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# Characterization and Detection of Yellow Vein Disease of Okra *(Abelmoschus esculentus* (L.) Moench) in Sri Lanka

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***ABSTRACT*:** *Begamoviruses transmitted by whitefly (Bemisia tabaci Genn, Family Aleyrodidae) cause severe damage to crop plants showing varying symptoms in different crop species. Okra Yellow Vein Mosaic Virus (OYVMV) is one of the most devastating diseases reported in okra cultivation worldwide. In this study, OYVMV disease severity and incidence of three recommended okra varieties were evaluated in field conditions at three different locations; Gannoruwa, Mahailuppalama and Angunakolapalasse. Similar disease symptoms were observed in Mahailuppallama and Angunakolapalasse. In Gannoruwa late infection caused lesser disease incidence and severity. Statistical analysis showed significant difference in severity and incidence of OYVMV among varieties as well as locations. MI 7 and MI 5 showed significantly high disease severity than Haritha while location Mahailluppallama showed significantly high disease severity followed by Angunakolapalasse. Diseased samples collected from these three locations and two additional locations i.e. Kilinochchi and Vauniya were subjected to PCR amplification with different begamovirus specific primers to identify the cross infection with other begamovirus diseases. All symptomatic samples collected from different locations showed positive results for OYVMV specific primer while none of the diseased samples showed positive results for Chili leaf curl virus, Tomato leaf curl virus, Okra leaf curl virus and Okra leaf curl crinkle virus disease specific primers. This study revealed that there is location and variety effect on OYVMV disease incidence and in these locations tested okra varieties were not cross infected with other tested viruses. Sequence analysis has to be done to identify genetic diversity of okra YVMV in different locations.*

***Keywords:*** *Disease incidence, disease severity, okra yellow vein mosaic virus*

## INTRODUCTION

Okra, *Abelmoschus esculentus* (L.) Moench belongs to the family Malvaceae and is an important vegetable crop grown throughout the tropical and subtropical parts of the world. It provides an important source of vitamins, calcium, potassium and other minerals that are often lacking in diets of developing countries (IBPGR, 1990). Among the different species of

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genus Abelmoschus, the most popularly grown species is *Abelmoschus esculentus* in Asia and has a great commercial demand due to its nutritional value. The major production constraint for okra is *okra Yellow vein mosaic virus* (OYVMV) disease and it causes losses with regard to the quality as well as the yield wherever the crop is grown (Venkataravanappa *et al.*, 2013). OYVMV was first reported from Mumbai in India (Kulkarni, 1924). This disease is transmitted through whitefly (*Bemisia tabaci* Genn, family Aleyrodidae). Okra plants infected by OYVMV show persistent symptoms of vein clearing followed by yellowing of whole leaf, reduction in leaf and fruit sizes and leading to a significant decrease of the produce. Based on the time of infection of the disease, up to 96 % yield loss has been reported (Pun and Doraiswamy, 1999). Common begamovirus infecting plants shows similar symptoms in many crop species and different begomoviruses or strains differ with respect to their host range. The choice of crops by the whitefly may directly affect their prevalence in a particular region. During a mixed infection, whether two viruses will interact with each other synergistically or antagonistically, depends upon the host plant in which they are interacting (Mendez-Lozano *et al.,* 2003). Plants usually offer resistance to geminiviruses through hypersensitive response (Mubin *et al.*, 2010a; Hussain *et al.*, 2005), and DNA- methylation (Raja, 2010; Raja *et al*, 2010) leading towards transcriptional gene silencing. Because of that, though the infection is there symptoms may not appear. Sattar, (2012) identified that okra in Africa is a host to many begomovirus complexes such as okra yellow crinkle virus and okra leaf curl cameroon virus. Further, strain differences of OYVMV were also identified by different research studies (Venkataravanappa *et al*, 2013). In 2015, Roy and co-workers observed alternate hosts of YVMV disease. Therefore, characterization and identification of okra infecting begamoviruses are important in crop improvement programmes.

In Sri Lanka, none of the recommended varieties showed consistent resistance to YVMV disease. Confirmation studies of OYVMV resistance in genotype of okra proved that the highly resistant genotypes possessed true and stable resistance reactions against this virus (Ali *et al.,* 2005). Therefore, utilization of crop genetic resources to enhance the resistance in cultivated okra is one of the solutions to overcome this problem. Incidence and severity of OYVMV disease are affected by environmental conditions. Decrease in minimum temperature was conducive for disease development while increase in relative humidity (Ali *et al.,* 2005) and high rainfall (Leite *et al.,* 2005) was detrimental to whitefly population. The objectives of this study were to evaluate three recommended okra varieties against OYVMV in three different locations, and to confirm the presence of OYVMV by virus specific primers and to test for the presence of other whitefly-transmitted geminiviruses as mixed infections in okra.

## MATERIALS AND METHODS

### Morphological characterization of yellow vein disease of okra

The study was conducted at research fields of Plant Genetic Resource Centre (PGRC), *Gannoruwa*, Field Crop Research and Development Institute (FCRDI), *Mahailuppallama* and Grain Legume and Oil crop Research and Development Centre (GLORDC), *Angunakolapalassa* during *Yala* (April to July) 2014.

Three recommended okra varieties (*MI5, MI7 and Haritha*) were grown in the field with the plot size of 1.8m x 1.8m in a randomized complete block design (RCBD) with three replicates. Plant spacing was 90cm x 90cm. All the cultural practices were done according to the recommendations of Department of Agriculture. YVMV disease pressure was increased

by cultivating highly susceptible variety; *MI-7* around the field as a boarder crop and natural infection was allowed. Disease symptoms and number of diseased plants were recorded 35, 45 & 55 days after planting. Disease severity was recorded according to the procedure given by Ali *et al.* (2005) with a slight modification. Table 1 summarizes the disease symptoms and respective severity scale used in this study. Based on that scale Percentage of Disease Incidence (PDI) (Sankara and Acharyya, 2012) and Disease Severity Index (DSI) (McKinney, 1923) were calculated.

**Table 1. Description of OYVMV disease symptoms used for scoring**

| Description of symptoms | Severity scale | Rating Scale | Severity Range |
|---|---|---|---|
| Absence of symptoms | 0 | HR | 0% |
| Very mild symptoms, initial vein clearing | 1 | R | 1-20% |
| Leaf veins completely yellow and inter-veinal regions remain green or normal | 2 | MR | 20-40% |
| Curly leaves and whole leaf get yellow color | 3 | MS | 40-60% |
| Whole leaf yellow coloured. Leaf Margin start drying | 4 | S | 60-80% |
| Yellowish and deformed pods with whole leaf yellow coloured. All leaves of the plant get affected | 5 | HS | 80-100% |

Adopted from: Ali *et al.,* (2005)

Note: R – Resistant, HR – Highly resistant, MR – Moderately resistant, MS – Moderately susceptible, S – Susceptible, HS – Highly Susceptible

$$PDI = \frac{\text{Number of diseased plants}}{\text{Total Number of plants observed}} \text{ X } 100$$

PDI= Percentage of Disease Incidence, (Sankara and Acharyya, 2012)

$$DSI = \frac{\Sigma \text{ Severity scale x Total of infected plants}}{5 \text{ x Total number of plants}} \text{ X } 100$$

DSI - Disease Severity Index (McKinney, 1923)

**Data analysis**

Data were transformed using Box-Cox transformation in Minitab (MINITAB 14) and Analysis of variance was done for transformed data of DSI. Mean separation was done to find out the location and variety differences according to the Tukey's Studentized Range test using Statistical Analysis System software (SAS, version 9.0).

**Molecular confirmation of yellow vein disease of okra**

Based on the type of symptoms observed diseased okra leaf samples were collected from five different locations; *Gannoruwa, Mahailuppallama, Angunakolapalassa, Vauniya* and *Kilinochchi*. Genomic DNA was extracted from both non-symptomatic and symptomatic okra leaf tissues using the method described by Kochko *et al*. (1990) with slight modifications; immature leaf samples were used instead of cotyledons and the time of incubation of ground leaf samples in water bath at 65 °C was increased by 10 minutes. Extracted DNA samples were quantified by Spectrophotometer (Model: Jennova, Jenway, United Kingdom) and samples were diluted to prepare the template DNA working solution of 15 ηg/µL with TE (Tris-EDTA) buffer. Symptoms of OYVMV in different locations were confirmed genotypically using OYVMV specific primers. To investigate the presence of a mixed infection of different viruses, molecular level identification was done for common begomovirus that cause diseases in vegetable crops using extracted DNA of all five locations. Primers were designed for OYVMV, Tomato leaf curl virus, Chili leaf curl virus, Okra leaf curl virus, and Okra leaf curl crinkle virus. For primer designing sequences were obtained from NCBI database and aligned using Bioedit software ver. 7.2.5.0. Primers were design manually based on conserved regions of each virus specific primers. PCR was performed using different virus specific primers (Table 2). For confirmation reference DNA samples from different crop species infected with each virus were used for respective diseases except okra leaf curl virus, and okra leaf curl crinkle virus.

**Table 2. Details of virus specific primers used for the study**

| Primer | Sequence | Annealing Temperature |
|---|---|---|
| OYVMV | F 5'- ATG TCG AAG CGA GCT GCC G 3'<br>R 5'- TCA ATT CGT TAC AGA GTC ATA AA 3' | 52 °C |
| Tomato Leaf Curl Virus | F 5'- AGC GAC CAG CAG ATA TAA TCA 3'<br>R 5'- CGA ATC ATA GAA ATA GAT CCG 3' | 56 °C |
| Chili leaf curl Virus | F 5'- TGC CAG AGC GGC ATC AGC GG 3'<br>R 5'- GTC CCC ATT GTC CCC CAT TCC 3' | 58 °C |
| Okra leaf curl virus | F 5' - CTG TGC ATG GGT TTC GTT GT 3'<br>R 5' - CGT CAT AGA TGG TGT TGA CC 3' | 55 °C |
| Okra leaf curl crinkle virus | F 5' - CCA TTA GTC AAC GAG TTC CC 3'<br>R 5' - ACC TCA AGT GTG GAT GCT CA 3' | 55 °C |

PCR reactions were performed in 15 µl total volume using 45 ηg template DNA, 1.5 units of *Taq* DNA polymerase (Promega, USA), 1x PCR buffer, 2 mM $MgCl_2$ 1 mM dNTPs, and 0.24 µM of each primer. The PCR was carried out in a Verity (Applied bio-systems) master cycler at 94 °C for 4 min, followed by 30 cycles at 94 °C for 1 min, annealing temperature specific to each primer for 1 min, 70 °C for 1 min, and final extension of 70 °C for 7 min. All PCR amplicons were resolved on 1.5 % agarose gel in 0.5 X TBE, stained with 0.5 µg/ml ethidium bromide and visualized under UV light in Gel documentation system (BIO RAD). Presence of disease was detected by PCR amplification using different virus specific primers shown in table 2.

## RESULTS AND DISCUSSION

### Morphological characterization of yellow vein disease of okra

Okra plants of all three locations were infected with OYVMV disease. *Mahailluppallama* and *Angunakolapelessa* locations showed heavy infestation while plants grown in *Gannoruwa* showed the least virus infection. PDI and DSI of OYVMV disease in each variety at *Mahailuppallama, Angunakolapalasse* and *Gannoruwa* are shown in figure 1. The disease symptoms at *Gannoruwa* appeared 90 days after sowing where very mild symptoms were observed in both variety *MI 7* and *MI 5* while variety *Haritha* was not affected with OYVMV disease until the experiment period is over.

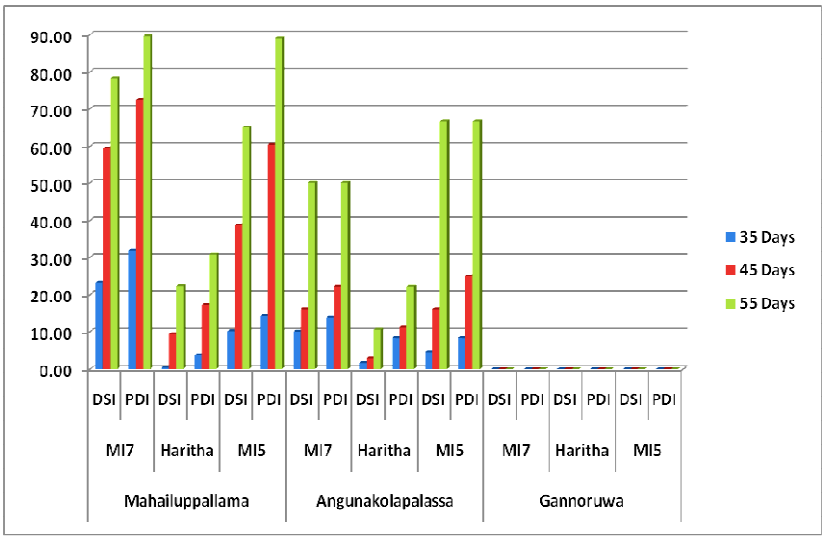


**Fig. 1. Percentage of Disease Incidence (PDI) and Disease Severity Index (DSI) of three different okra varieties in *Mahailuppallama, Angunakolapalasse* and *Gannoruwa***

There was OYVMV infection at early stage of crops grown in *Mahailuppallama* and *Angunakolapalasse* in all three recommended varieties. The severity and disease incidence were increased at an increasing rate. With time the yield of these two locations was very poor when compared to the yield at *Gannoruwa* (data not shown). The rate of increase in disease incidence was more at the early stages rather than that at later stages of the plant growth at *Mahailuppallama* and *Angunakolapalasse* in all tested varieties. Severity of the disease is high in *Mahailuppallama* than that of the other two locations in all three varieties. Disease symptoms observed in *Mahailuppallama* include vein and vein-let chlorosis, whole lamina becoming uniformly crinkled and yellow with regularly distributed green tissue in the inter venial regions and finally totally yellowish colour leaf with dried leaf margins and pods were complete yellow colour. Disease symptoms first observed in *MI 7* and then symptoms appeared in both upper and lower leaves of all three varieties. Symptoms observed in *Angunakolapalasse* were very much similar to the symptoms observed in *Mahailuppallama* such as vein and veinlet chlorosis, puckering of leaves, drying of leaf margins, yellowing of whole leaf. Deformed pods were also observed in these two locations. Two distinct vein clearing patterns were observed in *Gannoruwa*, one type was vein clearing occurred in

mosaic pattern and the other type was mid vein turned into yellow colour without mosaic symptoms (Plate 1). In *Mahailuppallama* and *Angunakolapalasse* variety *Haritha* showed late infection and lesser severity than two other tested varieties while at *Gannoruwa* no symptoms were observed in variety *Haritha*. The different symptoms observed in different locations were showed in Plate 1.

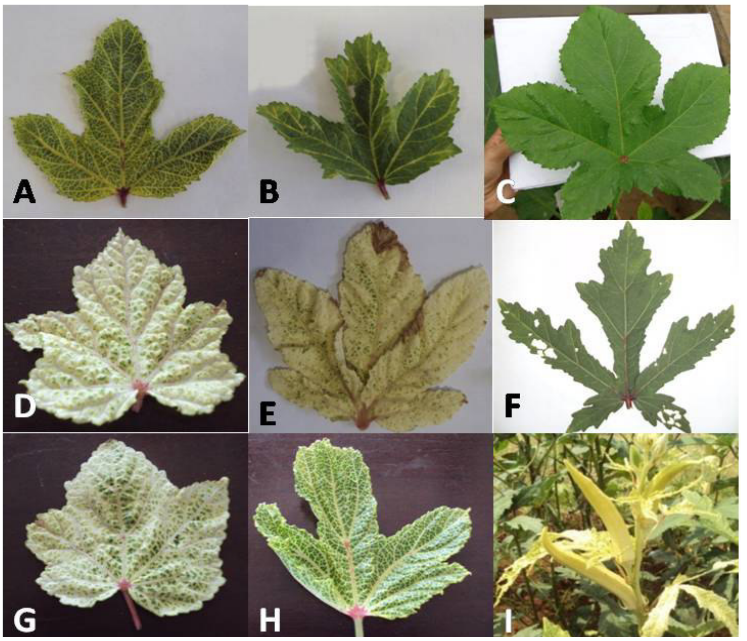


**Plate 1. Different symptoms of OYVMV observed in different locations (A) vein clearing occurred in mosaic pattern (*Gannoruwa*). (B) Mid vein turned into yellow colour without mosaic symptoms (*Gannoruwa*). (C) Non-symptomatic leaf collected from *Gannoruwa.* (D) Diseased okra leaves collected from *Mahailuppallama*, (E) Diseased okra leaves collected from *Angunakolapalasse*. (F) Non-symptomatic leaf collected from *Angunakolapalasse* (variety *Haritha* at 70 days after planting), (G) Diseased okra leaves collected from *Vauniya*, (H) Diseased okra leaves collected from *Kilinochchi*, (I) Complete yellow colour pods observed in *Mahailuppallama***

Analysis of variance of transformed data of DSI showed that there is significant difference of variety and location on DSI. Location and variety are significant at probability levels of p=0.0231 and p=0.006 respectively, while there is no significant location x variety interaction on disease severity. Results of Tukey's Studentized Range test revealed that the disease severity of variety *Haritha* is significantly lower than variety *MI 5* and *MI 7* in all tested locations. Disease severity of location *Mahailuppalama* was significantly high followed by *Angunakolapalasse.* Significantly least disease severity was observed at *Gannoruwa*.

**Molecular Confirmation of Yellow Vein Disease of Okra**

PCR products obtained with different primers were resolved in 1.5% agarose gel. Image of PCR products of okra YVMV primer, Tomato leaf curl virus primer and Chili leaf curl virus primer visualized under UV light in Gel documentation system is shown in Plate 2.

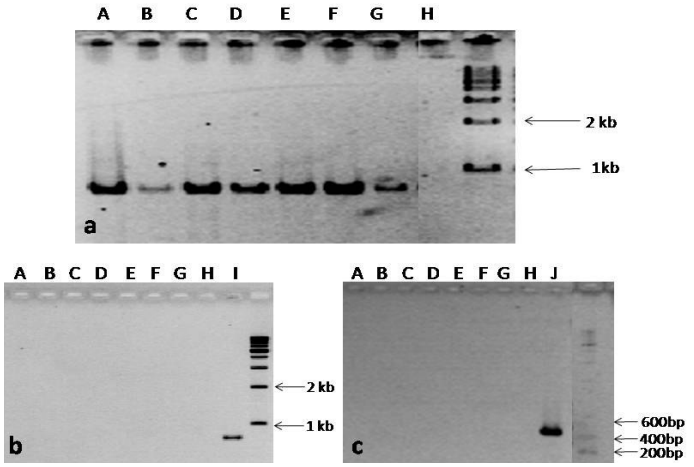


**Plate 2. Amplified PCR products of different virus specific primers in 1.5 % agarose gel for diseased okra samples collected from different locations, (a) OYVMV primer, (b) Tomato leaf curl virus primer and (c) Chili leaf curl virus primer, A- diseased leaf sample from *Gannoruwa* in which was vein clearing occurred in mosaic pattern, B- diseased leaves sample from *Gannoruwa* in which mid vein turned yellow colour without mosaic symptoms, C- Diseased okra leaf sample collected from *Mahailuppalama*, D -Diseased okra leaf sample collected from *Angunakolapalasse.* E- Diseased okra leaf sample collected from *Kilinochchi*, F- Diseased okra leaf sample collected from *Vauniya*, G – Non-symptomatic leaf collected from *Angunakolapalasse*, H- Non-symptomatic leaf collected from *Gannoruwa*, I - Tomato leaf curl virus disease sample, J- Chili leaf curl virus disease sample**

For OYVMV specific primer, all the tested samples from five locations gave corresponding fragment size of 800 bp for OYVMV proves that all the diseased samples collected from the five locations are infected with OYVMV. Samples, which showed vein clearing without mosaic symptoms collected from *Gannoruwa* and Non-symptomatic samples collected from *Angunakolapallasse* gave a light band for OYVMV primer while non-symptomatic leaves collected from *Gannoruwa* did not produce any amplification. It revealed that YVMV disease can be latent in the plant without showing symptoms and disease specific primers could help to identify the disease. Chili leaf curl virus specific primer and tomato leaf curl virus specific primer did not give any amplification for infected okra samples. However, positive results were shown for both leaf curl virus infected chili and tomato samples. Okra leaf curl virus and Okra leaf curl crinkle virus primers did not give any amplification for any of the samples tested. It proves that none of the samples collected from different locations were cross infected with respective begamoviruses.

According to the observations at different locations all three recommended varieties were infected by OYVMV to varying degree of intensities. None of the varieties evaluated were found to be resistant to OYVMV disease. The more susceptible responses have been observed in variety *MI 7* and *MI 5* in all three locations. Further, moderate resistant response was shown by variety *Haritha*. This may be due to inherent varietal character of *Haritha*. Presence of PCR amplicons in OYVMV primer for both symptomatic and non symptomatic samples revealed that there was infection with or without observable symptoms. Because molecular study showed that plants of variety *Haritha* has been infected with the disease though there was less symptom or no symptoms appear in field conditions. Direct expression of viral infectivity might be blocked at any of several stages of virus lifecycle. These blocks might be related to differences in biology of the host system (Ahlquist *et al.,* 1984). In addition, plants also induce hypersensitive and systemic acquired resistance responses, which together limit the viral infection and impart resistance to the non infected tissues. Disease incidence of plants grown in *Gannoruwa* was observed more than 75 days after planting. Therefore, less yield damage was observed. This may be due to differential response of varieties to the environmental conditions. In 2012, Ali and co workers showed that there was a significant correlation between environment and disease severity of OYVMV disease in okra. In *Gannoruwa* minimum average temperature is less than the two other locations and the rainfall was high during the studied period (data not shown). Minimum temperature and relative humidity had significant correlations with YVMV disease severity and whitefly population (Ali *et al.,* 2005). The disease incidence increased with the rise in minimum temperature and whitefly population decreased with increase in the relative humidity (RH) (Ali *et al.,* 2005) and total rainfall (Leite *et al.,* 2005). Therefore, variation in environmental conditions and its interaction could have affected to the differences in white fly population, then changing the disease incidence and severity at three locations.

## CONCLUSION

All three recommended okra varieties used in the study were infected with OYVMV disease at varying degree of disease severity and there is a significant difference in variety and location on disease severity. None of the tested okra diseased samples were cross infected with Chili leaf curl virus, Tomato leaf curl virus, Okra leaf curl virus and Okra leaf curl crinkle viruses.

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