reduce the number and the size of necrotic lesions caused by tobacco mosaic virus in tobacco leaves.

Ascophyllum nodosum (brown seaweed) extract in combination with chlorothalonil reduced Alternaria blight in tomato and it was hypothesized that the reduced disease incidence levels in treated plants might be due to induced resistance activated by elicitor compounds present in the seaweed extract (Ali *et al.,* 2013).

Sulfated fucans are common structural components of the cell walls of marine brown algae. Tobacco leaves treated with oligofucans accumulated salicylic acid locally and the phytoalexin (Scopoletin) and expressed several pathogenesis-related (PR) proteins. Fucan oligosaccharides induced the systemic accumulation of salicylic acid and the acidic PR protein PR-1, two markers of systemic acquired resistance. Fucan oligosaccharides strongly stimulated both local and systemic resistance to tobacco mosaic virus (Klarzynski *et al.,* 2003).

Ali *et al.* (2016) reported that foliar the application of *Ascophyllum nodosum* extract on tomato plants showed significantly higher levels of activity of defense enzymes (Polyphenol oxidase, phenylalanine ammonia lyase, peroxidase**,** chitinase and glucanase), and accumulated higher levels of phenols compared to control plants.

Investigation of transcript levels of defense pathway marker genes demonstrated the upregulation of JA/Ethylene pathway than SA pathway.

2.5.3 Induction of resistance using silicic acid

Marschner (1995) studied the role of silicon, its nature of interactions with boron. There was deposition of silicic acid and traces of boric acid in the cell walls and linings of xylem cells these silicic and boric acid complexes were considered inert.

The acquisition of silicic acid and its incorporation as silica is energetically inexpensive and offers an excellent possibility to plants to augment their defences. To build tailored silaceous defence structures on or within the body, plants first take up silicon from the soil in form of silicic acid $Si(OH)_4$ which is then loaded into the xylem from which it is transported to shoots to be deposited as insoluble silica (Ma and Yamaji, 2006).

Edward (2014) stated that the form of silicon taken up by plants is silicic acid, in agriculture silicon is usually supplied as potassium silicate (K_2SiO_4) or sodium silicate (Na_2SiO_4). Among the defence inducing molecules salicylic acid (0.002 %), silicic acid (0.2 %) and boric acid (0.2 %) were found effective in reducing the disease under field and glasshouse condition (Pushpa, 2014).

2.6 Effect of nanoparticles on virus multiplication

The word nanotechnology is generally used when referring to materials with the size of 0.1 to 100 nm (Morones *et al.,* 2005). Within this size range all the properties (chemical, physical and biological) changes in fundamental ways of both individual atoms/molecules and their corresponding bulk. Nanotechnology tenders prospects to explore the biological properties of already known antimicrobial compounds by manipulating their size to alter the effect. Based on enhanced effectiveness, the new age drugs are nanoparticles of polymers, metals or ceramics, which can display several biological applications.

2.6.1 Synthesis of silver nanoparticles using plant extracts

Biogenic gold nanotriangles and spherical silver nanoparticles were synthesized by using *Aloe vera* leaf extract as the reducing agent by Chandran *et al.* (2006). Reduction of silver ions by *Aloe vera* extract, led to the formation of spherical silver nanoparticles of 15.2 nm ± 4.2 nm.

Jain *et al.* (2009) synthesized silver nanoparticles from $AgNO_3$ solution using extract of papaya fruit as reducing as well as capping agent. Average particle size was 15 nm with cubic structure. These biologically synthesized nanoparticles were found to be highly toxic against different multi drug resistant human pathogens.

Elavazhagan and Arunachalam (2011) used aqueous leaf extract of *Memecylon edule* to synthesize silver nanoparticles. Upon treatment of aqueous solutions of silver nitrate and chloroauric acid with *M. edule* leaf extract, stable silver nanoparticles were rapidly formed. The silver nanoparticles were predominantly square shaped with uniform size range of 50-90 nm.

Awwad *et al.* (2013) reported that the use of carob leaf extract makes a fast and convenient method for the synthesis of silver nanoparticles. Formation of stable silver nanoparticles at different concentrations of $AgNO_3$ gave spherical particles with a diameter ranging from 5 to 40 nm.

Mostafa *et al.* (2014) synthesized silver nanoparticles (AgNPs) using hot water olive leaf extracts as reducing and stabilizing agent. Rate of formation of the nanosilver increased significantly in the basic medium with increasing temperature. The silver nanoparticles had an average size of 20-25 nm and were mostly spherical.

Silver nanoparticles from $AgNO_3$ were synthesized by Jain and Kothari (2014), using green synthesis process. X-ray diffraction and TEM analysis showed that average particle size was 15 nm with mixed (cubic and hexagonal) structure.

Aqueous leaf extract of *Azadirachta indica* was used for synthesis of silver nanoparticles by Ahmed *et al*. (2016). The plant extract acted both as reducing agent as

well as capping agent. Only 15 min were required for the conversion of silver ions into silver nanoparticles at room temperature, without the involvement of any hazardous chemical. Haroon *et al.* (2017), synthesized silver nanoparticles by green synthesis process using *Hybanthus enneaspermus* plant extract as a reducing agent.

2.6.2 Characterization of bio synthesized silver nanoparticles

Characterization refers to the study of material's features such as composition, colour, size, structure and various properties like physical, chemical, and magnetic properties. Characterization of nanoparticles is necessary to establish understanding and control of nanoparticle synthesis. Characterization could be done by a variety of different techniques.

2.6.2.1 Visual observation (colour change)

During bio-synthesis of silver nanoparticles, the silver ion solution changed from colorless to brownish color which indicated the formation of silver nanoparticles. Silver nanoparticles exhibited brown color in water due to excitation of surface plasmon vibrations in metal nanoparticles (Singh and Raja, 2011).

Ahmad *et al*. (2015), used leaf extract of *Rosa damascena* as a bioreductant to reduce silver nitrate. When the extract was added to the aqueous solution of $AgNO_3$ at room temperature, the color changed from colorless to brownish yellow and finally to dark reddish brown indicating the formation of silver nanoparticles (AgNPs).

2.6.2.2 UV-Vis spectroscopy analysis

UV-Vis spectroscopy is a technique used to quantify the light that is absorbed and scattered by a sample. The spectra recorded from silver nanoparticles solution showed an absorption peak at 420 nm which was specific for the silver nanoparticles (Mulvaney, 1996).

Fayaz *et al*. (2010) studied spectra of bio synthesized silver nano solution and found that the silver surface plasmon band occured at 405 nm in addition to prominent band at around 260 nm. Li *et al*. (2012) reported that Ultraviolet-Visible spectra of

Aspergillus terreus cell filtrate with $AgNO_3$ showed a strong broad peak at 440 nm (SPR band), which indicated the presence of AgNPs. Devi *et al.* (2013) screened 75 fungal isolates belonged to five *Trichoderma* species for bio synthesis of silver nanoparticles with high Plasmon band at 420 nm.

Soni and Prakash (2013) studied the UV-Vis spectra of silver nanoparticles synthesized by using *A. niger* and found a broad absorption band centered at 480 nm. The presence of broad resonance indicated an aggregated structure of the silver nanoparticles in the solution.

Azadirachta indica aqueous leaf extract was used for synthesis of silver nanoparticles (Ahmed *et al*., 2016). UV-Visible spectrophotometer showed absorbance peak in range of 436-446 nm. The formation of silver nanoparticle synthesized using green synthesis process, analyzed by UV-Vis spectra analysis showed a strong absorption peak at around 400 to 412nm as reported by Haroon *et al.* (2017).

2.6.2.3 Particle size analysis

The hydrodynamic diameter of the silver nanoparticles in the solution is measured by using the principle of Dynamic Light Scattering (DLS) technique. Mahl *et al*. (2011) obtained single peak indicated that the quality of the synthesized silver nanoparticles was good. Honary *et al.* (2013) studied green synthesis of silver nanoparticles induced by the fungus *Penicillium citrinum* and found that the Z - average size of the silver nanoparticles was 109 nm with 0.1 polydispersity index (PDI).

Roy *et al*. (2013) synthesized silver nanoparticles using the fungus *Aspergillus feotidus* MTCC8876 and found that the average size of synthesized silver nanoparticles was 104.9 nm which was measured using DLS technique. Similarly, green synthesis of silver nanoparticles from *Aspergillus terreus* with size ranging from 5 to 30 nm was described by Abeer *et al*. (2013).

Haroon *et al.* (2017) analyzed the formation of silver nanoparticle synthesized using green synthesis process by Dynamic light scattering measurement (DLS). The

particle size distribution showed high intensity of the maximum peak range from 20nm to 80nm and possessed an average size of 75.58nm with a zeta potential of -28.2.

2.6.3 Effect on nanoparticles on plant viruses

Different types of nanomaterials of copper, zinc, titanium, magnesium, gold, alginate and silver have been developed, but silver nanoparticles have proved to be most effective as they exhibit potent antimicrobial efficacy against bacteria, viruses and eukaryotic micro-organisms (Guo *et al.,* 2003)

Though there were few reports on effect of silver nanoparticles on plant viruses, antiviral activity of s ions has been recorded and interactions with S-H groups have been implicated in the mode of action (Thurmann and Gerba, 1989). The powerful antimicrobial effect of silver is believed to be brought about by enzyme inactivation (Kim *et al*., 1998).

Silver nanoparticles possess unique properties such as chemical stability, good conductivity, catalytic and most important antibacterial, anti-viral, antifungal properties (Joerger *et al.,* 2001 and Ahmad *et al.,* 2003). Nano silver particle had shown to have antibacterial, antifungal and antiviral effects (Nomiya *et al*., 2004; Sondi and Salopek, 2004).

Silver nanoparticles, had a high surface area and high fraction of surface atoms, have high antimicrobial effect as compared to the bulk silver. Silver in an ionic state exhibited high antimicrobial activity (Thomas and Mc Cubin, 2003).

Elechiguerra *et al.* (2005) reported that silver nanoparticles inhibited HIV-1 infectivity by binding to the disulfide bond regions of the CD_4 binding domain within gp120 glycoprotein subunit. Silver nanoparticles have been exploited in medicine for antibacterial, antifungal, antiviral and inflammatory therapy in human system, but its application in agricultural system against plant viruses had not been explored much (Gergerich and Dolja, 2006).

Park *et al.* (2006) reported on chemical injuries caused by a higher concentration of nanosized silica-silver on cucumber and pansy plant, when they were sprayed with a high concentration of 3200 ppm. Popularity of nano silver caused concern about regulating and classifying the nano silver as a pesticide (Anderson, 2009). Silver acts as an excellent plant growth stimulator and maximum number of patents are being filed for 'nano silver' for preservation and treatment of diseases in agriculture field (Sharon *et al*., 2010).

Commendable efforts have been made to explore antimicrobial property of silver nanoparticles against human pathogens, but insignificant research has been done to study its effects against phytopathogens (Kim *et al.,* 2012). Metallic nanoparticles are considered as the most promising as they contain remarkable antimicrobial properties due to their large surface area to volume ratio, which is of great interest due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains (Khalil *et al.,* 2013). The mechanisms of action of silver nanoparticles against various human and animal viruses suggested it as a novel therapeutic agent against some viruses bearing potentially fatal consequences (Khandelwal *et al.,* 2014).

Spray application of 50 ppm aqueous solution of silver nanoparticles on cluster bean leaves inoculated with sunhemp rosette virus (SHRV) showed complete suppression of the disease, suggesting that silver nanoparticles were effective antiviral agent (Jain and Kothari, 2014).

Since 1930, copper nanoparticles dissolved in water have been used as a fungicide for controlling diseases of grape and fruit trees (Hatschek, 1931). Nanocopper was reported to be highly effective in controlling bacterial diseases *viz.,* bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* and leaf spot of mung caused by *X. campestris* pv. *phaseoli* (Gogoi *et al.,* 2009).

2.7 Integrated management of PRSV

Among various constraints *viz*., lack of quality seed, inferior planting materials and lack of awareness on improved production technologies and poor disease management practices were the major contributing factors for low productivity of papaya. There is a great scope to increase the productivity by at least 50 per cent if only the ringspot virus disease is combated (Shikhamany, 2004).

No prophylactic or therapeutic option is available for the management of PRSV infection. The best solution is to minimize yield losses is to adopt an integrated approach involving different strategies. Due to the economic importance of the papaya crop and the damage caused by PRSV disease, substantial efforts were made to develop methods to manage or minimize the damage due to PRSV. An integrated approach towards PRSV management could be the best option for successful cultivation of papaya under PRSV infected regimes till PRSV resistant varieties are released. Major components of integrated disease management include host-plant resistance, cultural practices, biological and chemical options.

2.7.1 Components of integrated disease management

The solution to papaya ringspot lies in the strategies integration of several methods (chemical, cultural, mechanical and biological) to manage both viruses and their vectors. The different components of integrated management are reviewed here under.

2.7.1.1 Non-host barrier crops

Earlier workers (Difonzo *et al*., 1996; Fereres, 2000; Osakabe and Kenichiro, 2002) reported that barrier crops were effective in reducing virus transmission in different crops by blocking vectors from reaching the target plant.

Prasad and Kudada (2005) reported that intercropping maize with papaya reduced incidence and severity of PRSV in Bihar, India. Verghese *et al*. (2007) recommended intercropping or planting, all around the border with tall barrier crops like maize to obstruct aphid migrants.

Growing main crop in the midst of non-host crops reduced the spread of non-persistent viruses by avoiding influx of vector population on main crop (Kumar *et al*., 2010). Singh *et al*. (2010) also reported that growing border crop of maize in papaya orchards reduced the transmission efficacy of the aphid vector.

Banana and maize were recommended as border crops in papaya since they are not the primary or secondary hosts of virus and the aphid vector transmitting the virus (Chavan *et al*., 2010). Sharma *et al.* (2010) also observed that, border crop of banana reduced aphid-vector population inside the papaya plantation compared with outside the border crop throughout the year. Intercropping corn (*Zea mays* L.) as a barrier in papaya plantations reduced the damages caused by PRSV in endemic areas as reported by Mederos *et al*. (2013).

In PRSV disease management studies, growing papaya as intercrop with south African Tall maize (1:1) and Grand naine banana (2:1) as live barriers were found effective recording 60-90 per cent disease reduction (Pushpa, 2014)

2.7.1.2 Insect proof nylon mesh

In Taiwan papaya was protected against aphid vectors by insect-exclusion screening (IES-screen barrier) netting and was effective in producing marketable fruits, because late infection that occured when the net was removed after fruiting, resulted in little damage to the fruit yield (Kiritani and Su, 1999).

Covering crops with aphid proof netting in Taiwan enabled economic production of papaya for at least one season (Ray *et al*., 1999). Netting of papaya to prevent aphid landing till fruiting is a practise in Taiwan for the management of PRSV (Verghese *et al*., 2007). Kumar *et al*. (2010) observed delayed appearance of PRSV in papaya raised inside nylon net barrier compared to open field.

2.7.1.3 Reflective row covers

Moericke (1954) demonstrated that white surfaces reflecting ultraviolet or short wave light were unattractive to alighting aphids and were even avoided by them. For the first time Kring (1964) reported that short-wavelength radiation repelled aphids after a dispersal flight.

Jones and Chapman (1968) tested plastic sheets of nine different colors along with aluminum foil to determine their attractiveness to aphids. White, orange, light blue, aluminum foil and dark blue attracted less number of aphids while yellow was most attractive, followed in order by pink, green, red and black.

Vani *et al.* (1989) successfully minimized the mosaic disease incidence in muskmelon using mulches. James *et al*. (1993) found out that compared to other colours of plastic mulch, silver reflective mulch was superior in reducing aphid populations. UV reflective aluminum mulch was used successfully by Brown *et al.* (1993) to delay and reduce the incidence of aphid-borne virus diseases in squash. In South Africa, aphid-transmitted mosaic diseases of *Cucurbita pepo* were effectively reduced by use of white reflective mulches (Cradock *et al.,* 2001).

Several reports have indicated that reflective row covers and floating row covers delay the appearance of virus diseases by excluding or repelling the aphids by reflecting UV light (Santos *et al*., 1995; Gonsalves *et al*., 2010).

Under the conditions of high aphid populations and virus inoculum potential, the aphid numbers on leaves of cantaloupe plants (*Cucumis melo*) growing over reflective polyethylene mulches were consistently lower than on those growing over bare soil (Stapleton and Summers, 2002). Onset of symptoms of cucumber mosaic cucumovirus, watermelon mosaic and zucchini yellow mosaic potyviruses were delayed 3-6 weeks in plants growing over the mulches. The polyethylene mulch which completely covered each planting bed provided at least 9.5 fold increase in marketable yield.

Summers *et al.* (2004) tried plastic mulches of different colors in squash and achieved varied degrees of success in virus disease management. Plastic UV reflective mulch (metalized mulch) and wheat straw mulch delayed colonization by *Bemisia argentifolii*. Growing papaya with silver reflective mulch was found profitable with a benefit cost ratio of 1: 6.2, by reducing PRSV disease incidence (Pushpa, 2014).

2.7.1.4 Insecticides

Insecticides are known to induce behavioural changes in aphids during host selection, labial dabbing, test probing and also on duration of probing. These alterations of aphid/insect interface form the basis for the management of many non-persistent viruses when use of chemicals is an option.

Insecticides were reported to be successful in managing few aphidborne non-persistent viruses (Atiri *et al*., 1987; Perring *et al*., 1999; Martin *et al*., 2004). Host suitability of whitefly (*Bemisia tabaci*) could be overridden by systemic application of imidacloprid at sublethal levels (Isaacs *et al*., 1999).

Verghese *et al*. (2007) reported that systemic insecticide dimethoate (2 ml/l) gave the best result of 60 per cent reduction and delayed PRSV infection. Kalleshwaraswamy *et al.* (2009) reported that mineral oil (0.1 %) with imidacloprid and deltamethrin applications as alternative fortnightly sprays were effective in reducing PRSV incidence in papaya.

Out of seven treatments evaluated for management of PRSV, the most effective treatment comprising of neem oil 1 per cent + dimethoate 1.05 per cent showed the minimum disease incidence (76.66 %) as compared to the control showing 100 per cent incidence at 210 days after planting (Singh *et al*., 2010). Spraying with dimethoate (0.05 %) at fortnightly interval as a component of integrated management, recorded significantly less number of aphids per plant and minimum incidence of PRSV (Datar, 2012). Sprays of dimethoate reduced the chances of spread of disease either by its repelling or killing action on aphid.

2.7.2 Integrated management of PRSV

Use of plastic mulches, mineral oil plus insecticide sprays for management of PRSV-W in zucchini (*Cucurbita pepo*) in Australia, was evaluated by Pinese *et al.* (1994). Mulches with a reflective (silver) surface minimized losses by reducing aphid populations.

Revilla *et al.* (1995) showed how a complex set of strategies could increase papaya yield in Mexico by adapting integrated crop management practices. These strategies included seedbeds covered with an insect proof polypropylene mesh, high density papaya plantings (2222 plants ha^{-1}) which allowed roguing of diseased plants, foliage and soil nutrients to improve plant vigor, poisoned plant barrier (two lines of *Zea mays* and *Hibiscus sabdariffa* L.), two plastic strips (5 cm width and with a shiny gray-metallic color above each row of papaya plants), biweekly sprays with 1.5 per cent mineral oil. But these measures were only effective in regions where disease pressure was low.

Sharma *et al.* (2007) reported that use of Good Agricultural Practices (GAPs) which aimed at delaying PRSV infection could be the best option for successful cultivation of papaya in spite of PRSV infestation. It involved selection of the variety (Red Lady) that produced better yield, use of virus-free seedlings, planting a new papaya plantation away from other host plants like cucurbits and infected papaya plantation, rouging infected plants early, growing a border crop around papaya plantation, and adjusting the season of transplanting. By transplanting papaya during the lean period

(spring season), PRSV infection could be delayed till monsoon, by that time plants have crossed the fruit bearing stage.

A technological package to manage PRSV (covering crops, manure application and transplanting 3-4 month virus-free papaya plants, natural barriers and isolation distance of 200 m-from other papaya fields) was tried by Tenorio (2007). Use of the technological package delayed the virus infection by over 3 months, allowing a better commercial production.

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

Singh *et al*. (2010) had evaluated integrated approach involving seven treatments *viz.,* raising of papaya seedlings under nylon net (40-60 mesh) and spraying of dimethoate (1.05 %) 3 days before planting, use of two rows of maize as a border crop, application of neem seed kernel extract (2 %) at 15 days interval (30 days after planting), application of dimethoate (1.05 %), application of neem oil (1.0 %) + dimethoate (1.05 %), two rows of maize as a border crop + application of Zn (0.5 %) and B (0.1 %) along with control. The most effective treatment was application of neem oil (1 %) + dimethoate (1.05 %) with least disease incidence 6.66 per cent and 41.66 per cent respectively at 60 and 150 DAP.

Integrated approach of growing papaya plants with maize as a border crop and spraying with dimethoate (0.05 %) and azadirachtin (0.06 %) alternately at fortnightly interval recorded significantly least number of aphids per plant and minimum incidence of PRSV. The barrier crop of maize initially hindered the entry of alate aphids inside the main crop of papaya. Sprays of insecticides (synthetic and plant based) further reduced the chances of spread of disease either by its repelling or killing action (Datar, 2012).

III MATERIAL AND METHODS

As an attempt has been made to develop management measure against the deadly disease caused by papaya ringspot virus (PRSV) in papaya, the present investigations were carried out to study the variability among the isolates based on P1 proteinase gene and coat protein gene sequences, screen the available varieties for resistance, induction of resistance through defence inducing molecules and to evolve the strategies of integrated management practices.

The present investigations were carried out at Department of Plant Pathology, University of Agricultural Sciences, Bangalore (UASB); Main Research Station (MRS) farm, Hebbal and at farmer's field in Nelamangala, Doddaballapur taluk, Karnataka.

3.1 Survey for the collection of PRSV infected samples of papaya from Southern India

A survey was carried out in the selected locations of Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and Telangana during 2014-2015 where papaya was cultivated commercially. Survey covered kitchen gardens where commercial cultivation was not available. The observations on per cent disease incidence, type of symptoms expressed and variety of papaya cultivated were recorded. The per cent disease incidence was recorded using following formula:

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

3.1.1 Collection and maintenance of isolates

Papaya seeds (Red Lady) were sown in plastic sprouting trays. 20 days after germination seedlings were transplanted to polythene covers containing soil + sand + coir pith and FYM (1:1:1:1) and were maintained inside insect proof cages at Department of Plant Pathology glasshouse. These seedlings were taken to various locations during the survey. Leaf samples with characteristic PRSV symptoms were collected and sap

inoculated to healthy seedlings using standard mechanical transmission procedure in the papaya field. Papaya leaves infected by PRSV were collected, washed under tap water, blot dried and ground with 0.1M phosphate buffer (pH 7.5) using mortar and pestle. The sap was filtered through double layered muslin cloth and filtrate was mixed with a pinch of celite powder (600 mesh). For sap inoculation a piece of sterile non-absorbent cotton pad dipped in filtrate was rubbed in one direction starting from petiole towards the margin of papaya leaves. The inoculated leaves were washed five minutes after with a jet of water to remove the traces of celite, labeled, brought to glasshouse safely and maintained under insect proof cages at Department of Plant Pathology glasshouse. Plants were regularly observed till symptom expression.

3.1.2 Serological detection of PRSV by DAS-ELISA

Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA) technique with anti-PRSV capture antibody and ALP labelled anti-PRSV detection antibody, was used to detect PRSV in papaya samples (Agdia, USA).

3.1.2.1 Reagents used in DAS-ELISA

A) Carbonate buffer/coating buffer (pH 9.6): 1.59 g sodium carbonate anhydrous, 2.93 g sodium bicarbonate and 0.20 g sodium azide were dissolved in distilled water and volume was made up to 1000 ml. Buffer was stored at 4 ˚C.

B) PBS-T/wash buffer (pH 7.4): 8.00 g sodium chloride, 1.15 g sodium phosphate dibasic anhydrous, 0.20 g potassium phosphate monobasic anhydrous, 0.20 g potassium chloride and 0.5 ml Tween-20 were dissolved in distilled water and volume was made up to 1000 ml (Stored at 4 ˚C).

C) PBS/sample extraction buffer (pH 7.4): 8.00g sodium chloride, 1.15g sodium phosphate dibasic anhydrous, 0.20 g potassium phosphate monobasic anhydrous and 0.20 g potassium chloride were dissolved in distilled water and volume was made up to 1000 ml (Stored at 4 ˚C).

D) PBS-T-PO/blocking solution/antibody buffer (pH 7.4): 2.00 g bovine serum albumin (BSA), 20.00 g polyvinylpyrrolidone (PVP) and 0.20 g sodium azide were dissolved in PBS-T and volume was made up to 1000 ml (Stored at 4 ˚C).

E) PNP buffer/substrate buffer (pH 9.8): 0.10 g magnesium chloride hexahydrate, 0.20 g sodium azide and 97.00 ml diethanolamide were dissolved distilled water and volume was made up to1000 ml (Stored at 4 ˚C).

3.1.2.2 Protocol for DAS ELISA

A humid box was prepared by lining an airtight container with a wet paper towel in order to prevent evaporation of contents during incubation. The capture antibody (Anti-PRSV) was diluted with carbonate coating (1:200) buffer before use. The volume required was calculated based on number of samples to be tested. 100 µl of the prepared capture antibody was pipetted into each well. Coated plates were incubated overnight in the refrigerator (4 ˚C) or incubated in humid chamber at room temperature for 4 hours. After incubation, the wells were emptied into a sink or waste container. The tests wells were filled completely with PBST and then quickly emptied again. This step was repeated thrice. Plates were hold upside down and tapped firmly on paper towel to remove excess liquid.

PRSV infected and healthy plant tissue was ground using a mortar and pestle in PBS buffer at 1:10 ratio (tissue weight in g : buffer volume in ml). The homogenate was transferred to micro centrifuge tube of 1.5 ml and centrifuged at 8,000 rpm for 5-10 minutes. Following loading diagram, 100 µl of clear supernatant was loaded into sample wells. In addition, 100 µl of positive control was dispensed into positive control wells and 100 µl of sample extraction buffer was loaded into buffer wells. Plates were kept inside the humid box and incubated for 2 hours at room temperature or overnight in the refrigerator (4 ˚C). After incubation, the plates were washed with PBST. Enzyme conjugate (ALP labelled anti-PRSV) was freshly prepared by diluting in PBS-TPO buffer (1:200) and 100 µl of enzyme conjugate solution was dispensed into test wells. Plates were kept inside the humid box and incubated for 2 hours at room temperature or overnight in the refrigerator (4 ˚C).

After incubation, the plates were washed with PBST. Substrate solution was prepared by dissolving PNPP tablet (5 mg) in 5 ml of substrate buffer, at a concentration of 1 mg ml^{-1} (About 15 minutes before the end of the incubation step, 5 ml of substrate buffer was measured for each PNPP tablet and then without touching the tablets were added to the buffer). 100 µl of PNPP substrate was dispensed into each test well. The plate was incubated in a humid box for 60 minutes. Plates were protected from direct or intense light by incubating under darkness. The test wells were measured on a plate reader at 405 nm after one hour. The test wells showing positive for PRSV were recorded as infected.

3.2 Assessment of variability among PRSV isolates

PRSV isolates were characterized based on the symptoms developed as well as by sequencing P1 protein gene and coat protein (CP) gene.

3.2.1 Biological characterization of isolates based on symptoms

PRSV isolates collected during survey from different location were categorized based on symptoms associated with the disease. Selected representative isolates of PRSV were inoculated on Red Lady and reaction was recorded based on symptoms developed. The inoculated plants were maintained in insect proof glasshouse. Subsequently, the variation in symptoms expressed among isolates was studied based on visual observation at 60 days after inoculation (DAI).

3.2.2 Molecular variability of PRSV isolates

At 60 DAI, the virus isolates identified based on variability in symptoms on host differential variety Red Lady, were further characterized by partially sequencing the P1 proteinase gene and CP gene to study the molecular variability.

3.2.2.1 Isolation of RNA from PRSV infected papaya plants

Isolation of total RNA from selected isolates of PRSV was done using Trizol reagent. All the plastic wares and glasswares were washed thoroughly, dried and treated

with 0.1 per cent diethylene pyrocarbonate (DEPC) water by dipping for 24 hours and used after sterilization. The PRSV infected papaya leaf samples were brought under ice-cold condition and ground to a fine powder in sterilized and dried pre-chilled mortar and pestle using liquid nitrogen. About 100 mg of powdered leaf material was taken into a 1.5 ml micro centrifuge tube and homogenated partially using a homogenizer or a plastic pestle. Immediately 1ml of trizol was added to the homogenized tissue. The tubes were centrifuged at 9,000 rpm for 10 minutes. Without disturbing the pellet, supernatant was transferred to fresh tube and kept at room temperature. 200 µl of chloroform + phenol (1 ml chloroform : 1 ml phenol) was added to the supernatant. After 15 minutes of shaking, tubes were centrifuged at 12,000 rpm for 8 minutes. Three distinct layers were formed, from which only the top layer was transferred to fresh tubes. 0.5 ml of isopropanol was added to each tube, followed by 10-minute incubation at room temperature. The tubes were then centrifuged at 13,000 rpm for 5 minutes and the pellet was collected discarding supernatant. 75 per cent ethanol (750 µl + 250 µl H_2O) was added to the pellet and tubes were centrifuged at 12,000 rpm for 2 minutes. After centrifugation, ethanol was discarded, and pellet was vacuum dried for 10 minutes. 20 µl of DEPC treated water was added to each tube and they were incubated at 55-60 ˚C on a water bath to dissolve the pellet. RNA thus obtained was stored at -20 ˚C.

3.2.2.2 Reverse transcription

Total RNA from healthy and PRSV infected samples were taken for reverse transcription along with positive control and negative control (distilled water). 20 µl RT mixture was prepared by adding the following ingredients into the PCR tube. 5x RT buffer 4 µl, 25 mM $MgCl_2$ 1.0 µl, 10.0 mM dNTP mixture 2.0 µl, reverse Primer (10 µM) 2.0 µl, reverse transcriptase 25 units (TaKaRa primescript reverse transcriptase), viral RNA 5.0 µl (1:10 diluted with water), and finally volume was made with 5.5 µl DEPC treated distilled water. The RT-PCR mixture was reverse transcribed at 39 ˚C for 60 minutes and then at 94 ˚C for 5 minutes. The c-DNA thus obtained was used for performing PCR.

3.2.2.3 Primers used for amplification

Primers for amplification of P1 region were designed using NCBI primer blast tool with sequences from Hyderabad isolate (Accession No: KP743981).

Table 1: Details of the primers used for molecular characterization of P1 proteinase gene and CP gene

Gene/ primer name	Forward 5'-3'	Reverse 5'-3'	Annealing temperature (˚C)	Template size
CP (PRSVCP)	AGAAGCGTGGGT CAATGGA	CTCTCCAGTTTTT GTGCTAGTTG	53	500
P_1 (PRSV1P)	CAATTCGAAGCA ACCAAACAAAT (24-46)*	TCTTTCCGAACTT GAGTTGCT (1023-1003)	46	1,000
P_1 (PRSV2P)	TTGGAGTGCTAG CCTTGAGTT (940-960)	CCCCACACATTGT AACGTCCA (1832-1812)	54	893

*Figures in the parenthesis indicate sequence position

3.2.2.4 Polymerase chain reaction

The c-DNA obtained was subjected to PCR amplification using forward primer designed to amplify PRSV nucleotides from total RNA extracted from infected papaya plants. PCR amplifications were conducted using Eppendrof thermo-cycler in 15.0 µl reaction mixture that contained 2.0 µl c-DNA, 0.20 µl Taq DNA polymerase (1 unit), 2.5 µl of 10x PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 0.5 µl of 25 mM $MgCl_2$, 2.0 µl each primer (10 µM), 2.0 µl dNTPs mix (2.5 mM each) and DEPC treated distilled water to make up the volume. The mixture was subjected to one cycle of initial denaturation at 94 ˚C for 4 minutes followed by 35 cycles of denaturation at 94 ˚C for 60 seconds, annealing at primer specific temperature for 45 seconds, extension at 72 ˚C for 90 seconds and a final extension at 72 ˚C for 10 minutes. After the completion of the reaction, the products were kept at 4 ˚C till agarose gel electrophoresis.

3.2.2.5 Analysis of PCR products by agarose gel electrophoresis

Amplifications by polymerase chain reaction were confirmed by performing agarose gel electrophoresis.

3.2.2.6 Sequencing of amplified PCR product

After successful confirmation, the amplified PCR product was directly sequenced using ABI 3730Xl DNA analyzer available at Scigenome labs Pvt. Ltd., Cochin-Kerala, India. Sequencing was done in both directions using forward and reverse primers.

3.2.2.7 Construction of phylogenetic tree

The sequence homology obtained in BLAST (www.ncbi.nih.gov /BLAST) and Neighbor joining phylogenetic tree was generated using MEGA 6.06 software tool. In order to calculate the confidence limits placed in construction of phylogenetic tree, bootstrapping analysis was carried out using 1000 replicates, resulting in a boot strapped Neighbor joining tree.

3.3 Screening of papaya varieties against PRSV for identification of natural resistance

Papaya varieties were screened to identify resistance against PRSV both in greenhouse and field. Seeds of Bangalore dwarf, IHS selection-4 (Imperial agro genetics, Delhi, India), Madhu (Imperial agro genetics, Delhi, India), Mohini (Suvarna hybrid seeds Pvt. Ltd., Bangalore) Papita selection, Pune selection, Red Lady (Known you seeds India Pvt. Ltd., Pune), Sinta-1 (East west seeds India Pvt. Ltd., Aurangabad), Solo and Surya were collected from the local markets of Bangalore. Pusa dwarf and Pusa nanha seeds were collected from IARI New Delhi, Co-8 was collected from TNAU, Coimbatore. Arka prabhat and *Carica cauliflora* seeds were supplied by IIHR, Bangalore while seeds of Pant papaya 1 were collected from G. B Pant University, Pant Nagar. Healthy seedlings of each of these varieties were raised in 30 cm polyethylene bags.

3.3.1 Screening of papaya varieties under glasshouse condition

Fifteen varieties papaya *viz.,* Arka prabhat, Bangalore dwarf, Co-8, IHS selection-4, Madhu, Mohini, Pant papaya-1, Papita selection, Pune selection, Pusa dwarf, Pusa nanha, Sinta-1, Red Lady, Solo, Surya along with wild relative *Carica cauliflora* were screened against PRSV under glasshouse condition. The experiment was carried out during January 2015 to May 2015. Sixty days old seedlings with five plants of each variety were mechanically inoculated with PRSV GKVK Bangalore isolate and kept maintained inside glasshouse in completely randomized design (CRD) for symptom expression. Observations on days taken for symptom expression, varied symptoms observed on each variety were recorded at regular intervals. At 60 DAI, the experiment was terminated by subjecting apical leaves of all the varieties to DAS-ELISA to know the virus titre and were compared each other based on absorbance values.

3.3.2 Screening of papaya varieties under field conditions

Field trials were conducted from June 2015 to April 2017, to identify resistance against PRSV. Eleven varieties of papaya *viz.,* Bangalore dwarf, IHS selection-4, Mohini, Pant papaya-1, Papita selection, Pusa dwarf, Pusa nanha, Red Lady, Solo, Surya along with wild relative *C. cauliflora* were screened under field conditions. Sixty days old seedlings of each variety were planted in pits dug at a distance of 6'x6'. Each variety was replicated three times in randomized block design. Recommended dose of fertilizer NPK (250: 250: 500 Kg ha^{-1}) and farm yard manure (10 kg per pit) was applied. Per cent disease incidence at 100 per cent flowering, at final harvest, plant height (cm), number of fruits per plant (no.) and fruit yield per plant (kg) from each variety was recorded. Finally, the experiment was terminated by assaying apical leaves of all the varieties through DAS-ELISA to know the virus titre and was compared with each other based on absorbance values.

3.4 Assessment of efficacy of defence inducers and nanoparticles on PRSV multiplication in papaya

Effect of plant extracts, seaweed extracts and nanoparticles on multiplication of PRSV was assessed both under glasshouse and field conditions.

3.4.1 Assessment on efficacy of defence inducers on PRSV multiplication in papaya

Different defence inducers along with control were tested for their efficacy in inducing resistance against PRSV in papaya both under glasshouse and field conditions during August 2014 to January 2015. Crude extracts were prepared freshly by crushing of rhizome or root or leaf in distilled water (1:1) using a domestic blender. Pulp was strained through two folds of cheese cloth and the homogenate was clarified by centrifugation at 3000 rpm for 15 minutes. 50 ml of these extracts were dissolved in one liter of water (5 % extract). Required concentration of seaweed extract supplied by Sea6energy Pvt. Ltd., Bangalore, was mixed with water to prepare the spray solution. Different treatments evaluated were as follows:

T_1 : *Acorus calamus* (crude extract of rhizome-5 %)

T_2 : *Boerhavia diffusa* (crude extract of root-5 %)

T_3 : *Capsicum frutoscens* (crude extract of leaf-5 %)

T_4 : *Chenopodium amaranticolor* (crude extract of leaf-5 %)

T_5 : *Datura metel* (crude extract of leaf-5 %)

T_6 : *Kappaphycus alvarezii* extract (proprietary formulation KH-1 %)

T_7 : *Eucheuma spinosum* extract (proprietary formulation SH-1 %)

T_8 : Silicic acid (1 %)

T_9 : Control (water spray)

3.4.1.1 Assessment on efficacy of defence inducers on PRSV multiplication in papaya under glasshouse condition

An experiment was conducted to evaluate different defence inducing molecules individually for their efficacy against PRSV multiplication in papaya variety Red Lady

under glasshouse condition. Thirty days old papaya seedlings were mechanically inoculated with PRSV Bangalore isolate and kept in glasshouse for symptom expression. After 60 days of inoculation, the apical leaves of inoculated seedlings were assessed for detection of PRSV by DAS-ELISA following standard protocol. The initial absorbance of representative plant samples which showed positive reaction for PRSV infection were recorded and selected for evaluation of different molecules under glasshouse condition. Each treatment was replicated four times. All the treatments were imposed at 10 days intervals. A total of 10 sprays were given. At the end of the experiment, the apical leaves of all the treated plants were again subjected for DAS-ELISA to know the final virus titre and data was subjected for one way analysis. Per cent decrease in virus titre was calculated using the following formula,

$$\text{Per cent decrease in virus titter (\%)} = \frac{\text{Initial titre - final titre}}{\text{Initial titre}} \times 100$$

3.4.1.2 Assessment on efficacy of defence inducers on PRSV multiplication in papaya under field conditions

Sixty days old healthy papaya (Red Lady) seedlings were transplanted to the main field. The apical leaves of all plants were subjected for serological detection of PRSV by DAS-ELISA following standard protocol to eliminate any infected plants. The plants were sprayed with different defence inducing molecules at 10 days intervals. Each treatment was replicated four times. A total of 10 sprays were given. At the termination of experiment, the apical leaves of all the treated plants were again subjected for DAS-ELISA to know the final virus titre and obtained data was subjected for two way analysis.

3.4.2 Assessment on efficacy of nanoparticles on PRSV multiplication in papaya

Experiments were carried out both in the glasshouse and field conditions during February 2015 to June 2015 for the assessment of effect of nanoparticles on PRSV multiplication in papaya along with extract from *Boerhavia diffusa and Kappaphycus alvarezii*.

3.4.2.1 Synthesis and characterization of silver nanoparticles

Silver nanoparticles (AgNPs) were prepared by green synthesis process using neem (*Azadirachta indica* L.) leaves extract as a reducing agent and the formation of nanoparticles were confirmed by visual observation, UV-Vis spectrophotometer and Dynamic Light Scattering (DLS).

3.4.2.1.1 Green synthesis of silver nanoparticle

Neem (*Azadirachta indica* L.) leaves were collected from UAS, GKVK, campus and collected plant samples were washed thoroughly thrice with tap water to remove epiphytes and necrotic microbes. Then the leaves were rinsed with sterile distilled water to remove the associated debris if any. These clean, fresh leaves were shade-dried for two weeks and powdered using domestic blender. For the plant broth preparation, ten gram of the dried powder was boiled with 100 ml of deionised distilled water (Hot percolation method). The resulted infusion was filtered thoroughly until no insoluble material appeared in the broth.

Silver nitrate (17 mg) was dissolved in 100 ml distilled water (1mM). Ten ml of neem leaf extract was added to 90 ml of 10^{-3} M $AgNO_3$ solution for reduction of Ag^+ ions. The reduction of Ag^+ ions was monitored by measuring the color change and UV-Vis spectra of the solution at 24/72 hrs. The spectra of the surface plasmon resonance of silver nanoparticles in the reaction mixture were recorded using UV-Vis spectrophotometer (Shimodzu, UV-2450) at wavelengths between 200 to 800 nm. The bio-reduced aqueous component was used for measuring UV-Vis spectra of the solution.

3.4.2.1.2 Particle size analysis by Dynamic Light Scattering (DLS)

The aqueous suspension of the synthesized silver nanoparticles was filtered through a 0.22 µm syringe driven filter unit and the size of the distributed silver nanoparticles were measured by using the principle of dynamic light scattering technique made in a Nanopartica SZ-100 series compact scattering spectrometer.

After confirming the formation of nanoparticles, prepared nanosilver solution was mixed required concentration of double distilled water to obtain final concentration of 50 ppm which was sprayed on the inoculated papaya plants.

3.4.2.2 Assessment on efficacy of nanoparticles on PRSV multiplication in papaya

Experiments were carried out to study the effect of different nanoparticles against PRSV in papaya both under glasshouse and field conditions. Nano silver particles were synthesized by green synthesis method using *Azadirachta indica*. Colloidal silver nanoparticle (1200 ppm) was supplied by Resil chemicals Pvt. Ltd., Bangalore. Nano cupric oxide (~ 40 nm) and nano zinc oxide (~ 30 nm) were purchased from SRL chemicals Ltd., India. Required quantity of nanoparticles were mixed with deionised water and sprayed to the papaya plants. Different treatments evaluated were as follows:

T_1 : Silver nanoparticles (prepared by green synthesis process-50 ppm)
T_2 : Colloidal silver nanoparticles (50 ppm)
T_3 : Colloidal silver nanoparticles (100 ppm)
T_4 : Nano cupric oxide (50 ppm)
T_5 : Nano cupric oxide (100 ppm)
T_6 : Nano zinc oxide (50 ppm)
T_7 : Nano zinc oxide (100 ppm)
T_8 : *Kappaphycus alvarezii* extract (proprietary formulation LBS3-1 %)
T_9 : *Boerhavia diffusa* (crude extract of root-5 %)
T_{10} : Control (water spray)

3.4.2.2.1 Assessment on efficacy of nanoparticles on PRSV multiplication in papaya under glasshouse condition

An experiment was carried out to assess effect of nanoparticles on PRSV multiplication in papaya along with *K. alvarezii* extract and *B. diffusa* extract in papaya variety Red Lady under glasshouse condition. Thirty days old papaya seedlings were mechanically inoculated with PRSV GKVK Bangalore isolate and maintained in glasshouse till symptom expression. After 60 days of inoculation, the apical leaves of

inoculated seedlings were subjected for serological detection of PRSV by DAS-ELISA following standard protocol. The initial absorbance of representative plant samples which showed positive reaction for PRSV infection were recorded and selected for evaluation of different concentrations of nanoparticles under glasshouse condition. Each treatment was replicated four times. All the treatments were imposed at 10 days intervals and 10 sprays were given. At the end of the experiment, the apical leaves of all the treated plants were again subjected for DAS-ELISA to know the final virus titre by comparing with the control and data was subjected for one way analysis.

3.4.2.2.2 Assessment on efficacy of nanoparticles on PRSV multiplication in papaya under field conditions

An experiment was carried out to assess effect of nanoparticles on PRSV multiplication in papaya along with *K. alvarezii* extract and *B. diffusa* extract in papaya variety Red Lady under field conditions. Sixty days old healthy papaya seedlings were transplanted to the main field. The apical leaves of all seedlings were subjected for serological detection of PRSV by DAS-ELISA following standard protocol to eliminate any infected plants. Each treatment was replicated four times. The plants were sprayed with different concentrations of nanoparticles at 10 days intervals. A total of 10 sprays of different molecules were given. At the end of experiment, the apical leaves of all the treated plants were again subjected for DAS-ELISA to know the final virus titre and data was subjected for one way analysis.

3.5 Integrated management of PRSV

Integrated Disease Management (IDM) approach exploits several feasible options like mechanical, cultural, chemical and biological, which act synergistically or complementary to each other. In order to manage PRSV disease in field, an integrated management approach was laid out at farmer's field in Nelamangala, Doddaballapur taluk, Karnataka, during October 2014 to December 2015. Sixty days old seedlings were then transplanted in the main field by maintaining a spacing of 6'×6'. The recommended package of practices was followed till the end of experiment.

Four different integrated management modules were evaluated at farmer's field along with control using unreplicated block design. Each module consisted of 8 rows (6 plants per row). A total of 240 plants were planted in the field. Sixteen plants were collected from each module and data was subjected to two way ANOVA. The integrated management modules evaluated were as follows:

IDM Module I

a) Raising seedlings inside insect proof cage (40x mesh)

b) Barrier crop of maize (South African tall)

c) Spraying with

i. *Boerhavia diffusa* extract (5 %)

ii. *Kappaphycus alvarezii* extract (KH-1 %)

iii. Imidacloprid (0.05 %)

IDM Module II

a) Raising seedlings inside insect proof cage (40x mesh)

b) Barrier crop of maize (South African tall)

c) Spraying with

i. *A. calamus* extract (5 %)

ii. *K. alvarezii* extract (KH-1 %)

iii. Dimethoate (0.20 %)

IDM Module III

a) Raising seedlings inside insect proof cage (40x mesh)

b) Barrier crop of maize (South African tall)

c) Silver reflective row cover

d) Spraying with

i. *A. calamus* extract (5 %)

ii. *K. alvarezii* extract (KH-1 %)

iii. Imidacloprid (0.05 %)

IDM Module IV

a) Raising seedlings inside insect proof cage (40x mesh)

b) Priming with *K. alvarezii* extract (KH-1 %)

c) Silver reflective row cover

d) Spraying with

i. *A. calamus* extract (5 %)

ii. *K. alvarezii* extract (KH-1 %)

iii. Imidacloprid (0.05 %)

3.5.1.1 Raising of papaya seedlings inside insect proof cage

The papaya seedlings of variety Red Lady were raised in 6"×4" polyethylene covers and maintained inside insect proof nylon mesh of 40x gauge till transplanting.

3.5.1.2 Raising of maize as barrier crop

Two months before transplanting of papaya seedlings, fodder maize variety 'South African tall' was sown as a live barrier (2:1). Sowing was repeated every two months to maintain the barrier crop.

3.5.1.3 Priming with *K. alvarezii* extract (KH-1 %)

As an effort to induce resistance seedlings were sprayed with solution of *K. alvarezii* extract (KH-1 %) from the emergence of leaves till transplanting at ten days interval.

3.5.1.4 Growing papaya plants with silver reflective row cover

Sixty days old papaya seedlings of variety Red Lady were grown in row, with silver reflective mulch covering each papaya row.

3.5.1.5 Spraying of defence inducers and insecticides

Spraying of defence inducers and insecticides was started after transplanting and repeated at monthly intervals. A total of seven sprays were taken up.

3.5.1.6 Untreated control

Sixty days old papaya seedlings of variety Red Lady were planted in the main field. These plants were maintained untreated without imposing any of the above treatments. The normal agronomic practices were followed as in the earlier treatments.

The observations on per cent PRSV incidence, growth and yield parameters of papaya *viz.,* plant height (cm), number of fruits per plant (no.) and yield (kg) per plant were recorded.

3.5.2 Calculation of per cent disease incidence

Based on the ELISA absorbance values and yield per plant, per cent disease incidence, per cent disease reduction over control and per cent yield increase over control were calculated using the following formulae:

A) Per cent disease incidence (%) = $\frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$

B) Per cent disease reduction over control (%) = $\frac{C - T}{C} \times 100$

Where C = Per cent disease incidence in control plot

T = Per cent disease incidence in treated plot

C) Per cent yield increase over untreated (%) = $\frac{YT - YC}{YC} \times 100$

Where YT = Yield of the treated plant

YC = Yield of the untreated plant

3.5.3 Benefit Cost Ratio

Cost effectiveness of different modules and their influence on profit per acre were calculated by using formula

$$\text{B C ratio} = \frac{\text{Gross income}}{\text{Total cost of cultivatio n}}$$

IV RESULTS AND DISCUSSION

Papaya is the third most important traded fruit at the global level. India is one of the leading producers of papaya in the world. Papaya fruits are rich source of antioxidants, vitamins and minerals. Papaya is also the source of the enzyme papain which is used in various industrial processes like softening of leather, as well as in the production of pharmaceutical products (Evans and Ballen, 2012). Papaya ringspot virus (PRSV) is the cause of the widest spread and most destructive disease affecting papaya cultivation, production and quality. PRSV has gained global importance because of its devastating effects in all the papaya growing countries. PRSV incidence drastically reduces the fruit yield, fruit size, quality and can even result in the loss of the entire crop. In order to develop a solution for the PRSV disease menace the present investigation "Molecular variability and integrated management of papaya ringspot virus (PRSV)" was carried out and findings are presented below.

4.1 Survey for the collection of PRSV infected samples of papaya from Southern India

In view of the rapid spread, severity and economic losses caused by PRSV, a survey was conducted to study the incidence and variability of PRSV, if any. Survey was carried out in Southern states of India *viz.,* Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and Telangana (Fig. 1) during 2014-2015. Survey covered commercial gardens and kitchen gardens. Wherever commercial cultivation was not available kitchen gardens were included counting plants in the backyards to estimate the per cent incidence. Survey revealed that the disease incidence in different places showed wide range with varied symptoms in the surveyed areas of South India.

4.1.1 Symptoms of PRSV recorded during survey

PRSV incidence showed typical symptoms (Plate 1a and 1b) on papaya plants. Most of the infected papaya plants showed mosaic, chlorosis, mottling, leaf distortion, green islands, which were similar to earlier reports made by Linder *et al.* (1945), Holmes *et al*. (1948) and Jensen (1949). During advanced stage of infection puckering,

shoestring, dark green water soaked oily streaks on petiole and trunk appeared which were consistent with the findings of Tripathi *et al*. (2008) and Talukdar *et al.* (2013).

In severe cases, leaves were deformed and formation of shoestring occurred. Oily spots were visible on the petioles of the infected plants (Parameswari and Jain, 2016 and Navanath *et al.,* 2017). Fruits showed characteristic ringspots and in advanced stages small severely malformed fruits were observed. Some fruits were even beyond the point of recognition. In advanced stage of infection plants appeared bushy and stunted finally leading to wilting and death. Similar types of symptoms were also reported by Reddy (2000), Hemavati (2005), Kunkalikar *et al*. (2007), Verma *et al*. (2007), Mallikarjun (2009) and Reddy *et al*. (2011a).

4.1.2 Incidence of PRSV recorded during survey

The data presented in Table 2a and 2b depict that incidence of PRSV in Karnataka ranged from 0 to 100 per cent. Maximum of 100 per cent disease incidence was observed at different locations of Bangalore rural and Bangalore urban districts. Highest average disease incidence was noticed in Ramanagara district (82 %). Minimum PRSV incidence (0 %) was recorded in Kodagu district, followed by 4 per cent disease incidence in Raichur. Raichur also showed incidence of papaya leaf curl to an extent of 15 per cent. Incidence of different districts varied considerably; Chikkaballapura (67 %), Dharwad (70 %), Hassan (48 %), Mysore (40 %) and Vijayapura (47 %).

Papaya ringspot was severe in commercial orchards of Bangalore and Ramanagara, wherein papaya was grown under intensive cultivation for several years. Higher price and ever increasing demand from the markets of Bangalore encouraged farmers for extensive cultivation of papaya despite severe incidence of the disease. PRSV has been reported to be severe in Bangalore rural and Bangalore urban district with 75 to 100 per cent disease incidence (Reddy, 2000; Basha, 2002 and Mallikarjun, 2009). Reddy *et al.* (2011a) also observed high disease incidence (94 %) in Bangalore. Survey conducted in major papaya growing areas of Southern Karnataka during 2012-14 by Pushpa (2014) also revealed higher PRSV incidence in Bangalore rural (78 %) followed by Bangalore urban (69 %) and Chikkaballapura (53 %) districts.

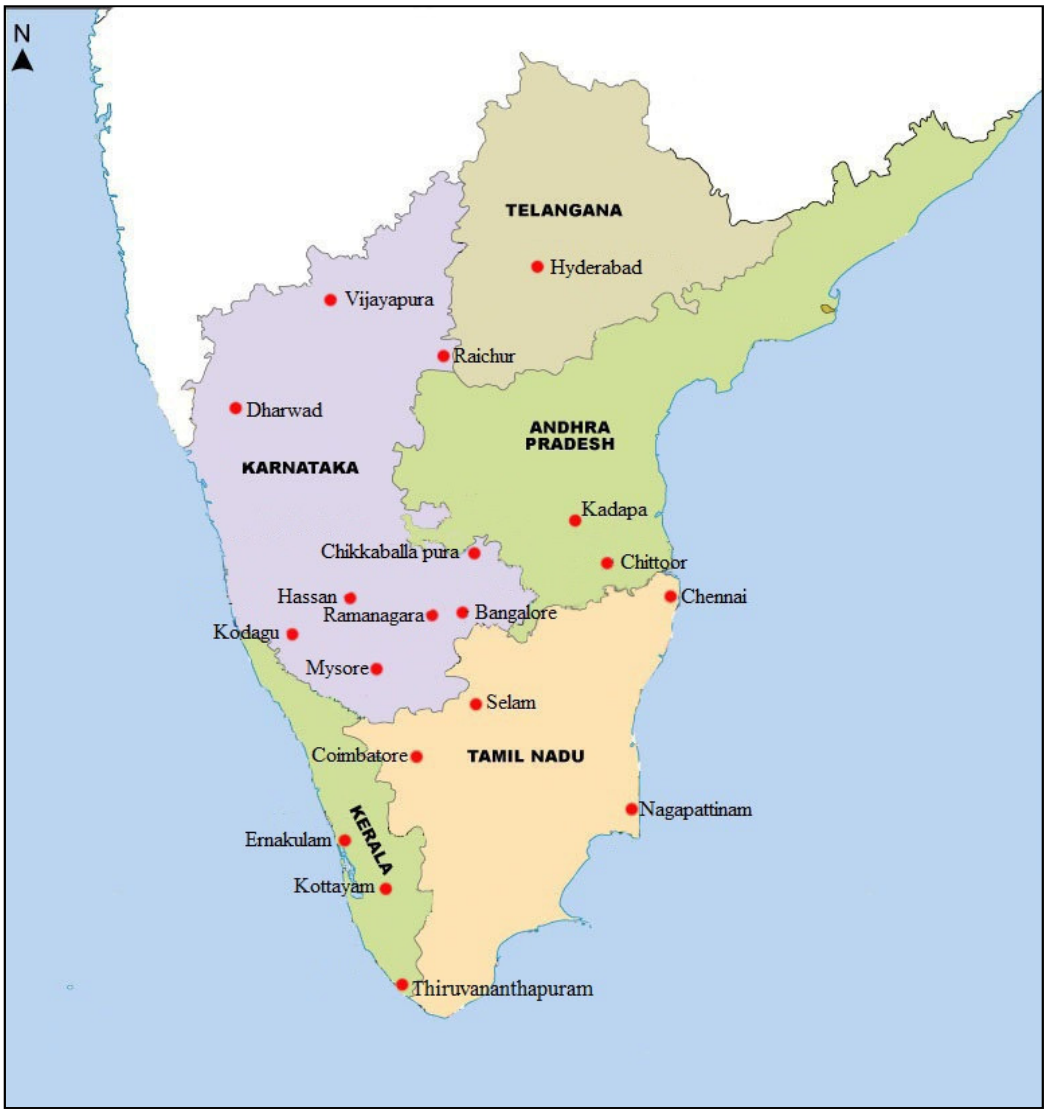


Figure 1: Locations surveyed during 2014-15 for assessment of PRSV incidence

A. Chlorosis of leaves

B. Oily streaks on petiole/stem

C. Distortion of fruits

D. Puckering of leaves

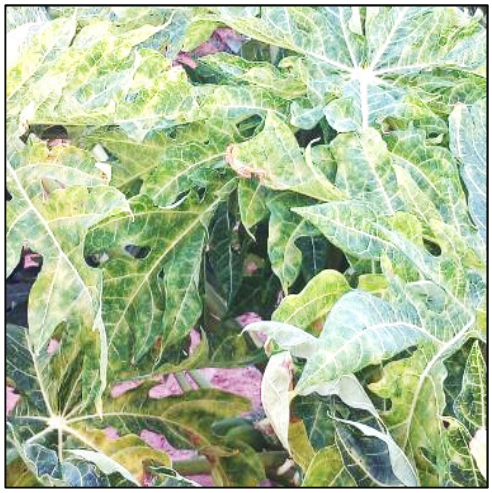
E. Green islands on leaves

F. Ringspots on fruits

Plate 1a: Different types of symptoms of PRSV observed on papaya during survey

G. Distortion of leaves

H. Shoestring on leaves



I. Mosaic on leaves

J. Stunted growth of the plant

K. Wilting of plants

Plate 1b: Different types symptoms of PRSV observed on papaya during survey

Table 2a: Incidence of PRSV at different locations in Karnataka*

Sl. No.	District	Location	Major variety	Place	Prominent symptoms	Disease incidence (%)
1	Bangalore rural	Devanahalli	RL	OR	Cl, Df, Mo, Os, Rs	44
		Doddaballapur	RL	OR	Cl, Df, Mo, Os, Rs	70
		Nelamangala	RL, Solo	OR	Cl, Df, Mo, Os, Rs, Ss	90
		Hoskotae	RL, Solo	OR	Cl, Df, Mo, Os, Rs	74
		Channahalli	RL	OR	Cl, Df, Mo, Os, Rs, St	100
					Mean	76
2	Bangalore urban	Hebbal	RL	OR	Df, Mo, Os, Rs, St, W	100
		Suggata	Solo	OR	Cl, Mo, Ld, Os, Rs	32
		Yelahanka	Local	KG	Cl, Df, Mo, Os, Rs	88
		Kengeri	Solo	OR	Cl, Df, Mo, Os, Rs, St	100
					Mean	80
3	Chikkaballa - pura	Paragodu	RL	OR	Cl, Df, Mo, Rs	58
		Kambalahalli	RL	OR	Cl, Df, Mo, Os, Rs	82
		Chintamani	RL, Solo	OR	Cl, Df, Mo, Os, Rs, Ss	60
					Mean	67
4	Dharwad	Karadigudda	Local	KG	Cl, Df, Mo, Os, Rs	80
		Narendra	Local	KG	Cl, Df, Mo, Os, Rs	80
		Hangaraki	Local	KG	Cl, Df, Mo, Pc, Rs	50
					Mean	70
5	Hassan	Hassan	Local	KG	Cl, Df, Mo, Os, Rs	70
		Arsikere	Local	KG	Cl, Df, Mo, Os, Rs	32
		Doddameti Kurkae	RL	OR	Cl, Df, Mo, Os, Rs, Ss	42
					Mean	48

Cl: Chlorosis
Df: Distortion of fruits
Gi: Green islands
Ld: Leaf distortion
Mo: Mosaic
Os: Oily streaks on petiole
Pc: Puckering
Rs: Ringspots on fruits
Ss: Shoestring of leaves
St: Stunted growth
W: Wilting
KG: Kitchen garden
OR: Orchard
RL: Red Lady
*During survey between 2014-15

Table 2b: Incidence of PRSV at different locations in Karnataka*

Sl. No.	District	Location	Major variety	Place	Prominent symptoms	Disease incidence (%)
6	Kodagu	Pollibetta	Local	KG	-	0
		Gonikoppal	Local	KG	-	0
		Siddapura	Local	KG	-	0
					Mean	0
7	Mysore	Srirampura	Local	KG	Cl, Df, Mo, Rs	40
		Mysore	Local	OR	Cl, Df, Mo, Rs	20
		Nanjangud	RL	OR	Cl, Df, Mo, Os, Rs	60
					Mean	40
8	Raichur	Askihal	Local	KG	Cl, Mo, Rs, Pc	9
		Rampur	Local	KG	Cl, Mo, Rs, Ld	0
		Raichur	RL	OR	Cl, Mo, Rs	4
					Mean	4
9	Ramanagara	Magadi	RL, Surya	OR	Df, Mo, Os, Rs, St, W	95
		Ramanagara	Local	OR	Cl, Df, Mo, Os, Rs, Ss	80
		Channapatna	Local	KG	Cl, Df, Mo, Os, Rs	72
					Mean	82
10	Vijayapura	Hittinahalli	Local	KG	Cl, Df, Mo, Os, Rs, Ss	67
		Hadagali	Local	KG	Cl, Mo, Rs	55
		Devara Hipparagi	Local	KG	Cl, Mo, Rs	20
					Mean	47

Cl: Chlorosis
Df: Distortion of fruits
Gi: Green islands
Ld: Leaf distortion
Mo: Mosaic
Os: Oily streaks on petiole
Pc: Puckering
Rs: Ringspots on fruits
Ss: Shoestring of leaves
St: Stunted growth
W: Wilting
KG: Kitchen garden
OR: Orchard
RL: Red Lady
*During survey between 2014-15

Mallikarjun (2009) recorded 81per cent incidence of PRSV in Vijayapura, 72 per cent incidence in Dharwad and 13 per cent in Shimogga. No incidence of PRSV was observed in Kodagu district. Since, no papaya orchards were located in surveyed places of Kodagu, only local varieties which were grown in kitchen gardens were free from PRSV infection. Similar observations were also reported by Kunkalikar *et al*. (2007) and; Kalleshwaraswamy and Kumar (2008) who reported absence of PRSV during repetitive surveys conducted in and around parts of Western ghats and Kodagu. The lesser incidence in these locations might be due to lesser availability of inoculum and cropping systems that do not support the virus and the vector.

In Andhra Pradesh, Kadapa recorded average disease incidence of 70 per cent while in Chittoor 42 per cent incidence of PRSV was observed (Table 3). Similar to Karnataka PRSV was a major problem in the commercial gardens of Andra Pradesh. Incidence of PRSV to the tune of 24 per cent was observed in Hyderabad.

In Kerala PRSV incidence (Table 4) was highest in Kottayam district (77 %) followed by Trivandrum (51 %) and Ernakulam (48 %). Commercial gardens could not be located in Kerala, hence the observations were recorded from research institute (College of Agriculture, Vellayani) and from local varieties grown in the home gardens, but the incidence was found to be relatively high. Papaya was very common in the backyards of Kerala which might have made inoculum readily available resulting in higher incidence. Verma *et al*. (2007) reported that PRSV was severe in Kerala with 35 to 66 per cent incidence.

In Tamil Nadu PRSV incidence was found to be minimum in Selam (36 %) and Nagapattinam (40 %) while Coimbatore (70 %) and Chennai (56 %) recorded relatively higher incidence (Table 5). Varied levels of PRSV incidence from different states of India were reported earlier including Maharashtra up to 75-100 per cent (Lokhande *et al.,* 1992), West Bengal up to 40 per cent (Verma *et al.,* 2007), Uttar Pradesh up to 45-85 per cent (Singh and Awasthi, 2007), Maharashtra up to 10 -100 per cent (Mallikarjun, 2009) and Assam up to 28-74 per cent (Talukdar *et al.,* 2013).

Table 3: Incidence of PRSV at different locations in Andhra Pradesh and Telangana*

Sl. No.	District	Location	Major variety	Place	Prominent symptoms	Disease incidence (%)
Andhra pradesh						
1	Kadapa	Kadapa	RL	OR	Cl, Mo, Gi, Os, Rs, Ss, St	71
		Rajampet	Local	KG	Cl, Mo, Os, Rs	50
		Kodur	RL	OR	Cl, Mo, Os, Rs, Ss, St	88
					Mean	70
2	Chittoor	Tirupati	Local	KG	Cl, Mo, Os, Rs	24
		Madanapalle	RL	OR	Cl, Mo, Os, Pc, Rs, Ss	62
		Rangampet	Local	KG	Cl, Mo, Os, Rs	40
					Mean	42
Telangana						
1	Hyderabad	Rjendra Nagar	Local	KG	Cl, Mo, Pc, Rs	40
		Secunderabad	Local	KG	Cl, Mo, Rs, Ld	10
		Ameerpet	Local	KG	Cl, Mo, Pc, Rs	20
					Mean	23

Cl: Chlorosis Mo: Mosaic Ss: Shoestring of leaves KG: Kitchen garden

Df: Distortion of fruits Os: Oily streaks on petiole St: Stunted growth OR: Orchard

Gi: Green islands Pc: Puckering W: Wilting RL: Red Lady

Ld: Leaf distortion Rs: Ringspots on fruits *During survey between 2014-15

Table 4: Incidence of PRSV at different locations in Kerala*

Sl. No.	District	Location	Major Variety	Place	Prominent symptoms	Disease incidence (%)
1	Thiruvanan-thapuram	Kalliyoor	Local	KG	Cl, Mo, Df, Ss, W	32
		Vellayani	Local	OR	Cl, Mo, Df, Gi, Ld, Rs, Ss, W	100
		Papanchani	Local	KG	Cl, Mo, Df, Rs, Ss, W	20
					Mean	51
2	Kottayam	Changanassery	Local	KG	Cl, Mo, Pc, Rs, Ss	80
		Kanjirappally	Local	KG	Cl, Mo, Pc, Rs	63
		Athirampuzha	Local	KG	Cl, Mo, Pc, Rs, Ss	87
					Mean	77
3	Ernakulam	Kalady	Local	KG	Cl, Mo, Pc, Rs, Ss	44
		Angamaly	Local	KG	Cl, Mo, Pc, Rs	36
		Kottamam	Local	KG	Cl, Mo, Pc, Rs, Ss	58
		Aluva	Local	KG	Cl, Mo, Pc, Rs, Ss	52
					Mean	48

Cl: Chlorosis
Df: Distortion of fruits
Gi: Green islands
Ld: Leaf distortion
Mo: Mosaic
Os: Oily streaks on petiole
Pc: Puckering
Rs: Ringspots on fruits
Ss: Shoestring of leaves
St: Stunted growth
W: Wilting
KG: Kitchen garden
OR: Orchard
RL: Red Lady
*During survey between 2014-15

Table 5: Incidence of PRSV at different locations in Tamil Nadu*

Sl. No.	District	Location	Major variety	Place	Prominent symptoms	Disease incidence (%)
1	Coimbatore	Coimbatore	-	OR	Cl, Mo, Os, Pc, Rs, Ss	93
		Velandipalayam	Local	KG	Cl, Mo, Os, Pc, Rs	40
		Malumichampatti	Local	KG	Cl, Mo, Os, Pc, Rs	77
					Mean	70
2	Chennai	Chennai	Local	KG	Cl, Mo, Rs	36
		Kodambakkam	Local	KG	Cl, Mo, Rs, Os, Pc	72
		Kolathur	Local	KG	Cl, Mo, Rs, Ss	60
					Mean	56
3	Nagapattinam	Nagapattinam	Local	KG	Cl, Mo, Os, Pc, Rs	50
		Velakanni	Local	KG	Cl, Mo, Rs	27
		Sirkazhi	Local	KG	Cl, Mo, Rs, Ss	43
					Mean	40
4	Salem	Salem	Local	KG	Cl, Mo, Os, Pc, Ld, Gi, Rs, Ss	44
		Mallamooppampatti	Local	KG	Cl, Mo, Rs	34
		Omalur	Local	KG	Cl, Mo, Rs	30
					Mean	36

Cl: Chlorosis
Df: Distortion of fruits
Gi: Green islands
Ld: Leaf distortion
Mo: Mosaic
Os: Oily streaks on petiole
Pc: Puckering
Rs: Ringspots on fruits
Ss: Shoestring of leaves
St: Stunted growth
W: Wilting
KG: Kitchen
OR: Orchard
RL: Red Lady
*During survey between 2014-15

Farmers adopted a wide range of cultural practices in papaya cultivation (Plate 2). Red Lady was found single most popular variety among farmers. It covered most of the commercial gardens due to its high yield potential and market value as stated by the farmers. Varieties *viz.,* Sunrise solo and Arka surya were also grown by the farmers due to their small fruits preferred by the nuclear families. In the kitchen gardens local varieties were found and their names were not known to the growers. They were much taller than the commercial varieties, often growing several meters tall and some of them had been surviving in the gardens for many years. None of the varieties were found resistant to PRSV disease. The plants in the backyards which escaped early infection remained almost free from disease, most probably attributed to the fact that vectors failed to reach the leaves later or may be due to very low inoculum availability.

Because of the high demand, farmers have been continuously cultivating papaya for several years in the commercial gardens. The high incidence of the disease in commercial areas might be due to intensive cultivation of the crop which helped for easy survival and dissemination of the virus. Cucurbits were found near to the papaya gardens in all the surveyed areas that also helped in survival, since members of Cucurbitaceae family act as alternate host for the virus. These observations were in agreement with the findings of Pushpa (2014).

4.1.3 Biological characterization of PRSV isolates based on symptoms

During the present investigation, nine PRSV isolates were collected from severely infected papaya plants of Tirupati, Kodur, Bangalore, Doddameti Kurkae, Ernakulam, Thiruvanthapuram, Coimbatore, Nagapttinam and Hyderabad. Seedlings of the papaya variety Red Lady were taken to survey locations, sap inoculated and brought back. The isolates collected during the survey were designated with code name using alphabets of the places from where they were collected (Table 6). Isolates were maintained by mechanically inoculating to 30 days old papaya seedlings of variety Red Lady raised under insect proof glasshouse (Plate 3).

Table 6: PRSV isolates used for biological characterization

Sl. No.	Place of collection	State	Isolate code
1	Tirupati	Andra Pradesh	PRSV-TPT
2	Kodur		PRSV-KOD
3	Bangalore	Karnataka	PRSV-BLR
4	Doddameti Kurkae		PRSV-DOD
5	Thiruvanthapuram	Kerala	PRSV-TVM
6	Ernakulam		PRSV-EKM
7	Coimbatore	Tamil Nadu	PRSV-CBE
8	Nagapttinam		PRSV-NAG
9	Hyderabad	Telangana	PRSV-HYD

A. Channahalli

B. Kambalahalli

C. Magadi (Red Lady)

D. Magadi (Surya)

E. Coimbatore

F. Madanpalle

Plate 2: Field view of papaya orchards visited during survey

Plate 3: Maintenance of PRSV isolates inside insect proof cages

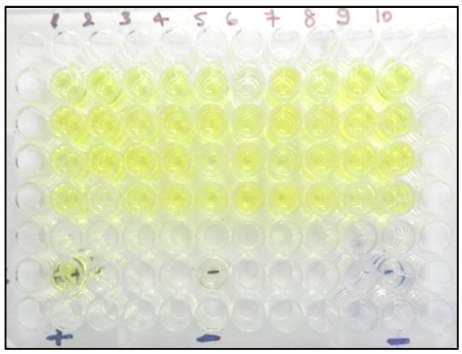


Plate 4: Confirmation of PRSV by DAS-ELISA

A. PRSV-BLR isolate

B. PRSV-CBE isolate

C. PRV-EKM isolate

D. PRSV-TPT isolate

Plate 5: Severe symptoms of PRSV observed on variety Red Lady inoculated with different isolates

Sixty days after sap inoculation all the isolates showed mosaic, severe puckering, shoestring, leaf distortion and stunted growth. None of the isolates showed mild symptoms and all of them finally lead to the deterioration and death of the inoculated plants. The isolates could not be differentiated based on the symptoms they produced (Plate 5).

Dhanam *et al.* (2011) reported that Coimbatore isolate of PRSV showed mottling, filiformity and oily streaks on stem within 18-22 days of mechanical inoculation while severe mottling, puckering, shoestrings, leaf distortion and stunted growth by Bangalore urban isolate was reported by Pushpa (2014). Similar observations were also made by Yeh and Gonsalves (1984); Gonsalves (1989) and Kenganal (2009) who failed to identify PRSV isolates that were mild in reaction. The entire field collected isolates produced severe symptoms on the inoculated plants during the advanced stages.

4.1.4 Confirmation of PRSV identity in isolates

Initially the identity of the virus was confirmed by pathogenicity tests on papaya. The presence of PRSV in different isolates was further verified using DAS-DAS-ELISA technique with anti-PRSV capture antibody and ALP labelled anti-PRSV detection antibody (Agdia, USA). All the isolates tested were found positive for PRSV (Plate 4). One isolate from each state *viz.,* PRSV- BLR (Bangalore), PRSV-CBE (Coimbatore), PRSV-EKM (Ernakulam) and PRSV-TPT (Tirupati) were maintained under insect proof condition till the end of the present investigation.

4.2 Molecular variability of PRSV isolates

Total RNA from PRSV infected papaya leaves of different isolates *viz.,* PRSV-BLR (Bangalore), PRSV-CBE (Coimbatore), PRSV-EKM (Ernakulam) and PRSV-TPT (Tirupati) were extracted using trizol reagent and part of P1 protein gene and coat protein gene were sequenced.

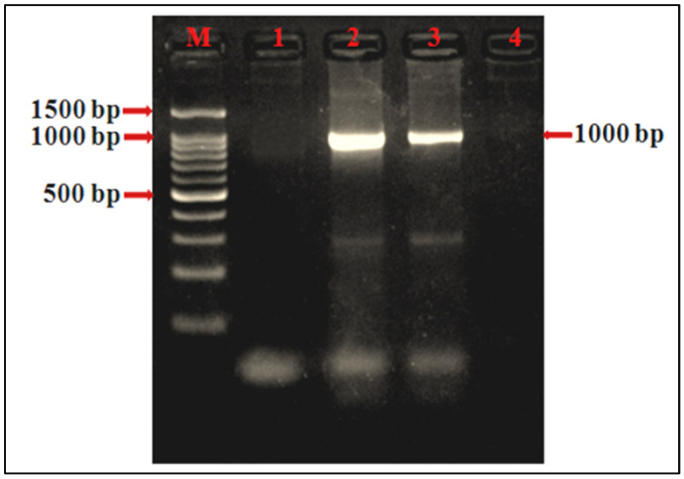
4.2.1 Molecular variability of PRSV isolates based on P1 protein gene sequence

Primer pair PRSV1PF (5'CAATTCGAAGCAACCAAACAAAT3')/ PRSV1PR (5'TCTTTCCGAACTTGAGTTGCT3') amplified part of P1 protein gene (Nucleotide position from 46 to 1003) of PRSV-BLR isolate only. Repeated effort to amplify P1 protein gene of other isolates failed. Hence another set of specific primer pair PRSV2PF (5'TTGGAGTGCTAGCCTTGAGTT3')/ PRSV2PR (5'CCCCACACATTGTAACGTCCA3') was designed which amplified part of P1 protein gene (nucleotide position from 960-1812) of all four PRSV isolates.

Primer pair PRSV1PF/PRSV1PR amplified part of P1 proteinase gene of PRSV-BLR isolate and an expected ~ 1000 bp band was confirmed by agarose electrophoresis (Plate 6A). No such amplification was observed from cDNA of healthy papaya tissue. The sequence homology obtained in BLAST (www.ncbi.nih.gov /BLAST) revealed the query matching with reported PRSV P1 protein gene sequences from different geographical locations. PRSV-BLR isolate showed highest nucleotide identity of 88 per cent with PRSV isolate from Hyderabad (Accession KP743981) and 87 per cent identity with PRSV-P isolate from Delhi (EF017707). It showed 84 per cent identity with PRSV-W isolate from India (EU475877) followed by 79 per cent identity with PRSV strain leaf deformation (DQ340769) and PRSV isolate pFT3NP (JX448373). BLAST revealed that it was distinct from the isolates of New Delhi and Hyderabad.

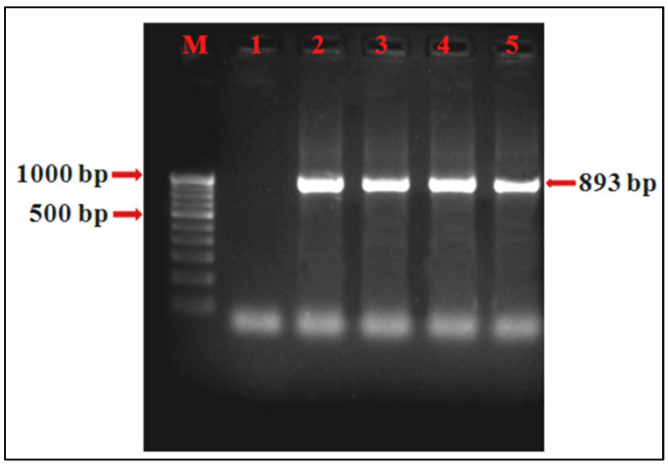
The nucleotide sequences of the PRSV-BLR isolate and nineteen other PRSV isolates available in the NCBI gene bank (Table 7) were used to construct phylogenetic tree. Indian isolates were separated from isolates of other geographical regions forming a separate sub-cluster in phylogenetic tree (Fig. 2). The close relationship among Indian isolates was noticed from the phylogenetic tree constructed based on available sequences and generally clustering pattern of isolates correlated well with their geographical origin except for one isolate PRSVR3 (KJ755852).

Primer pair PRSV2PF/PRSV2PR amplified part of P1 protein gene of four PRSV isolates which yielded a product of approximately 900 bp fragment (Plate 6B).The sequence homology obtained in BLAST (www.ncbi.nih.gov/BLAST) revealed the query



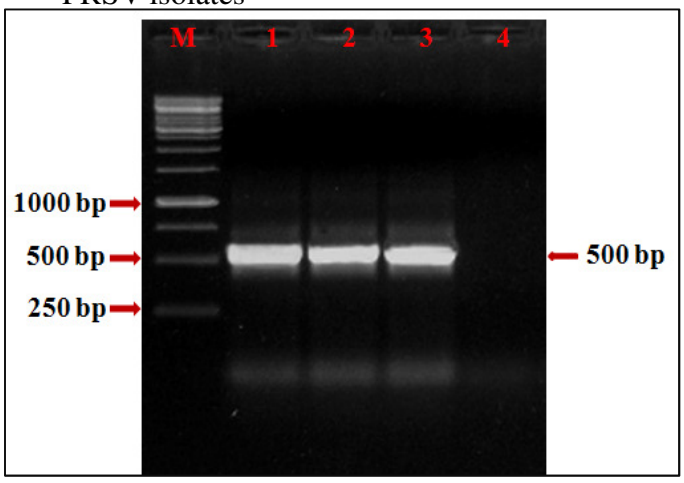
M. 100 bp DNA ladder
1.Healthy papaya sample
2. PRSV-BLR isolate
3. PRSV-BLR isolate
4. Water Control

A. Amplified PCR product of partial P1 proteinase gene of PRSV GKVK isolate



M. 100 bp DNA ladder
1. Healthy papaya sample
2. PRSV-BLR isolate
3. PRSV-CBE isolate
4. PRSV-EKM isolate
5. PRSV-TPT isolate

B. Amplified PCR product of partial P1 proteinase gene of South Indian PRSV isolates



M. 1 Kbp DNA ladder
1. PRSV-BLR isolate
2. PRSV-CBE isolate
3. PRSV-TPT isolate
4. Healthy papaya sample

C. Amplified PCR product of partial coat protein gene of PRSV isolates

Plate 6: Molecular characterization of PRSV isolates

Table 7: Details of the PRSV isolates obtained from NCBI GenBank (for P1 proteinase gene of PRSV-BLR isolate)

Sl. No.	Isolate	Pathotype	Location	Accession no.	Host	Reference
1	PRSV-BLR	P	Bangalore (India)	-	Papaya	Present study
2	PRSV-HYD	NS*	Hyderabad (India)	KP743981	Papaya	Unpublished
3	PRSV-DEL	P	India	EF017707	NS	Parameswari *et al.,*2007
4	PRSV W (India)	W	India	EU475877	NS	Mangrauthia *et al.,* 2008
5	Ping-tong leaf-deformation	NS	Taiwan	DQ340769	Papaya	Unpublished
6	pFT3-NP	P	Taiwan	JX448373	Papaya	Unpublished
7	pfT3-AX-D-Ph	P	Taiwan	JX448372	Papaya	Unpublished
8	prT3-AX-N-Ad6	P	Taiwan	JX448369	Papaya	Unpublished
9	prT3-AX-M-Ph	P	Taiwan	JX448371	Papaya	Unpublished
10	Ping-tong leaf-mottling	NS	Taiwan	DQ340771	Papaya	Unpublished
11	Ping-tong leaf-mottling and necrosis	NS	Taiwan	DQ340770	Papaya	Unpublished
12	PRSVR3	NS	India	KJ755852	Papaya	Unpublished
13	PRSV W (Thailand)	W	Thailand	AY010722	NS	Attasart *et al.,* 2002
14	PRSV W isolate CI	W	Taiwan	AY027810	*Luffa cylindrica*	Unpublished
15	prT3-AX-N-Ph	P	Taiwan	JX448370	Papaya	Unpublished
16	PRSV-LM	P	China	KT633943	Papaya	Unpublished
17	PRSV E2	NS	France	KC345609	*Cucurbita pepo*	Romay *et al.,* 2014
18	PRSV HA	NS	Taiwan	X67673	NS	Wang *et al.,* 1994
19	PRSV PG	NS	Hawaii (USA)	EU126128	NS	Unpublished
20	TUL15	W	USA	KY039583	Gourd	Ali., 2017

*NS: Not specified

matching with reported PRSV P1 protein gene sequences from different geographical locations. PRSV-BLR isolate showed highest nucleotide identity of 87 per cent with PRSV isolate from Hyderabad (Accession KP743981) and PRSV-P isolate from Delhi (EF017707). It showed 85 per cent nucleotide identity with PRSV isolate E2 from France (KC345609) and PRSV isolate Mex-VrPO from Mexico (AY231130). PRSV-CBE isolate showed maximum nucleotide identity of 87 per cent with PRSV isolate from Hyderabad (KP743981) followed by 86 per cent identity with PRSV-P isolate from Delhi (EF017707). This isolate showed 84 per cent nucleotide identity with PRSV Mex-VrPO from Mexico (AY231130) and 83 per cent identity with PRSV isolate PRSV-W-C from Brazil (DQ374152).

PRSV-EKM isolate showed 86 per cent nucleotide identity with PRSV isolate from Hyderabad (Accession KP743981) followed by 82 per cent identity PRSV-W-C (DQ374152) and PRSV E2 (KC345609). It showed 81 per cent nucleotide identity with PRSV-W-TUL 15 (KY039583). While PRSV-TPT isolate from Andhra Pradesh showed 86 per cent identity with PRSV isolate from Hyderabad (KP743981) and 85 per cent identity with PRSV-P isolate from Delhi (EF017707). It showed 83 per cent identity with PRSV isolate E2 from France (KC345609) and 82 per cent identity with accession PRSV CH from Colombia (KT275938). Overall the identity of the isolates of South India in this study ranged from 87 per cent to 72 per cent with other sequences of PRSV deposited in NCBI GenBank.

The nucleotide sequences of four isolates from South India (PRSV-BLR, PRSV-CBE, PRSV-EKM and PRSV-TPT) and sequences of eighteen other PRSV isolates available in the NCBI GenBank (Table 8) were used work out phylogenetic relationship. The close relationship among Indian isolates was noticed from the phylogenetic tree constructed based on available sequences (Fig. 3) and generally clustering pattern of isolates correlated well with their geographical origins except for isolate from Delhi (EF017707). Indian isolates were separated from isolates of other geographical regions forming a separate sub-cluster in phylogenetic tree. The isolate from Delhi was clustered separately indicating that the PRSV isolates from South India were diverse.

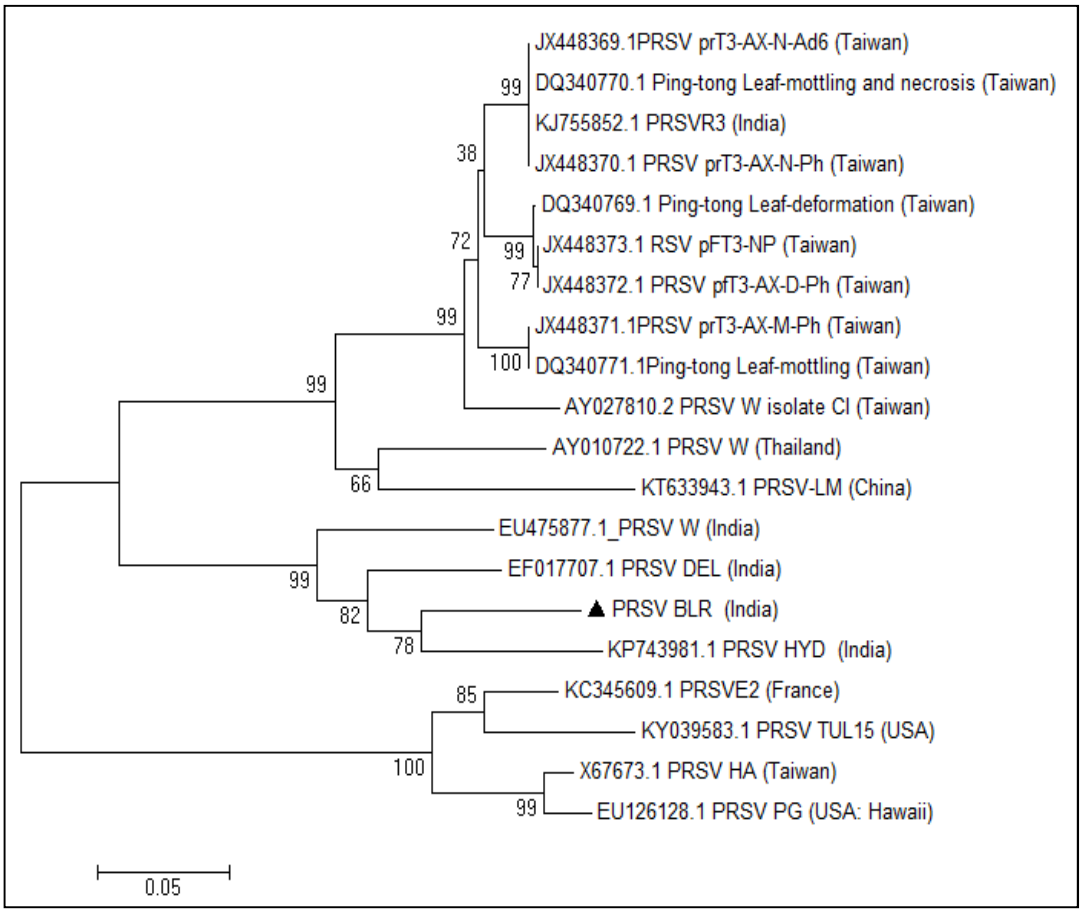


Figure 2: Bootstrapped neighbour joining phylogenetic tree showing the relationship of PRSV GKVK isolate based on partial sequences of P1 proteinase gene

Table 8: Details of the PRSV isolates obtained from NCBI GenBank (for P1 proteinase gene of South Indian isolates)

Sl. No.	Isolate	Pathotype	Location	Accession no.	Host	Reference
1	PRSV-BLR	P	Bangalore (India)	-	Papaya	Present study
2	PRSV-CBE	P	Coimbatore (India)	-	Papaya	Present study
3	PRSV-EKM	P	Ernakulam (India)	-	Papaya	Present study
4	PRSV-TPT	P	Tirupati (India)	-	Papaya	Present study
5	PRSV-HYD	NS	Hyderabad (India)		Papaya	Unpublished
6	PRSV-DEL	P	India	EF017707	NS	Parameswari *et al.,* 2007
7	PRSV E2	NS	France	KC345609	*Cucurbita pepo*	Romay *et al.,* 2014
8	Mex-VrPO	NS	Veracruz (Mexico)	AY231130	NS	Carrazana *et al.,* 2007
9	PRSV-W-C	W	Brazil	DQ374152	*Cucurbita pepo*	Nagata *et al.,* 2007
10	PRSV W (India)	W	India	EU475877	NS	Mangrauthia *et al.,* 2008
11	PRSV HA	NS	Taiwan	X67673	NS	Wang *et al.,* 1994
12	TUL15	W	USA	KY039583	Gourd	Ali., 2017
13	PRSV VR	P	Colombia	KT275937	Papaya	Rojas and Bedoya, 2017
14	FEV	NS	Federal District (Brazil)	KP462721	*Fevillea cordifolia*	Unpublished
15	Mex.QrFC-14.6	P	Quintana Roo (Mexico)	AY438010	NS	Unpublished
16	PRSV CH	P	Campo Hermoso (Colombia)	KT275938	Papaya	Rojas *et al.,* 2017
17	PRSV-W-1	W	Brazil	DQ374153	*Cucurbita pepo*	Nagata *et al.,* 2007
18	PRSV PG	NS	Hawaii (USA)	EU126128	NS	Unpublished
19	Mex.CpT-15.5	P	Tapachula (Mexico)	AY438009	NS	Unpublished
20	Mex.CpT-15.1	P	Tapachula (Mexico)	AY438007	NS	Unpublished
21	Mex.CpT-15.2	p	Tapachula (Mexico)	AY438008	NS	Unpublished
22	Mex.CpPM-14.10	P	Chiapas, Puerto (Mexico)	AY438006	NS	Unpublished

*NS: Not specified

A BLAST search of nucleotide using the genome of PRSV-W-TUL15 showed 83 to 92 per cent nucleotide sequence similarities with the published PRSV isolates (Ali, 2017). Between the P1 protein gene of Taiwanese and Hawaiian PRSV isolates there was only 70.90 per cent nucleotide identity (Wang and Yeh, 1997). Among ten putative proteins of PRSV, P1 was the most variable (73.90 per cent similarity) when compared to the other full PRSV sequences, while cytoplasmic inclusion (CI) protein was the most conserved with 99.10 per cent similarity (Gulsiri *et al.,* 2003). Sequence similarity among the type P and type W isolates suggested that the P type arose locally from type W. No significant difference between types P and W was discovered that would account for the host specificity. The comparative analysis of a severe isolate of PRSV (Mex-VrPO) of Mexico and five others reported before also confirmed that P1 was most variable with 13-33 per cent divergence while coat protein gene showed only 5-9 per cent divergence (Carrazana *et al.,* 2007).

High variability in the P1 protein gene has also been reported for other potyviruses like zucchini yellow mosaic virus (ZYMV). Lin *et al*. (2001) reported that in ZYMV isolate TW-TN3, P1 protein gene was most variable with amino acid identities of 59-93.20 per cent.

One reason for the high variability in the P1 protein gene may be the recombination events that occurred in the past. It has been proposed that P1 is related to infectivity, disease resistance, diversification and evolution of the virus (Valli *et al.,* 2007). However, recombination in PRSV is not confined solely to P1. Recently, recombination in the CP of PRSV was detected in isolates from Colombia (Bedoya and Rojas, 2015). With PRSV, most of the earlier works concentrated on the coat protein gene sequences while Romay *et al.* (2014) observed that pairwise sequence similarities in the CP coding region failed to unambiguously distinguish zucchini tigre mosaic virus (ZTMV) isolates from PRSV isolates.

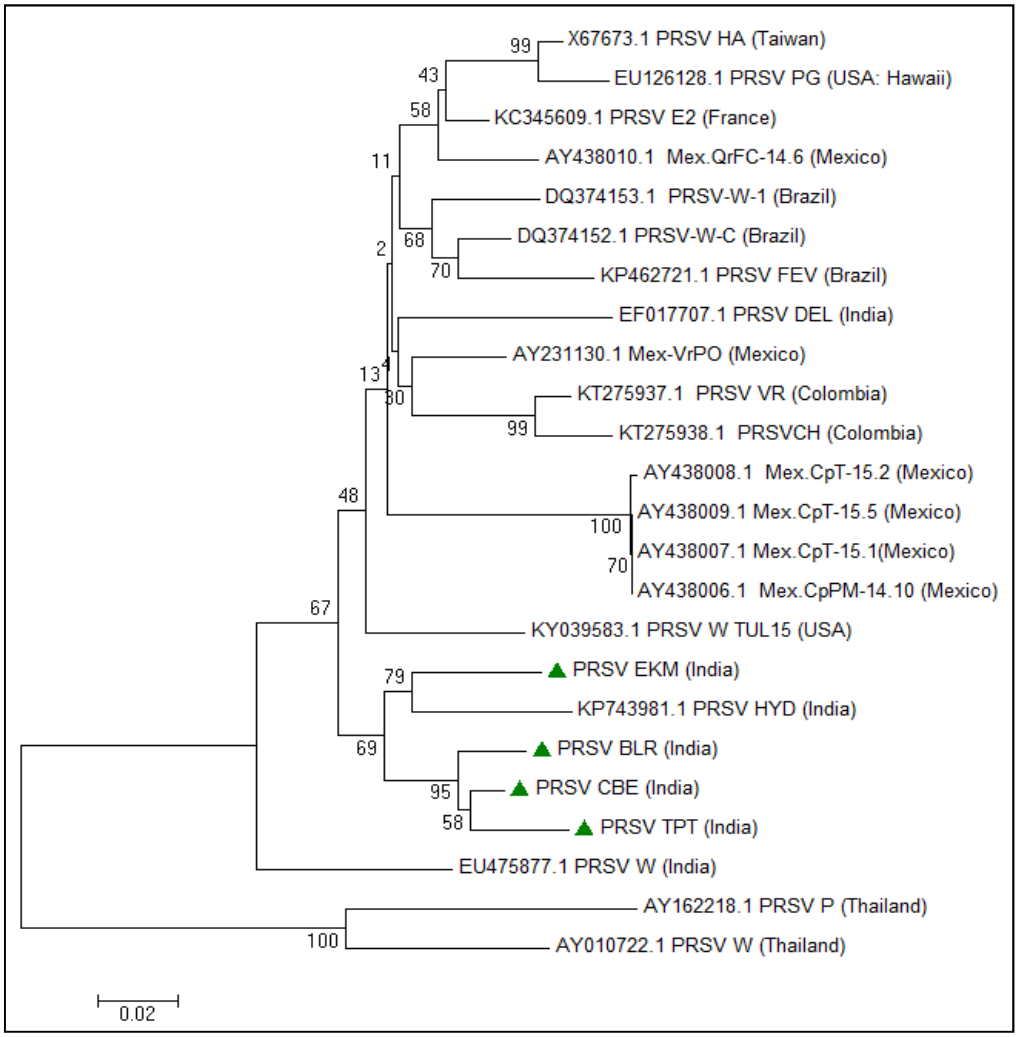


Figure 3: Bootstrapped neighbour joining phylogenetic tree showing the relationship of PRSV isolates based on partial sequences of P1 proteinase gene

4.2.2 Molecular variability of PRSV isolates based on CP gene sequence

Part of coat protein gene was amplified using specific PRSVCPF/PRSVCPR primer pair and ~ 500 bp band was confirmed by agarose gel electrophoresis. Three isolates *viz.,* PRSV-BLR, PRSV-CBE and PRSV-EKM were amplified at ~ 500 bp and sequences were obtained (Plate 6C). Isolate PRSV-TPT did not amplify after repeated attempts.

The sequence homology obtained in BLAST (www.ncbi.nih.gov/BLAST) revealed the query matching with reported PRSV coat protein gene sequences from different geographical locations. The CP nucleotide sequences of three South Indian PRSV isolates used in this study were compared to sequences of other PRSV isolates from different geographical locations in the Indian subcontinent (Table 9). The nucleotide sequence identity at nucleotide level of the three isolates used in the present study ranged from 97 per cent (PRSV-BLR) to 94 per cent (PRSV-CBE) with other Indian isolates deposited in the NCBI GenBank. PRSV-BLR isolate showed highest nucleotide identity of 94 per cent with CP gene sequences of accession AF323637 followed by 91 per cent with PRSV-TA Ti (DQ666641) and isolate PRSV-KE (DQ666639). PRSV-CBE isolate showed highest identity (95 %) with PRSV-TA Ti (DQ666641) PRSV-EKM isolate showed highest identity (97 %) with isolate PRSV-KE (DQ666640) followed by 94 per cent identity with PRSV-AP isolate (AF323637) and 91 per cent with HYD isolate (KP743981).

Phylogenetic tree (Fig. 4) was constructed based on the CP sequences of isolates from present investigation and sequences of twenty other PRSV isolates available in the NCBI GenBank (Table 9). The close relationship of isolates from respective states was evident from the phylogenetic tree and clustering pattern of isolates correlated fairly well with their geographical origins.

Table 9: Details of the PRSV isolates obtained from NCBI GenBank (for CP gene of South Indian isolates)

Sl. No.	Isolate	Pathotype	Location	Accession no.	Host	Reference
1	PRSV-BLR	P	Bangalore (Karnataka)	-	Papaya	Present study
2	PRSV-CBE	P	Coimbatore (Tamil Nadu)	-	Papaya	Present study
3	PRSV-EKM	P	Ernakulam (Kerala)	-	Papaya	Present study
4	PRSV-AP	P	Andhra Pradesh	AF323637	NS	Unpublished
5	PRSV-TA Ti	P	Tiruvallur (Tamil Nadu)	DQ666641	Papaya	Srinivasulu and Saigopal, 2011
6	PRSV-KA Gu	P	Gulbarga (Karnataka)	DQ666639	Papaya	Srinivasulu and Saigopal, 2011
7	PRSV-KE-Ca	P	Calicut (Kerala)	DQ666640	Papaya	Srinivasulu and Saigopal, 2011
8	PRSV-HYD	P	Hyderabad (Telangana)	KP743981	Papaya	Unpublished
9	PRSV-AP Ko	P	Kovvur (Andhra Pradesh)	DQ666638	Papaya	Srinivasulu and Saigopal, 2011
10	P-BR	P	Bangalore (Karnataka)	AF120270	Papaya	Unpublished
11	PRSV-AP-Te	P	Hyderabad (Telangana)	AY839864	Papaya	Srinivasulu and Saigopal, 2011
12	PRSV-AP-Ra	P	Rly Kodur (Andhra Pradesh)	AY839863	Papaya	Srinivasulu and Saigopal, 2011
13	PRSV-KA-Ho	P	Hospet (Karnataka)	AY839865	Papaya	Srinivasulu and Saigopal, 2011
14	KA-Dh	NS	Dharwad (Karnataka)	AY458618	NS	Jain *et al.,* 2004
15	Pune3	P	Pune (Maharashtra)	KC149502	Papaya	Unpublished
16	TN-Tr	NS	Trichy (Tamil Nadu)	DQ077175	NS	Unpublished
17	TNAU	P	Coimbatore (Tamil Nadu)	HM626464	Papaya	Unpublished
18	Avinashi	NS	Tamil Nadu	HM454196	NS	Unpublished
19	PRSV Tamil Nadu	NS	Coimbatore (Tamil Nadu)	EF104919	NS	Unpublished
20	Annur	NS	Tamil Nadu	HM454197	NS	Unpublished
21	Ellampillai	NS	Tamil Nadu	HM754218	NS	Unpublished
22	Dharapuram	P	Tirupur (Tamil Nadu)	HM626467	Papaya	Unpublished
23	Sathyamangalam	P	Erode (Tamil Nadu)	HM626466	Papaya	Unpublished

*NS: Not specified

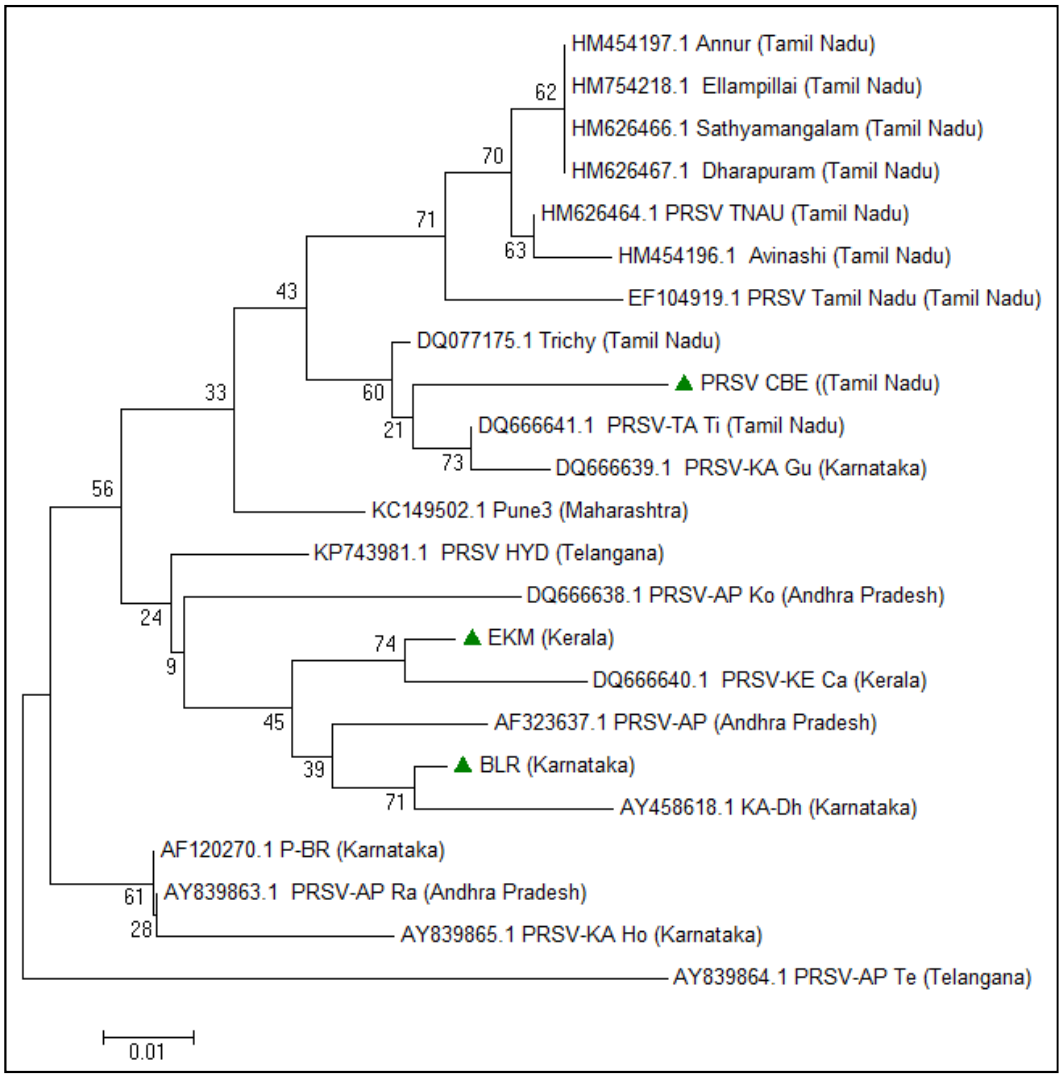


Figure 4: Bootstrapped neighbor joining phylogenetic tree showing the relationship of PRSV isolates based on partial sequences of coat protein gene

Partial characterization of isolate BUH-1 by CP gene showed maximum homology of 98 per cent with south Indian and 87-92 per cent with Asian isolates (Pushpa, 2014). Parameswari *et al*. (2007) found out that Indian isolate (PRSV-DEL) from New Delhi, shared sequence identity of 83-89 per cent at the nucleotide level with other PRSV isolates. The present study agrees with earlier reports that PRSV isolates from the Indian subcontinent are diverse (Bateson *et al.,* 2002; Hema and Prasad, 2004; Jain *et al.,* 2004; Sharma *et al.,* 2005 and; Srinivasulu and Saigopal, 2011). Similarly, when PRSV isolate from Uttar Pradesh was compared with isolates from other regions, maximum divergences of 14.50 per cent and 14.20 per cent was found with Karnataka and Tamil Nadu isolates (Singh *et al.,* 2017).

Mutation together with long distance movement is one of the reason contributing for the variation among isolates. Recombinations between the PRSV-P also contribute to sequence divergence especially with respect to the South Indian isolates (Hema and Prasad, 2004). While Jain *et al.* (2004) opinioned that higher sequence divergence within the PRSV population of the Indian subcontinent was due to wide range of cropping systems and cultivation practices followed in different geographical regions. This diversity might have resulted in different levels of selection pressure on PRSV. Introduction of isolates from other areas, mutations, local and long distance movement are the factors likely to contribute to the natural variation in PRSV-P populations.

Sequence variability has important implications for the use of genes to develop transgenic plants by pathogen derived resistance because such resistance could be highly sequence specific (Savenkove and Valkonen, 2001). The selection of the transgene would be vital step to develop long lasting virus resistant transgenic papaya. While designing transgenes for potyvirus resistance, it is essential to select regions of at least 90 per cent identity between strains to obtain a durable resistance (Moreno *et al.,* 1998). RNA mediated resistance to potyviruses has been reported with sequence identity of 88 per cent or greater (Mueller *et al.,* 1995), while Jones *et al*. (1998) reported that 89 per cent identity of the Nib gene was the minimum sequence identity for the specificity required to trigger gene silencing in the pea seed-borne mosaic potyvirus.

Genetic engineering is a viable option for managing viral diseases such as PRSV (Kung *et al.,* 2009; Mangrauthia *et al.,* 2010 and Yu *et al.,* 2011). Knowledge of the nucleotide sequence and genetic diversity is necessary to select a virus gene for the development of pathogen derived resistance. Recombinations occuring in the majority of RNA viruses are of great evolutionary importance and constitute one of the greatest forces that shape the virus genomes (Solinska *et al.,* 2011). Rodriguez *et al.* (2008) stated that the genetic diversity of PRSV isolates could be sufficiently large that it must be taken into account when designing management strategies such as transgenic resistance and cross-protection. Recent studies by Martinez *et al.* (2014) also confirmed that the due to variability in the coat protein genes, disease management using cross-protection and transgenic plants requires the selection of region specific virus isolates in each country.

The hot spots of recombination in PRSV were concentrated in the region encoding the P1 protein, P3 protein, cytoplasmic inclusion (CI) and the helper component proteinase (Mangrauthia *et al.,* 2008). Recombination events in the coat protein of PSRV appeared to be less frequent than in other regions of the genome (Bateson *et al.,* 2002). Zhu *et al*. (2016) reported that nucleotide BLAST analysis of the coat protein sequence of PRSV from China, showed highest identity of 99 per cent with four isolates from Taiwan. According to Srinivasulu and Saigopal (2011) coat protein gene of PRSV TA-Ti isolate seemed to be an ideal choice to develop transgenic papaya resistant to PRSV in south India. These evidences confirm that coat protein gene of PRSV is more conserved than P1 protein gene hence it is a suitable candidate to develop transgenic papaya resistant to PRSV using pathogen derived resistance.

4.3 Screening of papaya varieties against PRSV for identification of natural resistance

Papaya varieties along with a wild relative *Carica cauliflora* were screened under glasshouse and field conditions to identify natural resistance to PRSV if any.

4.3.1 Screening of papaya varieties under glasshouse condition

Fifteen varieties papaya *viz.,* Arka prabhat, Bangalore dwarf, Co-8, IHS selection-4, Madhu, Mohini, Pant papaya-1, Papita selection, Pune selection, Pusa dwarf, Pusa nanha, Sinta-1, Red Lady, Solo, Surya along with wild relative *Carica cauliflora* were evaluated under glasshouse to identify host-plant resistance against PRSV (Plate 7). The varieties differed slightly in respect of days taken for symptom expression and symptoms at 60 days after inoculation (Table 10).

Upon mechanical inoculation with PRSV-BLR isolate (BLR), all the varieties except wild relative *C. cauliflora* showed susceptibility with slight variation in the symptoms (Plate 8a and 8b). Varieties Mohini, Pusa dwarf, Sinta-1 and Surya developed initial symptoms 12 days post inoculation. IHS selection-4 and Pune selection, took 18 days to express initial symptoms while remaining nine varieties expressed symptoms within two weeks.

IHS selection-4, Papita selection, Pune selection, Pusa nanha, Solo and Surya showed symptoms such as chlorosis, mosaic and distortion of leaves. Six varieties (Arka prabhat, Bangalore dwarf, Co-8, Madhu, Mohini and Pant papaya-1) expressed chlorosis, mosaic, distortion of leaves, green islands and puckering. Pusa dwarf, Sinta-1 and Red Lady expressed severe symptoms including chlorosis, mosaic, distortion of leaves, green islands, puckering and shoestring of leaves. None of the cultivated varieties remained free of infection but wild relative *C. cauliflora* did not develop symptoms till the end of the experiment.

Among the cultivated varieties screened, none was found resistant to the disease and all the cultivated varieties developed symptoms within 12-18 days. Such findings have also been reported by Reddy *et al*. (2011b), wherein varieties like Sunrise solo, Red Lady, Surya, Pusa nanha, Pusa dwarf, Pusa gaint and Pant papaya-1 expressed symptoms 15 days after mechanical inoculation. Pushpa (2014) also found that Sunrise solo, Red Lady and Surya developed symptoms of infection within 10-15 days.

Table 10: Reaction of different papaya varieties to PRSV on mechanical inoculation

Variety	Disease incidence at 60 DAI (%)	Incubation period (Days)	Prominent symptoms	Average OD value at 405 nm
Arka prabhat	100	15	Cl, Mo, Ld, Gi, Pc	0.776
Bangalore dwarf	100	15	Cl, Mo, Ld, Gi, Pc	0.696
Co-8	100	15	Cl, Mo, Ld, Gi, Pc	0.663
IHS selection-4	100	18	Cl, Mo, Ld	0.836
Madhu	100	15	Cl, Mo, Ld, Gi, Pc	0.793
Mohini	100	12	Cl, Mo, Ld, Gi, Pc	0.726
Pant papaya-1	100	15	Cl, Mo, Ld, Gi, Pc	0.786
Papita selection	100	15	Cl, Mo, Ld	0.866
Pune selection	100	18	Cl, Mo, Ld	0.768
Pusa dwarf	100	12	Cl, Mo, Ld, Gi, Pc, Ss	0.643
Pusa nanha	100	15	Cl, Mo, Ld	0.788
Sinta -1	100	12	Cl, Mo, Ld, Gi, Pc, Ss	1.082
Solo	100	15	Cl, Mo, Ld	0.704
Surya	100	12	Cl, Mo, Ld	0.866
Red Lady	100	15	Cl, Mo, Ld, Gi, Pc, Ss	0.953
C. cauliflora	0	-	No symptoms	0.156
Healthy control				0.148

Cl: Chlorosis Ld: Leaf distortion Pc: Puckering
Gi: Green islands Mo: Mosaic Ss: Shoestring of leaves

Plate 7: Screening of papaya varieties under glasshouse condition

A. Arka prabhat

B. Bangalore dwarf

C. Co-8

D. IHS selection-4

E. Madhu

F. Mohini

Plate 8a: Symptoms of PRSV infection on papaya varieties under glasshouse condition

G. Pant papaya-1

H. Papita selection

I. Pune selection

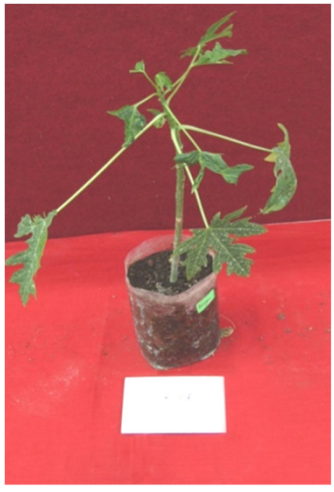
J. Pusa dwarf

K. Pusa nanha

L. Red Lady

M. Sinta-1

N. Solo



O. Surya

Plate 8b: Symptoms of PRSV infection on papaya varieties under glasshouse condition

At 60 days after inoculation (DAI) all the fifteen varieties recorded 100 per cent disease incidence based on the symptoms. All the plants were subjected for DAS-ELISA test to confirm the infection. Among the varieties, Sinta-1 recorded maximum absorbance value of 1.082 while *C. cauliflora* recorded least absorbance value of 0.156 (Table 10). Absorbance value of rest of the varieties ranged from 0.643 to 0.953. DAS-ELISA confirmed that none of the screened variety possessed natural to the disease at seedling stage while the wild relative *C. cauliflora* remained free of infection. Reddy *et al*. (2011b) and Pushpa (2014) also reported that upon mechanical inoculation none of varieties were found to possess resistance to PRSV. Similarly, Roff (2007) and Chavan *et al.* (2010) also failed to identify resistance against PRSV at seedling stage when several varieties were screened.

4.3.2 Screening of papaya varieties under field conditions

In order to evaluate varieties for their reaction to natural infection of PRSV, ten varieties along with wild relative *Carica cauliflora* were transplanted in field (Plate 9). Results presented in the Table 11 reveal that at 100 per cent flowering Pant papaya-1, Pusa nanha and *C. cauliflora* remained free of infection. Varieties Bangalore dwarf, IHS selection-4, Mohini, Pusa dwarf and Solo recorded 33.33 per cent disease incidence while Papita selection, Surya and Red Lady recorded 66.67 per cent infection. At harvest, all the varieties recorded 100 per cent incidence of the disease (Plate 10a and 10b) except *C. cauliflora* and none of the variety was found resistant to the disease.

All the ten varieties recorded chlorosis, severe mosaic, distortion of leaves, puckering, shoestring of leaves and ringspot on fruits. Varieties IHS selection-4, Mohini, Pant papaya-1 and Papita selection expressed chlorosis, severe mosaic, distortion of leaves, puckering, shoestring of leaves, ringspot on fruits, distortion of fruits, oily streaks on petiole and green islands. Consistent with the results of glasshouse studies *C. cauliflora* remained free of infection till the end of the experiment (Plate 12 and 13).

Table 11: Reaction of different papaya varieties to PRSV infection under field conditions

Variety	Disease incidence (%)		Prominent symptoms at harvest	Average OD value at 405 nm	Plant height (cm)	Fruit yield per plant (kg)	No. of fruits per plant (no.)	Average fruit weight (kg)
	At flowering	At harvest						
Bangalore	33	100	Cl, Mo, Ld, Pc, Ss, Rs	0.745	163.43^{b*}	7.96^{bc}	11.00^{bc}	0.72^{bc}
IHS selection-	33	100	Cl, Mo, Ld, Pc, Ss, Rs, Os, Df,	1.168	209.66^{a}	8.60^{bc}	12.67^{abc}	0.63^{d}
Mohini	33	100	Cl, Mo, Ld, Pc, Ss, Rs, Os, Df,	0.994	158.16^{b}	6.48^{cd}	10.33^{cd}	0.63^{d}
Pant papaya-1	0	100	Cl, Mo, Ld, Pc, Ss, Rs, Os, Df,	1.126	154.23^{b}	6.60^{cd}	10.00^{cd}	0.61^{d}
Papita	67	100	Cl, Mo, Ld, Pc, Ss, Rs, Os, Df,	0.673	161.47^{b}	8.19^{bc}	12.67^{abc}	0.65^{cd}
Pusa dwarf	33	100	Cl, Mo, Ld, Pc, Ss, Rs	0.760	168.47^{b}	9.94^{b}	11.67^{bc}	0.87^{a}
Pusa nanha	0	100	Cl, Mo, Ld, Pc, Ss, Rs	0.406	165.43^{b}	9.41^{b}	12.33^{abc}	0.77^{b}
Solo	33	100	Cl, Mo, Ld, Pc, Ss, Rs	0.636	170.63^{b}	5.37^{d}	14.00^{ab}	0.38^{e}
Surya	67	100	Cl, Mo, Ld, Pc, Ss, Rs	0.916	159.50^{b}	6.91^{cd}	10.67^{bcd}	0.65^{cd}
Red Lady	67	100	Cl, Mo, Ld, Pc, Ss, Rs	0.935	150.33^{b}	14.15^{a}	15.67^{a}	0.93^{a}
C. cauliflora	0	0	No symptoms	0.184	122.87^{c}	0.44^{e}	8.33^{d}	0.05^{f}
Healthy				0.151				
SEm±					10.35	0.89	1.47	0.04
CV					9.03	16.69	17.73	7.84
CD (5 %)					24.93	2.15	3.56	0.08
CD (1 %)					341	2.92	N. S	0.12

Cl: Chlorosis Ld: Leaf distortion Pc: Puckering

Df: Distortion of fruits Mo: Mosaic on leaves Rs: Ringspots on fruits

Gi: Green islands Os: Oily streaks on petiole Ss: Shoestring of leaves

*Means with same letter are not significantly different

Plate 9: Screening of papaya varieties under field conditions

A. Bangalore dwarf

B. IHS selection-4

C. Mohini

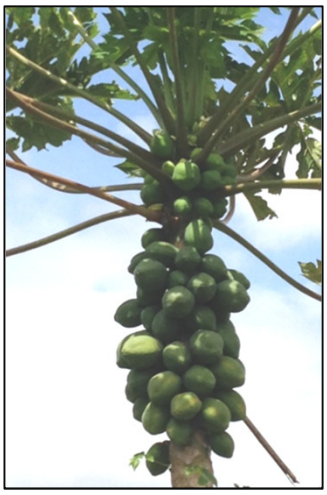
D. Pant papaya 1

E. Papita selection

F. Pusa dwarf

Plate 10a: Symptoms of PRSV infection on papaya varieties under field conditions

G. Pusa nanha



H. Solo

I. Surya

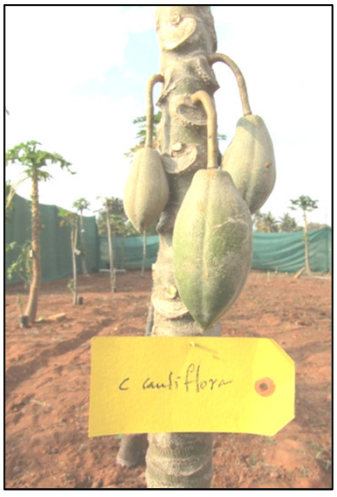
Plate 10b: Symptoms of PRSV infection on papaya varieties under field conditions

Plate 11: Tolerant variety Red Lady under field conditions

Plate 12: *C. cauliflora* 60 DAI under glasshouse condition

A. At seedling stage

B. At final harvest



C. Fruits

Plate 13: *Carica cauliflora* under field conditions

After 15 months of planting, plants were subjected to DAS-ELISA and consistent with results of glasshouse study none of the varieties were found resistant to PRSV under field conditions (Table 11). Highest absorbance value (1.168) was recorded in IHS selection-4 followed by Pant papaya (1.126) while lowest absorbance value (0.184) was recorded in *C. cauliflora*. The absorbance value of rest of the varieties varied from 0.406 to 0.994. Results of DAS-ELISA confirmed that only *C. cauliflora* remained free of infection.

Most of the efforts till date to identify natural resistance met little success (Kudada and Prasad, 2000; Singh *et al.,* 2005; Roff, 2007; Awasthi and Singh, 2009a; Balamohan *et al.,* 2010; Chavan *et al.,* 2010; Alviar, 2011; Reddy *et al.,* 2011b; Singh and Singh, 2013; and Pushpa, 2014). However, *C. cauliflora* was reported to be immune to PRSV (Capoor and Verma, 1961; Ram, 1993; Magdalita *et al.,* 1997; Valencia *et al.,* 2001; Chan and Ong, 2003; and Sudha *et al.,* 2015).

Out of 21 species in the genus *Carica*, only *C. papaya* bears edible fruits. Villegas (2001) observed that resistance to PRSV-P is not found in the cultivated papaya but was present in *C. cauliflora, C. pubescens* and *C. quercifolia while C. goudotiana, and C. parviflora* were susceptible.

C. cauliflora when inarched with *C. papaya* delayed PRSV symptom expression. The delayed PRSV symptom expression observed in *C. papaya* inarched with *C. cauliflora* could be attributed to a transmissible, water-soluble, low-molecular-weight protein factor that might have got transported from *C. cauliflora* to *C. papaya* (Valencia *et al.,* 2001). Roff (2007) stated that several species related to papaya were found to be resistant to PRSV-P, unfortunately crosses between these species have not been achieved due to strong cross incompatibility, while Davis *et al*. (2004) reported that variety Solo was very susceptible to PRSV.

Balamohan *et al*. (2010) reported that natural resistance to PRSV-P was not found in *Carica papaya*. Chavan *et al*. (2010) while screening eight commercial papaya varieties recorded 86 per cent disease incidence on widely grown Red Lady and 45 per cent on Pusa nanha. Result of the present investigation is in agreement with the earlier reports that cultivated varieties do not possess natural resistance to PRSV, while *C.*

cauliflora is immune to the disease. But utilization of *C. cauliflora* in breeding programme is difficult due to strong cross incompatibility evident from the earlier reports.

Average plant height was highest in IHS selection-4 (209.66 cm). Varieties Solo (170.63 cm), Pusa dwarf (168.47 cm), Pusa nanha (165.43 cm), Bangaloe dwarf (163.43 cm) and Papita selection (161.47 cm), Surya (159.50 cm), Mohini (158.16 cm), Pant papaya-1 (154.23 cm) and Red Lady (150.33 cm) were on par with each other in terms of average plant height. Variety Red Lady recorded highest fruit yield per plant (14.15 kg), maximum number of fruits per plant (15.67) and maximum average fruit weight (0.93 kg) among ten varieties despite severe infection (Plate 11). Red Lady was followed by Pusa dwarf (9.94 kg), Pusa nanha (9.41 kg), IHS selection-4 (8.06 kg) and Bangalore dwarf (7.96 kg) in per plant yield. On an average Red Lady produced 15.67 fruits per plant which was followed by Solo (14.00) Papita selection (12.67), IHS slection-4 (12.67) and Pusa nanha (12.33) and Pusa dwarf (11.67). Highest average fruit weight was recorded in Red Lady (0.93 kg) followed by Pusa dwarf (0.87 kg), Bangalore dwarf (0.72 kg) and Pusa nanha (0.77 kg). *C. cauliflora* produced very small fruits (50 g) which were not edible but remained completely free from infection (Plate 13).

Kudada and Prasad (2000) reported that factors such as number of fruits per plant, average fruit weight showed significant positive correlation with fruit yield per plant. Number of fruits per plant had maximum direct effect on fruit yield. Screening studies revealed that variety Red Lady was far superior in PRSV tolerance compared all other varieties as it was a heavy bearer, producing fairly good yield despite the infection. Pushpa (2014) reported that based on the average fruit yield per plant, the best level of tolerance was observed in Red Lady (22.54 kg per plant) as compared to other varieties. Tolerance of Red Lady was also evident from the reports of Crane *et al*. (1995) and Shaikh (1996). Reddy *et al*. (2011b) also reported that, among different varieties screened against PRSV, only Red Lady showed tolerance to disease when yield was taken into account.

4.4 Induction of resistance against PRSV

Red Lady has exhibited acceptable level of tolerance worldwide. In the present investigation an effort was made to test efficacy of bioextracts, seaweed extracts and

nanoparticles in increasing resistance level in variety Red Lady against PRSV under glasshouse and field conditions. The efficiency of treatments was assessed based on DAS-ELISA absorbance values compared to untreated control.

4.4.1 Assessment on efficacy of defence inducers on PRSV multiplication in papaya

Extracts of plants and seaweeds were reported to activate plant defence. Extracts of *Acorus calamus*, *Boerhavia diffusa*, *Capsicum frutoscens*, *Chenopodium amaranticolor* and *Datura metal* (Plate 14A-E) along with extract from *Kappaphycus alvarezii* (KH-1 %), *Eucheuma spinosum* (SH-1 %) and silicic acid were tested for their efficiency in inducing resistance against PRSV in papaya under glasshouse and field conditions. Plant extracts were freshly prepared just before the spray (Plate 14F) and required quantity was mixed with water.

4.4.1.1 Assessment on efficacy of defence inducers on PRSV multiplication in papaya under glasshouse condition

Different defence inducers were tested for their efficiency in inducing resistance against PRSV in papaya under glasshouse condition (Plate 15). Sixty days after mechanical inoculation, apical leaves of the papaya plants were subjected to DAS-ELISA and plants showing positive reaction were selected for the experiment (Table 12). Defense inducers were sprayed at ten days interval and at the end of the experiment apical leaves were subjected to DAS-ELISA.

Lowest absorbance value of 0.547 was recorded when plants sprayed with extract from *Boerhavia diffusa* which was on par with the plants sprayed with extract from *Acorus calamus* (0.572), KH from *Kappaphycus alvarezii* (0.570), silicic acid (0.588) and SH from *Eucheuma spinosum* (0.635). Spraying with extracts of *Datura metal* (0.675), *Chenopodium amaranticolor* (0.707) and *Capsicum frutoscens* (0.835) also recorded significant decrease in absorbance value when compared with control (1.472) indicating their inhibitory effect on the PRSV. Highest reduction in virus titre compared to initial value was observed when the plants were treated with *A. calamus* (43.15 %) followed by *B. diffusa* (35.45) silicic acid (35.18 %) and KH from *K. alvarezii* (32.49 %).

Table 12: Evaluation of bioextracts and seaweed extracts against PRSV in papaya variety Red Lady under glasshouse and filed conditions

Treatment No.	Treatment detail	Glasshouse condition			Field conditions	
		Average OD value at 405 nm		Per cent decrease (%)	Disease incidence (%)	Average OD value at 405 nm
		Before spray	After spray			
T_1	*Acorus calamus* (5 %)	1.015	0.572^{ab*}	43.15	100	0.422^{a}
T_2	*Boerhavia diffusa* (5 %)	0.885	0.547^{a}	35.45	100	0.417^{a}
T_3	*Capsicum frutoscens* (5 %)	1.005	0.835^{d}	16.20	100	0.672^{bc}
T_4	*Chenopodium amaranticolor* (5 %)	0.857	0.707^{c}	16.20	100	0.787^{cd}
T_5	*Datura metal* (5 %)	0.912	0.675^{bc}	25.84	100	0.655^{bc}
T_6	*Kappaphycus alvarezii* (KH-1 %)	0.867	0.570^{ab}	32.49	100	0.432^{a}
T_7	*Eucheuma spinosum* (SH-1 %)	0.857	0.635^{abc}	24.41	100	0.535^{ab}
T_8	Silicic acid (1 %)	0.947	0.588^{abc}	35.18	100	0.567^{ab}
T_9	Control (Water spray)	0.895	1.472^{e}	-71.03	100	0.857^{d}
	Healthy control	0.138	0.141			0.159
SEm±		0.079	0.048			0.075
CV		17.801	1213			20.395
CD (5 %)		N. S	0.129			0.172
CD (1 %)		N. S	0.177			0.242

*Means with same letter are not significantly different

A. *Acorus calamus* (rhizome)

B. *Boerhavia diffusa* (root)

C. *Capsicum frutoscens*

D. *Chenopodium amaranticolor*

E. *Datura metel*

F. Freshly prepared bioextracts

Plate 14: Plant extracts evaluated for induction of resistance against PRSV in papaya

Plate 15: Evaluation of bioextracts against PRSV under glasshouse condition

A. *Acorus calamus* (5 %)

B. *Boerhavia diffusa* (5 %)

C. *Kappaphycus alvarezii* (KH-1%)

D. Control

Plate 16: Effect of bioextracts against PRSV under glasshouse condition

4.4.1.2 Assessment on efficacy of defence inducers on PRSV multiplication in papaya under field conditions

An experiment was conducted to evaluate different defence inducing molecules to assess their efficacy against PRSV multiplication in papaya variety Red Lady under field conditions (Plate 17) and the results are presented in the Table 12. None of the treatments was effective in preventing PRSV infection at field. However, when titre values were compared with control (0.857) it was evident that spraying with *Boerhavia diffusa* (0.417), *Acorus calamus* (0.422), KH from *Kappaphycus alvarezii* (0.432), silicic acid (0.567) and SH from *Eucheuma spinosum* (0.535) significantly reduced absorbance value, indicating an inhibitory effect on virus. *Datura metal* (0.655) and *Capsicum frutoscens* (0.672) also significantly reduced absorbance value when compared with control (0.857). *Chenopodium amaranticolor* (0.787) was found to be not effective in reducing the virus titre at field conditions.

Viral diseases of plants are difficult to manage but may be minimized by some preventive measures like induced systemic resistance. Many higher plants are reported to contain endogenous proteins that act as virus inhibitors (Hansen, 1989; Chessin *et al.,* 1995; Parveen *et al.,* 2001 and Choudhary *et al.,* 2008). Antiviral properties present in the plants may be easily absorbed into the leaves on which it is sprayed and translocated in whole of the plant to induce the production or synthesis of some proteins which are actually antiviral and defend the plan against infection. Because of the plant origin, they have many other advantages over chemical as they are easily biodegradable do not leave any residue, eco-friendly, non phyto-toxic, easily absorbed by the plant and cheap.

Singh *et al.* (2011) reported that *Boerhavia diffusa* root extract was found to be significantly effective in inducing systematic resistance against PRSV in papaya. Glycoprotein from *B. diffusa* prevented 60-90 per cent of the virus infection of tomatoes, potatoes, pea and french bean (Awasthi and Mukerjee, 1980). The inhibitory effect of *B. diffusa* was attributed to the resistance inducer present, which activated systemic resistance against viruses (Verma and Singh 1994). Inhibitory action may be due to the blocking of host cell receptors or interference with virus multiplication in the host cells.

It may be hypothesized that virus inhibitor present in *Boerhavia diffusa* root extract, which was applied as foliar spray induced synthesis of translocable virus inhibitory or protective substances in papaya. The physiology of the host cell was affected in such way that the cells no longer supported virus multiplication. Verma *et al*. (1979) isolated a virus inhibiting glycoprotein from *B. diffusa* with a molecular weight of 16-20 kDa. When administered by foliar spraying in the field, this antiviral agent could protect economically important crops against natural infection by plant viruses. Awasthi and Menzel (1986) found that purified glycoprotein from *B. diffusa* exhibited strong antimicrobial activity against RNA (Ribonucleic acid) bacteriophages. Verma and Awasthi (1979) reported that when root, leaf, stem, flower and seed of *B. diffusa* were screened maximum antiviral activity was recorded with the aqueous extract of dried root powder applied before virus inoculation.

Several reports suggested that roots of *Boerhavia diffusa* were a rich source of a basic protein, which could be used for inducing systemic resistance in many susceptible plants against commonly occurring viruses (Verma and Awasthi, 1979; Verma *et al.,* 1979; Awasthi *et al.,* 1984 and Awasthi *et al.,* 1985). This antiviral agent was active against tobacco mosaic virus, sunnhemp rosette virus and gomphrena mosaic virus when applied a few hours (2-24 hours) before inoculation (Verma and Awasthi, 1979; and Awasthi *et al.,* 1984). Verma and Awasthi (1980) reported that following treatment with resistance inducing protein, the susceptible healthy host produced a virus inhibitory agent that reduced infectivity of the viruses both *in vitro* and *in vivo*. *B. diffusa* is found abundantly as a weed and if this plant could be used as an antiviral agent, the problem of PRSV management as well as weed management may be solved simultaneously with least financial input.

Khurana and Bhargava (1970) reported prevention of infection and spread of ringspot disease of papaya using leaf extracts of *Argemone mexicana*, *Carum capsicum* and seed extract of *Argemone mexicana*, *Datura fastuosa*, *Physachosia asnoris* and *Raphanus sativus*. Sharma *et al.* (2005) reported that commercial formulation Virex-H containing, water extract of *Acorus calamus*, *Dhatura metal*, *Boeharavia diffusa*,

Plate 17 : Evaluation of bioextracts against PRSV under field conditions

A. *Acorus calamus* (5 %)

B. *Boerhavia diffusa* (5 %)

C. *Kappaphycus alvarezii* (KH-1%)

D. Control

Plate 18: Effect of bioextracts against PRSV under field conditions

Capsicum frutoscens and *Chenopodium album* reduced severity of radish mosaic virus infecting radish.

A systemic resistance inducing protein was identified from *Clerodendrum aculeatum* against PRSV, which delayed development of symptoms of the disease. Vegetative growth of the treated plants was also significantly enhanced (Srivastava *et al.,* 2006). CAP-34, a protein from *Clerodendrum aculeatum* delayed appearance of symptoms of PRSV which could be due to suppressed virus replication (Srivastava *et al.,* 2009). Systemic antiviral resistance induced in papaya by CAP -34 was found to be associated with a proteinaceous virus inhibitory agent CP-VIA-34 (Srivastava *et al.,* 2015).

Plant extracts were found to be effective for the management of a number of viruses like brome mosaic virus (Picard *et al.,* 2005) mungbean yellow mosaic virus in mungbean and urdbean (Singh and Awasthi, 2006) yellow mosaic virus in mungbean and urdbean (Singh and Awasthi, 2009) tobamoviruses (Madhusudhan *et al.,* 2011) and watermelon mosaic virus in watermelon (Esam *et al.,* 2015).

The observed reduction in the virus titre may be attributed to defence inducing principles that were present in the plant extracts, which might have activated defence pathways. There is another possibility that they might have interfered with virus multiplication or movement.

Marine macroalgae and seaweeds are reported to contain a variety of unique polysaccharides (Kloareg and Quatrano, 1988) and some of these were shown to be potential sources of oligosaccharide elicitors of plant defence (Kobayashi *et al.,* 1993; Patier *et al.,* 1993; Klarzynski *et al.,* 2000 and Mercier *et al.,* 2001). Sulphated oligosaccharides from marine algae were found to stimulate plant defence responses and induce protection against plant pathogens including viruses (Klarzynski *et al.,* 2000; Klarzynski *et al.,* 2003; Laporte *et al.,* 2007 and Jimenez *et al.,* 2011).

Vera *et al.* (2011) reported that marine alga oligo-sulphated-galactan Poly-Ga induced a long-term protection against TMV in tobacco plants, mimicking a vaccination

effect. In plants binding of elicitors to specific receptors triggered a signal transduction cascade that activated defence genes, resulting in the synthesis and accumulation of compounds with potential antiviral activities (Zhang *et al.,* 2007). The existence of such receptors could explain the responses induced by the sulphated oligosaccharides in tobacco plants (Laporte *et al.,* 2007 and Vera *et al.,* 2011).

In plants as a response to elicitor-receptor interaction, activation of phenylalanine ammonia-lyase (PAL) and lipoxygenase (LOX) defence enzymes took place, which lead to the synthesis of phenylpropanoid compounds (PPCs) and oxylipins with antimicrobial activities (Blee, 2004 and Camera *et al.,* 2004). Vera *et al.* (2011) attributed involvement of phenylpropanoid pathway in the antiviral effect induced in tobacco plants by Poly-Ga from marine alga since increase in PAL activity showed a linear correlation with the decrease in necrotic lesions and decrease in TMV-CP transcript level.

There are several other reports of activation of enzymes involved in plant defence *viz.,* polyphenol oxidase, phenylalanine ammonia lyase, peroxidase, chitinase, glucanase after treatment with seaweed extracts or compounds derived from them (Laporte *et al.,* 2007; Vera *et al.,* 2011 and Ali *et al.,* 2016). Investigations on defense pathways have demonstrated the activation of salicylic acid and jasmonic acid/ethylene pathways involved in conferring resistance to pathogens (Klarzynski *et al.,* 2003 and Ali *et al.,* 2016). In one such case fucan oligosaccharides were shown to strongly stimulate both local and systemic resistance to tobacco mosaic virus in tobacco by the activation plant defence (Klarzynski *et al.,* 2003). Based on these evidences it may be hypothesized that extracts of seaweeds used in the present investigation *viz., Kappaphycus alvarezii* and *Eucheuma spinosum* activated the defence pathways in papaya which resulted in the significant reduction of virus titre.

The efficacy of silicic acid is in agreement with Pushpa (2014) who found that silicic acid (0.20 %) was effective in reducing the PRSV multiplication under field and glasshouse conditions. Ma and Yamaji (2006) reported that, the acquisition of silicic acid and its incorporation as silica offered an excellent possibility to plants to augment their defences. To build tailored silicaceous defence structures, plants take up silicon from the

soil in form of silicic acid $Si(OH)_4$ which is then translocated into the xylem from where it is transported to shoots to be deposited.

Considering the availability and performance of extracts of *Acorus calamus, Boerhavia diffusa* and *Kappaphycus alvarezii* both at glasshouse (Plate 16A-D) and field conditions (Plate 18A-D) they were included in the integrated management at field level.

4.4.2 Assessment on efficacy of nanoparticles on PRSV multiplication

Effect of nano silver, zinc and copper on the multiplication of PRSV in papaya was assessed in glasshouse and field conditions. Nano silver particles were synthesized by green synthesis process while zinc and copper nanoparticles were purchased.

4.4.2.1 Synthesis and characterization of silver nanoparticles

Silver nanoparticles were prepared by green synthesis process using neem (*Azadirachta indica*) leaf extract as a reducing agent and the formation of nanoparticles were confirmed different methods visual observation, UV-Vis spectroscopy analysis and dynamic light scattering.

4.4.2.1.1 Visual observation (colour change)

Silver nanoparticles were synthesized from 10^{-3} M $AgNO_3$ solution treated with neem leaf extract. Development of dark brown color was observed after 20 minutes (Plate 19). Similar color change was observed by Singh and Raja (2011) and Ahmad *et al*. (2015) after adding plant extract to silver nitrate solution. Silver nanoparticles exhibited brown color in aqueous solution due to excitation of surface plasmon vibrations (Krishnaraj *et al.,* 2010 and; Singh and Raja, 2011).

4.4.2.1.2 UV-Vis spectroscopy analysis

Formation and stability of silver nanoparticles (AgNPs) in aqueous solution was confirmed by UV-Vis spectral analysis. The spectra recorded from silver nanoparticles solution showed an absorption peak at 480 nm with the intensity of 3.00 that indicated the presence of scattered (not aggregated) nanoparticles in the solution (Fig. 5). Absorption

peak obtained was single and pointed, which indicated the presence of nanoparticles of uniform sizes. UV absorption peak of silver nanoparticles synthesized from bioextracts was observed at 400-480 nm by earlier workers (Mulvaney, 1996; Fayaz *et al.,* 2010; Li *et al.,* 2012; Devi *et al.,* 2013; Soni and Prakash, 2013; Ahmed *et al.,* 2016 and Haroon *et al.,* 2017).

4.4.2.1.3 Particle size analysis

The Dynamic Light Scattering (DLS) study provides the details about the particle size and dispersion *i.e.* monodispersed, or polydispersed and partricle size distribution of synthesized silver nanoparticles from $AgNO_3$ solution treated with neem leaf extract. It was observed that the silver nanoparticles synthesized were polydispersed mixtures in the range 60 to 100 nm. The average size of the silver nanoparticles in the solution was 80 nm (Fig. 6). In the data of DLS the obtained single peak indicated that the quality of synthesized silver nanoparticles was good (Mahl *et al.,* 2011).

Neem leaf extract was used as reducing and capping agent for the preparation of silver nanoparticles by Ahmed *et al*. (2016) which yielded nanoparticles with the average particle size of 34 nm. Similarly plant extracts of *Aloe vera* (Chandran *et al.,* 2006), Papaya fruit (Jain *et al.,* 2009), *Memecylon edule* (Elavazhagan and Arunachalam, 2011), Carob (Awwad *et al.,* 2013), olive leaf (Mostafa *et al.,* 2014) and *Hybanthus enneaspermus* (Haroon *et al.,* 2017) were successfully used for the production of silver nanoparticles.

Synthesis of silver nanoparticles using neem leaf extract in the present study was found to be simple and efficient. Chemical methods for nanoparticle synthesis usually involve toxic chemicals, which might be harmful to our environment. Although these methods successfully produced pure silver nanoparticles, they required the use of stabilizers. These methods were also found to be expensive and potentially harmful to the environment. Green synthesis is biocompatible, inexpensive, ecofriendly (Sadhasivum *et al*., 2010). In green synthesis, living organisms such as plants are used for the production of nanoparticles. The advantages of green syntheses over chemical and physical methods include eco friendly nature, cost effectiveness and it could be easily scaled up for large

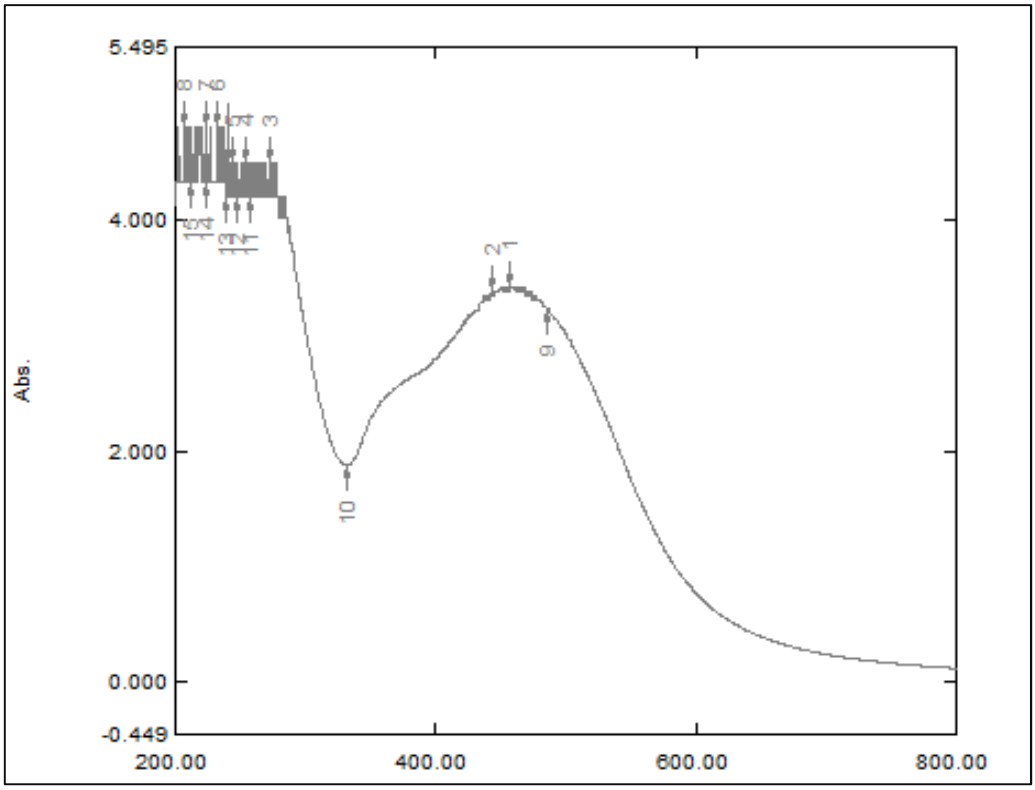


Figure 5: UV-Visible spectrum of silver nanoparticles synthesized from $AgNO_3$ solution treated with neem leaf extract

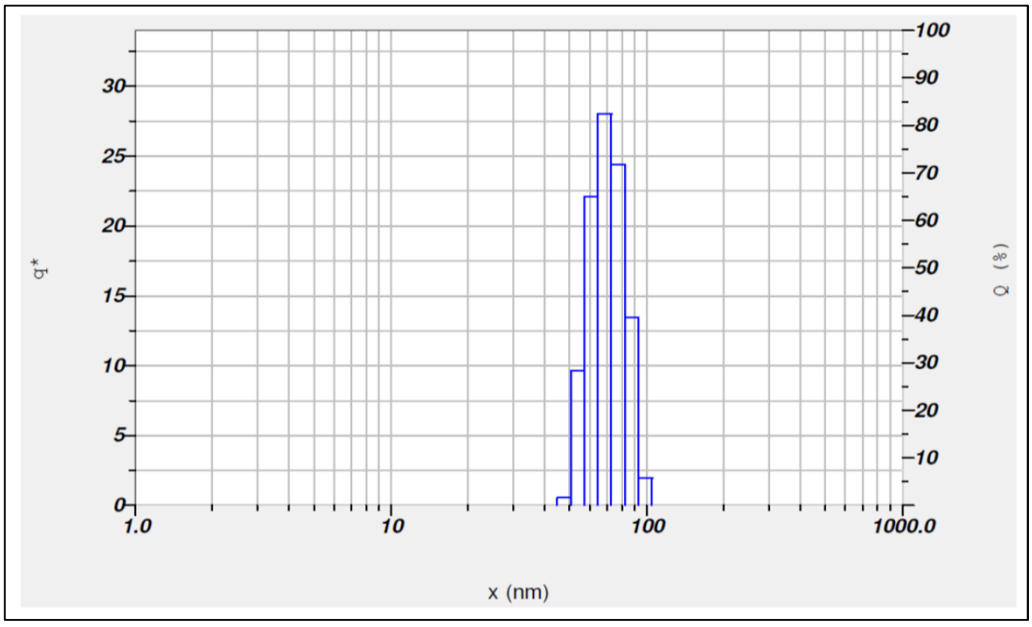


Figure 6: Dynamic Light Scattering (DLS) study of synthesized silver nanoparticles showing the size distribution

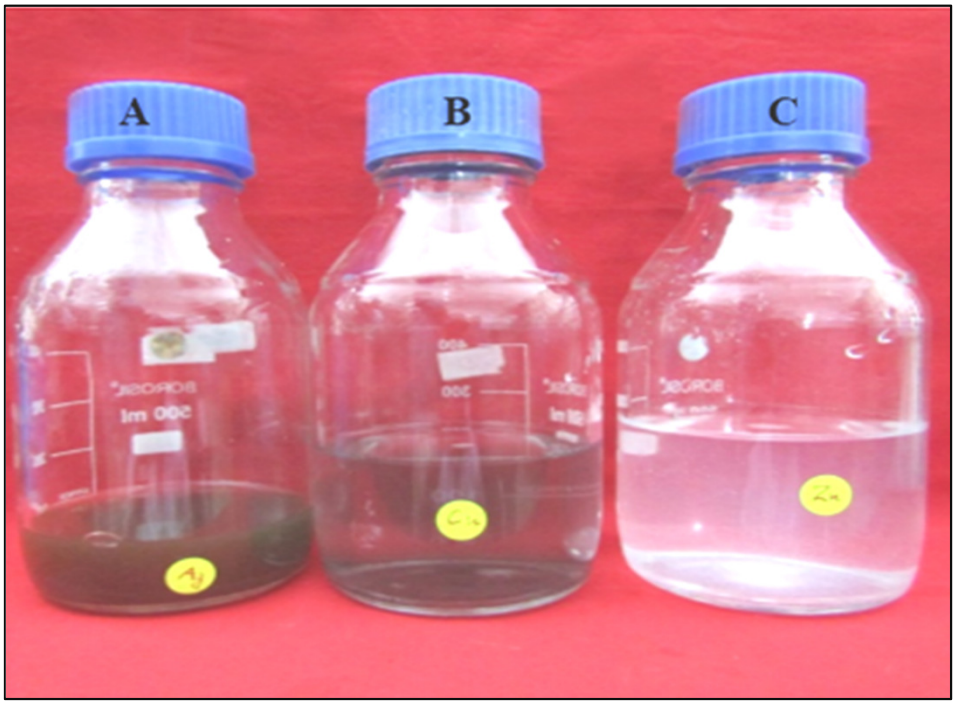


Plate 19: Development of dark brown color after the addition of neem leaf extract to $AgNO_3$ solution
Before addition and B) After addition

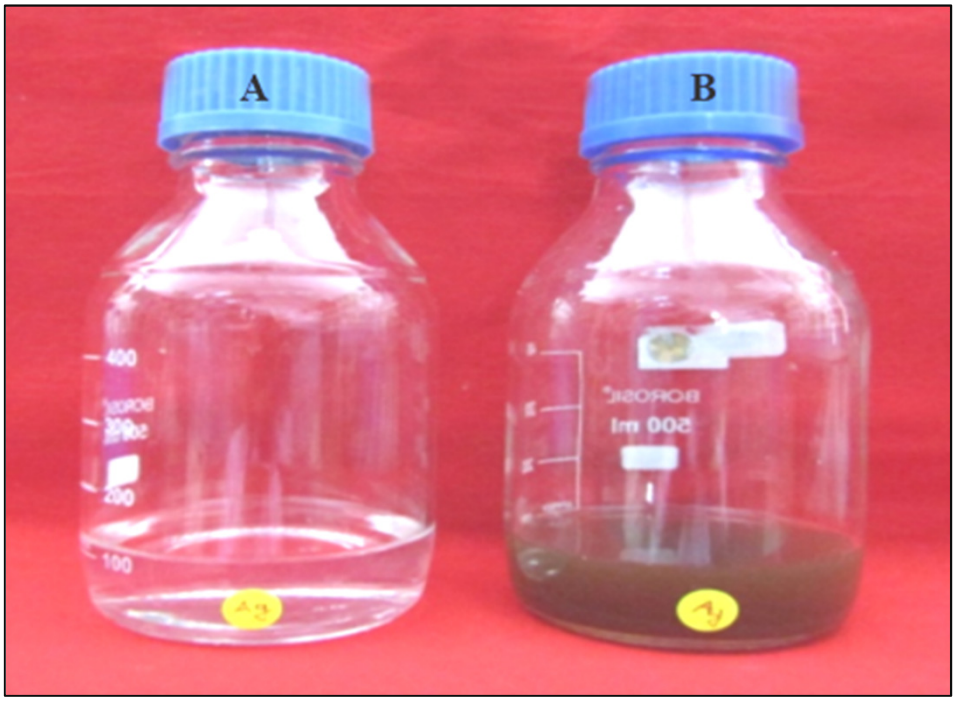


Plate 20: Freshly prepared nanoparticles solution
A) Silver B) Copper and C) Zinc

scale syntheses of nanoparticles, while there is no need to use high temperature, pressure, energy and toxic chemicals (Dhuper *et al.,* 2012).

Kalishwaralal *et al.* (2010) stated that use of plant extracts for synthesis of nanoparticles is advantageous over microorganisms due to the ease of improvement, less biohazard and there is no need of maintaining cell cultures. It is the best avenue for syntheses of nanoparticles; being free from toxic chemicals as well as providing natural capping agents for the stabilization of silver nanoparticles. Moreover, use of plant extracts also reduces the cost of isolation of micro-organisms and their culture media which enhance the cost competitive feasibility over nanoparticles synthesis by microorganisms. Present investigation confirmed that neem leaf extract is an excellent reducing agent for the production of silver nanoparticles which is in agreement with Ahmed *et al*. (2016).

4.4.2.2 Assessment on efficacy of nanoparticles on PRSV multiplication in papaya

Experiments were carried out to study the effect of different nanoparticles along with seaweed extract *Kappaphycus alvarezii* (LBS3-1 %) and extract from *Boerhavia diffusa* (5 %) against PRSV in papaya both under glasshouse and field conditions. Solutions were prepared by mixing required concentration of nanoparticles with water (Plate 20) and sprayed on the plants uniformly.

4.4.2.2.1 Assessment on efficacy of nanoparticles on PRSV multiplication in papaya under glasshouse condition

An experiment was carried out to assess the effect of nanoparticles on PRSV multiplication in papaya along with defence inducers in papaya variety Red Lady under glasshouse condition (Plate 21). Sixty days after sap inoculation apical leaves of the papaya plants were subjected to DAS-ELISA and plants showing positive reaction were selected for the experiment (Table 13). All the treatments were imposed at ten days interval and at the end of the experiment apical leaves were subjected to DAS-ELISA.

Table 13: Evaluation of nanoparticles and bioextracts against PRSV in papaya variety Red Lady under glasshouse and filed conditions

Treatment No.	Treatment detail	Glasshouse condition			Field conditions	
		Average OD value at 405 nm		Per cent decrease (%)	Disease incidence (%)	Virus titer
		Before spray	After spray			
T1	Silver nanoparticles (50 ppm)	0.865	0.645^{ab*}	23.08	100	0.702^{ab}
T2	Colloidal silver nanoparticles (50 ppm)	0.912	0.735^{b}	20.20	100	0.724^{bc}
T3	Colloidal silver nanoparticles (100 ppm)	0.812	0.612^{ab}	23.50	100	0.647^{ab}
T4	Nano cupric oxide (50 ppm)	0.847	0.165^{c}	-26.94	100	0.976^{de}
T5	Nano cupric oxide (100 ppm)	0.832	0.912^{c}	-15.21	100	0.957^{de}
T6	Nano zinc oxide (50 ppm)	0.775	0.954^{c}	-23.70	100	0.915^{cd}
T7	Nano zinc oxide (100 ppm)	0.962	0.117^{c}	-8.59	100	1.007^{de}
T8	*Kappaphycus alvarezii* (LBS3-1 %)	0.877	0.677^{ab}	21.16	100	0.727^{cd}
T9	*Boerhavia diffusa* (5 %)	0.913	0.575^{a}	367	100	0.512^{a}
T10	Control (Water Spray)	0.905	1.324^{d}	-47.21	100	1.132^{e}
	Healthy control	0.142	0.146			0.151
SEm±		0.084	0.061			0.080
CV		18.222	12.362			16.136
CD (5 %)		N. S	0.152			0.192
CD (1 %)		N. S	0.209			0.262

*Means with same letter are not significantly different

Plate 21: Evaluation of nanoparticles and bioextracts against PRSV under glasshouse condition

A. Nano silver (50 ppm)

B. Colloidal silver (100 ppm)

D. *Boerhavia diffusa* (5 %)

Control

Plate 22: Effect of nanoparticles and bioextracts against PRSV under glasshouse condition

After ten sprays *Boerhavia diffusa* (5 %) recorded lowest absorbance value of 0.575 compared to control (1.324) indicating inhibitory effect on the virus. Colloidal silver nanoparticles-100 ppm (0.612) nano silver-50 ppm (0.645), LBS3 (1 %) from *K. alvarezii* (0.677) were also found effective when compared to control. Highest reduction in virus titre was recorded when plants were treated with *B. diffusa* (36.07 %) followed by colloidal silver nanoparticles-100 ppm (23.50 %), silver nanoparticles-50 ppm (23.08) and LBS3 (1 %) from *K. alvarezii* (21.16 %). Data presented in Table 13 revealed that copper and zinc nanoparticles at 50 and 100 ppm failed to have significant impact on the PRSV and virus titre increased in these treatments compared to initial values.

4.4.2.2.2 Assessment on efficacy of nanoparticles on PRSV multiplication in papaya under field conditions

An experiment was carried out to assess effect of nanoparticles on PRSV multiplication in papaya along with bioextracts in papaya variety Red Lady under field conditions (Plate 23). At the end of the experiment apical leaves were subjected to DAS-ELISA and the results are presented in the Table 13.

All the treatments failed to prevent infection and 100 per cent disease incidence was observed. However, when DAS-ELISA values were compared it was revealed that highest absorbance value was recorded in control (1.132). Spraying with extract of *Boerhavia diffusa* (5 %) recorded lowest absorbance value of 0.512 followed by colloidal silver nanoparticles-100 ppm (0.647) and silver nanoparicles-50 ppm (0.702) indicating their inhibitory effect on PRSV. Colloidal silver nanoparticles-50 ppm (0.724) was found on par with colloidal silver nanoparticles-100 ppm (0.647) and silver nanoparicles-50 ppm (0.702). LBS3 (1%) from *Kappaphycus alvarezii* also significantly reduced virus titre (0.727). In consistent with the glasshouse studies copper and zinc nanoparticles at 50 and 100 ppm failed to reduce absorbance value at field level. *B. diffusa* (5 %), colloidal silver nanoparticles-100 ppm and silver nanoparicles-50 ppm were effective in significantly reducing the virus titre both at glasshouse (Plate 22) and field conditions (Plate 24).

The results of the present investigation are in agreement with Jain and Kothari (2014) who reported that spray application of 50 ppm aqueous solution of silver nanoparticles on cluster bean leaves inoculated with sunhemp rosette virus (SHRV) showed complete suppression of the disease.

Nanoparticles have at least one dimension in the range of 1 to 100 nm. When a solid material becomes very small, its specific surface area increases, which leads to an increase in the surface reactivity and quantum-related effects. Nanomaterials often show unique and considerably changed physical, chemical and biological properties compared to their counterparts at macro scale (Sharma *et al.,* 2009).

The antimicrobial properties of silver have been documented since 1000 B.C., when silver vessels were used to preserve water, but its applications in the field of agriculture have gained momentum very recently. Laudable efforts have been made to search antimicrobial property of silver nanoparticles against human pathogens, but insignificant research has been done to study its effects against devastating plant pathogens (Kim *et al.,* 2012). The present study was an attempt to assess the effect of nanoparticles on PRSV multiplication.

From the present investigation, it was revealed that silver nanoparticles were effective against PRSV. Guo *et al.* (2003) reported that among different nanomaterials developed (Silver, copper, zinc, titanium, magnesium, gold and alginate) silver nanoparticles proved to be most effective as they exhibited potent antimicrobial efficacy against bacteria, viruses and eukaryotic microorganisms. It has been reported that silver nanoparticles possesse unique properties such as chemical stability, good conductivity, catalytic and most important antibacterial, anti-viral, antifungal properties (Joerger *et al.,* 2001 and Ahmad *et al.,* 2003). Thomas and Mc Cubin (2003) reported that silver nanoparticles possessed high surface area and high fraction of surface atoms, have high antimicrobial effect as compared to the bulk silver. Silver in an ionic state exhibited high antimicrobial activity. Antibacterial, antifungal and antiviral effects nano silver particle had been reported in the past (Nomiya *et al*. 2004 and; Sondi and Salopek, 2004).

Plate 23: Evaluation of nanoparticles and bioextracts against PRSV under field conditions

A. Nano silver (50 ppm)

B. Colloidal silver (100 ppm)

C. *Boerhavia diffusa* (5 %)

D. Control

Plate 24: Effect of nanoparticles and bioextract against PRSV under field conditions

Interaction of silver particle with S-H groups has been implicated as the mode of action against pathogens (Thurmann and Gerba, 1989) while Kim *et al*. (1998) opinioned that powerful antimicrobial effect of silver was brought about by enzyme inactivation. Silver nanoparticles inhibited HIV-1 by binding to the disulfide bond regions of the CD_4 binding domain within the gp120 glycoprotein subunit (Elechiguerra *et al.,* 2005). It has been reported that silver nanoparticles and silver ions change the three dimensional structure of proteins by interfering with disulphide bonds and block the functional operations of the microorganism (Jia *et al.,* 2008; Rai *et al.,* 2009 and; Sadeghi and Gholamhoseinpoor, 2015). Based on the available evidence it may be hypothesized that silver nanoparticles interfered with PRSV multiplication in papaya which attributed to the reduction in virus titre.

When bulk silver is converted into nanoparticles, its effectiveness against viruses increased multifold, mainly because of the extremely large surface area possessed by nanoparticles compared to bulk silver that helped in increased contact with target pathogens. Silver is also an excellent plant growth stimulator and maximum number of patents are filed for 'nano silver' for preservation and treatment of diseases in agriculture field (Sharon *et al.,* 2010). The plant growth stimulation by silver might have also contributed to the recovery of papaya plants.

Several aspects of silver nanoparticles with relation to plants *viz.,* its half-life in soil and its toxicity effects on plants needs to be determined. There are some questions still remaining to be addressed, such as the exact mechanism of interaction of silver nanoparticles with viruses and how the surface area of nanoparticles influences killing activity.

There are also reports of deleterious efforts of silver on plants at high concentrations. Park *et al.* (2006) reported chemical injuries caused by a higher concentration of nanosized silica-silver on cucumber and pansy plant, when they were sprayed with a high concentration of 3200 ppm. Similarly, Patel *et al.* (2014) observed toxic effects of silver nanoemulsion on mung bean plants at higher concentrations. Thus,

before recommending the use of silver nanoparticles in agricultural fields, the toxic levels should be tested and an optimum concentration should be advised.

4.5 Integrated management of PRSV

Integrated disease management combines mechanical, cultural, chemical and biological methods to break away from relying on a single-technology and to adopt a more ecological approach (Plate 25). Four different integrated management modules along with control were evaluated at farmer's field. Based on the results obtained in glasshouse and filed studies, extracts of *Boerhavia diffusa* (5 %) and *Acorus calamus* (5 %) were included in the integrated management modules. Similarly, seaweed extract from *Kappaphycus alvarezii* (Proprietary formulation KH-1%) was included since it was found effective in glasshouse and field studies.

Four modules were evaluated at farmer's field. In all modules papaya seedlings were raised under insect proof mesh (40x mesh) to prevent early infection of PRSV. Module I comprised of maize (South African tall) as barrier crop and spraying with root extract of *Boerhavia diffusa* (5 %), *Kappaphycus alvarezii* extract (KH-1 %) and insecticide imidacloprid (0.05 %) at monthly intervals. Module II consisted of maize (South African tall) as barrier crop and spraying with extract of *A. calamus* (5 %), *K. alvarezii* (KH-1 %) and insecticide dimethoate (0.20 %). Module III was a combination of maize (South African tall) as barrier crop, silver reflective row mulch and spraying with extract of *A. calamus* (5 %), *K. alvarezii* (KH-1 %) and insecticide imidacloprid (0.05 %). Module IV comprised a combination of silver reflective row mulch and spraying with extract of *A. calamus* (5 %), *K. alvarezii* extract (KH-1 %) and insecticide imidacloprid (0.05 %). Control plants were raised with recommendation for general crop, without imposing any of the above treatments.

4.5.1.1 Effect of integrated management modules on the incidence of PRSV

The results of the experiment at farmer's field (Table 14 and Fig. 7) depict that integrated disease management modules delayed the incidence of PRSV. At 3 months

Table 14: Effect of different integrated disease management (IDM) modules on the incidence of PRSV

IDM Module	Disease incidence (%)			
	3 MAP	6 MAP	9 MAP	12 MAP
Module I	0.00 (0.82)[a]	8.25 (14.50)[a]	33.25 (35.13)[a]	87.50 (74.75)
Module II	0.00 (0.82)[a]	10.50 (16.48)[ab]	50.00 (45.00)[ab]	91.67 (80.57)
Module III	0.00 (0.82)[a]	4.00 (8.62)[a]	31.25 (33.87)[a]	72.92 (58.85)
Module IV	2.09 (4.73)[a]	25.00 (29.86)[b]	62.50 (52.64)[b]	91.67 (80.56)
Control	18.75 (25.19)[b]	60.50 (51.49)[c]	93.75 (79.39)[c]	100.00 (89.18)
SEm±	2.12	4.62	4.15	8.12
CV	65.32	38.2	16.9	18.41
CD (5 %)	6.52	14.25	12.81	N. S
CD (1 %)	9.15	19.97	17.97	N. S

Module I = Raising seedlings inside insect proof cage; Barrier crop of maize; Spraying with *Boerhavia diffusa* extract (5 %) + *Kappaphycus alvarezii* extract (KH-1 %) + imidacloprid (0.05 %) at 30 days interval

Module II = Raising seedlings inside insect proof cage; Barrier crop of maize; *A. calamus* extract (5 %) + *K. alvarezii* extract (KH-1 %) + dimethoate (0.20 %) at 30 days interval

Module III = Raising seedlings inside insect proof cage; Barrier crop of maize; Silver reflective row mulch; Spraying with *A. calamus* extract (5 %) + *K. alvarezii* extract (KH-1 %) + imidacloprid (0.05 %) at 30 days interval

Module IV = Raising seedlings inside insect proof cage; Priming with KH -1% ; Silver reflective row mulch; Spraying with *A. calamus* extract (5 %) + *K. alvarezii* extract (KH-1 %) + imidacloprid (0.05 %) at 30 days interval

Figures in the parenthesis are arcsine transformed values MAP: Months after planting

*Means with same letter are not significantly different

after planting (MAP) all the four modules recorded significantly lesser disease incidence compared to control (18.75 %). At 6 MAP lowest disease incidence was observed in Module III (4.00 %) which was on par with Module I (8.25 %) and Module II (10.50 %). Adoption of Module IV (25.00) also significantly reduced the incidence of PRSV compared to control (60.50 %).

Lowest disease incidence at 9 MAP was recorded in Module III (31.25 %) followed by Module I (33.25 %), Module II (50.00 %) and Module IV (62.50 %) while control recorded highest disease incidence (93.75 %). At 12 MAP lowest disease incidence was observed in Module III (72.92 %) followed by Module I (87.50 %). However, there were no significant differences among four modules and untreated control.

The results revealed that integrated management modules effectively reduced the incidence of PRSV (Fig. 8) This is in agreement with the findings of Pushpa (2014), who reported that growing papaya with South African tall (1:1) as live barrier was found effective, recording 60-90 per cent disease reduction and growing papaya with silver reflective mulch reduced the PRSV incidence by 90-100 per cent. Singh *et al*. (2010) reported that a combination of neem oil (1.00 %) + dimethoate (1.05 %) recorded least disease incidence 6.66 per cent and 41.66 per cent respectively at 60 and 150 days after planting. Significant disease reduction by adopting a combination of cultural, chemical and mechanical option has been reported by earlier workers (Sharma *et al.,* 2007 and Tenorio *et al.,* 2007). A technological package involving covering crops, manure application, transplanting 3-4 month old virus-free papaya plants, natural barriers and isolation distance of 200 m from other papaya fields delayed the virus infection by over 3 months, allowing a better commercial production (Tenorio *et al.,* 2007).

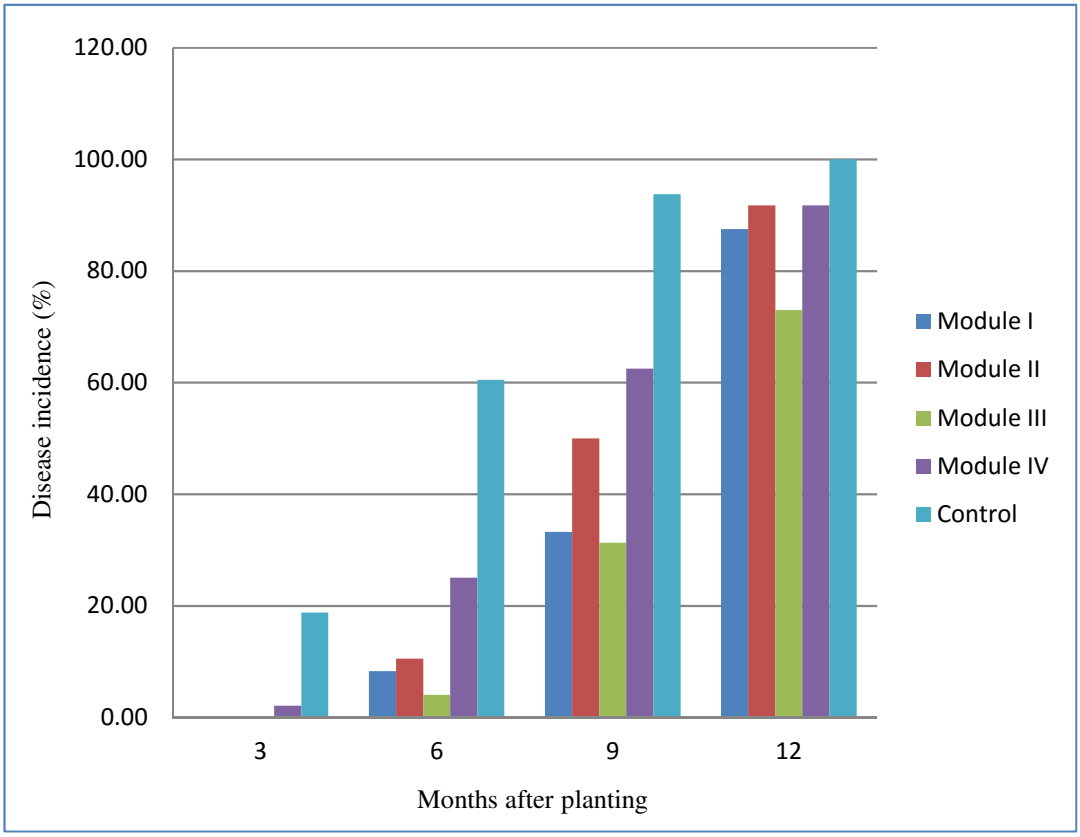


Figure 7: Effect of integrated disease management modules on the incidence of PRSV

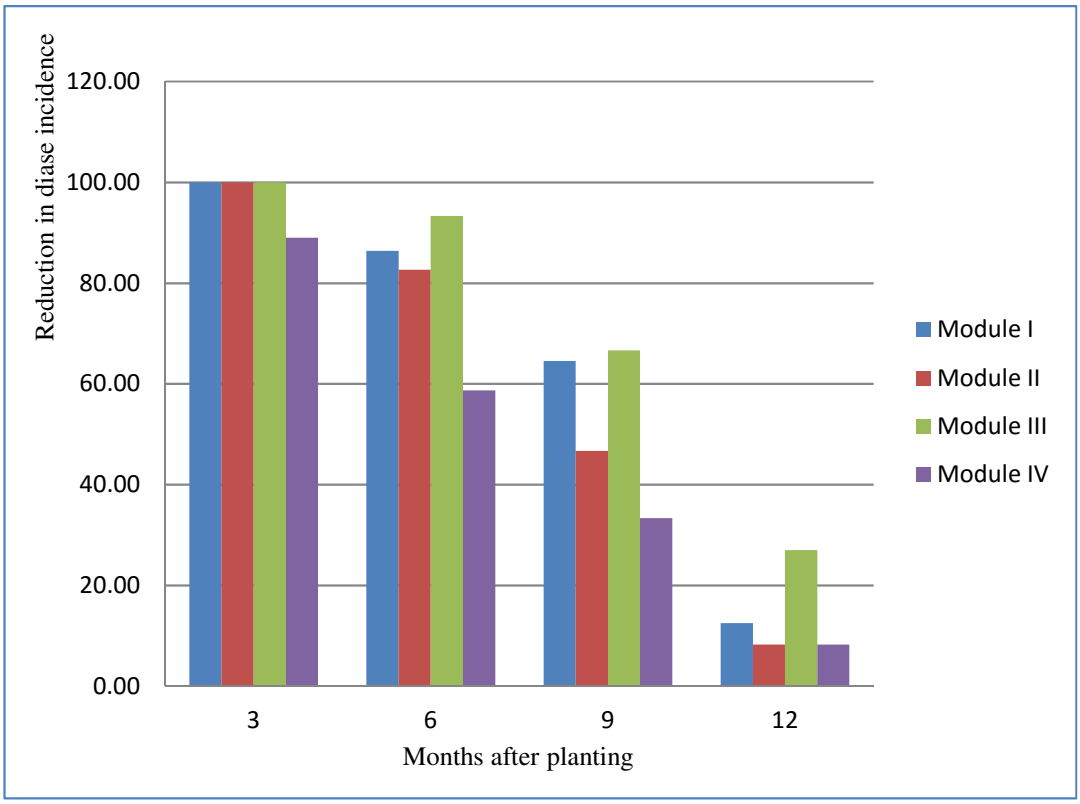


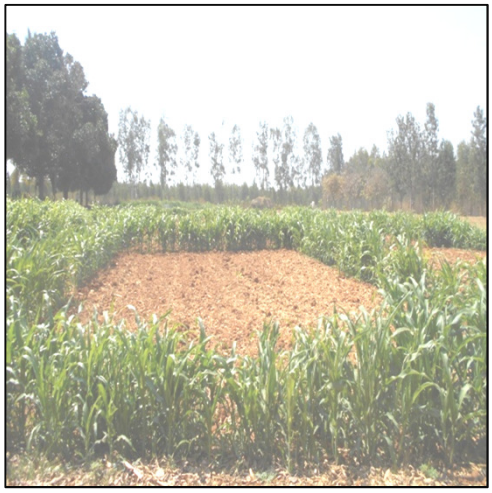
Figure 8: Reduction in disease incidence over control (%) in integrated disease management modules

A. Raising papaya seedlings inside insect proof cages

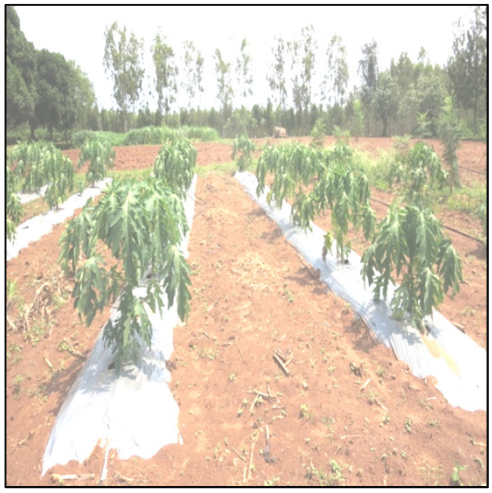
B. Use of silver reflective mulch row cover

C. Growing maize as barrier crop

D. Combination of silver reflective row mulch and barrier crop (Maize)



E. Barrier crop (Maize) sown before transplanting of papaya



F. Papaya plants grown with silver reflective mulch row cover

Plate 25: Components of IDM for PRSV management

4.5.1.2 Effect of integrated management modules on the yield and yield parameters of papaya

Total yield per plant (kg), plant height (cm), number of fruits per plant (no.) and fruit weight were recorded in each module at farmer's field. Highest yield per plant (28.68 kg) was recorded in Module III, followed by Module I (24.60 kg) and Module II (23.39 kg) that were on par with each other (Table 15). Module IV recorded 20.11 kg fruits per plant and performed on par with Module II (23.39 kg). Control recorded lowest per plant yield (11.83 kg).

Out of four modules, Module III recorded highest average plant height (143.94 cm). This was followed by Module I (133.91 cm) and Module II (132.16 cm) which were on par with each other. Module IV recorded average plant height of 120.60 cm while in control average plant height was 88.32 cm.

When average number of fruits per plant was considered Module III recorded maximum of 15.09 fruits per plant. Module I and module II were on par with each other by recording 13.25 and 13.24 fruits per plant followed by Module IV (13.33). In control plots, plants on an average produced 10.50 fruits per plant which was the least.

Average fruit weight was highest in Module III (1.93 kg) which was on par with Module I (1.92 kg) followed by Module II (1.74 kg). In Module IV average fruit weight was 1.50 kg while control recorded lowest average fruit weight of 1.09 kg.

Adoption of IDM Module III resulted in 142.39 per cent increase in yield over control which was followed by Module I (107.90 %), Module II (97.72 %) and Module IV (69.99 %). When yield and yield parameters were considered it was evident that all four modules performed significantly superior to control (Plate 26). Out of four modules, Module III, a combination of barrier crop and silver reflective mulch along with sprays of bioextracts, recorded highest fruit yield per plant with highest plant height, average number of fruits and average fruit weight and found significantly superior to all other modules. Pushpa (2014) also reported that on PRSV disease management, growing papaya with South African tall (1:1) as live barrier recorded maximum yield in Red Lady (33.28 kg per plant), while growing papaya with silver reflective mulch recorded fruit yield of 24.64 kg per plant.

Table 15: Effect of different integrated disease management (IDM) modules on yield and yield parameters of papaya

IDM Module	Average fruit yield/plant (kg)	Plant height (cm)	Average number of fruits/plant	Average fruit weight (kg)	Increase in yield over control (%)	Benefit cost ratio
Module I	24.60^{b*}	133.91^{b}	13.25^{b}	1.92^{a}	107.90	1.81:1
Module II	23.39^{bc}	132.16^{b}	13.24^{b}	1.74^{b}	97.72	1.66:1
Module III	28.68^{a}	143.94^{a}	159^{a}	1.93^{a}	142.39	1.91:1
Module IV	20.11^{c}	120.60^{c}	13.33^{b}	1.50^{c}	69.99	1.71:1
Control	11.83^{d}	88.32^{d}	10.50^{c}	19^{d}	-	1.32:1
SEm±	1.48	3.54	0.34	0.05		
CV	11.87	4.96	4.49	5.44		
CD (5 %)	3.95	9.44	0.91	0.19		
CD (1 %)	5.54	13.24	1.26	0.14		

Module I = Raising seedlings inside insect proof cage; Barrier crop of maize; Spraying with *Boerhavia diffusa* extract (5 %) + *Kappaphycus alvarezii* extract (KH-1 %) + imidacloprid (0.05 %) at 30 days interval

Module II = Raising seedlings inside insect proof cage; Barrier crop of maize; *A. calamus* extract (5 %) + *K. alvarezii* extract (KH-1 %) + dimethoate (0.20 %) at 30 days interval

Module III = Raising seedlings inside insect proof cage; Barrier crop of maize; Silver reflective row mulch; Spraying with *A. calamus* extract (5 %) + *K. alvarezii* extract (KH-1 %) + imidacloprid (05 %) at 30 days interval

Module IV = Raising seedlings inside insect proof cage; Priming with KH -1%; Silver reflective row mulch; Spraying with *A. calamus* extract (5 %) + *K. alvarezii* extract (KH-1 %) + imidacloprid (0.05 %) at 30 days interval

*Means with same letter are not significantly different

A. Module I

B. Module II

C. Module III

D. Module IV

E. Control

Plate 26: Effect of different integrated disease management modules

All four integrated management modules effectively delayed the incidence of PRSV, which resulted in better yields compared to control. Tenorio *et al.* (2007) reported that delayed virus infection by over 3 months, allowed a better commercial production. Similarly, Kalleshwaraswamy *et al*. (2009) reported that delayed PRSV infection resulted in significantly higher number of marketable papaya fruits per plant. Once plants were infected with PRSV, disease started spreading rapidly in the field. PRSV infection resulted in stunted growth and plants started producing small malformed fruits unfit for marketing.

Integrated disease management exploits several feasible options like mechanical, cultural, chemical and biological which act synergistically or complementary to each other. Effects of each component are briefed below.

4.5.2.1 Raising of papaya seedlings inside insect proof cage

One way to manage PRSV is to avoid the physical contact between aphids and plants. Landing of aphids on papaya crop could be prevented by the use of physical barriers. Insect-proof nets significantly reduced virus incidence and the need for insecticide applications. In Taiwan, papaya was protected from aphid vectors by insect-exclusion screening netting (IES-screen barrier) and was effective in producing marketable fruits, because late infection that occurred when the net was removed after fruiting resulted in little damage to the fruit yield (Kiritani and Su, 1999). Covering papaya crop with aphid proof netting enabled economic production of papaya for at least one season in Taiwan (Ray *et al.,* 1999). Netting of papaya to prevent aphid landing till fruiting is a practiced in Taiwan for the management of PRSV (Verghese *et al.,* 2007).

Raccah and Fereres (2009) reported that camouflaging nets greatly reduced insect landing and also virus infection. Kumar *et al.* (2010) also observed the delayed appearance of PRSV in papaya raised inside nylon net barrier compared to open field. Escape from early infection avoided severe reduction in yield and help to attain marketable fruits. Seedlings supplied by the nurseries may carry infection which may

result in early infection and rapid spread of PRSV. This could be avoided by raising seedlings inside insect proof cages.

4.5.2.2 Growing maize (South African tall) as live barrier

Barrier crops block aphids from reaching the target plant and thereby reduce virus transmission (Difonzo *et al.,* 1996; Fereres, 2000; Osakabe and Kenichiro, 2002). Growing papaya in the middle of barrier crops reduced the spread of viruses by avoiding invasion of vector on main crop (Kumar *et al.,* 2010). Hooks and Fereres (2006) stated that flora diversification reduced the incidence of a number of viruses transmitted by aphids. While selecting a border crop it should be kept in mind that the border crop should not be primary or secondary hosts of virus disease of main crop and vectors transmitting the disease. Otherwise, barrier crop would act as breeding ground for vectors. Therefore, maize was recommended as border crops (Chavan *et al.,* 2010).

Kalleshwaraswamy and Kumar (2008) stated that monoculturing of papaya should be avoided by planting papaya as an intercrop or mixed crop which could be one of the options for minimizing PRSV incidence disease prone places. Many synergistic and complementary roles are played by border crop in papaya. It forms a physical barrier between vectors and papaya plants. It moderates microclimate of the garden by functioning as a buffer for hot and cold air during summer and winters. It also functions as windbreak and prevents the uprooting of papaya plants under storms (Sharma *et al.,* 2010).

Prasad and Kudada (2005) reported that that intercropped maize barriers had a reducing effect on the incidence and final severity of PRSV in Bihar. Similarly, the practice of raising barrier crops (like maize) to obstruct aphid migrants was recommended by Verghese *et al*. (2007). Transmission efficacy of the aphid vectors got reduced when border crops like maize or sorghum were raised in papaya orchards (Singh *et al.,* 2010). Mederos *et al*. (2013) also demonstrated that the intercropped corn (*Zea mays* L.) in papaya plantations reduced the damages caused by PRSV in endemic areas.

Border crop of banana reduced aphid-population significantly as reported by Sharma *et al.* (2010). Recently, Pushpa (2014) reported that growing papaya as intercrop with South African tall (1:1) and Grand naine banana (2:1) as live barriers were found effective recording 60-90 per cent disease reduction.

It is evident that non-host barrier crop of maize minimized the spread of viruses by avoiding influx of vector population on main crop. Since South African tall can attain 9 feet height, it can be recommended as border crop with popular dwarf and semi dwarf varieties like Red Lady (3-5 feet) and Arka Surya (5-7 feet), but may not be effective for tall varieties like Sunrise solo which grows beyond 8 feet.

The problem with choosing South African tall as a border crop is that sowing has to be repeated every two months as staggered sowing, since the crop is completed in 100-120 days. Repeated sowing of maize every two month to maintain barrier is a tedious and cumbersome process for the farmers. This problem may be effectively addressed by the use of multicut fodder grasses which grow taller.

4.5.2.3 Effect of growing papaya plants with silver reflective row mulch

A number of studies on the role of reflective or repelling surface aluminum or plastic mulches were found to be effective against aphids, the vectors transmitting PRSV. Aphids react differently to various wave lengths of light, hence the exploitation of attractive colors as traps or repellents to avoid landing of the vector on susceptible crops, is advantageous in minimizing the spread and incidence of PRSV.

Reflective mulches have been used in several studies to reduce incidence of viruses transmitted by aphids in non-persistent manner in the field. Moericke (1954) reported that white surfaces reflecting ultraviolet or short wave light was found to be unattractive to descending aphids and were even avoided by them. Similarly, Kring (1964) reported that short wavelength radiation repelled aphids after a dispersal flight. After flying for varying periods, aphids enter the searching phase when they are repelled by the short wave light of the sky and are attracted to long wave light reflected from plants. At this point they descend, flying close to the ground. Mulches used in between

the rows reflect ultraviolet rays which the aphids perceive instead of blue-green light of the plants. As a result, aphids sense a signal to continue flying instead of descending and they fly further. Utilization of this principle in virus disease management is supported by the works of Moericke (1954), Kring (1964) and Cartier (1966). Using this principle Vani *et al.* (1989) effectively minimized the mosaic incidence in muskmelon using mulches, at New Delhi. Of late aphid-transmitted mosaic virus diseases were successfully reduced in cucurbits using white reflective mulches in South Africa (Cradock *et al.,* 2001).

Moericke (1954) noticed that when yellow pans were set on bare soil or on cotton cloths of different colors, aphid response varied according to species. Increased attractiveness to some colours was due to increased reflection of UV light from the surfaces. Repelling of aphids was observed by Kring (1964) when the aluminium pans were placed around yellow ones. Jones and Chapman (1968) found that yellow plastic sheets were most attractive to aphids followed by pink, green, red and black respectively, whereas white, orange, light blue, aluminum foil and dark blue attracted the fewest aphids. James *et al*. (1993) recorded that compared to other colors of plastic mulches; silver reflective mulch was superior in reducing aphid populations. It could be hypothesized that silver reflective mulch used in the present effectively repelled the vectors till the plant canopy masked the effect of silver mulches.

Results of the present investigation agree with the report of Summers *et al.* (2004) who compared the efficiency of spray mulches, film mulches and nets, in protecting zucchini squash from aphid-borne viruses transmitted in non persistent manner. Mulching was superior to netting because it covered more than 60 per cent of soil surface. Silver mulches were much more effective in repelling aphids than other colors (white, brown, green and black). It was also found that plants protected by silver mulches got infected in later stages when canopy cover significantly decreased the efficiency of mulches in repelling aphids. Infection at later stages is less deleterious than taking infection at early stage which may hamper production of marketable fruits. There are reports that reflective row covers and floating row covers delay the appearance of virus disease in plants by excluding or repelling the aphids by reflecting UV light (Santos *et al.,* 1995 and Gonsalves *et al.,* 2010).

It has been reported that aluminum mulches which reflect UV rays, effectively to minimized the incidence of aphid-borne viral diseases in squash and other crops (Brown *et al.,* 1993 and; Summers and Stapleton 1999; Stapleton and Summers 2002). These mulches reflected short-wave UV light, which confused and repelled incoming aphids thus minimizing their incidence of alighting on plants. In crops like bell pepper, tomato, squash, cucumber, lettuce and beet root, efforts were made to reduce the incidence of viral diseases by the use of aluminum mulches. These mulches were effective at least against twelve species of aphids (Smith *et al.,* 1964). Pushpa (2014) reported that *Aphis gossypii*, *A. craccivora* and *Myzus persicae* were transmitting PRSV throughout the year.

Insects like aphids are repelled from reflective surfaces and this effect is exploited in the use of metallic reflective surfaces. Stapleton and Summers (2002) observed that in late season cantaloupe, under conditions of high aphid populations and virus inoculum potential, the aphid numbers on leaves of plants growing over mulches were consistently lower than on those growing over bare soil. Onset of symptoms of cucumber mosaic cucumovirus, watermelon mosaic and zucchini yellow mosaic potyviruses were delayed 3-6 weeks in plants growing over the mulches. At least 9.5 fold increase in marketable yield was obtained using polyethylene mulch that totally covered planting bed. Due to increased reflection of shorter wavelength from silver reflective mulch used in the present investigation, aphids might have got repelled off from papaya resulting lesser disease incidence.

There are also reports that mulches were unsuccessful in protecting plants from infection when i) Very little mulch was used (Dickson and Laird, 1966; Hakkaart, 1967 and Rotham, 1967) ii) Number of vectors were relatively high (Rotham, 1967 and George and Kring, 1971) iii) the plants grew taller than the defensive border of the mulch (Hakkaart 1967) iv) Reflective surfaces were masked by the plant coverage before virus infection (Adlerz and Everett, 1968). UV reflectance of mulches was reduced due to application of certain fungicides and other plant protection chemicals which resulted in reduced the efficacy of the mulch. Reitz *et al.* (2003) reported that a single application of copper or mancozeb fungicide reduced the reflectance by approximately 49 per cent. The increased canopy coverage masking the reflectance of silver reflective mulch during later

stages, may be attributed to the higher incidence of PRSV during later stages of the papaya crop.

4.5.2.4 Effect of insecticides

Insecticides were reported to be successful in the management of aphidborne non-persistent viruses (Atiri *et al.,* 1987; Perring *et al.,* 1999 and Martin *et al.,* 2004). The reduced transmission by vectors was due to changes in the behaviour of aphids by prolonging the time taken for initiation of the probe on insecticide treated plants (Pirone and Harris, 1977). Devine *et al*. (1996) observed that behaviour of *Myzus nicotianae* and *M. persicae* was affected by systemically applied imidacloprid ensuing behavioural effect. Nauen (1995) also reported that imidacloprid prolonged the pre-probing period of *M. persicae* leading to reduced transmission and similar observations were made on *A. gossypii* by Kalleshwaraswamy *et al*. (2012). Sixty per cent inhibition in PRSV transmission was recorded when plants were treated with imidacloprid (Gajbhiye *et al.,* 2004). Effectiveness of imidacloprid in reducing PRSV incidence is also revealed from the studies carried out in India, by Kalleshwaraswamy *et al.* (2009).

Insecticides are known to induce behavioural changes in aphids during host selection, labial dabbing, test probing and also on duration of probing. These alterations of aphid/insect interface formed the basis for the management of many non-persistent viruses when insecticides were used. Different combination of insecticides differed in their effectiveness in delaying PRSV infection. Verghese *et al*. (2007) reported that systemic insecticide dimethoate (0.20 %) gave the best result of 60 per cent reduction and delayed PRSV infection. Singh *et al*. (2010) also found that neem oil (1.0 %) + dimethoate (1.05 %) was effective for management of PRSV in papaya. Dimethoate reduced the chances of spread of disease either by its repelling or killing action on aphid. Effectiveness of dimethoate is also evident from the works of and Datar (2012). The application of insecticides might have resulted in the prevention of rapid spread in the IDM modules.

There are reports of application of insecticides alone failing to manage the disease. Studies of the Kunkalikar (2003) revealed that none of the pesticides *viz*., dimethoate, acetamiprid, malathion, imidachloprid, acephate, trizophos, monocrotophos and endosulufon could check the spread of the PRSV disease. Kalleshwaraswamy *et al*. (2009) stated that failure of insecticide may be due to non-persistent nature of aphid vectors. Pesticides were effective in killing the aphids but they could not prevent few proboscis, the aphids make before the pesticides act. Hence virus gets transmitted during proboscis and disease spread continues (Hemavathi, 2005).

4.5.2.5 Integrated management of PRSV under field conditions

Shikhamany (2004) pointed out that among various constraints *viz*., lack of quality seed, use of inferior planting materials and lack of awareness on improved production technologies, viral diseases and poor disease management practices were the major factors responsible for low productivity of papaya. Productivity could be improved by at least 50 per cent if PRSV is managed. In the present invetigation a combination of different treatments was evaluated for the management of PRSV under field conditions and results indicated that integrated disease management modules significantly increased the production of marketable fruits which is in agreement with the earlier reports mentioned below.

By adapting integrated crop management practices in Mexico, Revilla *et al.* (1995) showed that papaya yield could be increased. These strategies included Seedbeds covered with an insect proof polypropylene mesh, High density papaya plantings (2222 plants ha^{-1}) which allowed roguing of diseased plants, foliage and soil nutrients to improve plant vigor, poisoned plant barrier (Two lines of *Zea mays* L. and *Hibiscus sabdariffa* L.), Two plastic strips (5 cm wide and with a shiny gray-metallic color above each papaya row of plants), Biweekly sprays with mineral oil (1.50 %). These measures were only effective in regions where disease pressure was low.

Sharma *et al.* (2007) reported that use of Good Agricultural Practices (GAPs) which involved selection of the variety (Red Lady) that produced better yield, use of virus-free seedlings, planting a new papaya plantation away from other host plants like

cucurbits and infected papaya plantation, rouging infected plants early, growing a border crop around papaya plantation, and adjusting the season of transplanting was a viable option for management of PRSV. It was found that by transplanting papaya during the lean period (spring season) PRSV infection could be delayed till monsoon, by that time plants have crossed the fruit bearing stage.

Tenorio *et al.* (2007) tried a technological package to manage PRSV (covering crops, manure application, and transplanting 3-4 month old virus-free papaya plants, natural barriers, isolation distance of 200 m from other papaya fields. Use of the technological package delayed the virus infection by over 3 months, allowing a better commercial production. Singh *et al*. (2010) reported that an integrated approach involving application of neem oil (1.0 %) + dimethoate (1.0 %) was effective in minimizing PRSV disease incidence.

A combination of reflective row cover, mineral oil (0.10 %) and imidacloprid (0.0053 % alternate fortnightly) spray was recorded as the most effective treatment in delaying the PRSV infection and produced significantly higher number of marketable papaya fruits per plant (Kalleshwaraswamy *et al.,* 2009). Datar (2012) also reported that integrated approach of growing papaya plants with maize as a border crop, spraying with dimethoate (0.05 %) and azadirachtin (0.06 %) fortnightly interval recorded significantly least number of aphids per plant and minimum incidence of PRSV.

Pinese *et al.* (1994) evaluated plastic mulches and mineral oil plus insecticide sprays for management of PRSV-W in zucchini (*Cucurbita pepo*) in Queensland, Australia and found that mulches with a reflective (silver) surface minimized losses by reducing aphid populations.

An integrated approach towards PRSV management could be the best option for successful cultivation of papaya under PRSV infected regimes till PRSV resistant varieties are released. Different components of integrated management are complementary to each other with strong interaction among and between them and the environment. The barrier crop of maize initially hinders the entry of alate aphids inside the main crop of papaya. Sprays of insecticides further reduce the chances of spread of

disease either by its repelling or killing action (Datar, 2012). Reflective mulches repel aphid vectors and also help in the fast growth of papaya plants probably suppressing the growth of weeds and minimizing competition. Application of plant extracts and seaweed extracts might trigger plant defence system or interfere with virus multiplication and movement.

Since PRSV is transmitted by aphids in nonpersistent manner it may be difficult to get benefits from integrated management at later stages of plant growth when disease pressure builds up. Revilla *et al.* (1995) reported that integrated crop management practices against PRSV were effective only in regions where disease pressure was low.

4.5.3 Benefit cost ratio

Papaya is a perennial crop capable of surviving up to twenty years, but infection by PRSV drastically reduces yield and it becomes extremely difficult and uneconomical to maintain the orchard (Mossler and Crane, 2009). The costs and returns associated with the first year of production involved in adopting integrated management approach against PRSV were calculated and presented as benefit-cost ratio over one year period (Table 15)

Highest returns per rupee invested were recorded in IDM Module III at field (1.91). The damage caused by PRSV was evident in control plots as it recorded least returns per rupee invested (1.32). Adoption of integrated management Modules I, II and IV also increased income as evident from the benefit cost ratio. At farmer's field adoption of Modules I, IV and II resulted in benefit cost ratio of 1.81, 1.71 and 1.66 respectively.

In PRSV disease management studies, growing papaya as intercrop with South African tall maize (1:1) as live barrier recorded maximum yield in varieties Arka surya (15.78kg per plant) and Red Lady (33.28 kg per plant) with benefit cost ratio (B:C ratio) of 3:1 in Surya and 9.2:1 in Red Lady respectively. Growing papaya with silver reflective mulch was recorded as next most profitable treatment with B:C ratio of 1.9:1 and 6.2:1 disease in Arka surya and Red Lady respectively (Pushpa, 2014). Migliaccio *et al.* (2010) reported that growing PRSV tolerant varieties farmers could increase their output three to

fourfold and extend the harvesting season by another year to compete and be profitable. Present investigation revealed that adoption of integrated management approach delayed the appearance of PRSV disease and helped in escape of early infection which resulted in the production of profitable yield.

VI SUMMARY

India is one of the leading producers of papaya in the world. At present papaya cultivation worldwide is threatened by papaya ringspot virus (PRSV), one of the devastating diseases of papaya. In order to develop a solution for the PRSV disease menace, the present investigation "Molecular variability and integrated management of Papaya Ringspot Virus (PRSV)" was attempted. Investigations were carried out to study the variability among isolates based on P1 proteinase gene and coat protein gene sequences, screening papaya varieties for PRSV resistance, to induce resistance through application of bioextracts and to develop strategies for integrated management practices. The results of various investigations undertaken in the present investigation are summarized as below.

During 2014-15, a survey was conducted in Southern states of India *viz.,* Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and Telangana. Survey revealed that, the incidence of PRSV in different places showed wide range of symptoms. Naturally infected papaya plants were found associated with symptoms of mosaic, chlorosis, green islands, leaf distortion, mottling and shoe strings on leaves. Grey oily streaks on petiole/stem, ringspots on fruits, deformed fruits and stunted growth of plants were also seen in advanced stages.

Incidence of PRSV ranged from 0 to 100 per cent at different locations. In Karnataka maximum of 100 per cent disease incidence was observed at some locations of Bangalore rural and Bangalore urban. Highest average disease incidence was noticed in Ramanagara district (82 %). Minimum PRSV incidence (0 %) was recorded in Kodagu district, followed by 4 per cent disease incidence in Raichur. Incidence of different districts varied considerably; Chikkaballapura (67 %), Dharwad (70 %), Hassan (48 %), Mysore (40 %), and Vijayapura (47 %), In Andhra Pradesh, Kadapa recorded average disease incidence of 70 per cent while in Chittoor 42 per cent incidence of PRSV was observed. In Telangana, low incidence of PRSV (24 %) was observed in Hyderabad. In Kerala PRSV incidence was highest in Kottayam (77 %) followed by Trivandrum (51 %) and Ernakulam (48 %). In Tamil Nadu PRSV incidence was found to be minimum in

Selam (36 %) and Nagapattinam (40 %) while Coimbatore (70 %) and Chennai (56 %) recorded relatively higher incidence

None of the isolates collected during survey produced mild symptoms on variety Red Lady. All the nine isolates recorded severe mosaic, puckering, shoestring, leaf distortion and stunted growth. The partial characterization of PRSV-BLR isolate using primer pair PRSV1PF/ PRSV1PR, recorded highest nucleotide identity of 88 per cent with PRSV isolate from Hyderabad (Accession KP743981) and 87 per cent identity with PRSV-P isolate from Delhi (EF017707).

Part of P1 proteinase gene of four PRSV isolates *viz.,* PRSV-BLR, PRSV-CBE, PRSV-EKM and PRSV-TPT was amplified using primer pair PRSV2PF/PRSV2PR. PRSV-BLR isolate showed highest nucleotide identity of 87 per cent with PRSV isolate from Hyderabad (Accession KP743981) and PRSV-P isolate from Delhi (EF017707). PRSV-CBE isolate showed maximum nucleotide identity of 87 per cent with PRSV isolate from Hyderabad (KP743981). PRSV-EKM isolate showed 86 % nucleotide identity with PRSV isolate from Hyderabad (Accession KP743981) followed by 82 per cent identity PRSV-W-C (DQ374152) and PRSV E2 (KC345609). While PRSV-TPT isolate from Andhra Pradesh showed 86 per cent identity with PRSV isolate from Hyderabad (KP743981) and 85 per cent identity with PRSV-P isolate from Delhi (EF017707). On the whole, the identity of South Indian isolates ranged from 87 to 72 per cent with sequences of PRSV deposited in NCBI GenBank, based on P1 proteinase gene sequences. The close relationship among the Indian isolates was observed from the phylogenetic tree constructed based on available sequences and clustering pattern of isolates correlated fairly well with their geographical origin.

When partial coat protein sequences were compared, PRSV-BLR isolate showed highest nucleotide identity of 94 per cent with CP gene sequence of accession AF323637, PRSV-CBE isolate showed highest identity (95 %) with PRSV-TA Ti (DQ666641) while PRSV-EKM isolate showed highest identity (97 %) with isolate PRSV-KE (DQ666640). Phylogenetic tree constructed from CP gene sequences revealed close relationship of

isolates among respective states and clustering pattern of isolates correlated with their geographical origin.

None of the cultivated papaya variety was found resistant to PRSV and all the cultivated varieties developed disease symptoms both under glasshouse and field conditions. However, variety Red Lady recorded highest fruit yield per plant (14.15 kg), maximum number of fruits per plant (15.67) and maximum average fruit weight (0.93 kg) among ten varieties despite severe infection. Wild relative *Carica cauliflora* did not produce any symptom and remained free from PRSV infection.

Extracts of *Acorus calamus* (5 %), *Boerhavia diffusa* (5 %), *Kappaphycus alvarezii* (KH-1%), *Eucheuma spinosum* (SH-1 %) and silicic acid (1 %) recorded significant inhibitory effect on PRSV.

Silver nanoparticles were prepared by green synthesis process from neem (*Azadirachta indica*) leaf extract and formation was confirmed by the nature of dark brown colour of the solution. UV-Vis spectral analysis showed an absorption peak at 480 nm with the intensity of 3.00 indicating presence of scattered nanoparticles in the solution. Absorption peak obtained was single and pointed which indicated the presence of nanoparticles of uniform size. Dynamic light scattering studies revealed that silver nanoparticles synthesized were polydispersed mixtures with size ranging from 60 to 100 nm.

Glasshouse and field studies revealed that extract of *Boerhavia diffusa* (5 %), colloidal silver nanoparticles (100 ppm), silver nanoparticles (50 ppm) prepared by green synthesis process and *Kappaphycus alvarezii* (LBS3-1 %) had significant inhibitory effect on PRSV.

In field studies, integrated disease management modules (IDM) significantly reduced the incidence of PRSV. Adoption of IDM Module III resulted in 142.39 per cent increase in yield over control followed by Module I (107.90 %), Module II (97.72 %) and Module IV (69.99 %). Out of four modules, Module III, the combination of maize (South African tall) as barrier crop, use of silver reflective mulch row cover and spraying with

extract of *A. calamus* (5 %), *K. alvarezii* extract (KH-1 %) and insecticide imidacloprid (0.05 %) recorded highest fruit yield per plant with highest plant height, average number of fruits and average fruit weight.

Highest return per rupee invested was recorded in IDM Module III (1.91). Adoption of integrated management modules I, II and IV increased net income as evident from the benefit cost ratio. Adoption of Modules I, IV and II resulted in benefit cost ratio of 1.81, 1.71 and 1.66 respectively. The damage caused by PRSV was evident in control as it recorded lowest returns per rupee invested (1.32).

Future line of work:

- Studies on epidemiology of the PRSV
- Validation of IDM modules and popularization among farmers for the management of PRSV
- Understanding the mechanism of resistance against PRSV in *Carica cauliflora*
- Development of transgenic papaya resistant to PRSV using CP gene mediated and Post Transcriptional Gene Silencing (PTGS) technology

VI REFERENCES

ABEER, R. M., ABD, E. A., MONIRA, R., OTHMAN, A., SALEH, A. E., MOHAMAD, A. M. AND MAJRASHI, M., 2013, Green synthesis of silver nanoparticles using the fungus *Aspergillus terreus* (KC462061). *Dig. J. Nanomater. Biostruct*., **8** (3): 1215-1225.

ADLERZ, W. C. AND EVERETT, P. H., 1968, Aluminum foil and white polyethylene mulches to repel aphids and control water melon mosaic. *J. Econ. Entomol*., **61**: 1276-1279.

AHMAD, A., MUKHERJEE, P., SENAPATI, S., MANDAL, D., KHAN, M. I., KUMAR, R. AND SASTRY, M., 2003, Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Colloids Surf. B.*, **28**: 313-318.

AHMAD, N., BHATNAGAR, S., ALI, S. S., AND DUTTA, R., 2015, Phytofabrication of bioinduced silver nanoparticles for biomedical applications. *Int. J. Nanomedicine*., 10: 7019-7030.

AHMED, S., SAIFULLAH, M., AHMAD, M., SWAMI, B. L. AND IKRAM, S., 2016, Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. *J. Radiat. Res. Appl. Sci.*, **9** (1): 1-7.

ALI, A., 2017, First complete genome sequence of papaya ringspot virus-W isolated from a gourd in the United States. *Genome Announc*., **5** (2): e01434-16.

ALI, N., RAMSUBHAG, A., FARRELL, A. AND JAYARAMAN, J., 2013, Effect of application of Ascophyllum extract on the growth, disease incidence and yield of field tomatoes in Trinidad. *Phytopathology*, **103** (9 Suppl. 3): 11.

ALI, N., RAMKISSOON, A., RAMSUBHAG, A. AND JAYARAJ J., 2016, Ascophyllum extract application causes reduction of disease levels in field tomatoes grown in a tropical environment. *Crop prot*., **83**: 67-75.

ALVIAR, A. N., 2011, Characterization of resistance to papaya ringspot virus (PRSV) in non transgenic papaya (*Carica papaya* L.) varieties and resistance evaluation of selected transgenic lines carrying the PRSV coat protein gene. *Ph.D. Thesis*, Univ. Philippines, Los Banos. p. 92.

ALVIZO, H. F. AND ROJKIND, C., 1987, Resistencia al virus mancha anular del papayo en *Carica cauliflora*. *Revista Mexicana De Fitopatologia*, **5**: 61-62.

ANDERSON, C. B., 2009, Regulating nanosilver as a pesticide, environmental defense fund, February-12.

ANONYMOUS, 2013, https://www.fao.org/corp/statistics.

ANONYMOUS, 2014, https://www.indiastat.com.

ANONYMOUS, 2015, https://www.indiastat.com.

ARAVIND, G., DEBJIT, B., DURAIVEL, S. AND HARISH, G., 2013, Traditional and medicinal uses of *Carica papaya*. *J. Med. Pl. Stud.,* **1** (1): 7-15.

ATIRI, G. I., THOTTAPPILLY, G. AND LIGAN, D., 1987, Effects of cypermethrin and deltamethrin on the feeding behaviour of *Aphis craccivora* and transmission of cowpea aphid borne mosaic virus. *Ann. Appl. Biol*., **110**: 455-461.

ATTASART, P., CHAROENSILP, G., KERTBUNDIT, S., PANYIM, S. AND JURICEK, M., 2002, Nucleotide sequence of a Thai isolate of papaya ringspot virus type W. *Acta Virol*., **46** (4): 241-246.

AWASTHI, L. P. AND MENZEL, G., 1986, Effect of root extract from *Boerhaavia diffusa* containing an antiviral principle upon plaque formation of RNA bacteriophages. *Zentralbl. Bakteriol*., **141**: 415-419.

AWASTHI, L. P. AND MUKERJEE, K., 1980, Protection of potato virus X infection by plant extracts. *Biologic Plantarum,* **22**: 205-209.

AWASTHI, L. P. AND SINGH, S., 2009a, Response of papaya (*Carica papaya* L.) cultivars against viral diseases under field conditions. *J. Pl. Dis. Sci*., **4** (1): 72-75.

AWASTHI, L. P. AND SINGH, S., 2009b, Management of ring spot disease of papaya through plant products. *Indian Phytopathol*., **62** (3): 369-375.

AWASTHI, L. P., CHOWDHURY, B. AND VERMA, R. N., 1984, Prevention of plant virus disease by *Boerhaavia diffusa* inhibitor. *Int. J. Trop. Plant. Dis*., **2**: 41-44.

AWASTHI, L. P., PATHAK, S. P. AND GAUTAM, N. C., 1985, Control of virus disease of vegetable crops by a glycoprotein isolated from *Boerhaavia diffusa*. *Indian J. Pl. Pathol*., **3**: 311-327.

AWWAD, A. M, NIDA, M. S. AND AMANY, O. A., 2013, Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. *Int. J. Ind. Chem.*, **4** (29): 1-6.

BADILLO, V. M., 1971, Monografia de la familie Caricaceae. Associacion de profesores, *Universidad central de Venezuela*. p. 221.

BAG, S., AGARWAL, S. AND JAIN, R. K., 2007, Sequence diversity in the coat proteins of papaya ringspot virus isolates originating from different locations in India. *Indian Phytopathol*., **60**: 244-250.

BALAMOHAN, T. N., AUXCILIA, J., THIRUGNANAVEL, A. AND MANORANJITHAM, S. K., 2010, CP-50: A papaya ring spot virus (PRSV) tolerant papaya genotype under field conditions. *Acta Hort.*, **851**: 153-156.

BASHA, J. R., 2002, Transmission approaches for management of papaya ring spot virus disease. *M.Sc. Thesis*, Univ. Agric. Sci., Bengaluru, p. 103.

BATESON, M. F., HENDERSON, J., CHALEEPROM, W., GIBBS, A. J. AND DALE, J. L., 1994, Papaya ringspot potyvirus: isolate variability and the origin of PRSV type P (Australia). *J. Gen. Virol.*, **75** (12): 3547-53.

BATESON, M. F., LINES, R. E., REVILL, P., CHALEEPROM, W., HA, C. V., GIBBS, A. J. AND DALE, J. L., 2002, On the evolution and molecular epidemiology of the potyvirus papaya ringspot virus. *J. Gen. Virol*., **83**: 2575-2585.

BEDOYA, C. G. AND ROJAS, O. L., 2015, Genetic variability of papaya ringspot virus isolates in Norte de Santander - Colombia. *Agron. Colomb*., **33** (2): 184-193.

BEDOYA, C. G., BARNEY, G. M. AND ROJAS, O. L., 2013, Genetic structure and evidence of putative Darwinian diversifying selection in the potato yellow vein virus (PYVV). *Agron. Colomb.*, **31** (2): 161-168.

BHANDARI, M. M., 1952, Abnormal papaya fruits. In: *Biology and Medicine*, p. 43.

BLEE, E., 2004, Impact of phyto-oxylipins in plant defense. *Trends Pl. Sci.,* **7**: 315-321.

BROWN, J. E., DANGLER, J. M., WOODS, F. M., HENSHAW, M. C., GRIFFY, W. A. AND WEST, M. W., 1993, Delay in mosaic virus onset and aphid vector reduction in summer squash grown on reflective mulches. *Hortic. Sci*., **28**: 895-896.

BYADGI, A. S., ANAHOSUR, K. H. AND KULKARNI, M. S., 1995, Ring spot virus in papaya. *The Hindu*, **118**: 28.

CAMERA, L. S., GOUZHERT, G., DOHNDT, S., HOFFMANN, L., FRITIG, B., LEGRAND, M. AND HEITZ, T., 2004, Metabolic reprogramming in plant innate immunity: the contribution of the phenylpropanoid and oxylipin pathways. *Immunol. Rev*., **198**: 267-284.

CAPOOR, S. P. AND VERMA, P. M., 1948, A mosaic disease on *Carica papaya* L. in the Bombay province. *Curr. Sci.*, **17**: 265-266.

CAPOOR, S. P. AND VERMA, P. M., 1961, Immunity of papaya mosaic virus in genus *Carica*. *Indian Phytopathol*., **14**: 96-97.

CARRAZANA, N. C., GONZALEZ-DE-LEON, D. AND SILVA-ROSALES, L., 2007, Molecular characterization of a severe isolate of papaya ringspot virus in Mexico and its relationship with other isolates. *Virus Genes*., **35** (1): 109-117.

CARTIER, J. J., 1966, Aphid responses to colors in artificial rearings. *Bull. Entomol. Soc. Am.,* **12**: 378-380.

CASTILLO, O. A., FERMIN, G., TABIMA, J., ROJAS, Y., TENNANT, P. F., FUCHS, M., SIERRA, R., BERNAL, A. J. AND RESTREPO, S., 2011, Phylogeography and molecular epidemiology of papaya ringspot virus. *Virus Res*., **159**: 132-140.

CHADHA, K. L., 2002, *Hand Book of Horticulture*. Directorate of information and publications of agriculture, ICAR, KAB, Pusa, New Delhi, 110012.

CHAN, Y. K. AND ONG, C. A., 1996, Resistance of some papaya varieties and their hybrids to ringspot virus disease. *Proc. Int. Conf. Tropical Fruits*, Kuala Lumpur, Malaysia, p. 137-49.

CHAN, Y. K. AND ONG, C. A., 2003, Field performance of papaya lines selected for tolerance to ringspot virus disease. *J. Trop. Agric. Fd. Sc.,* **31** (2): 129-137.

CHANDRAN, S. P, CHAUDHARY, M., PASRICHA, R., AHMAD, A. AND M., 2006, Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. *Biotechnol. Prog*., **22**: 577-583.

CHAUHAN, O. P. AND CHATTERJEE, T., 2005, A study on the development of papaya sauce. *Bever. Food World*, **32** (12): 28-30.

CHAVAN, V. M., TOMAR, S. P. S. AND DHALE, M. G., 2010, Management of papaya ring spot virus (PRSV-P) of papaya under Pune conditions. *Acta Hortic*., **851**: 447-452.

CHEEMA, S. S. AND REDDY, R. S., 1985, Studies on the transmission of papaya mosaic virus by *Rhopalosiphum maidis*. *Indian J. Virol*., **1**: 49-53.

CHEN, M. W., CHEN, C. C., WANG, D. N. AND CHEN, F. C., 1991, Somatic embryogenesis and plant regeneration from immature embryos of *Carica papaya* x *C. cauliflora* cultivated *in vitro*. *Can. J. Bot*., **69**: 1913-1918.

CHERIAN, B. AND CHERIYAN, S., 2003, Acceptability study on blended papaya leather. *J. Food Sci. Technol*., **40** (3): 293-295.

CHESSIN, M., BORDE, D. D. AND ZIPF, A., 1995, *Antiviral proteins in higher plants*. CRC Press, Inc., Boca Raton, Florida, USA.

CHOUDHARY, N., KAPOOR, H. C. AND LODHA, M. L., 2008, Cloning and expression of antiviral/ribosome inactivating protein from *Bougainvillea xbuttiana*. *J. Biosci*., **33**: 91-101.

CONOVER, R. A., 1962, Virus diseases of Florida. *Phytopathology*, 52: 6.

CONOVER, R. A., LITZ, R. E. AND MALO, S. E., 1988, *Cauliflora* - papaya ringspot virus tolerant papaya from South Florida and Caribbean. *Hort. Sci*., **2**: 1072.

CONWAY, K. E., MCCRAW, B. D., MOTES, J. E. AND SHERWOOD, J. L., 1989, Evaluation of mulches and row covers to delay virus disease and their effects on yellow squash. *Appl. Agric. Res.,* **4**: 201-207.

CRADOCK, K. R., GRACA, D. V. AND LAING, M. D., 2001, Control of aphid virus-vectors in *Cucurbita pepo* L. in KwaZulu-Natal, South Africa. *Subtrop. Plant. Sci.,* **53**: 49-54.

CRANE, J. H., DOREY, A. J., SCHAFFER, B. A. AND MC MILLAN, R. T., 1995, Comparison of papaya ringspot virus effects on 23 cultivars and 18 selections of papaya (*Carica papaya*) in South Florida. *Proc. Fla. State Hort. Soc.,* **108**: 354-357.

CROUCH, I. J. AND STADEN, V. J., 1994, Commercial seaweed products as bio stimulants in horticulture. *J. Home and Consumer Horti*., **1**: 19-76.

DAHAL, G., LECOQ, H. AND ALBRECHTSEN, S. E., 1997, Occurrence of papaya ringspot potyvirus and cucurbit viruses in Nepal. *Ann. Appl. Biol.,* **130** (3): 491-502.

DATAR, V. V., 2012, Integrated management of papaya ring spot virus in papaya (*Carica papaya*). *Indian Phytopathol*., **65** (1): 12-17.

DAVIS, M. J., WHITE, T. L. AND CRANE, J. H., 2003, Papaya variety development in Florida. *Proc. Fla. State Hort. Soc*., **116**: 4-6.

DAVIS, M. J., WHITE, T. L. AND CRANE, J. H., 2004, Resistance to papaya ringspot virus in transgenic papaya breeding lines. *Proc. Fla. State Hort. Soc*., **117**: 241-245.

DAVIS, R. I., MU, L., MAIREROA, N., WIGMORE, W. J., GRISONI, M., BATESON, M. F. AND THOMAS, J. E., 2005, First records of the papaya strain of papaya ringspot virus (PRSV-P) in French Polynesia and the Cook Islands. *Australas. Pl. Pathol*., **34** (1): 125-126.

DEVI, T. P., KULANTHAIVEL, S., KAMIL, D., BORAH, J. L., PRABHAKARAN, N. AND SRINIVASA, N., 2013, Biosynthesis of silver nanoparticles from *Trichoderma* species. *Indian. J. Exp. Biol.*, **51** (7): 543-547.

DEVINE, G., HARLING, Z. K., SCARR, A. W. AND DEVONSHIRE, A. L., 1996, Lethal and sublethal effects of imidachloprid on nicotine tolerant *Myzus nicotianae* and *Myzus persicae. Pesticide Sci*., **48**: 145-153.

DHANAM, S., SAVEETHAA, K., SANKARALINGAMA, A., KANNANA, R. AND PANT, R. P., 2011, Biological and molecular characterisation of Coimbatore isolate of papaya ringspot virus. *Arch. Phytopath. Pl. Prot*., **44** (10): 925-932.

DHUPER, S., PANDA, D. AND NAYAK P. L., 2012, Green synthesis and characterization of zero valent iron nanoparticles from the leaf extract of *Mangifera indica. Nano Trends: J. Nanotech. App*., **13** (2): 16-22.

DIALLO, H. A., MONGER, W., KOUASSI, N., YORO, D. T. AND JONES, P., 2007, First report of papaya ringspot virus infecting papaya in Côte d'Ivoire. *Pl. Pathol.*, **56** (4): 718.

DICKSON, R. C. AND LAIRD, E. F., 1966, Aluminum foil to protect melons from watermelon mosaic virus. *Pl. Dis. Rep*., **50**: 305.

DIFONZO, C. D., RAGSDALE, D. W., RADCLIFFE, E. B., GUDMESTAD, N. C. AND SECOR, G. A., 1996, Crop borders reduce potato virus Y incidence in seed potato. *Ann. Appl. Biol*., **129** (2): 289-302.

DUGGAR, B. M. AND ARMSTRONG, J. K., 1925, The effect of treating the virus of tobacco mosaic with juices of various plants. *Ann. Mo. Bot. Gard.,* **12**: 359-366.

EDWARD, E. F., 2014, *Leaf Defence*. books.google.co.in

EDWARD, E. F. AND CHRISTIE, R. G., 1986, Viruses infecting forage legumes. Florida agricultural experiment station, Monograph series, 4.

ELAVAZHAGAN, T. AND ARUNACHALAM, K. D., 2011, *Memecylon edule* leaf extract mediated green synthesis of silver and gold nanoparticles. *Int. J. Nanomed.,* **6**: 1265-78.

ELECHIGUERRA, J., BURT, J., MORONES, J., CAMACHO, B. A., GAO, X., LARA, H. AND YACAMAN, M., 2005, Interaction of silver nanoparticles with HIV-1. *J. Nanobiotech.* **3**: 1-10.

ESAM, K. F., ELBESHEHY, E., METWALI, M. R. AND OMAR, A. A., 2015, Antiviral activity of *Thuja orientalis* extracts against watermelon mosaic virus (WMV) on *Citrullus lanatus. Saudi J. Biol. Sci.,* **22**: 211-219.

EVANS, E. A. AND BALLEN F. H., 2012, An overview of global papaya production, trade and consumption. EDIS document FE913. http://edis.ifas.ufl.edu/pdffiles/FE/FE91300.pdf

FAUQUET, C. M., MAYO, M. A., MAINLOFF, J., DESSERLBERGER, U. AND BALL, L. A., 2005, Virus Taxonomy: 8th Report of the international committee on taxonomy of viruses. Elsevier, Academic Press, San Diego.

FAYAZ, M., TIWARY, C. S., KALAICHELVAN, P. T. AND VENKATESAN, R., 2010, Blue orange light emission from biogenic synthesized silver nanoparticles using *Trichoderma viride*. *Colloids Surf. B.,* **75** (1): 175-178.

FERERES, A., 2000, Barrier crops as a cultural control measure of non-persistently transmitted aphid-borne viruses. *Virus Res*., **71**: 221-231.

GAJBHIYE, V. T., GUPTA, S. AND GUPTA, R. K., 2004, Persistence of imidacloprid in/on cabbage and cauliflower. *Bull. Environ. Contam. Toxicol*., **72** (2): 283-288.

GARGA, R. P., 1963, Studies on virus diseases of plants in Madhya Pradesh - A serious virus disease of papaya. *Indian Phytopathol.*, **16**: 31-33.

GEORGE, W. L. AND KRING, J. B., 1971, Virus protection of late-season summer squash with aluminium mulch. Circular of the Connecticut agricultural experiment station No. 239.

GERGERICH, R. C. AND DOLJA, V. V., 2006, Introduction to plant viruses, the invisible foe. *The Plant Health Instructor,* DOI: 10.1094/PHI-I-2006-0414-01.

GIBBS, A. J., MACKENZIE, A. M., WEI, K. AND GIBBS, M. J., 2008, The potyviruses of Australia. *Arch. Virol*., **153**: 1411-1420.

GOGOI, R., DUREJA, P. AND SINGH, P. K., 2009, Nanoformulations: a safer and effective option for agrochemicals. *Indian Fmg.,* **59** (8): 7-12.

GOIA, D. V. AND MATIJEVIC, E., 1998, Preparation of monodisperse metal particles. *New. J. Chem.,* **22**: 1203-1215.

GONSALVES, D., 1989, Cross protection techniques for control of plant viruses in the Tropics. *Plant Dis.*, **73**: 592-597.

GONSALVES, D., 1994, Papaya ringspot virus. In: *Compendium to tropical fruit disease*, APS press, p. 67-68.

GONSALVES, D., 1998, Control of papaya ringspot virus in papaya: a case study. *Ann. Rev. Phytopathol.,* **36**: 415-437.

GONSALVES, D. AND ISHII, M., 1980, Purification and serology of papaya ringspot virus. *Phytopathology,* **70**: 1028-1032.

GONSALVES, D., TRIPATHI, S., CARR, J. B. AND SUZUKI, J. Y., 2010, Papaya ringspot virus. *The Plant Health Instructor,* DOI: 10.1094/PHI-I-2010-1004-01

GREER, L. AND DOLE, J. M., 2003, Aluminum foil, aluminum-painted, plastic and degradable mulches increase yields and decrease insect-vectored viral diseases of vegetables. *Hortic. Tech.,* **13**: 176-184.

GUEMADA, H., LHOSTIS, B., GONSALVES, REARDON, I. M., HEINRIKSON, R., SEIU, L. C. AND SLIGHTORRI, J. L., 1990, The nucleotide sequence of the 3'-terminal regions of papaya ringspot virus strains W and P. *J. Gen. Virol.,* **71**: 203-210.

GULSIRI, C., PONGSOPEE, A., MLLA, J., SAKOL, P. AND SUNEE, K., 2003, Sequencing and characterization of Thai papaya ring spot virus isolate type P (PRSVthP). *Sci. Asia*., **29**: 89-94.

GUO, W. Z., LI, J. J., WANG, Y. A. AND PENG, X. G., 2003, Conjugation chemistry and bioapplications of semiconductor box nanocrystals prepared via dendrimer bridging. *Chem. Mater.,* **15**: 3125-3133.

HAKKAART, F. A., 1967, Effects of aluminum stripes on the spread of two aphid borne chrysanthemum viruses. *Neth. J. Pl. Pathol*., **73**: 181-185.

HANSEN, A. J., 1989, Antiviral chemicals for plant disease control. *Plant Sci.,* **8**: 45-88.

HAROON, B. H., KULANDHAIVEL, M., ANBALAGAN, S., SANKARESWARAN, M., ABIRAMI, K., PRABHAVATHI, P. AND MANIKANDAN, A., 2017, Green synthesis of silver nanoparticles using *Hybanthus enneaspermus* plant extract against nosocomial pathogens with nanofinished antimicrobial cotton fabric. *Glob. J. Nanomed.,* **1** (1): 1-13.

HATSCHEK, E., 1931, Inventor: Electro chem processes Ltd., assignee Brouisol. British patent no. 392: 556.

HEGDE, P., 1998, Further studies on papaya ring spot virus. *M. Sc. Thesis*, Univ. Agric. Sci., Dharwad, p. 87.

HEMA, M. V. AND PRASAD, D. T., 2004, Comparison of the coat protein of a South Indian strain of PRSV with other strains from different geographical locations. *J. Pl. Pathol*., **86** (1): 35-42.

HEMAVATI, R., 2005, Detection, diagnosis and management of PRSV on cucurbits. *M. Sc. Thesis*, Univ. Agric. Sci., Dharwad, p. 109.

HENDERSON, J., 1999, Towards identification of host range determinants of papaya ringspot potyvirus. *Ph.D. Thesis*, Queensland Univ. Technology, Australia.

HEROLD, F. AND WEIBEL, J., 1962, Electron microscopic demonstration of papaya ringspot virus. *Virology*, **18**: 302-311.

HEU, R. A., NAGATA, N. M., FUKASA, M. T. AND YONAHARA, B. Y., 2002, Papaya ringspot virus. In: *New Pest Advisory*, Tersedia pada, p. 2-3.

HOLMES, F. O., HENDRIX, J. W., IKEDA, W., JENSEN, D. D., LINDNER, R. C. AND STOREY, W. B., 1948, Ringspot of papaya (*Carica papaya*) in the Hawaiian Islands. *Phytopathology*, **38**: 310-312.

HONARY, S., BARABADI, H., GHARAEI, E. AND NAGHIBI, F., 2013, Green synthesis of silver nanoparticles induced by the fungus *Penicillium citrinum*. *Trop J. Pharm. Res.,* **12** (1): 7-11.

HOOKS, C. R. R. AND FERERES, A., 2006, Protecting crops from non persistently aphid transmitted viruses: a review on the use of barrier plants as a management tool. *Virus Res*., **120**: 1-16.

HUSSAIN, S. AND VARMA, A., 1994, Occurrence of papaya ringspot virus from Amritsar (Punjab) India. *J. Phytopathol. Res*., **7**: 77-78.

ISAACS, R., CAHILL, M. AND BYRNE, D. N., 1999, Host plant evaluation behaviour of *Bemisia tabaci* and its modification by external or internal uptake of imidacloprid. *Physiol. Entomol*., **24**: 101-108.

JAIN, D. AND KOTHARI, S. L., 2014, Green synthesis of silver nanoparticles and their application in plant virus inhibition. *J. Mycol. Pl. Pathol.*, **44** (1): 21-24.

JAIN, R. K., PAPPU, H. R., PAPPU, S. S., VARMA, A. AND RAM, R. D., 1998, Molecular characterization of papaya ringspot potyvirus isolates from India. *Ann. Appl. Biol*., **132**: 413-425.

JAIN, R. K., SHARMA, J., SIVAKUMAR, A. S., SHARMA, P. K., BYADGI, A. S., VERMA, A. K. AND VARMA, A., 2004, Variability in the coat protein gene of papaya ringspot virus isolates from multiple locations in India. *Arch. Virol.,* **149**: 2435-2442.

JAIN, D., DAIMA, H. K, KACHHWAHA, S. AND KOTHARI, S. L., 2009, Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities. *Dig. J. Nanomater. Biostruct.,* **4**: 557-653.

JAMES, E. B., JAMES, M. D., FLOYD, M. W. AND KEN, M. T., 1993, Delay in mosaic virus onset and aphid vector reduction in summer squash grown on reflective mulches. *Hort. Sci*., **28** (9): 895-896.

JENSEN, D. D., 1949, Papaya virus diseases with special reference to papaya ringspot. *Phytopathology*, **39**: 191-211.

JIA, X., MA, X., WEI, D., DONG, J. AND QIAN, W., 2008, Direct formation of silver nanoparticles in cuttlebone derived organic matrix for catalytic applications. *Colloids Surf. A: Physico. Chem. Eng. Aspects.*, **30**: 234-40.

JIMENEZ, E., DORTA, F., MEDINA, C., RAMIREZ, A., INGRID, R. AND HUGO, P. C., 2011, Anti-phytopathogenic activities of macro-algae extracts. *Mar. Drugs.*, **9**: 739-756.

JOERGER, K. T., JOERGER, R., OLSSON, E. AND GRANQVIST, C., 2001, Bacteria as workers in the living factory: metal accumulating bacteria and their potential for materials science. *Trends Biotechnol.*, **19**: 15-20.

JONES, F. R. AND CHAPMAN, R. K., 1968, Aluminum foil and other reflective surfaces to manipulate the movement of aphid vectors of plant viruses. *Proc. North Central Branch. Entom. Soc. Am.*, **23**: 146-148.

JONES**,** A. L., JOHANSEN, I. E., BEAN, S. J., BACH, I. AND MAULE, A. J., 1998, Specificity of resistance to pea seed-borne mosaic potyvirus in transgenic peas expressing the viral replicase (NIb) gene. *J. Gen. Virol.*, **79**: 3129-3137.

KALISHWARALAL, K., DEEPAK, V., PANDIAN, S. K., KOTTAISAMY, M., MANIKANTH, S. B., KARTIKEYAN, B. AND GURUNATHAN, S., 2010, Biosynthesis of silver and gold nanoparticles using *Brevibacterium casei*. *Colloids Surf. B: Biointerfaces.*, **77**: 257-262.

KALLESHWARASWAMY, C. M. AND KUMAR, K. K., 2008, Transmission efficiency of papaya ringspot virus by three aphid species. *Phytopathology*, **98** (5): 541-546.

KALLESHWARASWAMY, C. M., KUMAR, K. K., DINESH, M. R., CHANDRASHEKAR, K. N. AND MANJUNATHA, M., 2009, Evaluation of insecticides and oils on aphid vectors for the management of papaya ringspot virus (PRSV). *Kar. J. Agric. Sci.,* **22** (3): 552-553.

KALLESHWARASWAMY, C. M., KUMAR, K. K., CHANDRASHEKARA, K. N. AND VANI, A., 2012, Efficacy of insecticides and oils on feeding behaviour of

Aphis gossypii Glover and transmission of papaya ringspot virus (PRSV). *Kar. J. Agric. Sci*., **25** (1): 63-67.

KENGANAL, M. Y., 2009, Investigations on mild strains and transformation of PRSV-cp gene in papaya, *Ph.D. Thesis*, Univ. Agric. Sci., Dharwad.

KHALIL, K. A., FOUAD, H., ELSARNAGAWY, T. AND ALMAJHDI, F. N., 2013, Preparation and characterization of electrospun PLGA/silver composite nanofibers for biomedical applications. *Int. J. Electro. chem. Sci.,* **8**: 3483-3493.

KHANDELWAL, N., KAUR, G., KUMAR, N. AND TIWARI, A., 2014, Application of silver nanoparticles in viral inhibition: a new hope for antivirals. *Dig. J. Nanomater. Biostruct*., **9**: 175-186.

KHURANA, S. P. AND BHARGAVA, K. S., 1970, Induced apocarpy and double papaya fruit formation in papaya with distortion ringspot virus infection. *Pl. Dis. Rep*., **54**: 181-183.

KIM, T. N., FENG, Q. L., KIM, J. O., WU, J., WANG, H., CHEN, G. C. AND CUI, F. Z., 1998, Antimicrobial effects of metal ions (Ag^{+}, Cu^{2+}, Zn^{2+}) in hydroxyapatite. *J. Mater. Sci. Mater. Med*., **9**: 129-134.

KIM, S. W., JUNG, J. H., LAMSAL, K., KIM, Y. S., MIN, J. S. AND LEE, Y. S., 2012, Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi. *Mycobiol.*, **40**: 53-58.

KIRITANI, K. AND SU, H. J., 1999, Papaya ringspot, banana bunchy top and citrus greening in the Asia and Pacific region: occurrence and control strategy. *JARQ.,* 33: 23-30.

KLARZYNSKI, O., PLESSE, B., JOUBERT, J. M., YVIN, J. C., KOPP, M., KLOAREG, B. AND FRITIG, B., 2000, Linear beta-1,3 glucans are elicitors of defense responses in tobacco. *Pl. Physiol.,* **124**: 1027-1038.

KLARZYNSKI, O., DESCAMPS, V., PLESSE, B., YVIN, J. C., KLOAREG, B. AND FRITIG, B., 2003, Sulfated fucan oligosaccharides elicit defense responses in tobacco and local and systemic resistance against tobacco mosaic virus. *MPMI.*, **16** (2): 115-122.

KLOAREG, B. AND QUATRANO, R. S., 1988, Structure of the cell walls of marine algae and ecophysiological functions of the matrix polysaccharides. *Oceanogr. Mar. Biol. Annu. Rev.,* **26**: 259-315.

KOBAYASHI, A., TAI, A., KANZAKI, H. AND KAWAZU, K., 1993, Elicitor-active oligosaccharides from algal laminaran stimulate the production of antigungal compounds in alfalfa. *Z. Naturforsch. C.*, **48**: 575-579.

KOWSHIK, M., ASHTAPUTRE, S., KHARRAZI, S., VOGEL, S., URBAN, J., KULKARNI, S. K. AND PAKNIKAR, K. M., 2003, Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. *Nanotechnology*, **14**: 95-100.

KRING, J. B., 1964, New ways to repel aphids. *Frontiers Sci. Conn. Agric. Exp. Stn.*, **17**: 6-7.

KRISHNARAJ, C., JAGAN, E. C., RAJASEKHAR, S., SELVAKUMAR, P., KALAICHELVAN, P. T. AND MOHAN, N., 2010, Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water born pathogens. *Colloids Surf. B: Biointerfaces.*, **76**: 50-56.

KUDADA, N. AND PRASAD, S. M., 2000, Screening of commercial papaya (*Carica papaya*) varieties for tolerance against papaya ringspot virus. *J. Res. Birsa Agric. Univ.*, **12**: 51-56.

KUMAR, K. K., SINGH, H. S. AND KALLESHWARASWAMY, C. M., 2010, Aphid (Aphididae: Homoptera) vectors of papaya ringspot virus (PRSV), bionomics, transmission efficiency and factors contributing to epidemiology. *Acta Hortic.*, **851**: 431-442.

KUMAR, S., SANKARLINGAM, A. AND RABINDRAN, R., 2014, Characterization and confirmation of papaya ringspot virus-W Strain infecting *Trichosanthese cucumerina* at Tamil Nadu, India. *J. Pl. Pathol. Microb.,* **5** (2): 1-4.

KUNG, Y. J., BAU, H. J., WU, Y. L., HUANG, C. H., CHEN, T. M. AND YEH. S. D., 2009, Generation of transgenic papaya with double resistance to papaya ringspot virus and papaya leaf-distortion mosaic virus. *Phytopathology*, **99**: 1312-1320.

KUNKALIKAR, S., 2003, Molecular characterization, cloning of coat protein gene, epidemiology and management of papaya ringspot virus. *Ph.D. Thesis*, Univ. Agric. Sci., Dharwad, p. 109.

KUNKALIKAR, S., BYADGI, A. S., KULKARNI, V. R. AND REDDY, K. M., 2007, Survey for papaya ringspot virus incidence in Karnataka and its molecular detection. *J. Mycol. Pl. Pathol*., **37** (2): 269-271.

LAPORTE, D., VERA, J., CHANDIA, N. P., ZUNIGA, E. A., MATSUHIRO, B. AND MOENNE, A., 2007, Structurally unrelated algal oligosaccharides differentially stimulate growth and defense against tobacco mosaic virus in tobacco plants. *J. Appl. Phycol.,* **19**: 79-88.

LI, G., HE, D., QIAN, Y., GUAN, B., GAO, S., CUI, Y., YOKOYAMA, K. AND WANG, L., 2012, Fungus-mediated green synthesis of silver nanoparticles using *Aspergillus terreus*. *J. Mol. Sci*., **13**: 466-476.

LIMA, R. A., SOUZA, M. T., PIO-RIBEIRO, G. AND LIMA, J. A., 2002, Sequences of the coat protein gene from Brazilian isolates of papaya ringspot virus. *Fitopatol. Bras.*, **27** (2): 174-180.

LIN S. S., ROGER, F. H. AND SHYI D. Y., 2001, Complete genome sequence and genetic organization of a Taiwan isolate of zucchini yellow mosaic virus. *Bot. Bull. Acad. Sin*., **42**: 243-250.

LINDER, R. C., JENSON, D. D. AND IKEDA, W., 1945, Ring spot new papaya plunderer. *Hawaii Farm Home*, **8**: 10-12.

LOKHANDE, N. M. AND MOGHE, P. G., 1992, Influence of nutrients and hormones on fruit quality traits and their correlation with yield in PRSV infected papaya cv. Honeydew (*Carica papaya* L.). *The Punjab Hort. J*., **32** (1): 102-106.

LOKHANDE, N. M., MOGHE, P. G., MATTE, A. D. AND HIWARE, B. J., 1992, Occurrence of papaya ring spot virus (PRSV) in Vidharbha regions of Maharastra. *J. Soils and Crops*., **2**: 36-39.

LOUIS, V. AND BALAKRISHNAN, S., 1995, Screening of certain medicinal plant extracts against pumpkin mosaic virus. *Proc. VII Kerala sci. cong.*, Palakkad, p. 182-183.

LOUIS, V. AND BALAKRISHNAN, S., 1996, Effect of application of selected medicinal plant extracts on the incidence of pumpkin mosaic virus. *Indian Phytopathol.*, **49** (4): 373-377.

MA, J. F. AND YAMAJI, N., 2006, Silicon uptake and accumulation in higher plants. *Trends Pl. Sci*., **11**: 392-397.

MADHUSUDHAN, K. N., VINAYARANI, G., DEEPAK, S. A., NIRANJANA, S. R., PRAKASH, H. S., SINGH, G. P., SINHA, A. K. AND PRASAD, B. C., 2011, Antiviral activity of plant extracts and other inducers against Tobamoviruses infection in bell pepper and tomato plants. *Int. J. Pl. Pathol.,* **2**: 35-42.

MAGDALITA, P. M., PERSELY, D. M., GODWIN, I. D., DREW, R. A. AND ADKINS, S. W., 1997, Screening *Carica papaya* x *C. cauliflora* hybrids for resistance to papaya ringspot virus-type P. *Plant Pathol*., **46**: 837-841.

MAHL, D., DIENDRORF, J., MAYER-ZAIKA, W. AND MATTHIAS., 2011, Epple colloids and surfaces physicochem. *Eng. Aspects*., p. 377- 386.

MALLIKARJUN, Y. K., 2009, Investigations on mild strains and transformation of PRSV-CP gene in Papaya. *Ph.D. Thesis,* Univ. Agric. Sci., Dharwad, p. 121.

MANGRAUTHIA**,** S. K., PARAMESWARI, B., JAIN, R. K. AND PRAVEEN, S., 2008, Role of genetic recombination in the molecular architecture of papaya ringspot virus. *Biochem. Genet.,* **46** (11&12): 835-846.

MANGRAUTHIA, S. K., SINGH, P. AND PRAVEEN. S., 2010, Genomics of helper component proteinase reveals effective strategy for papaya ringspot virus resistance. *Mol. Biotechnol*., 44: 22-29.

MAOKA, T., KAWANO, S. AND USUGI, T., 1995, Occurrence of the P strain of papaya ringspot virus in Japan. *Jpn. J. Phytopathol*., **61**: 34-37.

MARILIA, G. V., LUIS, E. C. AND JORGE, A. R., 2003, Nucleotide sequence comparison of the capsid protein gene of severe and protective mild strains of papaya ringspot virus*. Fitopatol. Bras.,* **28** (6): 678-681.

MARSCHNER, H., 1995, *Mineral Nutrition of Higher Plants* (2nd Ed.). Academic press, San Diego, CA, p. 889.

MARTIN, B., VARELA, I. AND CABALEIRO, C., 2004, Effects of various oils on survival of *Myzus persicae* Sulzer and its transmission of cucumber mosaic virus on pepper. *J. Hort. Sci. Biotech.*, **79** (6): 855-858.

MARTINEZ, D. R., DE SOUSA, P., DUARTE, G., OLMEDO, J. G. AND FIGUEIRA, A. R., 2014, Molecular and biological studies of papaya ringspot virus isolates from Brazil and Cuba. *American J. Agric. Fores.*, **2** (5): 209-218.

MEDEROS, D. C., HERNANDEZ, D. G., GONZALEZ, J. G. AND ORELVIS, P. O., 2013, Epiphyte management of papaya ringspot virus using *Zea mays* L. barriers in *Carica papaya* L*. Rev. Prot. Veg.*, **28** (2): 127-131.

MERCIER, L., LAFITTE, C., BORDERIES, G., BRIAND, X., ESQUERRE-TUGAYE, M. T. AND FOURNIER, J., 2001, The algal polysaccharide carrageenans can act as an elicitor of plant defence. *New Phythol.,* **149**: 43-51.

MIGLIACCIO, K. W., SCHAFFER, B., CRANE, J. H. AND DAVIES, F., 2010, Evapotranspiration and soil water sensor irrigation scheduling methods for papaya production in South Florida. *Agri. Water Manage.*, **97**: 1452-1460.

MIN, J. S., KIM, K. S., KIM, S. W., JUNG, J. H., LAMSAL, K., KIM, S. B., JUNG, M. AND LEE, Y. S., 2009, Effects of colloidal silver nanoparticles on sclerotium-forming phytopathogenic fungi. *J. Pl. Pathol*., **25**: 376-380.

MISHRA, J. N. AND JHA, A., 1955, Mosaic of papaya (*Carica papaya* L.) in Bihar. *Proc. Bihar Acad. Agric. Sci.*, **4**: 102-103.

MOERICKE, V., 1954, New investigations on color in relation to Homoptera. *Proc. 2nd conference on potato virus diseases*. Lisse Wageningen, 25-29th June, p. 55-69.

MORENO**,** M., BERNAL, J. J., JIMENEZ, I. AND RODRIGUZE, C. E., 1998, Resistance in plants transformed with the P1 or P3 gene of tobacco vein mottling potyvirus. *J. Gen. Virol.,* **79**: 2819-2827.

MORONES, J. R., ELECHIGUERRA, J. L., CAMACHO, A., HOLT, K., KOURI, J. B., RAMIREZ, J. T. AND YACAMAN, M. J., 2005, The bactericidal effect of silver nanoparticles. *Nanotechnol.,* **16** (10): 2346-2353.

MOSSLER, M. A. AND CRANE, J. H., 2009, Florida crop/pest management profile: papaya. Electronic Data Information Source (EDIS) PI053 (CIR 1402). Univ. Florida, Gainesville.

MOSTAFA, M. K., EMAN, H. I., KHALED, Z. B. AND MOHAMED, D., 2014, Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. *Arabian J. Chem.*, **7**: 1131-1139.

MUELLER, E., GILBERT, J., DAVENPORT, G., BRIGNETI, G. AND BAULCOMBE, D. C., 1995, Homology-dependent resistance: transgenic virus resistance in plants related to homology-dependent gene silencing. *Pl. J*., **7**: 1001-1013.

MULVANEY, P., 1996, Surface plasmon spectroscopy of nanosized metal particles**.** *Langmiur*, **12**: 788-800.

MURPHY, F. A., FAUQUET, C. M., BISHOP, D. L., GHABRIAL, S. A., JARVIS, A. W., MARTELLI, G. P., MAYO, M. A. AND SUMMERS, M. D., 1995, Virus Taxonomy: 6th Report of the international committee on taxonomy of viruses. Springer-Verlag, Vienna and New York, p. 586.

NAGATA, I. K., FRANCO, C. M., MARTIN, D. P., REZENDE, J. A., FERREIRA, G. B., DUTRA, L. S. AND NAGATA, T., 2007, Genome analysis of a severe and a mild isolate of papaya ringspot virus-type W found in Brazil. *Virus Genes.,* **35** (1): 119-127.

NAUEN, R., 1995, Behaviour modifying effects of low systemic concentrations of imidacloprid on *Myzus persicae* with special reference to an antifeeding response. *Pest Sci*., **44**: 145-153.

NAVANATH, M. D., ANITHA. P., GAHUKAR S. J. AND AKHARE A. A., 2017, Biological indexing of papaya ring spot virus (PRSV) in *Carica papaya. Int. J. Agr. Sci.,* **9** (4): 3728-3730.

NOMIYA, K., YOSHIZAWA, A., TSUKAGOSHI, K., KASUGA, N. C., HIRAKAVA, S. AND WATANABE, J., 2004, Synthesis and structural characterization of silver (I), aluminium (III) and cobalt (II) complexes with 4-isopropyltropolone (hinokitiol) showing noteworthy biological activities. Action of silver (I)-oxygen bonding complexes on the antimicrobial activities. *J. Inorg. Biochem*., **98**: 46-60.

OSAKABE, M. H. AND KENICHIRO, H., 2002, Influence of trap and barrier crops on occurrence of and damage by stink bugs and lepidopterous pod borers in soybean fields. *Japanese J. Appl. Entmol. Zool.*, **46** (4): 233-241.

OSAMA, A. A. AND ALI, A., 2012, Genetic diversity in the 3'-terminal region of papaya ringspot virus (PRSV-W) isolates from watermelon in Oklahoma. *Arch. Virol.,* **157**: 405-412.

PARAMESWARI, B., MANGRAUTHIA, S. K., PRAVEEN, S. AND JAIN, R. K., 2007, Complete genome sequence of an isolate of papaya ringspot virus from India. *Arch. Virol.,* **152** (4): 843-845.

PARAMESWARI, B. AND JAIN, R. K., 2016, Biological characterization of papaya ringspot virus pathotypes. *J. Prog. Agric.*, **7**(2): 67-73.

PARK, H. J., KIM, S. H., KIM, H. J. AND CHOI, S. H., 2006, A new composition of nanosized silica-silver for control of various plant diseases. *Pl. Pathol. J*., **22**: 295-302.

PARVEEN, S., TRIPATHI, S. AND VARMA, A., 2001, Isolation and characterization of an inducer protein (Crip-31) from *Clerodendrum inerme* leaves responsible for induction of systemic resistance against viruses. *Pl. Sci*., **161**: 453-459.

PATEL, N., DESAI, P., PATEL, N., JHA, A. AND HEMANT, K. G., 2014, Agro-nanotechnology for plant fungal disease management: a review. *Int. J. Curr. Microbiol. App. Sci*., **3** (10): 71-84.

PATIER, P., YVIN, J. C., KLOAREG, B., LIENART, Y. AND ROCHAS, C., 1993, Seaweed liquid fertilizer from *Ascophyllum nodosum* contains elicitors of plant D-glucanases. *J. Appl. Phycol.*, **5**: 343-349.

PERRING, T. M., GRUENHAGEN, N. M. AND FARRAR, C. A., 1999, Management of plant viral diseases through chemical control of insect vectors. *Annu. Rev. Entomol*., **44**: 457-481.

PICARD, D., KAO, C. C. AND HUDAK, K. A., 2005, Pokeweed antiviral protein inhibits brome mosaic virus replication in plant cells. *J. Biol. Chem*., **280**: 20069-20075.

PINESE, B., LISLE, A. T., RAMSELY, M. D., HALFPAPP, K. H. AND DEFAVERI, S., 1994, Control of aphid-borne papaya ringspot potyvirus in zucchini marrow

(*Cucurbita pepo*) with reflective mulches and mineral oil-insecticide sprays. *Int. J. Pest Manage.*, **40**: 81-87.

PIRONE, T. P. AND HARRIS, K. F., 1977, Non-persistent transmission of plant viruses by aphids. *Annu. Rev. Phytopathol*., 15: 55-73.

PLOBNER, L. AND LEISER, R. M., 1990, Induction of virus resistance by carnation proteins. *Proc. Int. Cong. Virol.*, Berlin, p. 26-21.

PRASAD, S. M. AND KUDADA, N., 2005, Effect of barrier and inter crops on natural incidence of papaya ringspot virus disease and fruit yield of papaya. *Indian J. Virol*., **16** (1&2): 24-26.

PROWIDENTII, R., 1996, A Taiwan strain of papaya ring spot virus causing prominent symptoms on cultivated cucurbits. Report cucurbit genetics co-operative, **19**: 83-84.

PURCIFULL, D. E., EDWARDSON, J., HIEBERT, E. L. AND GONSALVES, D., 1984, Papaya ringspot virus. CMI/AAB Descr. Plant Viruses, No. 292**.**

PUSHPA, R. N., 2014, Molecular characterization, epidemiology and integrated management of papaya ringspot virus (PRSV) in papaya (*Carica papaya* L.). *Ph.D. Thesis* (Unpub.), Univ. Agric. Sci., Bengaluru, p. 54-133.

QUEMADA, H., LHOSTIS, B., GONSALVES, D., REARDON, I. M., HEINRIKSON, R., HEIGERT, E. L., SIEU, L. C. AND SLIGHTOM, J. L., 1990, The nucleotide sequences of the 3' terminal regions of papaya ringspot virus strains W and P. *J. Gen. Virol.,* **71**: 203-210.

RACCAH, B. AND FERERES, A., 2009, Plant virus transmission by insects. In: *Encyclopedia of life sciences* (ELS). John Wiley and sons, Ltd., Chichester, DOI: 10.1002/9780470015902.A0021525.a0000760.pub2

RAI, M., YADAV, A. AND GADE, A., 2009, Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. Adv*., **27**: 76-83.

RAM, D. D., 1993, Papaya ringspot, research on virus, virus like and mycoplasma diseases of plants. Bull. IARI regional station, Pune (India).

RAO, R. P., 1988, Studies on papaya ring spot virus infecting *Carica papaya*. *Ph. D. Thesis*, Marathwada Agric. Univ., Parbhani, p. 89.

RAY, P. K., YADAV, J. P. AND KUMAR, A., 1999, Effect of transplanting dates and mineral nutrition on yield and susceptibility of papaya to ring spot virus. *The Hort. J.*, **8**: 16-18.

REDDY, C. L., 2000, Studies on papaya ringspot virus disease. *M.Sc. Thesis*, Univ. Agric. Sci., Bengaluru, p. 105.

REDDY, V. P., GOWDA, N. V., RAJESH, A. M., YATHINDRA, H. A. AND HARSHAVARDHAN, M., 2011a, Survey for the incidence and variability of symptoms on papaya ringspot virus in Southern Karnataka, India. *Pl. Archives*, **11** (2): 1147-1149.

REDDY, V. P., GOWDA, N. V., RAJESH, A. M., YATHINDRA, H. A. AND HARSHAVARDHAN, M., 2011b, Screening of commercial papaya genotypes for resistance against papaya ringspot virus at seedling stage. *Pl. archives*, **11** (2): 1017- 1019.

REDDY, G., JOY, J. M., MITRA, T., SHABNAM, S. AND SHILPA, T., 2012, Nano silver - a review. *Int. J. Adv. Pharm.,* **2** (1): 09-15.

REITZ, S. R., YEARBY, E. L., FUNDERBURK, J. E., STAVISKY, J., MOMOL, M. T. AND OLSON, S. M., 2003, Integrated management tactics for *Frankliniella* thrips (Thysanoptera: Thripidae) in field-grown pepper. *J. Econ. Entomol.,* **96**: 1201-1214.

REVILLA, F. C., GARCIA, E., NIETO, A. D., TELIZ, O. D. AND VILLANUEVA, J. A., 1995, Integrated management of papaya in Mexico. *Acta Hortic.,* **370**: 15-158.

ROBERTO, C. A., LIMA, M., SOUZA, T., GILVAN, P. R. AND ALBERSIO, J. L., 2002, Sequences of the coat protein gene from Brazilian isolates of papaya ringspot virus. *Fitopatol. Bras*., **27** (2): 174-180.

RODRIGUEZ, F. T., RUBIO, L., CARBALLO, O. AND MARYS, E., 2008, Genetic variation of papaya ringspot virus in Venezuela. *Arch. Virol.,* **153** (2): 343-349.

ROFF, M. N., 2007, Disease rating of papaya cultivars to papaya ringspot virus in Malaysia. *Acta Hort*., **740**: 277-281.

ROJAS**,** O. Y. AND BEDOYA, C. G., 2017, Molecular characterization of two papaya ringspot virus isolates that cause devastating symptoms in Norte de Santander, Colombia. *Eur. J. Pl. Pathol*., DOI:10.1007/s10658-016-1143-z.

ROMAY, G., LECOQ, H. AND DESBIEZ, C., 2014, Zucchini tigre mosaic virus is a distinct potyvirus in the papaya ringspot virus cluster: molecular and biological insights. *Arch. Virol.,* **159** (2): 277-289.

ROTHAM, P. G., 1967, Aluminum foil fails to protect winter oats from aphid vectors of barley yellow dwarf virus. *Pl. Dis. Rep*., **51**: 354-355.

ROY, S., MUKHERJEE, T., CHAKRABORTY, S. AND DAS, T. K., 2013, Biosynthesis, characterisation and antifungal activity of silver nanoparticles synthesized by the fungus *Aspergillus foetidus* MTCC8876. *Dig. J. Nanomater. Bios.*, **8** (1): 197-205.

SADEGHI, B. AND GHOLAMHOSEINPOOR, F., 2015, A study on the stability and green synthesis of silver nanoparticles using *Ziziphora tenuior* (Zt) extract at room temperature. *Spectrochim Acta A Mol. Biomol. Spectrosc.*, **134**: 310-315.

SADHASIVUM, S., SHANMUGAM, P. AND YUN, K., 2010, Biosynthesis of silver nanoparticles by *Streptomyces hygroscopicus* and antimicrobial activity against medically important pathogenic microorganisms. *Colloids Surf. B: Biointerfaces*, **81**: 358-362.

SANTOS, O. M., ZAMORA, P. O. AND ARRIAGA, L. O., 1995, Floating row cover and transparent mulch to reduce insect populations, virus diseases and increase yield in cantaloupe. *Florida Entmol*., **78**: 493-501.

SARAVANAN, K., GODARA, R. K., GOYAL, R. K. AND SHARMA, R. K., 2004, Preparation and evaluation of squash from papaya fruit. *Indian J. Hill Fmg*., **17** (1&2): 59-65.

SAVENKOVE, E. I. AND VALKONEN, J. P., 2001, Coat protein gene mediated resistance to potato virus-A in transgenic plants is suppressed following infection with another potyvirus. *J. Gen. Virol.,* **82**: 2275-2278.

SHAIKH, N. B., 1996, Studies on papaya ringspot virus disease. *M.Sc. Thesis*, Univ. Agric. Sci., Dharwad, p. 71.

SHARMA, U., CHOWLA, S. C., THAKUR, P. D., HANDA, A. AND GUPTA, B., 2005, Management of radish mosaic disease in Himachal Pradesh. *Indian J. Environ. Ecplan.*, **7** (2): 75-77.

SHARMA, S. K., CHAVAN, V. M., KADAM, U. M., TOMAR, S. S. AND DHALE, M. G., 2007, Good Agricultural Practices (GAPs) for successful management of papaya ringspot virus (P) in papaya - a case study of IARI regional station, Pune, India. *Int. Pl. Virus Epid. Symp*., ICRISAT, Patancheru, India. October 15-19, p. 37.

SHARMA, V. K., YNGARD, R. A. AND LIN, Y., 2009, Silver nanoparticles: green synthesis and their antimicrobial activities. *Adv. Colloid Interface Sci.,* **145**: 83-96.

SHARMA, S. K., ZOTE, K. K., KADAM, U. M., TOMAR, S. S., DHALE, M. G. AND SONAWANE, A. U., 2010, Integrated management of papaya ringspot virus. *Acta Hortic*., **851**: 473-480.

SHARON, M., CHOUDHARY, A. K. AND KUMAR, R., 2010, Nanotechnology in agricultural diseases and food safety. *J. Phytology*, **2** (4): 83-92.

SHIKHAMANY, S. D., 2004, Strategies to improve productivity, quality and post harvest management in fruit crops. In: *Souvenir, first Indian horticultural congress on improving productivity, quality, post harvest management and trade in horticultural crops*, p. 5-14.

SHUKLA, D. D., WARD, C. W. AND BRUNT, A. A., 1994, *The potyviridae*. Cab International, Wallingford, Oxon UK.

SINGH, I. D., 1990, *Papaya*. Oxford and IBH publishing company Pvt. Ltd., New Delhi, p.192.

SINGH, S. J., 2003, *Virus and phytoplasma diseases of papaya, passion fruit and pineapple*. Kalyani Publishers, Ludhiana, p. 147.

SINGH, S. AND AWASTHI, L. P., 2006, Protection of mungbean and urdbean crops against vector-borned Mungbean yellow mosaic virus through botanicals. Abstract In: *XVI Annu. convention of IVS and int. Sympo. management of vector-borne viruses*. ICRISAT, Patancheru, Hyderabad, India.

SINGH, S. AND AWASTHI, L. P., 2007, Survey and serodiagnosis of viruses infecting papaya (*Carica papaya* L.) plants in Uttar Pradesh, India. *Int. Pl. Virus Epidemiology Symp*., ICRISAT, Patancheru, India. October 15-19, p. 63.

SINGH, S. AND AWASTHI. L. P., 2009, Plant products for the management of yellow mosaic disease of mungbean and urdbean. *J. Pl. Prot. Sci.*, **1** (1): 87-91.

SINGH, P. AND RAJA, R. B., 2011, Biological synthesis and characterization of silver nanoparticles using the fungus *Trichoderma harzianum. Asian J. Exp. Biol. Sci.,* **2** (4): 600-605.

SINGH, S. K. AND SINGH, R., 2013, *Screening* of papaya (*Carica papaya* L.) varieties for resistance to viral diseases. *Pl. Archives*, **13** (1): 481-483.

SINGH, R. K., SINGH, D. AND SINGH, J., 2003, Incidence, distribution and detection of a virus infecting papaya (*Carica papaya* L.) in Eastern Uttar Pradesh. *Indian Phytopathol*., **21** (1&2): 51-56.

SINGH, V., RAO, G. P. AND SHUKLA, K., 2005, Response of commercially important papaya cultivars to papaya ringspot virus in eastern U.P. conditions. *Indian Phytopathol*., **58** (2): 212-216.

SINGH, S. K., JHA, P. K. AND RAY, P. K., 2010, Integrated management of papaya ring spot virus (PRSV) in agro ecological conditions of Bihar. *Acta Hortic*., **851**: 487-494.

SINGH, S., AWASTHI, L. P. AND SINGH, R. K., 2011, Induction of systemic resistance through antiviral agents of plant origin against papaya ring spot disease (*Carica papaya* L.). *Archives Phytopathol. Pl. Prot.*, **44** (17): 1676-1682.

SINGH, S., JANGRE, A., KUMAR, P., AWASTHI, L. P., 2017, Studies on the molecular variability in Indian isolates of papaya ringspot virus. *Virol. Res. J*., **1** (1): 10-16.

SINGHAL, V., 1990, Indian agriculture, Indian economic data research centre, Mayapuri, New Delhi, p. 625-629.

SMITH, F. F., JOHNSON, G. V., KAHN, R. P. AND BING, A., 1964, Repelling of aluminum to transient aphid virus vectors. *Phytopathology,* **54**: 748.

SOHEYLA, H., HAMED, B., FATHABAD, E. G. AND FARZANEH, N., 2013, Green synthesis of silver nanoparticles induced by the fungus *Penicillium citrinum. Trop. J. Pharm. Res.,* **12** (1): 7-11.

SOLINSKA, S. J., URBANOWICZ, A., FIGLEROWICZ, M. AND BUJARSKI, J. J., 2011, RNA-RNA recombination in plant virus replication and evolution. *Annu. Rev. Phytopathol*., **49**: 415-443.

SONDI, I. AND SALOPEK, B., 2004, Silver nano particles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J. Colloid Interface Sci*., **275**: 177-182.

SONG, J. Y. AND KIM, B. S., 2009, Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess Biosyst. Eng.,* **32**: 79-84.

SONI, N. AND PRAKASH, S., 2013, Fungus generated novel nanoparticles: a new prospective for mosquito control. *Int. J. Recent Sci. Res*., **4**: 1481-1487.

SRINIVASULU, M. AND SAIGOPAL, D. R., 2011, Coat protein sequence comparison of south Indian isolates of papaya ringspot virus with other Indian subcontinent isolates. *Phytopathol. Mediterr.*, **50**: 359-367

SRIVASTAVA, A., SRIVASTAVA, S. AND VERMA, H. N., 2006, Induction of antiviral resistance in *Carica papaya* by systemic resistance inducing protein from *Clerodendrum aculeatum*. Abstract In: XVI *Ann. convention of IVS and Int. symp. management of vector-borne virus*es, ICRISAT, Patancheru, Hyderabad, India.

SRIVASTAVA, A., TRIVEDI, S., KRISHNA, S. K., VERMA, H. N. AND PRASAD, V., 2009, Suppression of papaya ringspot virus infection in *Carica papaya* with CAP-34, a systemic antiviral resistance inducing protein from *Clerodendrum aculeatum*. *Eur. J. Pl. Pathol*., **123**: 241-246.

SRIVASTAVA, A., SRIVASTAVA, S. AND PRASAD, V., 2015, Systemic antiviral resistance induced in papaya by CAP-34, a resistance inducing protein from *Clerodendrum aculeatum*, is associated with a proteinaceous virus inhibitory activity. *J. Plant. Pathol*., **97** (1): 45-54.

STAPLETON, J. J. AND SUMMERS, C. G., 2002, Reflective mulches for management of aphids and aphid-borne virus diseases in late-season cantaloupe (*Cucumis melo* var. *cantalupensis*). *Crop Prot*., **21**: 891-898.

SUDHA, R., BALAMOHAN, T. N., SOORIANATHASUNDARAM, K., RABINDRAN, R. and MANORANJITHAM, S. K., 2015, Studies on resistance

to papaya ringspot virus (PRSV) in intergeneric population of *Carica papaya* L. and *Vasconcellea cauliflora. SABRAO J. Breed. Genet.,* **47** (2): 113-123.

SUMMERS, C. G. AND STAPLETON, J. J., 1999, Management of aphids, silverleaf whiteflies, and corn stunt leafhoppers using reflective plastic mulch and insecticides: 1998 season review. *Pl. Prot*., **9**: 2-7.

SUMMERS, C. G., MITCHELL, J. P. AND STAPLETON, J. J., 2004, Management of aphid borne viruses and *Bemisia argentifolii* (Homoptera: Aleyrodidae) in zucchini squash by using UV reflective plastic and wheat straw mulches. *Environ. Entomol*., **33**: 1447-1457.

SUREKHA, S. K., MATHUR, K. AND SHUKLA, D. O., 1977, Virus disease of papaya (*Carica papaya*) in Udaipur. *Indian J. Mycol. Pl. Pathol.*, **7**: 115-121.

SUSAN, J. K., 1985, Studies on mosaic disease of papaya (*Carica papaya*). *M. Sc. Thesis*, Acharaya N.G Ranga Agric. Univ., Hyderabad, p. 43.

SUZUKI, J. Y., TRIPATHI, S. AND GONSALVES, D., 2007, Virus-resistant transgenic papaya: commercial development and regulatory and environmental issues. In: Biotechnology and plant disease management. [(Eds.) Z. Punja, S. H. De Boer and H. Sanfacon]. UK, CABI, p. 436.

TALUKDAR, B., SAIKIA, A. K. AND NATH, P. D., 2013, Detection of papaya ring spot virus through double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) in Assam. *J. Mycol. Plant Pathol*., **43** (4): 442-446.

TENNANT, P. F., FERMIN, G. A. AND ROYE, R. E., 2007, Viruses infecting papaya (*Carica papaya* L.): etiology, pathogenesis and molecular biology. *Pl. Viruses*, **1**: 178-188.

TENORIO, J., SALAZAR, J., FUENTES, S., AGUILAR, C., MALPARTIDA, C., MULLER, G., CHUQUILLANQUI, C., CABRERA, A., TORRE, D. C., MARIN, J., CAMPOVERDE, E.V., BARKER, I. AND SALAZAR, L. F., 2007,

Integrated management of papaya ringspot virus (PRSV) in Peru. *Int. Pl. Virus Epidemiology Symp*., ICRISAT, Patancheru, India. October 15-19, p. 67.

THOMAS, M. B., 1999, Ecological approaches and the development of truly integrated pest management. *Proc. Nation. Acad. Sci., USA*. **96**: 5944-5951.

THOMAS, J. E. AND DODMAN, R. L., 1993, The first record of papaya ringspot virus type -P from Australia. *Australas. Plant Pathol.*, **22** (1): 2-7.

THOMAS, S. AND MC CUBIN, P., 2003, A comparison of the antimicrobial effects of four silver containing dressings on three organisms. *J. Wound Care,* **12**: 101-107.

THURMANN, R. B. AND GERBA, C. P., 1989, The molecular mechanisms of copper and silver ion disinfection of bacteria and viruses. *Critic. Rev. Environ. Cont*., **18**: 295-315.

TORDO, V. J., CHACHULSKA, A. M., FAKHFAKH, H., ROMANCER, M. L., ROBAGLIA, C. AND ASTIER, M. S., 1995, Sequence polymorphism in the P1 coding region of potato virus Y genomic RNA. *J. Gen. Virol*., **76**: 939-949.

TRIPATHI, S., JON, Y. S., STEPHEN, A. F. AND GONSALVES, D., 2008, Papaya ringspot virus-P: characteristics, pathogenicity, sequence variability and control. *Mol. Pl. Pathol.,* **9** (3): 269-280.

URCUQUI-INCHIMA, S., HAENNI, A. AND BERNARDI, F., 2001, Potyvirus proteins: a wealth of functions. *Virus Res*., **74**: 157-175.

VALENCIA, L. D., GARCIA, R. N., LAURENA, A. C., VILLEGAS, V. N. AND BAJET, N. B., 2001, Delayed ring spot virus symptom expression in *Carica* interspecific grafts and inarches., *Philipp. J. Crop Sci.,* **26** (2): 51-54.

VALLI, A., LOPEZ-MOYA, J. J. AND GARCIA, J. A., 2007, Recombination and gene duplication in the evolutionary diversification of P1 proteins in the family Potyviridae. *J. Gen. Virol*, **88**: 1016-1028.

VANI, S., VARMA, A., MORE, T. A. AND SRIVASTAVA, K. P., 1989, Use of mulches for the management of mosaic disease in muskmelon. *Indian Phytopathol*., **42**: 227-235.

VERA, J., CASTRO, J., GONZALEZ, A., BARRIENTOS, H., MATSUHIRO, B., ARCE, P., ZUNIGA, G. AND MOENNE, A., 2011, Long-term protection against tobacco mosaic virus induced by the marine alga oligo-sulphated-galactan poly-Ga in tobacco plants. *Mol. Pl. Pathol*., **12** (5): 437-447.

VERDAGUER, A. E., GOUDOU, U. C., DUBERN, J., BEACHY, R. N. AND FAUQUET, C., 1997, Analysis of the sequence diversity of the P1, HC, P3, NIb and CP genomic regions of several yam mosaic potyvirus isolates: implications for the intraspecies molecular diversity of potyviruses. *J. Gen. Virol.*, **78** (6): 1253-64.

VERGHESE, A., KALLESHWARASWAMY, C. M. and KUMAR, A. R., 2007, Pests of papaya and their management with special reference to aphid vectors. *Acta Hortic*., **740**: 259-264.

VERMA, H. N. AND AWASTHI, L. P., 1979, Antiviral activity of *Boerhaavia diffusa* root extract and physical properties of virus inhibitor. *Can. J. Bot*., **57**: 926-932.

VERMA, H. N. AND AWASTHI, L. P., 1980, Occurrence of a highly antiviral agent in plants treated with *Boerhaavia diffusa* inhibitor. *Can. J. Bot*., **2**: 41-44.

VERMA, A. AND SINGH, R. B., 1994, *Clerodendrum aculeatum*: a possible prophylactic agent against natural viral infection in mungbean. *Ann. Pl. Protec. Sci.*, **2** (2): 60-63.

VERMA, H. N., AWASTHI, L. P. AND SAXENA, K. C., 1979, Isolation of the virus inhibitor from the root extract of *Boerhaavia diffusa* inducing systemic resistance in plants. *Can. J. Bot.,* **57**: 1214-1218.

VERMA, R., RAM, R. D. AND TOMER, S. S., 2007, Survey and surveillance of papaya ringspot virus disease in India. *J. Maharashtra Agric. Univ*., **32** (2): 277-278.

VILLEGAS, V. N., 1997, *Carica papaya* L. In: *Edible fruits and nuts* [(Eds.) E.W.M. Verheij and R. E. Coronel]. Wageningen University, Netherlands, p. 2:12.

VILLEGAS, V. N., 2001, Approaches in developing ringspot virus resistant papaya. *Transactions of the national academy of science and technology*, Philippines. **23**: 63-70.

WAHAB, N., 1991, Papaya ring spot virus disease is there in Malaysia. *MAPPS Newsltr*., **15**: 36.

WAN, S. H. AND CONOVER, R. A., 1983, Incidence and distribution of papaya virus in Southern Florida. *Pl. Dis.*, **67**: 353-356.

WANG, D. N., 1982, Screening of papaya varieties for ringspot tolerance. *J. Agric. Res*., **31**: 162-168.

WANG, C. H. AND YEH, S. D., 1997, Divergence and comparison of the genomic RNAs of Taiwan and Hawaii strains of papaya ringspot potyvirus. *Arch. Virol*., **142**: 271-285.

WANG, C., BAU, H. AND YEH, S., 1994, Comparison of the nuclear inclusion b protein and coat protein genes of five papaya ringspot virus strains distinct in geographic origin and pathogenicity. *Phytopathology,* **84** (10): 1205-1210.

WATSON, B., 1997, Agronomy/agroclimatology notes for the production of papaya. *MAFFA*, *Australia*, **6**: 45-47.

WEBB, R. E. AND SCOTT, H. A., 1965, Isolation and identification of watermelon mosaic viruses 1 and 2. *Phytopathology*, **55**(8): 895-900.

WISLER, G. C., PURCIFULL, D. E. AND HIEBERT, E., 1995, Characterization of the P1 protein and coding region of the zucchini yellow mosaic virus. *J. Gen. Virol.*, **76** (1): 37-45.

WU, F. C., PENG, X. X. AND XU, S. H., 1983, Preliminary studies on identification, purification and properties of papaya ringspot virus in South China. *Acta Phytopathol. Sin.*, **13**: 21-28.

YEH, S. D, 1994, Comparison of genetic organization of papaya ringspot virus with other poty viruses. *Pl. Pathol. Bull.*, 3: 54-64.

YEH, S. D. AND GONSALVES, D., 1984, Evaluation of induced mutants of papaya ringspotvirus for control by cross protection. *Phytopathology*, **74**: 1086-1091.

YEH, S. D. AND GONSALVES, D., 1985, Translation of papaya ringspot virus RNA: *in vitro* detection of a possible polyprotein that is processed for capsid protein, cylindrical inclusion protein and amorphous inclusion protein. *Virology*, **143**: 260-271.

YEMEWAR, S. I. AND MALI, V. R., 1980, On the identity of a sap transmissible virus of papaya in Marathawada. *Indian J. Mycol. Pl. Pathol*., **10**: 155-160.

YU, T. A., CHIANG, C. H., WU, H. W., LI, C. M., YANG, C. F., CHEN, J. H., CHEN, Y. W. AND YEH, S. D., 2011, Generation of transgenic watermelon resistant to zucchini yellow mosaic virus and papaya ringspot virus type W. *Pl. Cell Rep*., **30**: 359-371.

ZEE, F., 1985, Breeding of papaya virus tolerance in solo papayas, *Carica papaya* L. *Ph.D. Thesis*, Univ. Hawaii, Honolulu, p. 105.

ZHANG, Z. K., OUYANG, M. A., WU, Z. J., LIN, Q. Y. AND XIE, L. H., 2007, Structure activity relationship of triterpenes and triterpenoid glycosides against tobacco mosaic virus. *Planta Med*., 73: 1457-1463.

ZHU, L., YANG, T., CHEN, L. J., LIN, H. H. AND XI, D. H., 2016, First report of papaya ringspot virus infecting bitter gourd in China. *Pl. Proc. Dis.*, **100** (11): 2338.

APPENDICES

Appendix I: Agarose gel electrophoresis

Reagents for agarose gel electrophoresis

A) 10x TBE (Tris borate EDTA buffer, 1 L): 108.00 g Tris base, 55.00 g boric acid and 20.00 ml of 0.50 M EDTA (pH 8.00); volume made up to 1000 ml with distilled water and stored at room temperature.

B) 6x loading dye: 10.00 mM Tris HCl (pH 7.60) 0.03 per cent bromo phenol blue, 0.03 per cent xylene cyanol, 60.00 per cent glycerol, 60.00 mM EDTA.

C) 0.1 per cent ethidium bromide stock solution: 100 mg ethidium bromide dissolved in 10 ml distilled water; Stored in dark bottle at 4 ˚C.

D) DNA ladder: 10.00 µl DNA ladder of either 100 b or 1 Kb (0.5 µg μl^{-1}), 10 µl 6x DNA loading dye, 40 µl deionized water. Six microlitre of loading mixture was loaded for an agarose gel lane.

Agarose gel electrophoresis procedure

The gel-casting tray was cleaned by washing and subsequent wiping with 70 per cent ethanol. Required volume of 1 per cent agarose was prepared by melting agarose in 1x TBE buffer (300 mg in 30 ml 1x TBE buffer) in a microwave oven. Once agarose solution was cooled up to 45-50 ˚C, ethidium bromide was added at the rate of 0.5 µg ml^{-1} of agarose. Gel casting tray was prepared with the desired number of wells and taping the ends. After comb was placed in the boat, molten agarose was poured into the boat without forming air bubbles and allowed for 30 minutes for proper solidification. Five microliter of each PCR product was separately mixed with 2 µl of loading dye on a clean polythene strip. After removing the tapes from the ends of the casting tray, tray was placed in the electrophoresis tank filled with 1x TBE buffer maintaining the buffer level at 2 mm above the gel slab. The comb was removed carefully. Samples of PCR amplified products were loaded into wells of the gel along with DNA ladder and run at 60 volts for 1-2 hours until the dye front reached the other end of the gel. Gel was removed from the casting tray, visualized under UV and photographed in a gel documentation unit.

Appendix II: Cost of cultivation involved in adoption of IDM modules for one year

Sl. No.	Module	I	II	III	IV	Control
I	**Variable cost***					
	Material input					
1	Planting material	10608	10608	10608	14520	14520
2	Farm yard manure	14400	14400	14400	14400	14400
3	Red earth	3150	3150	3150	3150	3150
4	Chemical fertilizer	8575	8575	8575	8575	8575
5	Irrigation	2440	2440	2440	2440	2440
6	Transportation of materials	3694	3694	3694	3694	3694
7	Insect proof cage/ Border crop (SA tall) /Mulching	8466	8466	1496	9460	-
8	Bioextract and seaweed extract	5738	9739	8065	11,049	-
A	**Sub total**	57071	61072	65878	67288	46779
	Labour input					
1	Land preparation with tractor	4884	4884	4884	4884	4884
2	Pit opening /Mulching	4500	4500	6000	6600	4500
3	Manuring and silt application	2000	2000	2000	2000	2000
4	Planting	1906	1906	1906	1906	1906
5	Chemical fertilizer application	969	969	969	969	969
6	Weeding	2867	2867	2867	2867	2867
7	Irrigation	1719	1719	1719	1719	1719
8	Labour for plant protection operations	5586	5586	5586	5586	-
B	**Sub total**	24431	24431	25931	26531	18845
C	Interest on working capital @ 10% per annum	8150	8550	9181	9382	6562
D	**Total variable cost**	89652	94053	100990	103201	72186
II	**Fixed cost**					
15	Depreciation	269	269	269	269	269
16	Rental value of land	23990	23990	23990	23990	23990
17	Land revenue	35	35	35	35	35
18	Managerial cost @10% of working capital	8150	8550	9181	9382	6562
19	Drip for irrigation	5247	5247	5247	5247	5247
E	Total fixed cost	37691	38091	38722	38923	36103
F	**Total cost (I+II)**	**127343**	**132145**	**139712**	**142124**	**108290**

* Input costs are calculated at prevailing market rate

Appendix III: Returns per rupee invested in adoption of integrated management modules against PRSV in the first year

Particulars	Number of plants per acre (no.)	Yield per acre (kg)	Income from papaya (Rs.)	Income from fodder maize (Rs.)	Total income (Rs.)	Total cost (Rs.)	B:C ratio
Module I	884	21746	217460	13160	230620	127343	1.81
Module II	884	20677	206770	13160	219930	132145	1.66
Module III	884	25353	253530	13160	266690	139712	1.91
Module IV	1210	24333	243330	0	243330	142124	1.71
Control	1210	14362	143620	0	143620	108290	1.32

Price of papaya Rs. 10 kg^{-1}

Price of fodder maize Rs. 1.5 kg^{-1}

Mysore J. Agric. Sci., 51(3) : 631-636, 2017

Partial Sequence Comparison of P1 Proteinase Gene of PRSV Infecting Papaya from Southern Karnataka

ANIL PAPPACHAN AND N. NAGARAJU
Department of Plant Pathology, College of Agriculture, UAS, GKVK, Bengaluru-560 065
E-mail: agrico.pappachan@gmail.com

ABSTRACT

GKVK-Bengaluru isolate of Papaya Ring Spot Virus (PRSV) showed highest nucleotide identity of 88 per cent with isolate from Hyderabad (Accession KP743981) and 87 per cent nucleotide identity with PRSV P isolate from Delhi (Accession EF017707) when the partial sequence of P1 proteinase gene was compared. Close relationship among Indian isolates was noticed from the phylogenetic tree constructed based on available sequences and Indian isolates were separated from isolates of other geographical regions forming a different sub-cluster in the phylogenetic tree. The clustering pattern of isolates correlated well with their geographical origins except for one isolate PRSVR3 (Accession KJ755852).

Keywords: Papaya ring spot virus (PRSV), proteinase, phylogeny and genetic diversity

PAPAYA (*Carica papaya* L.) belongs to family Caricaceae, is an important fruit crop because of its great economic potential. Papaya Ring Spot Virus disease (PRSV) caused by PRSV-P has gained global importance in all the papaya growing countries. The ring spot virus disease is posing a major threat to papaya cultivation throughout India by rendering orchards economically unproductive. Infected Papaya plants shows yellowing, leaf distortion and severe mosaic. Oily or water-soaked spots and streaks appear on the trunk and petioles. The fruits exhibit bumps and the classic ringspot symptoms. Severe isolates are known to cause tissue necrosis (Gonsalves *et al.*, 2010). PRSV infection drastically reduces fruit yield, fruit size and quality. The disease is found to cause losses up to 100 per cent in some parts of the globe (Tennant *et al*., 2007). There is a great scope to increase the papaya productivity if the ring spot virus disease is combated.

PRSV has a monopartite linear single-stranded positive sense RNA genome and is about 10,326 nucleotides long, excluding a poly-A-tract found at its 3' end. PRSV genome encodes a single large protein which is subsequently cleaved into smaller proteins *viz.*, P1, HC-Pro, P3, CI, 6K, NIa-Pro, NIb and CP. P1 codes for a 63 K.D protein which is a proteinase and also possibly involved in cell-to-cell movement (Gonsalves *et al.*, 2010).

Knowledge of sequence diversity among isolates of a virus, their distribution has the potential to deepen our understanding of viral origin, disease etiology, development and dispersal. This information would be useful in the development of effective management programmes against viral diseases. Sequence data needs to be generated from isolates of different regions of the country to provide the complete PRSV population profile which could allow critical assessment of sequence divergence within the PRSV population. The comparative studies of isolates in respect to genome sequences would provide data about the complexity in PRSV populations in the country and also help to trace strain phy-logeny for better understanding of the evolution and molecular epidemiology of PRSV. With PRSV, most of the studies have been focused on examining variation in the coat protein gene sequences and data on the evolution and molecular epidemiology of PRSV based on P1 proteinase gene are limited. Hence, the present study reports sequence comparison analysis P1 proteinase gene to ascertain the ge-netic diversity of PRSV isolate in the Southern Karnataka (Bengaluru).

MATERIAL AND METHODS

Virus isolate and maintenance: Papaya seeds (Red lady) were sown in plastic sprouting trays. Twenty days after germination, seedlings were transplanted

to polyethylene covers containing soil + sand + coir pith and FYM (1:1:1:1) and were maintained inside insect proof cages at Department of Plant Pathology glass house. The leaf samples with characteristic PRSV symptoms were collected and sap inoculated to healthy seedlings using standard mechanical transmission procedure. Papaya leaves infected by PRSV were collected, washed under tap water, blot dried and ground with 0.1M phosphate buffer (pH 7.5 at 1gm / ml) using mortar and pestle. The sap was filtered through double layered muslin cloth and filtrate was mixed with a pinch of celite powder (600 mesh). A piece of sterile non-absorbent cotton pad was dipped in filtrate and was rubbed unidirectionally from petiole towards the margin of papaya leaves. The inoculated leaves were washed after 5 minutes with a jet of water to remove the traces of celite. Sap inoculated seedlings were brought to glass house and individually maintained under insect proof cages. Plants were observed daily for symptom development and symptoms expressed were recorded.

Symptomatology and confirmation of PRSV infec-tion in isolate: The identity of the virus was initially confirmed by symptoms developed on papaya. The presence of PRSV in the different isolates was further verified by double antibody sandwich ELISA with anti-PRSV capture antibody and ALP labelled anti-PRSV detection antibody.

Isolation of RNA: Isolation of total RNA from infected plants was done by using Trizol reagent. All the plastic wares and glasswares were washed thoroughly, dried and treated with 0.1 per cent DEPC (Diethylene pyrocarbonate) water by dipping for 24 hours and used after sterilization. The PRSV infected papaya leaf samples maintained under glasshouse were brought under ice-cold condition and ground to a fine powder in sterilized and dried pre chilled mortar and pestle using liquid nitrogen. About 100 mg of powdered leaf material was taken into a 1.5 ml micro centrifuge tube and homogenated partially using a homogenizer. Immediately 1ml of Trizol was added to the homogenized tissue. The tubes were centrifuged at 9,000 rpm for 10 minutes to remove extracellular material. Without disturbing the pellet, supernatant was transferred to fresh tube and kept at room temperature. 200 µl of chloroform + phenol (1 ml chloroform : 1 ml phenol) was added to the supernatant. After 15 minutes of shaking, tubes were centrifuged at 12,000 rpm for 8 minutes. Three distinct layers were formed, from which only the top layer was transferred to fresh tubes. 0.5 ml of Isopropanol was added to each tube, followed by 10 minutes incubation at room temperature. The tubes were then centrifuged at 13,000 rpm for 5 minutes and the pellet was collected discarding supernatant. 75 per cent ethanol (750 µl+250 µl H_2O) was added to the pellet and tubes were centrifuged at 12,000 rpm for 2 minutes. After centrifugation, ethanol was discarded, and pellet was vacuum dried for 10 minutes. 20 µl of DEPC treated water was added to each tube and they were incubated at 55-60 °C on a water bath to dissolve the pellet. RNA thus obtained was stored at -20 °C.

Reverse Transcription: Total RNA from healthy and PRSV infected samples were taken for reverse transcription along with negative control (distilled water). Primers for amplification of P1 proteinase gene were designed using NCBI primer blast tool with sequences from Hyderabad isolate (Accession No: KP743981). 20 µl RT mixture was prepared by adding the following ingredients into the PCR tube. 5X RT buffer 4 µl, 25 mM $MgCl_2$ 1.0 µl, 10.0 mM dNTP mixture 2.0 µl, reverse primer 5'TCTTTCCGAACTTGAGTTGCT3' (10 µM) 2.0 µl, Reverse Transcriptase 0.5 µl (50 units), Viral RNA 5.0 µl (1:10 diluted with water) and finally volume was made with 5.5 µl DEPC treated distilled water. The RT-PCR mixture was reverse transcribed at 39 °C for 60 min. and then at 94 °C for 5 min. The c-DNA thus obtained was used for performing PCR.

Polymerase chain reaction: The c-DNA obtained was subjected to PCR amplification using forward primer designed to amplify PRSV nucleotides from total RNA extracted from infected papaya plants. PCR amplifications were conducted using Eppendrof thermo-cycler in 15 µl reaction mixture that contained 2.0 µl c-DNA, 0.2 µl Taq DNA polymerase (5 U/µl), 2.5 µl of 10X PCR buffer, 0.5 µl of 25 mM $MgCl_2$, 2.0 µl forward primer 5'CAATTCGAAGCAACCAAACAAAT3' (10 µM), 2.0 µl dNTPs mix (2.5 mM each) and DEPC treated distilled water to make up the volume. The mixture was subjected to one cycle of initial Denaturation at

94 ^{0}C for 4 minutes followed by 35 cycles of denaturation at 94 ^{0}C for 60 seconds, annealing at 46.00 °C for 45 seconds, extension at 72 ^{0}C for 90 seconds and a final extension at 72 ^{0}C for 10 min. After the completion of the reaction, the products were kept at 4 ^{0}C prior to gel analysis.

Analysis of PCR products by agarose gel electrophoresis: Amplification was confirmed by agarose gel electrophoresis.

Sequencing of amplified PCR product and sequence analysis: After successful confirmation, the amplified PCR product was directly sequenced using ABI 3730Xl DNA Analyzer available at Scigenome Labs Pvt. Ltd., Cochin-Kerala, India. Sequencing was done in both directions using forward and PRSV reverse primers.

Construction of Phylogenetic tree: The sequence homology obtained in BLAST (www.ncbi.nih.gov / BLAST) and Neighbor joining phylogenetic tree was generated using MEGA 6.06 software tool. In order to calculate the confidence limits placed in construction of phylogenetic tree, bootstrapping analysis was carried out using 1000 replicates, resulting in a boot strapped Neighbor joining tree.

Results and Discussion

Symptomatology and confirmation of PRSV infection: Virus induced symptoms typical of PRSV infection on papaya with severe mosaic and leaf distortion on papaya cv. Red lady. The isolate strongly reacted in DAS-ELISA with PRSV-P polyclonal antibody confirming the identity of PRSV in the inoculated plants. After confirming the identity, isolate was maintained on their natural host pa-paya in an insect-proof glasshouse by mechanical inoculation.

Amplification and sequencing of P1 proteinase gene: Total RNA from PRSV infected papaya leaves was extracted using Triazole method and cDNA was synthesized through reverse transcription. Part of P1 proteinase gene was amplified and an expected ~1000 bp band was confirmed through electrophoresis (Plate 1). After confirmation further PCR amplifications were carried out with 50 µl reaction mixture and products were send for sequencing.

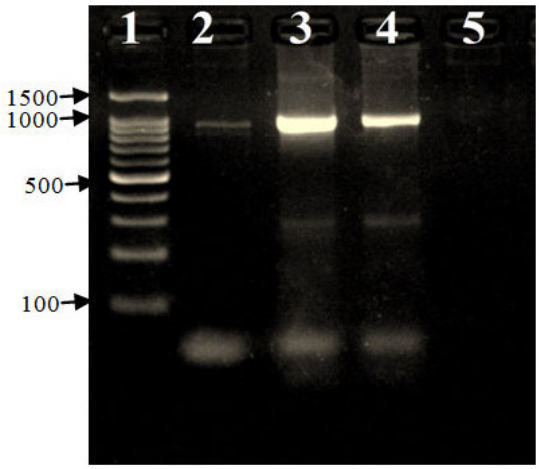


Plate 1: Amplified PCR product of P1 proteinase gene of GKVK-Bengaluru isolate

Lane 1: 100bp DNA ladder, 2: Healthy Papaya sample, 3&4: GKVK Bengaluru isolate and 5: Water Control

The sequence homology obtained in BLAST revealed the query matching with reported PRSV P1 proteinase gene sequences from different geographical locations. GKVK-Bengaluru isolate showed highest identity of 88 per cent PRSV isolate from Hyderabad (Accession KP743981) and 87 per cent identity with PRSV P isolate from Delhi (Accession EF017707). It showed 84 per cent identity with PRSV W isolate from India (Accession EU475877) followed by 79 per cent identity with PRSV strain Leaf deformation (Accession DQ340769) and PRSV isolate pFT3NP (Accession JX448373). BLAST revealed that it was distinct from the isolates of New Delhi and Hyderabad.

The nucleotide sequences of the GKVK-Bengaluru isolate (PRSV BLR) was compared to sequences of nineteen other PRSV isolates available in the gene bank (Table I). Indian isolates were separated from isolates of other geographical regions forming a separate sub-cluster in phylogenetic tree (Fig. 1) and clustering pattern of isolates correlated well with their geographical origins. The close relationship among Indian isolates was noticed from the phylogenetic tree constructed based on available sequences and generally clustering pattern of isolates correlated well with their geographical origins except for one isolate PRSVR3 (Accession KJ755852).

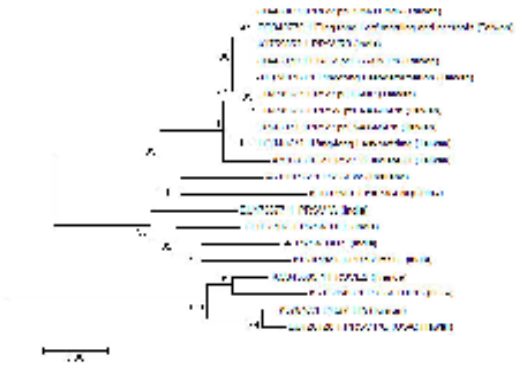


Fig.1: Bootstrapped neighbor joining phylogenetic tree showing the relationship of PRSV isolates

A nucleotide BLAST search using the complete genome of PRSV-W-TUL15 showed 83 to 92 per cent nucleotide sequence similarities with the published PRSV isolates (Ali, 2017). Between the P1 proteins of Taiwanese and Hawaiian PRSV isolates there was only 70.9 per cent nucleotide identity (Wang and Yeh, 1997). The comparative analysis of a severe isolate of papaya ringspot virus (Mex-VrPO) of Mexico and five others reported before showed that P1 was most variable with 13-33 per cent divergence while coat protein showed only 5-9 per cent divergence (Noa-Carrazana *et al.*, 2007). High variability in the P1 proteinase gene has also been reported for other

TABLE I

Details of the Papaya ring spot virus isolates obtained from NCBI database

Isolate	Pathotype	Location	Genbank accession no.	Host	References
PRSV-BLR	P	GKVK-Bangalore	-	Papaya	This study
HYD	Not Specified	Hyderabad: India	KP743981	Papaya	Unpublished
DEL	P	India	EF017707	Not Specified	Parameswari *et al*, 2007
PRSV W (India)	W	India	EU475877	Not Specified	Mangrauthia *et al*, 2008
Ping-tong Leaf-deformation	Not Specified	Taiwan	DQ340769	Papaya	Unpublished
pFT3-NP	P	Taiwan	JX848373	Papaya	Unpublished
pfT3-AX-D-Ph	P	Taiwan	JX848372	Papaya	Unpublished
prT3-AX-N-Ad6	P	Taiwan	JX848369	Papaya	Unpublished
prT3-AX-M-Ph	P	Taiwan	JX848371	Papaya	Unpublished
Ping-tong Leaf-mottling	Not Specified	Taiwan	DQ340771	Papaya	Unpublished
Ping-tong Leaf-mottling and necrosis	Not Specified	Taiwan	DQ340770	Papaya	Unpublished
PRSVR3	Not Specified	India	KJ755852	Papaya	Unpublished
PRSV W (Thailand)	W	Thailand	AY010722	Not Specified	Attasart *et al*, 2002
PRSV W isolate CI	W	Chiayi: Taiwan	AY027810	*Luffa cylindrica* (Linn.) Roem.	Unpublished
prT3-AX-N-Ph	P	Taiwan	JX848370	Papaya	Unpublished
PRSV-LM	P	Hainan, Lingshui, China	KT633943	Papaya	Unpublished
PRSVE2	Not Specified	France	KC345609	*Cucurbita pepo*	Romay *et al*, 2014
PRSVHA	Not Specified	Taiwan	X67673	Not Specified	Wang *et al*, 1994
PRSVPG	Not Specified	USA: Hawaii	EU126128	Not Specified	Unpublished
TUL15	WUSA	KY039583	Gourd	Ali, 2017	

Potyviruses like Zucchini yellow mosaic virus (ZYMV). Lin *et al.* (2001) reported that in ZYMV isolate TW-TN3 P1 proteinase gene was most variable, with amino acid identities of 59.0-93.2 per cent.

With PRSV most of the earlier works concentrated on the coat protein gene sequences while Romay *et al.,* 2014 observed that pairwise sequence similarities in the coat protein (CP) coding region failed to unambiguously distinguish Zucchini tigre mosaic virus (ZTMV) isolates from PRSV isolates. Sequence variability has important implications for the use of genes to develop transgenic plants by pathogen derived resistance and such resistance could be highly sequence specific. The selection of the transgene would be vital step to develop long-lasting virus resistant transgenic papaya. It has been suggested that when designing transgenes for potyvirus resistance, it is essential to select regions of at least 90 per cent identity between strains to obtain a durable resistance (Moreno *et al.,* 1998). RNA mediated resistance to potyviruses has been reported with sequence identity of 88 per cent or greater (Mueller *et al.,* 1995), while Jones *et al.* (1998) reported that 89 per cent identity of the Nib gene was the minimum sequence identity for the specific-ity required to trigger gene silencing in the pea seed-borne mosaic potyvirus.

Genetic engineering is a viable option for managing viral diseases such as PRSV (Kung *et al.,* 2009; Mangrauthia *et al.,* 2010 and Yu *et al.,* 2011). Knowledge of the nucleotide sequence and genetic diversity is necessary to select a virus gene for the development of pathogen derived resistance. Recombinations occur in the majority of RNA viruses, are of great evolutionary importance and constitute one of the greatest forces that shape the virus genomes (Sztuba-Solinska *et al.,* 2011). The hot spots of recombination in PRSV were concentrated in the region encoding the P1 proteinase, P3 protein, cytoplasmic inclusion (CI) and the Helper component proteinase (Mangrauthia *et al.,* 2008). Recombination events in the coat protein of PSRV appeared to be less frequent than in other regions of the genome (Bateson *et al.,* 2002). Zhu *et al.* (2016) reported that nucleotide BLAST analysis of the coat protein sequence of PRSV from China, showed highes tidentity of 99 per cent with four isolates from Taiwan. According to Srinivasulu and Sai Gopal (2011) coat protein gene of PRSV TA-Ti isolate seemed to be an ideal choice to develop transgenic papaya resistant to PRSV in south India. These evidences confirm that coat protein gene of PRSV is more conserved than P1 proteinase gene hence it is a suitable candidate to develop transgenic papaya resistant to PRSV using Pathogen derived resistance.

Identification of gene sequences in PRSV could provide valuable information on the sequence of events that lead to infection and will lead to a better understanding of the significance of changing hosts during molecular evolution of PRSV, an essential requirement for the development of long-term sustainable management strategies against PRSV. The present study confirms the observation that P1 gene is highly variable, hence it does not seem to be an ideal choice to develop transgenic papaya resistant PRSV using Pathogen derived resistance. However, sequences of more isolates are needed to ascer-tain the complete population profile and to draw strong conclusions on sequence divergence within the PRSV population in the Indian sub-continent.

References

Ali, A., 2017, First Complete Genome Sequence of Papaya ringspot virus-W Isolated from a Gourd in the United States. *Genome Announc.*, 5 (2) : e01434 - 16.

Attasart, P., Charoensilp, G., Kertsundit, S., Panyim, S. and Jupimek, M., 2002, Nucleotide sequence of a Thai isolate of Papaya ringspot virus type W. *Acta Virol.*, **46** (4) : 241 - 246.

Bateson, M. F., Lines, R. E., Revill, P., Chaleeprom, W., Ha, C. V., Gibbs, A. J. and Dale. J. L., 2002, On the evolution and molecular epidemiology of the potyvirus Papaya ringspot virus. *J. Gen. Virol.*, **83** : 2575 - 2585.

Gonsalves, D., Tripathi, S., Carr, J. B. and Suzuki, J. Y., 2010, Papaya Ringspot virus. *The Plant Health Instructor* DOI: 10.1094/PHI-I-2010-1004-01.

Jones, A. L., Johansen, I. E., Bean, S. J., Bach, I. and Maule, A. J., 1998, Specificity of resistance to pea seed-borne mosaic potyvirus in transgenic peas expressing the viral replicase (NIb) gene. *J. Gen. Virol.*, **79** : 3129 - 3137.

KUNG, Y. J., BAU, H. J., WU, Y. L., HUANG, C. H., CHEN, T. M. AND YEH. S. D., 2009, Generation of transgenic papaya with double resistance to Papaya ringspot virus and Papaya leaf-distortion mosaic virus. *Phytopathology*, **99** : 1312 - 1320.

LIN SHIH- SHUN, ROGER, F. H. AND SHYI-DONG, Y., 2001, Complete genome sequence and genetic organization of a Taiwan isolate of Zucchini yellow mosaic virus. *Bot. Bull. Acad. Sin.*, **42** : 243 - 250.

MANORAUTHIA, S. K., PARAMESWARI, B., JAIN, R. K. AND PRAVEEN. S., 2008, Role of genetic recombination in the molecular architecture of Papaya ringspot virus. *Biochem. Genet*, **46** : 835 - 846.

MANORAUTHIA, S. K., SINGH, P. AND PRAVEEN. S., 2010, Genomics of helper component proteinase reveals effective strategy for papaya ringspot virus resistance. *Mol. Biotechnol.*, **44** : 22 - 29.

MORENO, M., BERNAL, J. J., JIMENEZ, I. AND RODRIGUEZ-CEREZO, E., 1998, Resistance in plants transformed with the P1 or P3 gene of tobacco vein mottling potyvirus. *J. Gen. Virol.*, **79** : 2819 - 2827.

MUELLER, E., GILBERT, J., DAVENPORT, G., BRIGNETI, G. AND BAULCOMBE, D. C., 1995. Homology-dependent resistance: transgenic virus resistance in plants related to homology-dependent gene silencing. *Plant J.*, **7**(6) : 1001-1013.

NOA-CARRAZANA, J. C., GONZALEZ-DE-LEON, D. AND SILVA-ROSALES, L., 2007, Molecular characterization of a severe isolate of papaya ringspot virus in Mexico and its relationship with other isolates. *Virus Genes.*, **35** (1) : 109 - 117.

PARAMESWARI, B., MANORAUTHIA, S. K., PRAVEEN, S. AND JAIN, R. K., 2007, Complete genome sequence of an isolate of Papaya ringspot virus from India. *Arch. Virol.*, **152** (4) : 843 - 845.

ROMAY, G., LECOQ, H. AND DESBIEZ, C., 2014, Zucchini tigre mosaic virus is a distinct potyvirus in the papaya ringspot virus cluster: molecular and biological insights. *Arch. Virol.*, **159** (2) : 277 - 289.

SRINIVASULU, M. AND SAI GOPAL, D. V. R., 2011, Coat protein sequence comparison of south Indian isolates of Papaya ringspot virus with other Indian subcontinent isolates. *Phytopathol. Mediterr.*, **50** : 359 - 367.

SZTUBA- SOLINSKA, J., URBANOWICZ, A., FIGLEROWICZ, M. AND BUJARSKI, J. J., 2011, RNA-RNA recombination in plant virus replication and evolution. *Annu. Rev. Phytopathol.*, **49** : 415 - 443.

TENNANT, P. F., FERMIN, G. A. AND ROYE, R. E., 2007, Viruses infecting papaya (*Carica papaya* L.): Etiology, pathogenesis and molecular biology. *Plant Viruses*, **1** : 178 - 188.

WANG, C. H., AND YEH, S. D., 1997, Divergence and comparison of the genomic RNAs of Taiwan and Hawaii strains of Papaya ringspot Potyvirus. *Arch. Virol.*, **142** : 271 - 285.

WANG, C., BAU, H. AND YEH, S., 1994, Comparison of the nuclear inclusion b protein and coat protein genes of five papaya ringspot virus strains distinct in geographic origin and pathogenicity. *Phytopath.*, **84** (10) : 1205 - 1210.

YU, T. A., CHIANG, C. H., WU, H. W., LI, C. M., YANG, C. F., CHEN, J. H., CHEN, Y. W. AND YEH, S. D., 2011, Generation of transgenic watermelon resistant to Zucchini yellow mosaic virus and Papaya ringspot virus type W. *Plant Cell Rep.*, **30** : 359 - 371.

ZHU, L., YANG, T., CHEN, L. J., LIN, H. H. AND XI, D. H., 2016, First Report of *Papaya ringspot virus* infecting bitter gourd in China. *Plant Dis.*, **100** (11) : 2338.

(*Received* : May, 2017 *Accepted* : August, 2017)

Trends in Biosciences 10(34), Print : ISSN 0974-8431, 7247-7249, 2017

Screening of Papaya Genotypes for Resistance Against Papaya Ringspot Virus Under Glasshouse Condition

ANIL PAPPACHAN AND N. NAGARAJU

Department of Plant Pathology, College of Agriculture, UAS, GKVK, Bangalore, Karnataka
email: agrico.pappachan@gmail.com

ABSTRACT

Fifteen varieties papaya *viz*, Arka prabhat, Bangalore dwarf, Co-8, IHS selection-4, Madhu, Mohini, Pant papaya-1, Papita selection, Pune selection, Pusa dwarf, Pusa nanha, Sinta-1, Red Lady, Solo, Surya along with wild relative *Carica cauliflora* were screened under glasshouse condition for host-plant resistance against papaya ringspot virus. None of the cultivated papaya variety was found resistant to PRSV and all the cultivated varieties developed disease symptoms within 12-18 days after inoculation, while *C. cauliflora* did not develop symptoms till the end of the experiment.

Keywords *Papaya ringspot virus (PRSV), Screening, Resistance*

Papaya (*Carica papaya* L.) belongs to family Caricaceae, is an important fruit crop because of its great economic potential. Papaya Ringspot Virus disease (PRSV) caused by PRSV-P has gained global importance in all the papaya growing countries. The ringspot virus disease is posing a major threat to papaya cultivation throughout India by rendering orchards economically unproductive. Infected papaya plants shows yellowing, leaf distortion and severe mosaic. Oily or water-soaked spots and streaks appear on the trunk and petioles. The fruits exhibit bumps and characteristic ringspot symptoms. Severe isolates are also known to cause tissue necrosis (Gonsalves *et al.*, 2010). PRSV infection drastically reduces fruit yield, fruit size and quality. The disease is found to cause losses up to 100 per cent in some parts of the globe (Tennant *et al*., 2007). There is a great scope to increase the papaya productivity if the ringspot virus disease is effectively managed.

Papaya ringspot virus spreads rapidly in the field by many species of aphids in a non-persistent manner and it is also readily transmitted mechanically through sap (Suzuki *et al*., 2007). At present, management is mainly focused on insecticides to prevent the movement of insect vectors. But chemicals and insecticides are causing environmental pollution and health hazards. The best way to manage a disease is to identify resistant germplasm and incorporation of the resistance into adapted cultivars resulting in decreased production costs, improved product quality and reduced detrimental effects of chemicals on ecosystem. Genetic resistance is considered to be the most economical and eco-friendly approach for virus management (Shukla *et al.*, 1994).

Plant breeding aims to evolve genotypes suitable for a given agro-climatic situation to optimize the level of genetic potentiality. Use of these resistant varieties is non hazardous, eco-friendly and easily adoptable approach by the farmers for the management of plant diseases. The success of any crop improvement programme mainly depends on the strength and high yielding potential of the germplasm. Hence it is important to screen the available genotypes against the papaya ringspot disease before including genotypes in hybridization programme. In view of the above, an investigation was undertaken to screen some of the available genotypes against PRSV.

MATERIAL AND METHODS

The present investigation was carried out at Department of Plant Pathology, University of Agricultural Sciences, Bangalore (UASB) and Main Research Station (MRS) farm, Hebbal, Bangalore Karnataka.The experiment was carried out during January 2015 to May 2015.Seeds of papaya varieties Bangalore dwarf, IHS selection-4 (Imperial agro genetics, Delhi, India), Madhu (Imperial agro genetics, Delhi, India), Mohini (Suvarna hybrid seeds Pvt. Ltd., Bangalore) Papita selection, Pune selection, Red Lady (Known you seeds India Pvt. Ltd., Pune), Sinta-1 (East west seeds India Pvt. Ltd., Aurangabad), Solo and Surya were collected from the local markets of Bangalore. Pusa dwarf and Pusa nanha seeds were collected from IARI New Delhi, Co-8 was collected from TNAU, Coimbatore. Arka prabhat and *Carica cauliflora* seeds were supplied by IIHR, Bangalore while seeds of Pant papaya 1 were collected from G. B Pant University, Pant Nagar.

Papaya seeds were sown in plastic sprouting trays. 20 days after germination seedlings were transplanted to 30 cm polythene covers containing soil + sand + coir pith and FYM (1:1:1:1). Sixty days old seedlings each variety were mechanically inoculated with PRSV-GKVK Bangalore isolate and kept maintained inside glasshouse in completely randomized design (CRD) with five replications. Observations on days taken for symptom expression and varied symptoms observed on each variety were recorded at regular intervals. Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA**)** technique with anti-PRSV capture antibody (Agdia, USA) and ALP labelled anti-PRSV detection antibody, was used to confirm infection by PRSV in papaya plants. At 60 DAI (Days after inoculation), the experiment was terminated by subjecting apical leaves of all the plants to DAS-ELISA to confirm the infection.

RESULTS AND DISCUSSION

None of the cultivated papaya variety screened in the study was found resistant to PRSV and all the cultivated varieties developed disease symptoms under glasshouse condition. The cultivars differed slightly in respect of days

Table 1. Reaction of different papaya varieties to PRSV on mechanical inoculation

Variety	Average OD value at 405 nm	Disease reaction	Disease incidence at 60 DAI (%)	Incubation period (Days)	Prominent symptoms
Arka prabhat	0.776	+	100	15	Cl, Mo, Ld, Gi, Pc
Bangalore dwarf	0.696	+	100	15	Cl, Mo, Ld, Gi, Pc
Co-8	0.663	+	100	15	Cl, Mo, Ld, Gi, Pc
IHS selection-4	0.836	+	100	18	Cl, Mo, Ld
Madhu	0.793	+	100	15	Cl, Mo, Ld, Gi, Pc
Mohini	0.726	+	100	12	Cl, Mo, Ld, Gi, Pc
Pant papaya-1	0.786	+	100	15	Cl, Mo, Ld, Gi, Pc
Papita selection	0.866	+	100	15	Cl, Mo, Ld
Pune selection	0.768	+	100	18	Cl, Mo, Ld
Pusa dwarf	0.643	+	100	12	Cl, Mo, Ld, Gi, Pc, Ss
Pusa nanha	0.788	+	100	15	Cl, Mo, Ld
Sinta -1	1.082	+	100	12	Cl, Mo, Ld, Gi, Pc, Ss
Solo	0.704	+	100	15	Cl, Mo, Ld
Surya	0.866	+	100	12	Cl, Mo, Ld
Red Lady	0.953	+	100	15	Cl, Mo, Ld, Gi, Pc, Ss
C. cauliflora	0.156	-	0	-	No symptoms
Healthy control	0.148				

Cl: Chlorosis Ld : Leaf distortion Pc: Puckering

Gi: Green islands Mo: Mosaic Ss: Shoestring of leaves

taken for symptom expression and symptoms at 60 days after inoculation (Table 1).

Upon mechanical inoculation with PRSV-Bangalore isolate, all the varieties except wild relative *C. cauliflora* showed susceptibility to PRSV with slight variation in the symptoms. Varieties Mohini, Pusa dwarf, Sinta-1 and Surya developed initial symptoms 12 days post inoculation. IHS selection-4 and Pune selection, took 18 days to express initial symptoms while remaining varieties expressed symptoms within two weeks.

IHS selection-4, Papita selection, Pune selection, Pusananha, Solo and Surya showed symptoms such as chlorosis, mosaic and distortion of leaves. Six varieties (Arka prabhat, Bangalore dwarf, Co-8, Madhu, Mohini and Pant papaya-1) expressed chlorosis, mosaic, distortion of leaves, green islands and puckering. Pusa dwarf, Sinta-1 and Red Lady expressed severe symptoms including chlorosis, mosaic,distortion of leaves, green islands, puckering and shoestring of leaves. However, wild relative *C. cauliflora*did not develop symptoms till the end of the experiment.

Among the cultivated varieties screened under glasshouse condition, none was found resistant to the disease and all the cultivated varieties studied developed symptoms within 12-18 days. Such findings have also been reported by Reddy *et al.* (2011), wherein papaya varieties expressed symptoms 15 days after mechanical inoculation. Pushpa (2014) also found that Sunrise solo, Red Lady and Surya developed symptoms of infection within 10-15 days.

At 60 DAI all the fifteen varieties recorded 100 per cent disease incidence based on the symptoms. All the plants were subjected for DAS-ELISA test to confirm the infection. DAS-ELISA confirmed that none of the screened variety possessed natural to the disease at seedling stage while the wild relative *C. cauliflora*re mained free of infection indicating the presence of resistance. Reddy *et al.* (2011) and Pushpa (2014) also reported that upon mechanical inoculation none of varieties were found to possess resistance to PRSV. Similarly Roff (2007) and Chavan *et al.* (2010) also failed to identify resistance against PRSV at seedling stage when several varieties were screened.

Most of the efforts till date to identify natural resistance met little success (Kudada and Prasad, 2000; Singh *et al.,* 2005; Roff, 2007; Awasthi and Singh, 2009; Balamohan *et al.,* 2010; Chavan*et al.,* 2010; Alviar, 2011; Reddy *et al.,* 2011; Singh and Singh,2013 and Pushpa, 2014). However *C. cauliflora*was reported to beimmune to PRSV (Magdalita*et al.,* 1997; Valencia *et al.,* 2001; Chan and Ong, 2003; and Sudha*et al.,* 2015).Balamohan*et al.* (2010) also reported that natural resistance to PRSV-P was not found in *C. papaya*.Chavan*et al.* (2010) while screening eight commercial papaya cultivars recorded 86 per cent disease incidence on widely grown Red Lady and 45 per cent on Pusa nanha.

Villegas (2001) observed that resistance to PRSV-P was not found in the cultivated papaya but was present in *C.cauliflora*.Several species related to papaya were found to be resistant to PRSV-P, unfortunately crosses between

these species have not been achieved due to strong cross incompatibility (Roff, 2007).Results of the present investigation are in agreement with the earlier reports that cultivated varieties do not possess natural resistance to PRSV, while *C. cauliflora* is immune to the disease. But utilization of *C. cauliflora*in breeding programme appears to be very difficult due to strong cross incompatibility as evident from the earlier reports.

CONCLUSION

Out of the fifteen cultivated varieties of papaya screened, none of the variety was found resistant to PRSV and all the cultivated varieties developed disease symptoms within 12-18 days after inoculation, while *C. cauliflora* did not develop symptoms indicating the presence of natural resistance against papaya ringspot virus.

LITERATURE CITED

Alviar, A. N., 2011, Characterization of resistance to papaya ringspot virus (PRSV) in non transgenic papaya (*Carica papaya* L.) varieties and resistance evaluation of selected transgenic lines carrying the PRSV coat protein gene. *Ph.D. Thesis*, Univ. Philippines, Los Banos. p. 92.

Awasthi, L. P. and Singh, S., 2009, Response of papaya (*Carica papaya* L.) cultivars against viral diseases under field conditions. *J. Plant Dis. Sci*., **4** (1): 72-75.

Balamohan, T. N., Auxcilia, J., Thirugnanavel, A. and Manoranjitham, S. K., 2010, CP-50: A papaya ring spot virus (PRSV) tolerant papaya genotype under field conditions. *Acta. Hortic*., **851**: 153-156.

Chan, Y. K. and Ong, C. A., 2003, Field performance of papaya lines selected for tolerance to ringspot virus disease. *J. Trop. Agric. and Fd. Sc.,* **31** (2): 129-137.

Chavan, V. M., Tomar, S. P. S. and Dhale, M. G., 2010, Management of papaya ring spot virus (PRSV-P) of papaya under Pune conditions. *Acta. Hortic*., **851**: 447-452.

Gonsalves, D., Tripathi, S., Carr, J. B. and Suzuki, J. Y., 2010, Papaya Ringspot virus. *The Plant Health Instructor.* DOI: 10.1094/PHI-I-2010-1004-01.

Kudada, N. and Prasad, S. M., 2000, Screening of commercial papaya (*Carica papaya*) varieties for tolerance against papaya ringspot virus. *Journal of Research, BBirsa Agricultural University.*,**12**: 51-56.

Magdalita, P. M., Persely, D. M., Godwin, I. D., Drew, R. A. and Adkins, S. W., 1997, Screening *Carica papaya* x *C. cauliflora* hybrids for resistance to papaya ringspot virus-type P. *Plant Pathol*., **46**: 837-841.

Pushpa, R. N., 2014, Molecular characterization, epidemiology and integrated management of papaya ringspot virus (PRSV) in papaya (*Carica papaya* L.). *Ph.D. Thesis*, Univ. Agric. Sci., Bengaluru, p. 54-133.

Reddy, V. P., Gowda, N. V., Rajesh, A. M., Yathindra, H. A. and Harshavardhan, M., 2011, Screening of commercial papaya genotypes for resistance against papaya ringspot virus at seedling stage. *Plant archives*, **11** (2): 1017- 1019.

Roff, M. N., 2007, Disease rating of papaya cultivars to papaya ringspot virus in Malaysia. *Acta. Hortic*.,**740**: 277-281.

Shukla, D. D., Ward, C. W. and Brunt, A. A., 1994, *Thepotyviridae*. Cab International, Wallingford, Oxon UK.

Singh, V., Rao, G. P.and Shukla, K., 2005, Response of commercially important papaya cultivars to papaya ringspot virus in eastern U.P. conditions. *Indian Phytopathol*., **58** (2): 212-216.

Singh, S. K. and Singh, R., 2013, *Screening* of papaya (*Carica papaya* L.) varieties for resistance to viral diseases. *Plant Archives*, **13** (1): 481-483.

Sudha, R., Balamohan, T. N., Soorianathasundaram, K., Rabindran, R. and Manoranjitham, S. K., 2015, Studies on resistance to papaya ringspot virus (PRSV) in intergeneric population of *Carica papaya* L. and *Vasconcellea cauliflora, SABRAO J. Breed. Genet.,***47** (2): 113-123.

Suzuki, J. Y., Tripathi, S. and Gonsalves, D., 2007, Virus-resistant transgenic papaya: commercial development and regulatory and environmental issues. In : Biotechnology and plant disease management. [(Eds.) Z. Punja, S. H. De Boer and H. Sanfacon]. UK, CABI, p. 436

Tennant, P. F., Fermin, G. A. and Roye, R. E., 2007, Viruses infecting papaya (*Carica papaya* L.): Etiology, pathogenesis and molecular biology. *Plant Viruses*, **1**:178-188.

Valencia, L. D., Garcia, R. N., Laurena, A. C., Villegas, V. N. and Bajet, N. B., 2001, Delayed ring spot virus symptom expression in *Carica* interspecific grafts and inarches., *Philipp. J. Crop Sci.,* **26** (2): 51-54.

Villegas,V. N., 2001, Approaches in developing ringspot virus resistant papaya. *Transactions of the national academy of science and technology*, Philippines. **23**: 63-70.

Received on 11-09-2017 Accepted on 14-09-2017