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# Prevalence of Okra Yellow Vein Mosaic Viral Disease and its Correlation with Vector Population in Sindh, Pakistan

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**Abstract**

Okra (*Abelmoschus esculentus* (L.) Moench) being an important vegetable crop is widely grown in tropical and subtropical regions of the world. Whitefly (*Bemisia tabaci*) is not only a major insect pest of okra in Pakistan but also the vector insect of *Begomoviruses* including okra yellow vein mosaic virus which causes okra yellow vein mosaic viral disease (OYVMVD). Regional monitoring of insect vectors and disease prevalence is being emphasized for proper management, therefore this study was focused on determining the intensity of OYVMVD in the fields and the correlation between the disease and vector population. For this purpose, the virus infection was confirmed with DNA sequencing. The disease and vector population was visually assessed approximately 65-70 days after sowing of okra under field conditions at commercial farmer fields in district viz., Hyderabad, Tando Allahyar, Matiari, and Sanghar of Sindh, Pakistan during the growing season of 2016. Ten okra plants with three replications ($N$ = 30) at ten different locations of each district were assessed. Results of nucleotide alignment of DNA amplified from diseased plants confirmed that yellowing and vein clearing were due to the virus infection. The visual assessment showed that the disease was prevailing throughout all region monitored in this study and the disease was significantly correlated with the vectors population. However, maximum disease incidence and severity were recorded in Tando Allahyar. Therefore, it is recommended to validate various management practices at such high disease risk points for better management.

**Keywords:** Okra (*Abelmoschus esculentus*); Whiteflies (*Bemisia tabaci*); Okra yellow vein mosaic virus; disease prevalence; visual assessment

## INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) is one of the most important vegetable crop grown in tropical and sub-tropical regions in the world, including Pakistan. Okra crop also known as lady's finger and bhindi, is highly nutritious and suitable for cultivation under home gardening as well on large commercial fields. Okra crop in Pakistan is adversely infected by numerous viruses such as Okra leaf curl virus (OLCV), okra mosaic virus (OMV), and okra yellow vein mosaic virus (OYVMV) (Rehman & Ahmed 1996a; Rehman & Ahmed 1996b; Mansoor et al. 2001; Ali et al. 2014). Among these OYVMV (Genus *Begomovirus* and Family *Geminiviridae*) causing okra yellow vein mosaic viral disease (OYVMVD) is considered as most severe and widely distributed disease (Jose & Usha 2003; Ali et al. 2014). The virus is transmitted by whitefly (*Bemisia tabaci*) in a persistent circulative manner, which is acquired from contaminated sap from the phloem in the body of whitefly through style and is introduced into new plant after latent period of a few hours which may continue throughout life of insect (Lapidot & Polston 2010). The disease may appear at any time during all growth stags. Variety of symptoms may appear on successful infection such as, intermingled patches of green and yellow colour, vein clearing and leaves chlorosis, and deformed, malformed and yellow fruits (Baghat et al. 2001). The minimum number of whiteflies required to induce 100% infection is 10 insects/plant, although a single whitefly can transmit the OYVMV effectively (Sanwal et al. 2014). It is predicated that the virus may infect 100 % okra plants with range of yield losses between 50 to 94 % which is highly influenced by stage of crop being infected (Sastry & Singh 1975).

The assessment of the amount of the disease on okra plants in field condition is essential in any quantitative epidemiological study and so far to conduct field based experiments for managing the disease at high disease rick points. Thus there is need to enhance regional forecasts to identify the field at greatest risk of OYVMVD in Sindh province, Pakistan. Disease can be assess visually direct on or in plant material using descriptive or pictorial keys and indirectly by monitoring the propagative unit of infectious agent with help of various modern

techniques (Gonzalez-Perez et al. 2011; Hajano et al. 2016). Obviously visual assessment of disease in term of incidence, severity is simpler, cost effective and more strongly correlated with yield losses in the crop (Cooke et al. 2006; Gonzalez-Perez et al. 2011).

Aim of this study was to determine intensity of OYVMVD in the fields and correlation between the disease and vector population, so that the outcome of the study could be used as a guidance for screen critical management practices for controlling this disease at high disease risk points.

## Materials and Methods

**The disease assessment:** During the growing season of 2016, the disease was visually assessed under field conditions at commercial farmer fields at Hyderabad, Tando Allahyar, Sanghar, and Matiari regions of Sindh, Pakistan. It was not possible to choose fields according to a stratified random sampling plan; rather, the selection was based on attempting to achieve coverage of the main arable areas, coupled with the willingness of farmers to participate in the survey. Fields were managed by growers as farming practices recommended for okra production. Approximately 65-70 days after sowing, ten different locations of each district viz., Hyderabad, Tando Allahyar, Matiari, and Sanghar were assessed for disease development. Ten okra plants with three replications ($N$ = 30) in an area of about 43560 $ft^2$ were observed for the okra yellow vein mosaic virus symptoms. The disease incidence percentage was calculated by dividing the number of okra plants showing vein-clearing symptoms by the total number of plants examined for the disease. Disease severity was measured by using a 0-6 rating scale (Ali et al. 2005) where, 0 indicates complete absence of disease symptoms, 1 = Vein Clearing 1-10 %, 2 = Vein Yellowing of small leaves 11-25 %, 3 = Yellow network on some leaves 25-50 %, 4 = Yellow network on all leaves 51-60 %, 5 = Complete leaves turn Yellow or cream color 60-70 % and 6 = Plant stunted, deformed and small fruits and the whole plants become colorless > 70 %.

**The virus identification:** Total DNA was extracted from leaves showing typical symptoms of the disease using CTAB method with necessary modifications (Ghosh et al. 2009). A degenerated primer set (GEM-F: 5'-ATRRTHTGGATGGAYGARAACAT-3'; GEM-R: 5′- AAATCCCCTNTATTTCAAARAT-3′) designed by Roy et al. (2015) was used to amplify 760 bps. Amplified PCR products were purified using the UltraClean® PCR Clean-Up kit (Mo-Bio, USA) and were sent to Sogo Ltd. for nucleotide sequencing. The obtained sequence was aligned on the NCBI website (http://www.ncbi.nlm.nih.gov/).

**Whitefly scouting:** Whitefly population (both adults & nymphs) was recorded from, the upper leaf (first plant), middle leaf (second plant), and lower leaf (third plant) at the time of visual disease assessment (Akram et al. 2013) to correlate with incidence and severity of the symptoms.

**Statistical analysis:** The mean disease incidence percentage, disease severity, and white fly population per plant at each location were compared for significant differences analysis among variance using LSD test ($\alpha$ = 0.005) of completely randomized design using STATISTIX v. 8.1 software (Analytical Software). A nonparametric Spearman rank correlation test was used for the correlation analysis between the disease severity with disease incidence percentage and the white fly population at each location of four districts using PRISM v. 5.01 (GraphPad Software).

## Results

**The virus identification:** Typical symptoms of OYVMV such as yellowing and vein clearing in the leaf were observed in the diseased plants (Figure 1 A&B). Amplification of degenerated geminiviruses sequence-specific primers showed a band of required length of 760 bps after gel electrophoresis of PCR products (Figure 1C). Furthermore, sequence alignment showed 88% nucleotide identity to DNA-A segment of Bhendi yellow vein mosaic virus isolate OK309-RAJ (complete sequence ID KT 390325-1). These results clearly showed the plants were infected with the virus.

**The disease incidence, severity, and whitefly population at different regions:** The incidence of OYVMVD was studied during 2016 growing season in four different regions of Sindh, Pakistan. A total of 10 commercial farmer fields for each region were visited at the hub of vegetable production. A maximum disease incidence of 88.33 % was recorded in the Tando Allahyar region followed by the Sanghar region 81.0 %. At Tando Allahyar region the disease incidence ranged between 73.3 – 100 % (Table 1). Plants at L1, L2, L3 showed 100 % incidence of the disease, whereas, only L1 showed 100 % disease incidence in the Sanghar region (Table 1). In other remaining regions, the disease incidence was 73.3 % with a range of 50 – 93.3 % in Matiari and 71.67 % the disease incidence with a range of 50 -96.6 % in Hyderabad (Table 1). The disease severity was also assessed in the same plants evaluated for the disease incidence. Statistically, there was no significant difference in the disease severity among all four regions (Table 1). However, the disease severity was higher (5.20) in the Tando Allahyar region followed by Sanghar, Hyderabad, and Matiari with the disease severity of 5.18, 4.98, and 4.77, respectively (Table 1). This result indicates that the disease is prevailing throughout okra cultivation in surveyed regions with severe levels.

Whitefly population was scouted out at plants used for visual disease assessment. An average of 8.84 and 8.49 whiteflies per leaf were recorded in Tando Allahyar and Sanghar regions, respectively (Table 1). Whereas, 8.01 whiteflies per leaf were observed in the Matiari region and the lowest population was 7.48 per leaf in the Hyderabad region (Table 1). This indicates

whiteflies are playing a major role in the disease epidemic in these okra production regions.

Table 1 Okra yellow vein mosaic viral disease incidence, severity and whitefly population at different regions of Sindh.

| **Parameter** | **Region** | **Locations** | | | | | | | | | | **Average** | **C-Value** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | L1 | L2 | L3 | L4 | L5 | L6 | L7 | L8 | L9 | L10 | | |
| Incidence (%) | Hyderabad | 96.6 | 80 | 80 | 76.6 | 76.6 | 70 | 60 | 60 | 66.6 | 50.0 | 71.67b | 15.79 |
| | Tando Allahyar | 100 | 100 | 100 | 96.6 | 90 | 86.6 | 86.6 | 76.6 | 73.3 | 73.3 | 88.33a | |
| | Sanghar | 100 | 93.3 | 90 | 80 | 80 | 73.3 | 73.3 | 86.6 | 70 | 63.3 | 81.00ab | |
| | Matiari | 93.3 | 90 | 76.6 | 80 | 76.6 | 70 | 70 | 53.3 | 73.3 | 50 | 73.33b | |
| Severity | Hyderabad | 5.7 | 5.3 | 5.2 | 5.2 | 5.1 | 5.1 | 4.7 | 4.6 | 4.5 | 4.0 | 4.98a | 10.50 |
| | Tando Allahyar | 6.0 | 5.7 | 5.6 | 5.4 | 5.0 | 5.0 | 5.0 | 4.9 | 4.6 | 4.5 | 5.20a | |
| | Sanghar | 6.0 | 5.8 | 5.3 | 5.1 | 5.1 | 5.0 | 4.9 | 4.9 | 4.7 | 4.7 | 5.18a | |
| | Matiari | 5.7 | 5.6 | 5.2 | 5.2 | 5.0 | 4.5 | 4.3 | 4.1 | 4.1 | 3.7 | 4.77a | |
| White fly population | Hyderabad | 8.8 | 8.1 | 6.5 | 7.8 | 7.7 | 7.4 | 7.6 | 7.6 | 6.5 | 6.5 | 7.48b | 12.12 |
| | Tando Allahyar | 10.9 | 9.7 | 10.9 | 7.8 | 9.7 | 9.6 | 8.3 | 8.6 | 6.5 | 6.2 | 8.84a | |
| | Sanghar | 9.7 | 9.1 | 8.5 | 8.8 | 8.5 | 9.2 | 7.7 | 7.6 | 8.1 | 7.4 | 8.49a | |
| | Matiari | 8.4 | 8.2 | 8.2 | 8.1 | 8.2 | 7.6 | 8.0 | 7.6 | 7.8 | 7.7 | 8.01ab | |

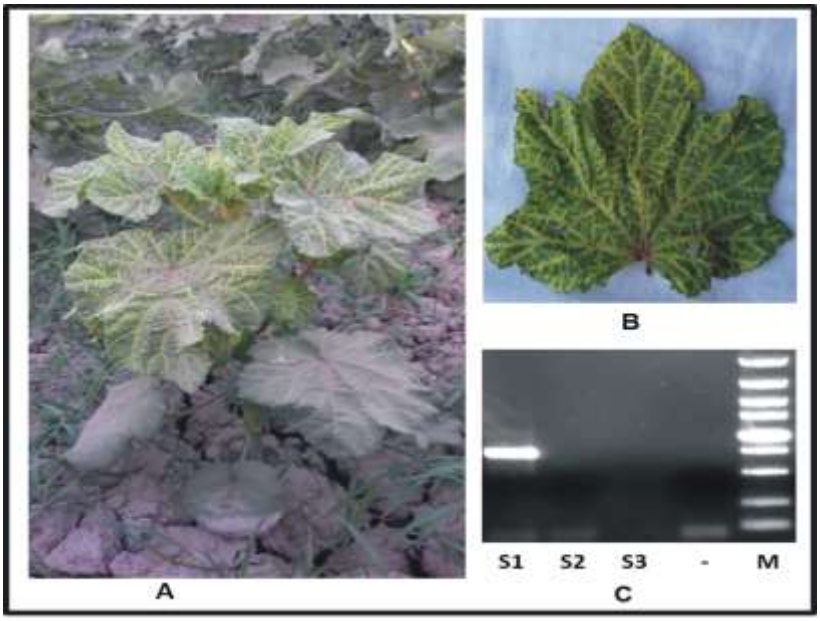


**Figure 1**. Symptoms of okra yellow vein mosaic viral disease and the virus detection. (A) Plant of okra in the field showing yellowing (B) the diseased leaf showing vein clearing and (C) ethidum bromide stained agarose gel showing 760 bps band amplified from DNA, extracted from diseased leaf samples. Lane "S1-3" is indicating amplification of 760 bps DNA band from the diseased leaf samples, "-" is indicating water template and "M" is indicating DL2000 DNA marker.

**Relationship between the disease and whitefly population:** Of the 40 selected fields for recording the disease incidence and severity in four regions of Sindh province, no field was found free of the disease. It was predicted the disease may occur in these regions but not be severe, therefore correlation analysis was performed to explore the relationship between the disease severity and incidence. Correlation analysis showed the disease severity at all regions was significantly and strongly correlated with the disease incidence. The data showed the relationship of the disease severity with the incidence at Hyderabad $r = 0.9565$; $P < 0.0001$, Tando Allahyar $r = 0.9416$; $P < 0.0001$, Sanghar $r = 0.8913$; $P = 0.0005$ and Matiari region was $r = 0.9073$; $P = 0.0003$ (Figure 2). Similarly, the relationship between the disease severity and whitefly population was also good and significantly correlated in all the regions (Figure 3). sThis indicates that the disease development and vector population were directly correlated in these regions. Such a strong relation may severely reduce crop productivity or may destroy entire plants within time.

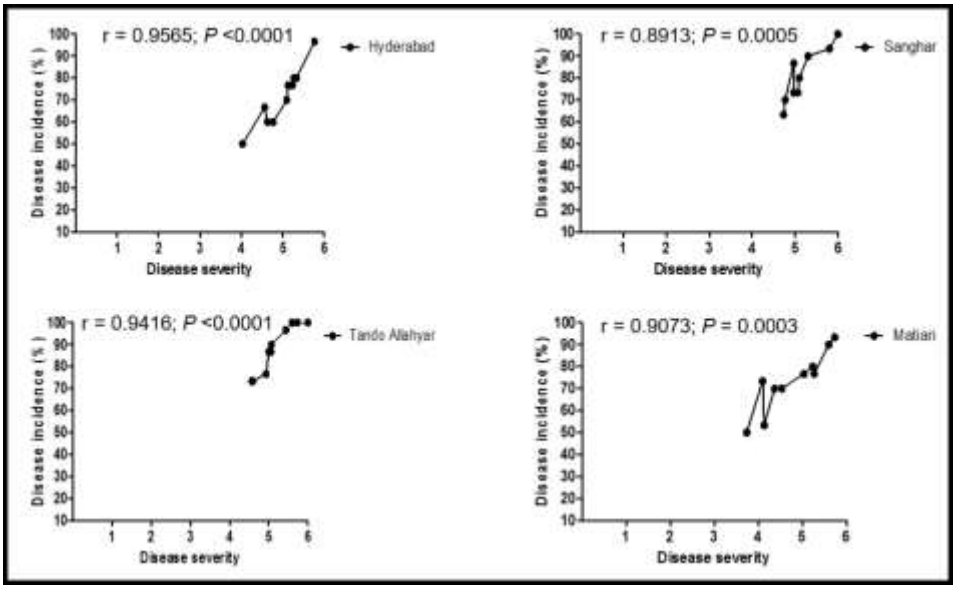


**Figure 2.** Scatter plot showing correlation coefficients for okra yellow vein mosaic viral disease severity with the disease incidence at different regions under field conditions

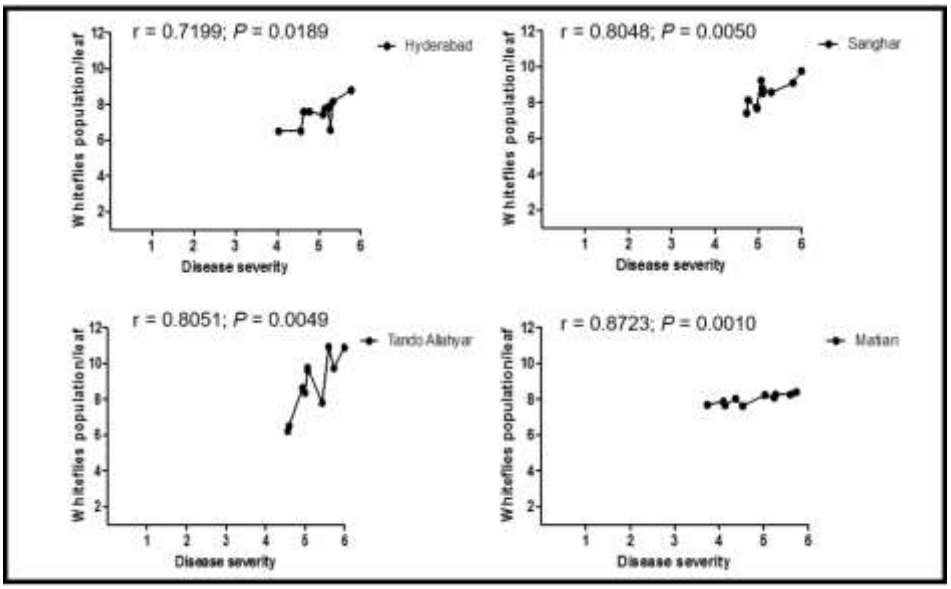


**Figure 3**. Scatter plot showing correlation coefficients for okra yellow vein mosaic viral disease severity with the whiteflies population at different regions under field conditions

## Discussion

This study presents extensive data on the disease development and vector population in Sindh, demonstrating that it is widespread in all of the regions studied. During the survey, diagnosis was based only on visual symptom expression and sequence analysis. Yellowing of vein encircling green tissue is a known characteristic symptom of the disease (Bhagat et al. 2001). Moreover, sequencing results confirmed the presence of the virus. In this study, the diseased plants were also showing such severe symptoms under field conditions. The disease was observed at all the locations of the four regions, more than 50 % of plants were infected. The average of 5.0342 severity of the disease was found in all regions focused on this study. However, highest the disease incidence and severity were recorded at Tando Allahyar. Some locations of the Tando Allahyar region were showing 100 % incidence with chronic and severe conditions of the disease, such specific locations may be selected for field-based experiments, including resistance screening. Moreover, management practices can be evaluated to overcome such a divesting situation of the disease in the future. A variety of crops has been surveyed for viral diseases in specific regions to determine epidemic levels (Foster et al. 2004; Ntawuruhunga et al. 2007; Iqbal et al. 2012; Aliyu et al. 2012; Shelat et al. 2014). Furthermore, there was a strong relationship between the disease incidence, severity, and whitefly abundance, as has been demonstrated from studies conducted elsewhere for other diseases (Foster et al.

2004; Ali et al. 2005; Sindhumole & Manju 2013). However, our study showed a severe OYVMVD epidemic situation exists in the surveyed areas. Even though, due to capriciousness in the whitefly population, latent period, and proper symptom expression it is difficult to find such a relationship. Therefore, surveys should be conducted frequently after some interval of time for a better understanding of the relationship between whiteflies and OYVMV occurrence at the hub of vegetable production.

In conclusion, these data show that OYVMVD vectored by whitefly is widely distributed in Sindh, Pakistan, with higher severity levels. This survey first time is conducted to provide baseline information on the OVYMVD spread in Sindh province, Pakistan. It is important to take into consideration that to widely cultivation of the disease susceptible cultivars and the unavailability of management practices would be perilous threats to okra production of Pakistan in future. Therefore, here is immediate need to validate management practices at such high disease risk points and to control the disease spread at these vegetable production hub.

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