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Research Article

BIOLOGICAL INDEXING OF PAPAYA RING SPOT VIRUS (PRSV) in *Carica papaya*

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Abstract- India holds the forth position in the world in producing Papaya (56, 39,000 metric tons in 2014). The production has lowers not increased in proportion in the area because of severe infection of PRSV which causes 60 to 100 present losses in the field. Which is responsible for not only reduced in production also quality of fruit is deteriorated and market value has been lowered. The export of papaya and its related products has been stopped because of PRSV infection. So, the present investigation was carried out to confirm presence of virus in infected sample without any advanced technology and skilled techniques with short period of time. There are ample of techniques were developed still but they are supported with sophisticated instrumentation and costly chemicals and these are having hazardous effect on workers. So in these work the presence of virus was tested by using indicator plant i.e. live plant samples of summer squash. Zucchini (summer squash) which is indicator plant of PRSV used for the bio-assay. After infection of virus to the four leaf seedlings it was found that after manual infection in controlled house inoculated plants shows symptoms like dark green spots accumulated on leaf surface after one week. In this way within a week, we can confirm presence of virus in a seedling material which is major carrier of PRSV from nursery to field.

Keywords- Zucchini, PRSV(Papaya Ring spot Virus), Bioassay, ELISA, PCR, Indexing.

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Introduction

In India papaya is mainly cultivated in Uttar Pradesh, Bihar, Assam, Andhra Pradesh, Tamil Nadu, Karnataka, Gujarat, Maharashtra, West Bengal, Orissa, Manipur and Meghalaya [1].Papaya is widely cultivated despite its natural enemies. It was introduced to India in sixteenth century, spread to South India and throughout the Caribbean with Spanish explorations. By the middle of seventeenth century, papaya was distributed pan tropically [2]. The high production of the crop has supported development of industries in the beginning of twentieth century in India but declined rapidly with the introduction of viral disease that threaten papaya everywhere.

India stands fourth in production (56.39 Lakh Tonnes) contributing 12 per cent of global production followed by Brazil, Mexico, and Nigeria. The area during 2013-14 increased to 133.4 million hectares and the production stood at 5639.3 metric tons. Among the states in India, Karnataka ranks fourth with a production of 4757100 MT grown over an area of 6750 ha with an average yield of 70.5 MT/HA during 2013-14 which is reduced from 2011-12 productivity of 72 MT/HA[1]. The reason behind reduced in production and area is PRSV, in karnataka the major zone of Papaya growing is Hoskote where nine out of ten papaya orchards are seriously infected with PRSV and causing severe damage to filed and its production.

The crop is being infected by 12 viral, 8 bacterial, 33 fungal, 6 nematodal and 2 phytoplasma diseases [3] of which, ring spot disease caused by Papaya Ring Spot Virus (PRSV) has gained global importance in all the papaya growing countries. Papaya ring spot disease is aphid transmitted (*Myzus persicae*), PRSV is a *potyvirus* and it is a major problem in papaya cultivation, the virus drastically reducing the fruit yield, fruit size and quality [4]. Crop loss ranging from 48 to 100% was observed indifferent parts of India and world [5]. There are two isolates of the virus, PRSV-P infecting papaya and cucurbits and PRSV-W infecting cucurbits but not papaya. Eight of 10 fruiting orchards are infected in Karnataka with PRSV causing serious loss to economy and papaya industry. Papaya ring spot disease, a deadly disease of papaya has become prevalent in all papaya growing regions. No part of any papaya orchard has been left without this disease across the globe. It has threatened entire papaya industry to the tune of major impact on national economies of many papaya growing nations.

In southern India, earlier PRSV was restricted to Karnataka and Andhra Pradesh, the states adjoining Tamil Nadu. However, the virus had entered into Tamil Nadu in 2003 [6] and wide spread occurrence of papaya ring spot disease was noticed in plantations around Coimbatore and now it has been transferred to Maharashtra region. Management of crop viral diseases is challenging and also conventional methods are limited, early and accurate diagnosis of viral disease in plants and vector plays a critical role in its management. Serological and molecular diagnosis are important modern techniques, which are used to achieve this as they are highly sensitive, safe, specific, fast, reproducible and affordable. Several scientific group have worked on the serological and PCR based detection and relatedness of PRSV using techniques like Dot Enzyme Linked Immunosorbant Assay (DIBA), plate Enzyme Linked Immunosorbant Assay (ELISA) , RT-PCR, PCR [4], [7] Bi-Directional PCR. The 950 bp size coat protein of PRSV has been reported [8, 9] for its confirmation.

Material and Methodology

Virus isolation, purification and electron microscopy

Virus isolate was collected from PRSV infected papaya plants from a Papaya

garden in Hoskote, Bangalore, Karnataka. The virus was ultra-purified from systemically infected papaya leaves using a slightly modified protocol [10] using 0.5M potassium phosphate buffer containing 0.01 M EDTA (pH 7.5). The virus was confirmed by electron microscopy as given in [4].

Biological Indexing

For further study zucchini plants were planted in soil pot in controlled condition at green house, where Healthy summer squash seedlings of three to four leaves raised were selected and uniformly dusted with carborundum (600 meshes). The leaves were pin pricked and immediately a small piece of sterilized absorbent cotton wool dipped in the inoculums extract containing Ultrapurified PRSV in phosphate buffer, which was gently rubbed on the upper surface of the leaves. Healthy plants inoculated only with phosphate buffer were used as the control. The inoculated leaves were washed immediately after 15 min to remove the excess inoculums of the extract with a jet of distilled water and the plants were kept in insect proof net house for symptom expression. During the course of maintenance, the plants were sprayed periodically with systemic insecticide (0.2% Dimethoate) to avoid possible cross contamination by aphids. The plants were observed periodically for symptom development as shown in [Fig-1] Together With zucchini seedlings the test was carried out on papaya var. Pusa Nanha seedling from IIHR, Bangalore and results were shown in [Fig-2]. The study was carried out in triplicates on zucchini and papaya seedlings. After finalization of experiment the material was burned and properly disposed.

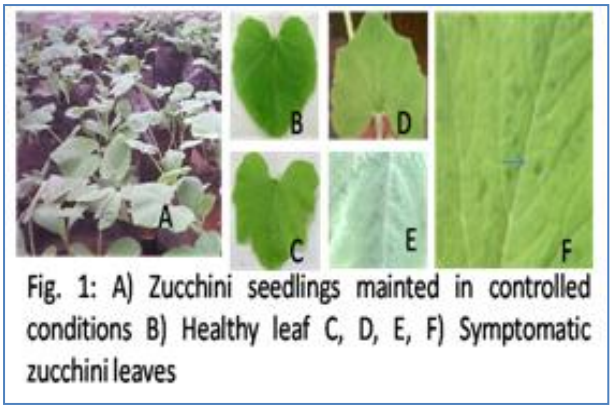

Fig. 1: A) Zucchini seedlings mainted in controlled conditions B) Healthy leaf C, D, E, F) Symptomatic zucchini leaves

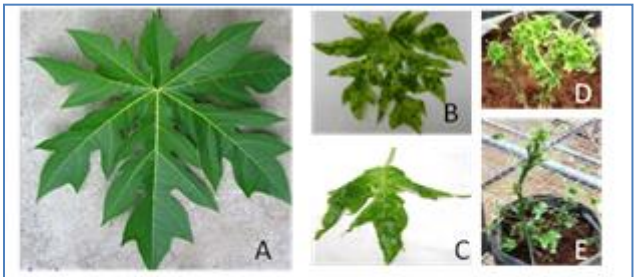

Fig .2: A) Healthy papaya leaf, B,C) Papaya leaf infected with PRSV, D, E) Papaya seedlings after inoculation of PRSV with specific symptoms

Results and Discussion

The purification of virus was carried out using infected plant samples showing typical symptoms of PRSV such as chlorotic mottling, blistering of leaf tissue, leaf distortion and stunting. Ultra-purified virus was confirmed by electron microscopy and used in further study.

Electron Microscopy

The size of the virus particles isolated from zucchini plants and papya seedlings artificially infected measured was 700nm in length and 12-13 nm in width [Fig-3]. This closely resembled the size observed by other group of scientists namely, 700-720nm [11], 760-800 nm [12], reported that PRSV virions are filamentous, non enveloped and flexuous, measuring 760–800 X 12 nm [4].

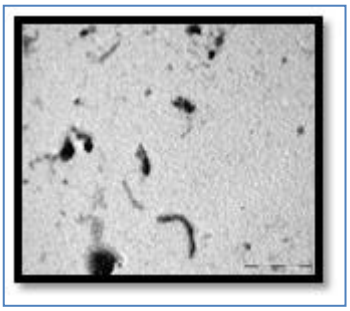

Fig-3 Electron micrographs of PRSV showing flexuous rods 75 nm long and 12-13 nm in diameter (X200).

Bioassay for confirmation of PRSV from infected orchard

Symptomatology

Mechanically inoculated seedlings of zucchini expressed symptoms after 7-10 days of inoculation. Mosaic, leaf blistering, Dark green spots and combinations of these symptoms were observed on leaves of inoculated seedlings as well as Symptoms such as mild mosaic, mosaic, puckering, mottling, vein clearing, vein banding, blistering, distortion chlorophyll lobing, filiformity and shoe strings in severe case appear on leaves after two to three weeks of infection on zucchini. And with same symptoms Oily streaks on petioles, ring spots on leaves was observed. In advanced case infected plants appear bushy, back headed, tapering and finally death was noticed in papaya seedlings as shown in [Fig-1] and [Fig-2], similar results was found by [13] in papaya and *Cucumis*. Further the virus was confirmed with ELISA using antiserum raised in rabbit and PCR using coat protein gene of PRSV[4].

Host range

The isolate of the present study was tested for its host range. Different cucurbitaceous plants *viz*., ash gourd (*Benincasa hispida*), bitter gourd (*Momordicacharantia*), bottle gourd (*Lagenaria siceraria*), ridge gourd (*Luffa acutangula*), snake gourd (*Trichosanthes cucumerina*) cucumber (*Cucumis sativus*), zucchini orsummer squash (*Cucurbita pepo*) and Chenopodium amaranti color, Chenopodium quinoa were used for symptom bioassay. The plants were sap inoculated with PRSV as mentioned in mechanical transmission and found symptomatic results on them.

Conclusion

PRSV is a divesting virus, which causes major economic losses to filed, to avoid this there is need of proper, accurate and safe diagnosis method. For the same purpose the study was carried out and it was found that biological indexing is an easy accurate and time limiting detection method for PRSV. Zucchini is an indicator plant where it helps in the early and accurate virus indicator prominently than other host plant.

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Conflict of Interest: None declared

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