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Short Communication

First Record of Papaya Ring Spot Virus (PRSV) Strain in Malir District Sindh and in Islamabad Pakistan

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Abstract

The papaya strain of *Papaya ring spot virus* (PRSV), that causal agent of PRSV disease was confirmed in Malir District Sindh and in Islamabad by double antibody sandwich enzyme linked immunosorbant assay (DAS-ELISA). In Malir district, the virus attacked on the papaya trees growing in field induced great losses to the farmer's income. Later it was also reported in National Agriculture Research Centre (NARC) field, Islamabad. A survey was conducted in Malir district and samples were brought to NARC Plant Virology laboratory. In contrast, PRSV has only recently appeared in Malir district and is now the subject of eradication campaign. © 2015 Friends Science Publishers

Keywords: ELISA; DAS-ELISA; PRSV; Papaya.

Introduction

Papaya (*Carica papaya* L.), is the well-known and widely distributed species of family Caricaceae, grown in tropical and subtropical regions and is mostly used for its highly nutritious fruit value (Bhattacharya and Khuspe, 2001). On the basis of FAO statistics for area harvested, yield and production (2010), worldwide 59 countries are producing about 9,095,875 MT of papaya in 2008 over the last 48 years in different countries papaya production is increasing steadily in exponential fashion. However, analysis of the production level in single country during this period discloses significant variations. Due to improved yields production of Papaya is increased by 687.58% or almost 7 times, presumably (that amounted to ca.203.21 increase or 2 times) and an extended area fixed to papaya cultivation (3.38 times increase) (FAO, 2010). Amongst causative agents of *papaya ring spot virus* (PRSV) is highly damaging threat to the papaya cultivation (Fermin *et al.,* 2010) through the damage up to 100% described in some regions (Tennant *et al.,* 2007). Though PRSV first described in Hawaii in 1945, it has been documented as major harmful factor to papaya growth in many tropical and subtropical areas including South and Central America, Africa Asia and Caribbean Islands (Tripathei *et al.,* 2008) before 1940s. Moreover, PRSV still continue to be reported "for the first time "in many countries, where most likely, it has been ignored for decades, as in the case of Maxico (Noa-Carrazana *et al.,* 2006).

PRSV attacks all cultivars of papaya including commercial and non-commercial papaya types, respectively. Infected plants develop the typical symptoms of stunting, radically reduced yield and fruits with the indicative of ring spots (Heu *et al*., 2002). The pathogen spreads mainly by numerous species of aphids in non-persistent way and is not measured as seed borne (Gibbs *et al*., 2010). Bayot *et al.* (1990) elevated the chance that PRSV might be spread through seeds (0.15%), which would help explain the easy dispersal of the virus throughout the tropics. Even if not transferred via papaya seeds, seeds of cucurbits or other hosts may show a role in the epidemiology of the disease. In addition there is at least one report on the potential spread of PRSV by birds (Gonsalves and Trujillo, 1986) efforts at management of PRSV by conventional means have proven difficult, particularly under high disease severity. Resistance against the virus does not exist in *carica papaya r*esistance genes from species belonging to other genera in the caricaceae family have been identified but the resistance appears to be variable and dependent on the geographic origin of the virus and environmental conditions (Gonsalves *et al*., 2005). Therefore, it was highly desirable to diagnose and characterize the virus causing disease in papaya in infected area and to provide necessary help to farmers without further spread of disease and its losses.

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Materials and Methods

Survey of Fields and Symptoms Identification

In 2009 a survey was conducted in different region of Pakistan. Firstly disease symptoms like chlorosis, shoe string rings spots on leave and fruit as identified in Malir district as disease abrupted over there. Later on disease was reported in NARC field papaya that was grown in PGRI Field. But there were no disease symptoms identified in plants from other regions as in Baluchistan, Khaiber Pakhtoon Khawa and Azad Kashmir.

Collection of Samples

Samples of severe symptoms from Malir district were collected in the form of infected leaves and fruit and carried to plant transformation lab (NARC Islamabad). Samples were stored at -72°C for further investigation. Later on severely infected plants from NARC was also identified and carried to lab for further investigation.

Identification of Viruses with Enzyme–linked Immunosorbent Assay (ELISA)

Double antibody sandwich enzyme linked immunosorbant assay (DAS-ELISA) method was used for virus confirmation as described by Mowat and Dawn (1987). Leaves of papaya with severe disease symptoms were collected from diseased plants of different regions (Malir district and NARC). Desiccated and surface sterilized leaves samples were used. 190 plants were tested by DAS-ELISA by using one –ve control (water) and +ve control (healthy leave from green house). Tissue (100 mg) was grounded in 1 mL of 50 mM carbonate coating buffer pH 9.6 using eppendorf TM micropestle grinder and 200 uL aliquots were coated on to the wells immunosorbant microtitre plate (Nunc). The plate was incubated at 4°C overnight. Primary antibody, raised in a rabbit to a glass house isolate of PRSV-P (Bateso, 1995), was diluted 1:1000 in BST/PVP and 200 µL of the primary antibody solution was added and incubated for 1.5 h at room temperature. The wells were washed thoroughly 4-5 times with PBST and 200 µL of swine anti rabbit I gG Horseradish peroxide

Plate 1: Showing ELISA result first is positive samples and last is negative control

Plate 2: Complete necrosis and curling of papaya leaves

Plate 3: Mosaic on papaya leave

Plate 4: Vein clearing of leaves

Plate 5: Leaf distortion

Plate 6: Green islands

Plate 7: Shoe string

Plate 8: Shoe string

Plate 9: Ring spots on petiole and fruits

Plate 10: Infected plant with ringspots on fruits and symptomatic leaves

conjugate (DAKO), diluted 1:2000 in PBST/PVP with 0.2% BSA, was added and incubated at room temperature for 1 h. Plates were washed again and reaction measured calorimetrically using *o*-phenyleneediamine (OPD) Sigma and Hydrogen per oxide according to the manufacture instruction. Results were recorded as +/- colour reaction compared to the buffer and healthy plant tissue to control or measured spectrophotometrically at 460 nm after 10 min using Beckman Biomerck plate reader. Reading was considered positive when absorbance values were at least three times greater than the healthy control.

Results

Survey of PRSV Incidence in Malir District

The incidence of PRSV was found higher in Malir districts of Province Sindh. The maximum disease incidence of 100% was observed in places from Malir district Sindh and Islamabad districts of Punjab. No incidence was noticed from places of Khaiber Pakhtoon Khawa and Azad Jammu and Kashmir. The disease incidence in different districts had wide range with varied symptoms as followed (Plate 1-10) (Table 1) (Fig. 1). In provinces, incidence of PRSV ranged from 0 to 100%. The places visited were Islamabad (100%), Malir (100%), Wahcant (0%), Peshawar (0%), Kahota (0%) and Bhimber (AJK) (0%), with symptoms ranging from mosaic, chlorosis, mottling, leaf distortion, shoestrings, green islands, vein clearing, ring spots and wilting were observed. Severely attacked samples were collected bearing disease symptoms.

Association of Papaya Age with PRSV Incidence in Different Region

In all provinces of Pakistan the incidence of PRSV was found more in 1-2 years papaya. The observations recorded during the survey were presented in Table 2 and Fig. 2.

PRSV Detection by DAS ELISA

DAS-ELISA was performed 5-10 times and it has been found that plants with symptoms from both Malir and Islamad showed 100% infection (Table 3; Fig. 3) result was analysed through T-test (Table 4) and was found that Disease %age is same non-significant in Malir district and in Federal area at 5% level of significant.

Discussion

The origin of disease outbreak is not unknown. Since its identification by Lindner *et al.* (1945) from Hawaii, it has been reported from all parts of the world. The virus causes variety of symptoms ranging from mosaic and chlorosis to varied degrees of leaf destruction and fruit malformation causing 100% yield losses under early and severe infections

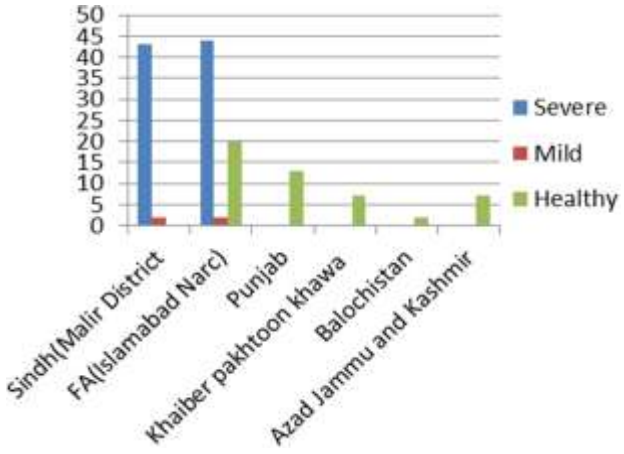

Fig. 1: Shows that 1-2 Y and 2 Years plants are mostly affected

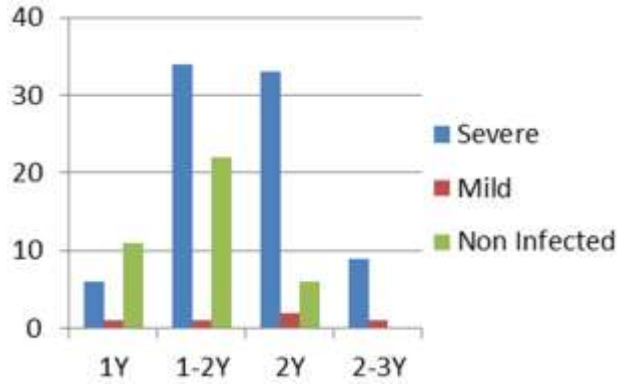

Fig. 2: Shows disease effect with respect to age

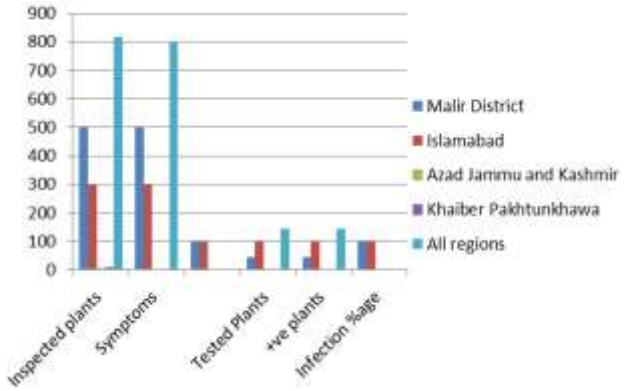

Fig. 3: ELISA Result in 2009

(Husain and Verma, 1994; Dahal *et al.*, 1997). PRSV-Type W was reported on cucurbitaceous plant in Brazil exhibiting symptoms of mosaic and leaf malformation were found in plants (Jadao *et al.*, 2010). Recently disease has also been reported in Sudan. Symptoms leaf mosaic, malformation

Table 1: Distribution of disease in different regions

Region	Severe	Mild	Healthy
Sindh(Malir District)	43	2	0
FA(Islamabad NarcARC)	44	2	20
Punjab	0	0	13
Khaiberpakhtoonkhawa	0	0	7
Balochistan	0	0	2
Azad Jammu and Kashmir	0	0	7

Table 2: Shows disease effect with respect to age

Age of papaya	Severe	Mild	Non Infected
1Y	6	1	11
1-2Y	34	1	22
2Y	33	2	6
2-3Y	9	1	0

Table 3: Result of *papaya ring spot virus* (PRSV) detection by DAS ELISA

			2009			
Regions of sampling	Inspected plants	Symptoms	Symptomatic/inspected (%)	Tested plants	+ve plants	Infection (%)
Malir District	500	500	100	45	45	100
Islamabad	300	300	100	100	100	100
Azad Jammu and Kashmir	7	0	0	0	0	0
Khaiber Pakhtun Khawa	10	0	0	0	0	0
All regions	817	800	97%	145	145	100%

and blistering were observed on young plants (Mohammed *et al.*, 2012). In Pakistan the virus was reported 2004 in Cucurbits grown throughout the North-West Frontier Province (Now KPK) of Pakistan as summer and winter crops (Ali *et al*., 2004). Viruses include Cucumber green mottle mosaic virus (CGMMV), Zucchini yellow mosaic virus (ZYMV), Watermelon mosaic virus (WMV) and PRSV were found infecting cucurbits. Besides cucurbit viruses in NWFP, another very severe disease of melon (*Cucumis melon* L.) was characterized by leaf curling, chlorotic spots, vein clearing, mosaic, leaf distortion and enations on the upper leaf surface and was identified by DAS-ELISA (Malik *et al.*, 2010).

Conclusion

The PRSV-Pak isolate was close to Indian isolates of PRSV possibly reflecting the geographical relationship between these isolates.

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