SHORT COMMUNICATION

DETECTION AND PHYLOGENETIC RELATIONSHIP OF *PAPAYA RINGSPOT VIRUS*-P IN PAKISTAN

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SUMMARY

Four commercial papaya orchards in the Sindh province of Pakistan were surveyed for the presence of *Papaya ringspot virus* (PRSV) in February and May of 2012. Leaf samples with mottling, yellowing and dark streaking were collected from trees with fruits showing concentric C-shaped rings and tested by ELISA with virus-specific antibodies. Results showed that 70 samples tested positive to PRSV, nine of which were characterized by RT-PCR using primers designed in the sequence encoding the nuclear inclusion b (NIb) and the coat protein (CP) genes. Phylogenetic analyses of the partial coat protein sequence showed a close relationship of the isolates from Pakistan to one other and to an isolate from India (99% identity) but a relative divergence with other isolates from different geographical locations. This is the first report of PRSV on papaya in Pakistan.

Key words: *Papaya ringspot virus,* Pakistani isolate, phylogeny, polyprotein (NIb-CP) gene.

Papaya (*Carica papaya,* family *Caricaceae*) is native to Central and South America and is grown primarily in the tropical and subtropical areas of the world (Gonsalves and Ishii, 1980; Tripathi *et al*., 2008). In Pakistan, commercial papaya orchards are located in the coastal areas of the Sindh province (Panhawar, 2005). *Papaya ringspot virus* (PRSV; genus *Potyvirus*, family *Potyviridae*) is the most devastating pathogen infecting papaya and a major limiting factor for its production worldwide (Davis *et al*., 2005; Gonsalves *et al*., 2010; Persley, 2003; Yap *et al*., 2009). PRSV was first described in 1945 in Hawaii (Jensen, 1949), where it severely affected the local papaya industry in the 1980s (Davis *et al*., 2005). There are two PRSV biotypes that can be distinguished by their host range, i.e. PRSV-P which naturally infects papaya and cucurbits while PRSV-W (formerly *Watermelon mosaic virus* 1) infects only cucurbits (Purcifull *et al*., 1984; Bateson *et al*., 1994; Tripathi *et al*., 2008). The virus is non-persistently transmitted by several aphid species, including *Myzus persicae* and *Aphis gossypii* (Kalleshwaraswamy *et al*., 2007). PRSV genome consists of a single stranded positive sense RNA that encodes a single open reading frame (ORF) (Gonsalves *et al*., 2010).

In Pakistan, PRSV was reported in cucurbits (Ali *et al*., 2004) but not papaya. Thus, the aim of this study was to identify PRSV on papaya in Pakistan and to analyze the phylogenetic relationship of the partial coat protein gene of Pakistani isolates with other isolates from different geographic origin.

During a survey of papaya-growing areas for PRSV in the Sindh province of Pakistan, symptomatic leaf samples were collected from four commercial orchards (Belli farm, Darsamo, Tando Allayar and Tandojam), together with samples from apparently healthy greenhouse-grown plants. Leaf samples were processed for DAS-ELISA with a PRSV antiserum kindly provided by Dr. S. Winter or stored at -80°C for further testing. Out of 70 symptomatic PRSV-positive samples, nine were further characterized by RT-PCR using total RNA extracted by following the standard protocol from Life Technologies (USA, Cat No. 12183018A), oligo dT primer, and the Invitrogen kit (USA, Cat No. 11734-050). The resulting cDNA was amplified by PCR by using the PRSV coat protein gene upstream forward and reverse primers described by Ali *et al.* (2004). The PCR products (720 bp) were extracted with the Gene Jet PCR purification kit (K0701) and custom sequenced (Macrogen, Korea)**.** The sequence contained approximately 55% of the full-length CP gene at the 5' end. Sequences were submitted to GenBank and used for further analysis. The source of the PRSV isolates and their corresponding GenBank accession numbers are given in Table 1. PRSV sequences obtained through BLAST search were selected for phylogenetic analyses on the basis of maximum identity (up to 90%) and minimum expected value (e-value) (Jenkins *et al*., 2002). The sequences were aligned using Clustal W (Thompson *et al*., 1994) in MEGA5 software and the neighbor joining method was used in for the construction of phylogenetic tree with a bootstrap value of 1000 (Tamura *et al*., 2011).

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Table 1. Sources of *Papaya ringspot virus* isolates.

Country	PRSV Isolate	Type	Origin	GenBank accession no.
	TJ-11	P	Tandojam	JX024999
	TJ-12	P	Tandojam	JX025000
	TJ-13	P	Tandojam	JX025001
	TJ-14	P	Tandojam	JX025002
Pakistan (PK)	Belli-1b	P	Belli Farm	JX661503
	Dar 2	P	Darsamo	JX661504
	Dar 3	P	Darsamo	JX661505
	Belli 1c	P	Belli Farm	JX661506
	TR	P	TandoAllayar	JX661507

Phylogenetic analyses on the basis of the partial NIb-CP nucleotide sequence of nine Pakistani PRSV isolates and other isolates from distinct geographical locations resulted in four lineages. PRSV-P Pakistani isolates grouped into two clusters, though showing little diversity among themselves. Clade I contained isolates TJ11-TJ14 which are highly similar to each other while isolates in clade II showed 93% similarity with the isolates of group I. Pakistani PRSV-P isolates showed the highest similarity (up to 99%) with an Indian PRSV-P isolate (AF323638.1). Phylogenetic group III comprises PRSV-P isolates from Vietnam and Japan, whereas isolates from USA grouped in cluster IV (Fig. 1). The PRSV-P isolate from Thailand was used as an outgroup. The single PRSV-W strain from Pakistan was very divergent and grouped by itself in the phylogenetic tree (Fig. 1), conforming to previous findings (Ali *et al.*, 2004). This PRSV-W isolate is considered as an atypical species in the evolutionary history of potyviruses (Gibbs *et al.*, 2008).

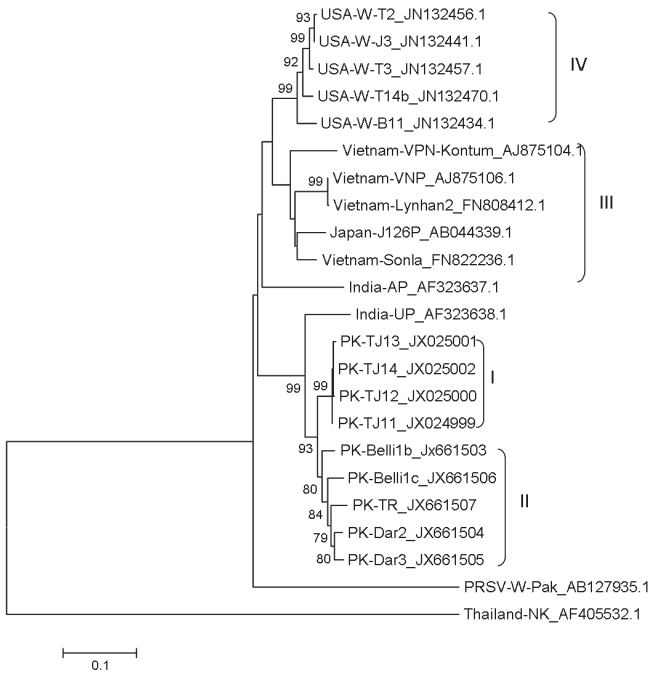

Fig. 1. Neighbor-joining tree representing phylogenetic relationships of *Papaya ringspot virus* (PRSV) polyprotein sequence from Pakistan (PK) and other related sequences selected via BLAST search. The tree is based on pairwise comparisons using the Jukes-Cantor parameter on nucleotide sequences. Upper and lower branch points show bootstrap values (1,000 replicates) supporting a particular phylogenetic group. The scale bar represents nucleotide substitutions per site. All nucleotide sequences are identified according to the isolate name and the GenBank accession number.

On the basis of partial NIb-CP gene sequences, the PRSV isolates from the coastal area of the Sindh province Pakistan are divergent from those of India and Vietnam. This indicates that PRSV-P isolates exist as a defined subpopulation in Pakistan.

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