



## Detection of natural infection and reaction of tomato lines to potato virus Y in Pakistan

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### Abstract

Tomato (*Solanum lycopersicum* L.) is an important solanaceous crop worldwide including Pakistan. In Pakistan the successful production of tomato is hampered due to many viral diseases including PVY which causes havoc and colossal yield losses. For management of plant viruses, accurate and proper identification of plant viruses and resistance sources is very significant. In present study, a total of 595 tomato samples with symptoms like mosaic, vein chlorosis and mild mottling were collected from tomato fields in Pakistan. All symptomatic samples were screened for the presence of *Potato virus Y* by DAS-ELISA using virus specific polyclonal antiserum (Bioreba AG, Switzerland). Among symptomatic samples, 104 were positive for PVY infection, of which, only eight were further screened for the presence of PVY by RT-PCR using primer pair PVYPK-F/R, that resulted in amplification of 1050 bp fragments. A total of 1050 nucleotides were obtained by sequencing each amplicon comprising a full length coat protein gene including 300 bases of UTR. The sequences of two isolates were submitted to Genbank under accession number KX816568 and KX816570. The isolate AARTPK (KX816568) was used in screening of 11 tomato cultivars. The cultivars; Kalam, NSC-92, Yaqui were found resistant (R), Rio-grandis as moderately resistant (MR) and Super-SPC and Giant-cluster as moderately susceptible (MS). Similarly, the response of BSS-30 and Gala was recorded as susceptible (S) and of Junny-2144, CKD-267 and Jagular as highly susceptible (HS). The identified resistant cultivars can be used as genetic source in developing resistant varieties against PVY in future.

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## Introduction

Tomato (*Solanum lycopersicum* L.) is one of the extensively cultivated solanaceous vegetables worldwide including Pakistan. Being a crucial part of our daily diet, it is 2<sup>nd</sup> most consumed vegetable after potato in Pakistan (Kamran *et al.*, 2012). Beside an excellent source of vitamin A, B and C (Kothari *et al.*, 2010), the tomatoes also contain minerals like iron, phosphorous and carotenoids having a high oxygen-radical quenching and scavenging capability (Babalola *et al.*, 2010). Because of low input costs, short duration crop and inelastic demand, the growers are attracted to cultivate tomato in Pakistan (Lohano and Mari, 2005; Tahir *et al.*, 2012). A diverse range of tomato varieties and cultivars of various size, shape, quality and yield are grown globally (Georgiev *et al.*, 1988). Adaptability to versatile environmental conditions makes tomato a successful crop worldwide (Tiwari *et al.*, 2012). At present about 100 million tonnes of fresh tomatoes are produced on 3.7 million hectares worldwide (FAOSTAT, 2014). In Pakistan, the production of good quality tomatoes is favoured by diversified climatic conditions throughout the year (Chohan *et al.*, 2016). With an annual production of 574,052 tons from an area of 58,196 ha, the Pakistan stands at 33<sup>rd</sup> position globally (Aslam *et al.*, 2017). Per acre yield of tomato in Pakistan is hampered by several fungal (Iqbal and Mukhtar, 2014; Iqbal *et al.*, 2014), viral (Ashfaq *et al.* 2014, 2015), bacterial (Tiwari *et al.*, 2012) and nematode diseases (Kayani *et al.*, 2017). Tomato is infected by more than 146 viruses worldwide, of which 27 are potyviruses, (Green and Kim, 1991). In Pakistan, among potyviruses, only *Chilli veinal mottle virus* (Ahmad and Ashfaq, 2017), has been reported to infect tomato crop.

The *Potato virus Y* (PVY) is a destructive potyvirus among tomato infecting viruses (Lorenzen *et al.*, 2006). PVY has a wide hosts range, the virus is transmissible in approximately 120 species belonging to 5 families (Horvath, 1983). Solanaceous crops like Potato (*Solanum tuberosum*), Pepper (*Capsicum anum*), Tobacco (*Nicotinia tobacum*) and Tomato (*Lycopersicom esculentum*) are most affected crops (Shukla, *et al.*, 1994).

The infected plants exhibit symptoms like mottling, chlorosis, necrosis, leaf drop and premature plant death. The virus infection can cause a yield loss up to 50% in tomato (Alam *et al.*, 2013). Because of furious nature and huge losses PVY is ranked at 5<sup>th</sup> position in term of economic damages worldwide (Gray *et al.*, 2010). The transmission of *Potato virus Y* is attributed to aphids in non-persistent, stylet borne and non-circulative manner (Dombrovsky *et al.*, 2005). Moreover, the virions are also thought to be transmitted by plant material with infection like cuttings, tubers and seed etc. (Revers and Gracia, 2015). Till now, five strains of PVY are known (Abbas *et al.*, 2012), while some newly emerged recombinant strains have been recorded as well (Ali *et al.*, 2010).

Management of plant viruses depends on proper identification, understanding of their ecology and epidemiology, and resistance sources. For proper identification of plant viruses, conventional methods like symptomology or serology are occasionally insufficient (Fauquet *et al.*, 2003), as viruses may possess high levels of intraspecific variability and a number of species have serological association. Hence, the molecular detection has become essential for accurate identification of plant viruses (Danci *et al.*, 2009). The knowledge on host virus interaction and their adoptability to different hosts are prerequisites to develop different environment friendly and sustainable management strategies. In case of plant viruses, development of resistant varieties is the only promising and reasonable approach of disease management, which requires desired resistant sources and continuous screening against the pathogen. Unfortunately, the information about resistance for plant viruses in available tomato germplasm is scanty in Pakistan, hence, the present research aimed to detect and identify natural infection of *Potato virus Y* in tomato, to develop and standardize the molecular techniques for detection of Pakistani PVY isolates and to assess the degree of resistance in available tomato varieties against *Potato virus Y*. So that the resistant cultivars can be used as a significant element in integrated disease Management approaches.

## Materials and methods

### *Detection and identification of Potato virus Y*

In 2013-14, symptomatic tomato leaf samples showing mosaic, vein chlorosis and mild mottling from selected tomato growing districts of Pakistan were collected. Potyvirus infection was detected by PTA ELISA (Potygroup test), Bioreba and PVY infection was detected triple antibody sandwich (TAS) ELISA (Karasev *et al.*, 2010), following the manufacturer's instructions. The infection was further confirmed by molecular detection. Total RNA from PVY positive samples was isolated using Tri Reagent (Life Technologies, Carlsbad, CA) as per manufacturer's instruction and used in cDNA synthesis by MMLV-RT (Invitrogen) using oligo (dT) reverse primer (Gibbs *et al.*, 2003). PCR amplification was performed using degenerate primer pair; oligo (dT)/Poty3 (Gibbs *et al.*, 2003). The PCR products were sequenced directly in both directions. The consensus sequences were subjected to BLASTn analysis and presence of PVY was confirmed. The complete CP gene of Pakistani isolates of PVY was amplified by a newly designed primer pair PVYPK-F/R (PVYPK-F: 5'-AACTGTGATGAATGGGCTTATG-3'; PVYPK-R: 5'-TGTTATGACGAAATCACAAACACC-3'). PCR was performed in a 25  $\mu$ L reaction volume of cyber green PCR master mix (Invitrogen) with 2  $\mu$ L of total RNA, and conditions were standardized as; first cycle at 94°C for 3 min, followed by 35 cycles at 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min, and a final cycle at 72°C for 5 min.

### *Screening of tomato germplasm*

Leaf sap of selected sample infected with PVY isolate-AARTPK (NCBI Acc. No. KX816568), homogenized (1/5 w/v) in 0.05 M phosphate buffer, pH 7.2 (Ashfaq *et al.*, 2014) at dilution rate 1:10 (w:v), was mechanically inoculated to 11 virus free tomato genotypes, obtained from Federal Seed Certification and Registration department of Pakistan and Department and Horticultural Research Institute, NARC, Islamabad. The nursery was raised in a controlled glass house at daily temperature of 25-27 °C. The inoculation was done at 2-3 leaf stage of tomato seedlings raised in 10x15 cm pots containing sterilized potting mixture of sand, silt and compost at the ratio 3:1:1, respectively.

The pots were kept in glasshouse maintained at 25 °C and moistured in alternate days. The experiment was performed in three replications with positive and negative controls. The plants were regularly monitored until the onset of symptoms. The association of potato virus Y with symptom development was confirmed after 3-4 weeks of inoculation by triple antibody sandwich (TAS) ELISA (Karasev *et al.*, 2010), and RT-PCR (Gibbs *et al.*, 2003). The symptoms were visually scored according to the disease rating scale as used by Ashfaq *et al.* (2014) (Table 1).

## Results

### *Detection and identification of potato virus Y*

Of the 595 tested tomato samples, 104 samples were found positive with Potato virus Y infection in DAS ELISA. The polymerase chain reaction by primer pair Poty3/Oligo (dT) (Gibbs *et al.*, 2003) amplified the DNA fragment of ~750 base pairs (Fig. 1). The primer pair PVYPK-F/R, amplified a DNA fragment of ~1 kb. The BLASTn analysis revealed the sequences as 3' genomic region of PVY comprised of complete CP gene and 3'UTR. This sequence was submitted to Gen Bank under accession number KX816568.

### *Response of tomato germplasm to potato virus Y*

The response of tomato germplasm is summarised in Table 2. The results revealed that none of the understudied tomato cultivars was found immune or highly resistant to *Potato virus Y*. Three cultivars named as Kalam, NSC-92, Yaqui responded as resistant (R). Rio-grandi responded as moderately resistant (MR) and two varieties viz. Super-SPC and Giant-cluster reacted as moderately susceptible (MR). Similarly, the response of BSS-30 and Gala was recorded as susceptible (S) and of Junny-2144, CKD-267 and Jagular as highly susceptible (HS).

The susceptible and moderately susceptible inoculated tomato plants showed mild leaf mottling combined with rugosity and leading to distortion in case of highly susceptible plants. Some of the moderately resistant plants were also observed as droopy with curved petioles and downward leaf rolling (Fig. 2).

Symptoms were appeared 4-5 days after inoculation in susceptible (S) and highly susceptible (HS) cultivars, while in moderately resistant cultivars it

took 10-15 days in the onset of symptoms. No symptoms were observed on fruits later on.

**Table 1.** Disease rating scale for mechanical inoculation of plant viruses (Ashfaq *et al.*, 2014).

Sr. No.	Disease reaction	Disease severity index (DSI)	Reaction
1	Highly Resistant (0% infection, All plants free of symptoms)	0	HR
2	Resistant (1-10% plants infected)	1	R
3	Moderately resistant (11-20% plants infected)	2	MR
4	Moderately susceptible (21-30% plants infected)	3	MS
5	Susceptible (31-40% plants infected)	4	S
6	More than 40% plants infected, with all plants showing severe symptoms	5	HS

### Discussion

In present study, the primer pair PVYPK-F/R was designed from aligned PVY sequences obtained from NCBI Genbank. The PCR conditions were standardised by repeated reactions. This primer pair is capable of amplifying complete capsid protein gene of Potato virus Y, of Pakistani isolate. The CP gene is considered as focal gene for the identification,

characterization and classification of potyviruses (Spetz *et al.*, 2003). For proper and accurate identification, of plant viruses, biological and serological characterization is occasionally not enough to distinguish strains from species (Fauquet *et al.*, 2003). Modern techniques like PCR and Blotting, etc. have taken up the use of genomic composition of viruses.

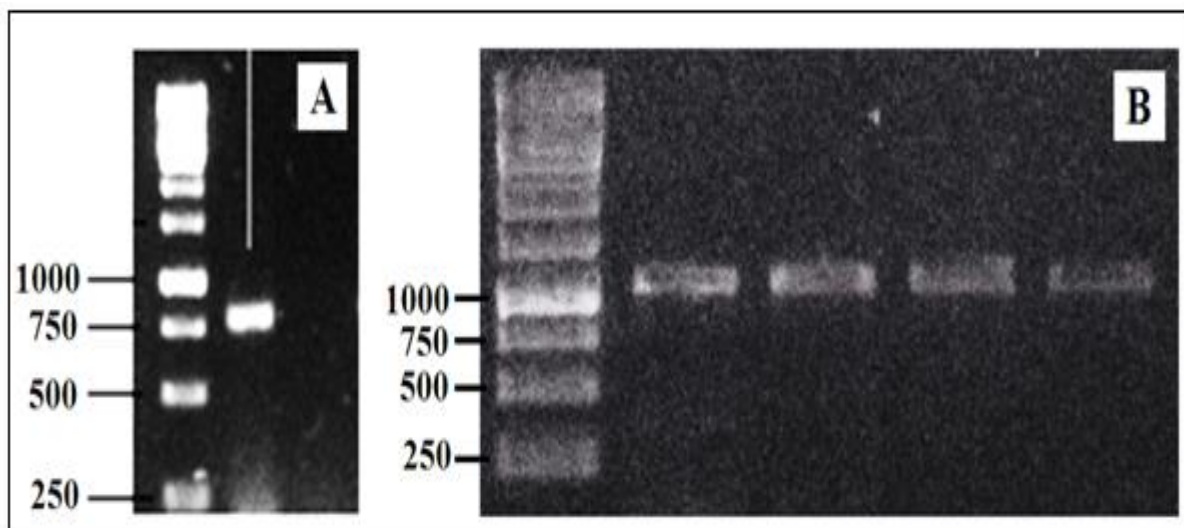
**Table 2.** Reaction of tomato germplasm against potato virus Y.

Sr. No.	Genotypes	Disease severity	Reaction
1	Nil	0	HR
2	Kalam, NSC-92, Yaqui.	1	R
3	Rio-Grandi.	2	MR
4	Super-SPC, Giant-cluster.	3	MS
5	BSS-30, Gala.	4	S
6	Junny-2144, CKD-267, Jagular.	5	HS

I present research, 11 tomato cultivars were screened for resistance against PVY, of which three were found resistant (R), one as moderately resistant (MR), two as moderately susceptible (MS), two as susceptible (S) and three as highly susceptible (HS). Resistance to potato virus Y in tomato genotypes has been reported all over the world (Aramburu *et al.*, 2006). Use of resistant cultivars is considered as one of the economical, environment friendly and sustainable disease management approach. Likewise other potyviruses, PVY also undergoes a continuous

diversification through mutation and recombination at nucleotide and genome level, which leads towards to the emergence of novel strains/isolates bearing versatile in degrees of pathogenicity in their host species and breaking their resistance (Nieet *et al.*, 2013; Karasev and Gray 2013).

hence the search for new source of resistance sources remains scoped all the time. Search for resistance sources against potato virus Y in various vegetables has been pursued in several countries worldwide.



**Fig. 1.** PCR products amplified by poty3/oligo (dT) (A) and PVYPK-F/R (B) primer pair.



**Fig. 2.** Symptoms caused by PVY on tomato varieties. (a) Vein chlorosis, (b) rugosity, (c) Mild mosaic, (d) Leaf crinkling.

In present study, the symptoms were observed to appear after 4 days in susceptible genotypes, and 14 days in resistant genotypes. Association of resistance and susceptibility with symptom development has been studied in a number of studies. The findings are in line with the findings of Anith *et al.* (2004).

The present study concludes that newly designed primer pair PVYPK-F/R is capable of detecting Pakistani PVY isolates at conditions as mentioned in materials and methods. The study also reveals that distinctions were observed in response of understudied tomato cultivars to *Potato virus Y* infection. None of the cultivars was found immune or highly resistant. The cultivars; Kalam, NSC-92 and Yaqui were found as resistant (R) and Rio-grandi found as moderately resistant (MR). Hence, are suggested for cultivation under integrated production systems and in developing new resistant cultivars under different field conditions as environmental factors may affect their response to appear as susceptible at one place and resistant at another place.

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