



## Incidence and distribution of begomoviruses from pothwar region of Pakistan

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### Abstract

Begomoviruses are single stranded whitefly transmitted plant viruses which are responsible for huge economic losses across the world. Several begomovirus infections have been reported from Pakistan in last two decades but there are very less reports of begomoviruses from Pothwar region which lies on the north-eastern part of Pakistan with very diverse vegetation. The study was aimed to estimate the incidence and distribution of begomoviruses in Pothwar region. Plant samples from 17 different plant families were collected and followed by PCR analysis by different primer sets in order to identify the presence of begomoviruses in tested plant samples. The presence of begomoviruses was confirmed in 15 plant families with 77.5% of an overall incidence of begomoviruses and uneven distribution was observed with maximum 32 % in Rawalpindi district of Pothwar region. The incidence was also calculated across plant families where family *Solanaceae* topped the list with 92 % incidence. The overall picture in Pothwar region was much favourable for begomoviruses spread and their vector whitefly. In present study the results is a glimpse of the huge damage to plants caused by begomoviruses. The region must be thoroughly combined for begomoviruses with large amount of plant samples covering more plant families.

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## Introduction

Pakistan lies on the hot belt of begomoviruses in terms of their diversity and severity. The over-cropping, increased recombination between begomoviruses and friendly environmental conditions for the whiteflies are main contributing factors towards the rapidly evolving begomoviruses which cause huge losses to national economy. Around one million hectares of cotton have been destroyed by cotton leaf curl viruses in Pakistan (Mansoor *et al.*, 1993) whereas estimated losses from 1992 - 1997, because of Cotton leaf curl disease (CLCuD), were at \$ 5 billion (Bridson *et al.*, 2000).

The Pothwar region, with coordinates latitude  $32^{\circ} 10$  to  $34^{\circ} 9$  N and longitude  $71^{\circ} 10$  to  $73^{\circ} 55$  E, is located in north-eastern part of Pakistan making the northern part of Pakistan's Punjab province with an area of 22,254 km<sup>2</sup>. The region is home to diverse vegetation which includes cereal crops, fruits, vegetables and small forests with an annual rainfall of 380mm-510mm. The Pothwar region includes four districts namely Attock, Chakwal, Jhelum, Rawalpindi and a capital territory Islamabad (Fig. 1) (Edwards, 2007).

Geminiviruses are ssDNA viruses having circular genome and double-capsid morphology and are persistently transmitted by whiteflies, leaf hoppers

and tree hoppers infecting broad range of monocotyledonous and dicotyledonous plants. The most important genus of family *Geminiviridae* is *Begomovirus* which are further divided into two types based on their genome organization i.e Monopartite begomoviruses (DNA-A) and Bipartite begomoviruses (DNA-A and DNA-B) of equal size (~2.8 kb) (Stanley *et al.*, 2005) and only infect dicotyledonous plants (Brown *et al.*, 2012). The present study was focused on accessing the incidence of begomoviruses in Pothwar region and their distribution in four districts of the region.

## Materials and methods

### Plant sampling

All four districts of Pothwar region *viz.* Attock, Chakwal, Jhelum and Rawalpindi including capital territory Islamabad, where varieties of horticultural, agricultural and wild plants are present, were surveyed. Leaf samples were collected from 2011-2013. These samples were kept in polythene bags in an ice bucket and brought to lab for further use. The samples were washed with distilled water in order to remove any superficial materials or contamination. Half of the each sample was kept at  $-80^{\circ}\text{C}$  and the other half was dried in desiccator containing silica gel.



Fig. 1. pothwar Region's location in Pakistan.

*Total nucleic acid extraction*

Total DNA was extracted from 100 mg leaf samples by using CTAB (Cetyl trimethyl ammonium bromide) method (Doyle and Doyle, 1987) with little modifications. DNA was quantified using Nanodrop (Thermo Scientific, Germany). Working dilution of 500ng/μl was prepared to utilize in experiments. The integrity of DNA was observed in 0.7 % Agarose gel prepared in 1X TAE.

*Polymerase chain reaction*

The presence of begomoviruses leaf samples were confirmed through nucleic acid based technique Polymerase Chain Reaction (PCR) with molecular detection of DNA-A was done by primer pairs CPF/CPR 5' (Haider, M. S. 1996) and prV324F/prC889R (Wyatt and Brown, 1996).

The PCR reactions were carried out in Ependorf 96 well thermocycler. All amplifications were performed in volumes of 50 μL containing final concentration of

1X PCR buffer (75mM Tris-HCL pH 8.8 at 25° C, 1X KCl buffer, 100-200 μM dNTPs, 1.5-2 mM magnesium sulphate, 2.5 μM of each primer, 2.5 U of Taq Polymerase (Thermoscientific, Germany) and approx. 100 ng of total nucleic acid. The disease incidence and distribution were recorded using formulae:

$$\text{Begomoviruses Incidence} = \frac{\text{No. of positive/infected samples}}{\text{Total no. of samples}} \times 100$$

$$\text{Begomoviruses Distribution} = \frac{\text{No. of positive samples in a district}}{\text{Total no. of positive samples in the region}} \times 100$$

**Results**

The Pothwar region consisting of Attock, Chakwal, Jhelum and Rawalpindi including capital city Islamabad was surveyed from 2010-2013 and a total of 138 symptomatic plant leaf samples (Fig. 2) belonging to 17 different plant families, were collected.

**Table 1.** District and Plant Family-wise Incidence of Begomoviruses in Pothwar region.

S. No.	Family	% of No. of plants infected with begomoviruses (No. of plants infected/No. of plants collected)					
		Total	Districts				
			Attock	Chakwal	Jhelum	Rawalpindi	Islamabad
1.	<i>Euphorbiaceae</i>	82 (09/11)	2/2	1/2	0/1	3/3	3/3
2.	<i>Malvaceae</i>	90 (19/21)	7/8	4/4	1/1	5/6	2/2
3.	<i>Caricaceae</i>	83 (05/06)	0/0	0/0	0/0	3/4	2/2
4.	<i>Solanaceae</i>	92 (22/24)	6/7	7/7	2/3	5/5	2/2
5.	<i>Ranunculaceae</i>	50 (01/02)	0/0	1/2	0/0	0/0	0/0
6.	<i>Cucurbitaceae</i>	88 (23/26)	5/6	8/8	1/3	7/7	2/2
7.	<i>Geraniaceae</i>	50 (01/02)	0/0	0/0	0/0	0/0	1/2
8.	<i>Asteraceae</i>	71 (05/07)	0/0	1/2	0/0	2/3	2/2
9.	<i>Lamiaceae</i>	0 (0/02)	0/0	0/0	0/0	0/0	0/0
10.	<i>Amaranthaceae</i>	67 (02/03)	0/0	0/0	0/0	1/2	1/1
11.	<i>Verbenaceae</i>	77 (07/09)	2/2	1/2	1/1	3/3	0/1
12.	<i>Acanthaceae</i>	60 (03/05)	2/3	1/1	0/0	0/1	0/0
13.	<i>Apocynaceae</i>	50 (02/04)	0/0	0/2	0/0	2/2	0/0
14.	<i>Fabaceae</i>	75 (06/08)	0/0	5/6	0/0	1/2	0/0
15.	<i>Vitaceae</i>	0 (0/03)	0/0	0/0	0/0	0/0	0/0
16.	<i>Rosaceae</i>	33 (01/03)	0/0	0/1	0/0	1/1	0/1
17.	<i>Brassicaceae</i>	50 (01/02)	0/0	0/1	0/0	1/1	0/0
		77.5 (107/138)	85.7 (24/28)	81 (29/38)	55.5 (5/9)	85 (34/40)	83.3 (15/18)

These plant leaf samples were photographed and taken back to lab into a polythene bag placed in an ice box. Samples were stored at -80° C for further use. All of the 138 plant leaf samples were subjected to

PCR using begomoviruses specific degenerate primer set for the confirmation of begomoviruses infection which amplified 750bp band in the tested plant samples.

The incidence of begomoviruses in Pothwar region was quite high i.e. 77.5 % while maximum incidence was observed in Attock (85.7%) followed by Rawalpindi (85%) , Islamabad (83.3%), Chakwal (81%) and Jhelum (5%). Furthermore it was also noted that the family *Solanaceae* had maximum number of plants infected with an

incidence of 92% followed by *Malvaceae* (90%), *Cucurbitaceae* (88%), *Caricaceae* (83%), *Euphorbiaceae* (82%), *Verbenaceae* (77%), *Fabaceae* (75%), *Asteraceae* (71 %), *Amaranthaceae* (67%), *Acanthaceae* (60%) and 50% in *Apocynaceae*, *Brassicaceae*, *Geraniaceae* and *Ranunculaceae*.



**Fig. 2.** Plants infected with begomoviruses in Pothwar Region; A: *Duranta repens* B: *Malvastrum coromandelianum* C: *Zinnia elegans* D: *Capsicum annum* E: *Snapis alba* F: *Solanum lycopersicum* G: *Calotropis acia* H: *Cucumis sativus* I: *Abelmoschus esculentus* J: *Cucurbita pepo* K: *Euphorbia pulcherrima* L: *Althea rosea*.

No plant was found infected belonging to families *Lamiaceae* and *Vitaceae* (Table 1).

It was observed that begomoviruses are not evenly distributed in Pothwar region and maximum distribution was recorded Rawalpindi (32 %) followed by Chakwal (27 %), Attock (22.4 %), Islamabad (14 %) and Jhelum (5 %) (Fig. 3).

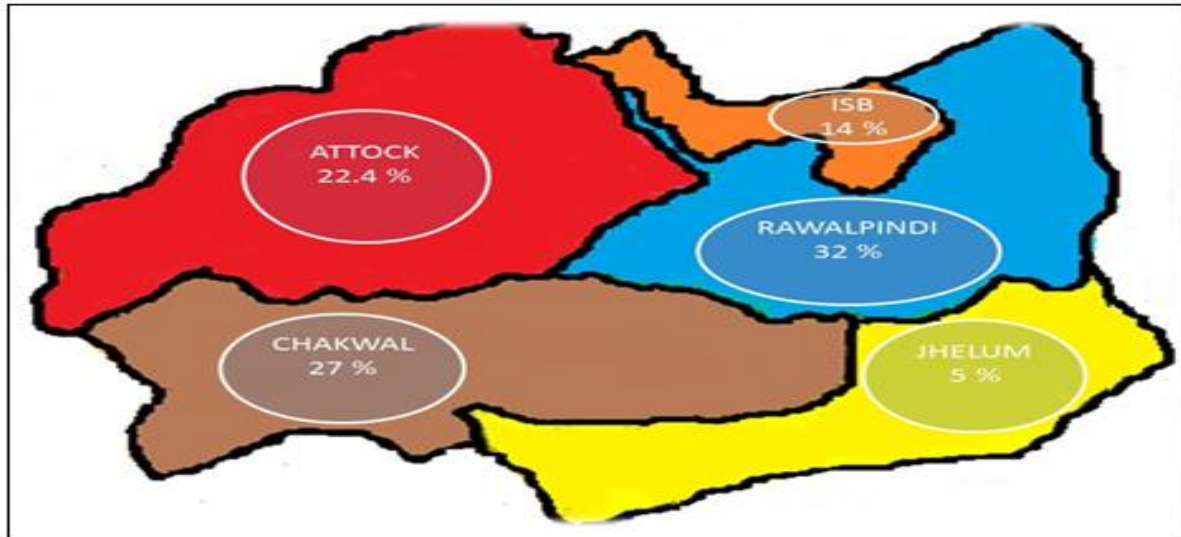
### Discussion

Pothwar region is a home of diverse vegetation which includes herbs, shrubs, weeds, vegetable and field crops, fruit crops and small forests (Edwards, 2007). The topography of Pothwar plateau is undulating and hosts valleys, rivers, small forests and plains which provide a conducive environment for the rearing and



spread of begomoviruses vector whitefly. The whiteflies are the only mode of begomoviruses transmission with an exception of *Tomato yellow leaf curl virus* (TYLCV) which has recently been established as a seed-borne virus along with whitefly

transmission (Kil *et al.*, 2015). The availability of numerous types of dicotyledonous plants and hospitable environment for whiteflies in Pothwar region are the main reasons for higher incidence of begomoviruses.



**Fig. 3.** Distribution of Begomoviruses in Pothwar Region.

The begomoviruses are known to infect plants belonging to families *Asteraceae* (Tahir *et al.*, 2015), *Apocynaceae* (Ilyas *et al.*, 2013), *Fabaceae* (Hameed and Robinson, 2004), *Malvaceae* (Nawal-ul-Rehman *et al.*, 2012; Tahir *et al.*, 2011; Zhou *et al.*, 1998), *Solanaceae* (Akhtar *et al.*, 2009) and many other reports which are not published yet but sequences are submitted in genetic database.

Unfortunately there was no systematic study carried out on begomoviruses from Pothwar region located in the northern Punjab of Pakistan because the main focus of research groups, working on begomoviruses, was on cotton crop grown in Southern Punjab. A large number of full genome sequences of begomoviruses are available on global genomic databases with a total of 3,123 begomoviruses isolates have been rectified by ICTV so far and out of these 3,123 isolates 174 have been reported from Pakistan (Brown *et al.*, 2015). Almost all of the 174 isolates are from Central and Southern Punjab province with only 7 isolates from Northern Punjab (Shih *et al.*, 2003; Ilyas *et al.*, 2010; Mustujab *et al.*, 2015;) which clearly shows that genetic diversity and distribution of begomoviruses was never systematically studied from Pothwar region.

### Conclusion

The present study gave a detailed picture of begomoviruses infection covering 17 plant families covering vegetable crops, ornamentals and weeds. The results of the current study is a tip of an iceberg and if the Pothwar region is thoroughly combed for begomoviruses, employing large resources, the results may give higher incidence and distribution of begomoviruses along with an increased genetic diversity which could become a vital source for understanding of begomoviruses evolution particularly in this part of the world and globally in general.

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