



# Study on Evaluation of Forty-Two Pepper Lines and Races for Resistance to Virus Diseases Under Field Conditions

Okun, K. E., Adetula, O. A., Idowu-Agida, O. O., Akinfaseye, J. O., Kayode, A. B., Orkpeh, U., Oyediji, E. O., Abdul-Rafiu, A. M., Taiwo, O. S., Dixon, H. G., Adediji, O. E., Ahmed, O., Modupeola, T. O., Olatun, B. O., Adediji-Adelani, Yemisi

National Horticultural Research Institute Idi-Ishin Jericho Ibadan

+Department of Crop Protection, Faculty of Agriculture, University of Ibadan Nigeria.

Correspondence author okun@jcrs1965@gmail.com

## Abstract

Forty-two pepper lines seeds obtained from the surveillance locations with desirable characteristic of high yield were screened for resistance to virus diseases under field conditions. The seedlings were prepared in an insect tight screen house in National Horticultural Research Institute Ibadan (NHRI) and the pepper lines were transplanted in February 2017 and repeated in February 2018. The experiment was laid out in Randomised Complete Block Design (RCBD) with three replicates. The incidence of infection was visually observed with symptoms description and the percentage incidence grouping of infection is taken in the scale of category of I-IV. The results revealed that the viral incidences vary between 0-63.88% and severity 1-3.00. The pepper lines were categorized into resistant (R), tolerant (T), moderately susceptible (MS) and susceptible (S). The Pepper lines that were resistant were 40.48% (17 out of 42) which include RFK<sub>1</sub>, RFK<sub>2</sub>, RFK<sub>3</sub>, RFK<sub>4</sub>, RFK<sub>5</sub>, RFK<sub>6</sub>, RFK<sub>7</sub>, RFK<sub>8</sub>, RFK<sub>9</sub>, RFK<sub>10</sub>, RMY<sub>1</sub>, RMY<sub>2</sub>, RMY<sub>3</sub>, RMY<sub>4</sub>, RMY<sub>5</sub>, Ojokarodo, Oke-isi, Oremba, Esosa-south and Tobo-odo. On the other hand, 13 of 42 (30.95%) were tolerant, 19.05% (8 out of 42) of the lines were moderately susceptible and 4 of 42 (9.52%) highly susceptible. The leaf samples collected from infected pepper plant were subjected to Double antibody sandwich ELISA and CMV incidence 88.33% cut across all the areas, PVY 50% and mixed infection was also found CMV+PVY 33.3%.

**Keywords:** DAS ELISA, Tomato lines, Cucumber mosaic virus, Tomato mosaic virus, Potato virus Y

## Introduction

Fruit vegetable crops such as pepper tomato, eggplant, water melon are attacked by large number of diseases. Control of these diseases through the agronomical measures and chemical control leads to several disadvantages like higher cost of production, environmental pollution, development of vector resistance to the pesticides and sometimes pesticides has carcinogenic effect to the human being (Dhalwal and Sharma, 2016). Hence development of disease resistant variety is only solution to overcome these problems most especially viral diseases. *Capiscumfrutescens* is cultivated widely in Africa and it is considered as hot pepper and traditional spices. Nigeria is the current leading producer in Africa and ranked 7<sup>th</sup> in the world. Average yield per hectare stands at 9 tons per hectare, the world average yield per hectare stand at 13.4 tons ha<sup>-1</sup> (FAO, 2005). This yield trend is associated with many problems such as pest and diseases and most especially viral diseases. Virus diseases annually reduce yield and quality of all kind of pepper (Alonso et al., 1991). Symptoms of virus infection widely vary in expression and severity including mild mottle, mosaic, vein banding, ring spots, necrosis, leaf discoloration, deformation and blistering and severe stunting of the whole plant (Tomimilson, 1989; Zitter et al., 2005). Viruses could

not just be identified based on symptoms, because symptoms could vary with respect to the strain of the virus, the host cultivar, the age of the host, environmental conditions and co-infection with other viruses (Villalon, 1981; Zitter et al., 2005). Different viruses may cause similar symptoms, as well as insect damage, particularly by thrips, mites, and aphids may be similar to virus symptoms. To date, about 50 viruses have been reported to infect peppers (Beunt et al., 1996). The genus *Potyvirus* (family *Potyviridae*) containing about 200 species account for almost 25% of known plant viruses (Shukla et al., 1994). Many potyviruses cause economically significant yield losses in pepper (*Capiscum* spp.) crops throughout the world (Percin et al., 2003). *Potato virus Y* (PVY) is the most common *Potyvirus* infecting pepper (Giles et al., 2002). Although this virus occurs worldwide, but it mostly appears in warmer climates (Mills and Abdil-Magid, 1987). In some areas, disease incidence may be as high as 100%, resulting in considerable crop loss (Green and Kim, 1991). *Pepper vein mottle virus* (PVMV) is another member of *Potyvirus* genus which infects pepper plant and has been frequently reported in western Nigeria (Aitri and Dele, 1985; Arogundade et al., 2012). Tobamoviruses including *Pepper mottle virus* (PmMV) and *pepper mild mottle virus* (PMMV) are stable and highly infectious which



easily spread from plant to plant by mechanical contact. These viruses have been mentioned responsible for significant economic losses on pepper across the world (Alonso *et al.*, 1991; Moyer, 1999). Pepper crop is strongly affected by Cucumber mosaic virus (CMV) from genus *Cucumovirus* (Oke *et al.*, 2009). These viruses are estimated to cause up to 50% losses in potential production of pepper varieties (Francki *et al.*, 1979). This study was carried out with the aim to develop high yielding pepper lines of good qualities, resistance and tolerance to virus diseases.

#### Materials and Methods

Forty two pepper lines with desirable characteristics that were land races obtained from farmers' fields during surveillance were raised in insect tight screen house at National Horticultural Research Institute Idirho Jericho Ibadan (NIHORT) and four weeks after sowing, the seedlings were laid out on the experimental field of NIHORT research farm by Randomized Complete Block Design under open field condition in February 2017/2018. Land was prepared by ploughing, harrowing and manual bedding into 1.5m x 1.5m with 0.5m x 1.0m inter row spacing of the beds. The seedlings were planted on the prepared beds 0.5m x 0.5m with a population of 16 seedlings planted per plot and total plots size 14m x 40.5m. N.P.K 15:15:15 compound fertilizer were applied at the rate of 200kg per hectare in two equal doses using the rig method, first dose 3 weeks after transplanting (WAT) and the second dose at fruit set; weeding were carried out 3 and 7 (WAT) and the spraying of appropriate pesticides was avoided to enhance the diseases control under a natural field environment. Viral incidence and severity indexing was carried out fortnightly from 2 WAT till 18WAT by visual observation. The viral incidence was categorized in the scale 1-4, while symptoms severity scoring was done based on extent of symptoms and damage observed on leaves on the scale of 1 - 5 as shown below.

#### Viral Incidence Scoring

Virus disease incidence, defined as the extent of infection in the field, and calculated according to Alegbejo (2006); Taiwo *et al.* (2006) formula:

$$\text{Number of infected plants} = \frac{\text{Disease incidence (\%)} \times 100}{100}$$

Number of plants in the field

The percentage incidence and severity were rated as stated in Table 1.

The symptoms were described and leaf samples collected from the plants (WAT) and kept on CaCl<sub>2</sub> pellet in samples bottles. These included both symptom and symptomless samples were subjected to serological diagnosis

#### Serological diagnosis:

This was by Double antibody sandwich ELISA as described by Clark and Adams (1977) which was carried out at the Biotechnology Laboratory, Federal University of Agriculture, Abeokuta Nigeria. The ELISA kits was purchase from Leibniz-Institut DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH Germany. The tubes containing coating antibody IgG and IgG-AP conjugate liquid were spanned down by short centrifugation of 3000rpm for 10seconds before the tubes were opened.

This was used to test for the presence of Cucumber mosaic virus (CMV), Pepper vein mottle virus (PVMV), Potato virus Y (PVY) and Tomato mosaic virus (ToMV) on the leaf samples. Hundred and twenty leaf samples were subjected to the test. 24ul IgG was diluted in 24ml of the coating buffer at a dilution of 1:1000. Two hundred microlitres of IgG were dispensed into each well of the microtitre plate. The plates were incubated at 37°C for 2 hours. The plates were washed with PBS- Tween soaked for 3 minutes and repeated the washing three times. The plates were tapped dry upside down on tissue paper. 200ul aliquots of the plant test samples obtained from the fields of 1mg in 10ml extraction buffer was dispensed into each of the wells then incubated overnight at 4°C. The plates were then washed three times. 200 ul antiviral conjugate, diluted 50ul in 50ml of conjugate buffer were dispensed into each well and incubated at 37°C for 2 hours. The plates were washed three times. 200 ul aliquots of a freshly prepared substrate with 50 mg. p - nitrophenyl phosphate (Sigma, Fluka) dissolved in 50 ml of substrate buffer were dispensed into each well incubated at room temperature for 60 min. The result was assessed by Visual observation and a MR-96 MINDRAY microtitre plate reader photometer was used to measure the optical densities at 405nm and blanked against air samples with values exceeding twice the reading of the healthy control were considered positive.

#### Results

The observation from the field screened pepper lines showed that there was presence of virus diseases on the field. The level of resistance, tolerance and susceptibility varies among the pepper lines. The results revealed that the viral incidences vary between 0-63.88% and severity 1-3.00. The trend of infection in 2017 and 2018 were quite similar to each other, but the percentage incidence and severity are relatively higher in 2018 than 2017. The pepper lines were categorized into resistant (R), tolerant (T), moderately susceptible (MS) and susceptible (S). The Pepper lines that were resistant were 40.48% (17 out of 42) which include RFK<sub>2</sub>, RFK<sub>3</sub>, RFK<sub>4</sub>, RFK<sub>5</sub>, RFK<sub>6</sub>, RFK<sub>7</sub>, RFK<sub>8</sub>, RFK<sub>9</sub>, RFK<sub>10</sub>, RFK<sub>11</sub>, RFK<sub>12</sub>, RFK<sub>13</sub>, RFK<sub>14</sub>, RFK<sub>15</sub>, RFK<sub>16</sub>, RFK<sub>17</sub>, RFK<sub>18</sub>, RFK<sub>19</sub>, RFK<sub>20</sub>, RFK<sub>21</sub>, RFK<sub>22</sub>, RFK<sub>23</sub>, RFK<sub>24</sub>, RFK<sub>25</sub>, RFK<sub>26</sub>, RFK<sub>27</sub>, RFK<sub>28</sub>, RFK<sub>29</sub>, RFK<sub>30</sub>, RFK<sub>31</sub>, RFK<sub>32</sub>, RFK<sub>33</sub>, RFK<sub>34</sub>, RFK<sub>35</sub>, RFK<sub>36</sub>, RFK<sub>37</sub>, RFK<sub>38</sub>, RFK<sub>39</sub>, RFK<sub>40</sub>, RFK<sub>41</sub>, RFK<sub>42</sub>.



RFK<sub>12</sub>, RFK<sub>13</sub>, RFK<sub>14</sub>, RFK<sub>15</sub>, RMY<sub>2</sub>, RMY<sub>3</sub>, RMY<sub>4</sub>, Ojikanrodo, Oke-isi, Orumba, Eosa-south and Tobarodo. On the other hand, 13 of 42 (30.95%) were tolerant, 19.05 % (8 out of 42) of the lines were moderately susceptible and 4 of 42 (9.52%) highly susceptible. The leaf samples collected from infected pepper plant were subjected to Double antibody sandwich ELISA and CMV incidence 88.33% cut across all the areas, PVY 50% and mixed infection was also found CMV+PVY 33.3%.

#### Discussion

The results of the investigation demonstrated that of all the 42 of pepper lines screened for resistance to viral diseases 17 of 42 are potential resistant lines to various kinds of viruses under field condition. This favours the finds of Oke *et al.* (2009). The symptoms exhibited on the diseased plants on the fields resembled those reported elsewhere (Arogundade *et al.*, 2012). High incidence observed in 2018 could be attributed to high density of aphids and whiteflies that ravage the plots which could have enhanced the spread of disease (Alegbejo 1987; Oke *et al.* 2009). The disease incidence varies from 0-63.88% and severity 1-3.00 in both seasons, this suggest that the lines under screening are resistant, tolerant, and susceptible may be influenced by inheritance genetic makeup individual lines, the presence of alternative host of the disease and viruliferous insect vectors population dynamics in the environment (Añri ,2004; Taiwo *et al.*, 2006; Oke *et al.*, 2012). Most of the lines screened during the investigation are susceptible to viruses which implies that the disease will continue to be a treat in pepper growing areas. However, there is need for further investigation to ascertain resistance levels of the 17 lines that are resistant in this trial, have been slated for further breeding work. Further screening of more local lines combined with good cultural practices, and effective IPM method will enhance pepper productivity.

#### References

- Alegbejo, M. D. (2006). Moderate resistance of tomato yellow leaf curl virus genus begomovirus among commercial tomato cultivars in Northern Nigeria. Horticon proceedings of 24<sup>th</sup> Annual Conf. 2006. Uni of Gombe. Page 37-42.
- Alonso, E., Garcia-Lague, I., de la Cruz, A., Wicke, B., Avila-Rincon, M.J., Serra, M. T., Castresana, C. & Diaz-Ruiz, J.R. (1991). Nucleotide sequence of the genomic RNA of pepper mild mottle virus, a resistance-breaking tobamovirus. in pepper. *Journal of General Virology*. 72: 2875-2884.
- Alegbejo MD (1987). Identification of a weed host of Pepper vein mottle virus in Northern Nigeria. *Samaru J Agric Res*. 1987;5(1 and 2):65-70.
- Arogundade O, Balogun OS, Kareem KT (2012). Occurrence and distribution of pepper vein mottle virus and cucumber mosaic virus in pepper in Ibadan, Nigeria. *Virology*. 1: 9-19.
- Atari G. I. (1984). The occurrence of okra mosaic virus in Nigeria weeds. *Annals of Applied Biology*. 104; 261-265.
- Atari, G.I., & Dele, H.W. (1985). Pepper vein mottle virus infection, host reaction, yield and aphid transmission in pepper plants. *Trop. Agr.* 62:190-192.
- Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L. & Zurcher, E.J. (eds.) (1996). *Plant Viruses Online: Descriptions and Lists from the VIDE Database*. Version: 20th August 1996. URL: <http://biology.amsu.edu.au/Groups/MES/vide/>
- Clark, M.F. & Adams, A. (1977). Characteristics of microplates method of enzyme-linked immunosorbent assay for detection of plant viruses. *Journal of General Virology*. 34: 475-483.
- Dhaliwal, M.S. and Shama, A., 2016. Breeding for Resistance to Virus Diseases in Vegetable Crops. *Innovations in Horticultural Sciences*, pp. 303-327 © 2016, New India Publishing Agency, New Delhi, India Edited by Prof. K.V. Peter
- FAO, (2005) Crop production ranking by Countries. [faostat.fao.org](http://faostat.fao.org)
- Francki, R.L.B., Mosseop, D.W. & Hatta, T. (1979). Cucumber mosaic virus. CMI/AAB. Description of Plant Viruses No.213.
- Green, S.K. & Kim, J.S. (1991). Characteristics and control of virus infecting Pepper: A literature review. Asian vegetable research and development centre. Technical Bulletin, 18: 60p.
- Glaiz, L., Kerlan, C. & Robaglia, C. (2002). Variability and evolution of Potato virus Y, the type species of the Potyvirus genus. In *Plant viruses as Molecular Pathogens*. pp. 225-253. Edited by J. A. Khan & J. Dijkstra. New York: Haworth Press.
- Mills, P.R., & Abdill-Magid, A.G. M. 1987. Infection of *Capsicum frutescens* with potato virus Y and tobacco etchvirus in the Sudan. *Plant Dis.* 71:557. (Abstr.)
- Moyer, A. (1999). *Togaviruses (Bunyaviridae)*. In: A. Granoff and R.G. Webster (eds) *Encyclopedia of Virology*. 2nd edn pp. 1803-1807. Academic Press San Diego.



- Oke, K. E., Adetula, O. A., Chikadeke, V. A., Taiwo, S. O., Kareem, K. I., Orike, A., Arogundade, O. and Ogunla, C. A. (2012). Evaluation of eleven early Maturing Okra lines for resistance to Okra mosaic virus disease and yield. *Continental Journal of Agronomy* 6(1):38-41.
- Oke, K. E., Idowu-Agida, O. O., Kareem, K. T. and Arogundade, O. (2009). Horticultural society of Nigeria, Proceedings of the 27<sup>th</sup> Annual conf. Kano 2009. Held at Royal Tropicana Hotel Kano state between 11<sup>th</sup>-16<sup>th</sup> October. 2009. p 139-142.
- Pennetny, K., Robert, P. D., Murphy, J. F. & Goldberg, N. P. (2003). Compendium of pepper diseases. The American Phytopathological society mimeo. 24-25 p.
- Shukla, D. D., Ward, C. W. & Brunt, A. A. (1994). The Potyviridae. Cambridge, UK: CAB International.
- Taiwo, M. A., Hughes, J. A. and Oke, K. E. (2006). Studies on Maize streak virus and Maize mottle/chlorotic stunt virus in Lagos Virus. *Plant Disease* 90:199-202.
- Tomlinson O. (1987). Epidemiology and control of virus disease of vegetable. *Ann. Appl. Biol.* 100:661-681.
- Zitter, T. A., Florin, D. and Providenti, R. (2005). Viruses of vegetable crop of pepper. Cornell university <http://www.vegetableonline.edu/factsheet/virus>.

Table 1: The percentage incidence and severity was graded as follows

| Grade                  | % Incidence | Disease severity | Category |
|------------------------|-------------|------------------|----------|
| Resistant              | 1-15        | 1.1-2.0          | IV       |
| Tolerant               | 16-30       | 2.1-3.0          | III      |
| Moderately Susceptible | 31-50       | 3.1-4.0          | II       |
| Highly Susceptible     | 51-100      | 4.1-5.0          | I        |

Source: Oke et al., 2009, Alegbeyo, 2006

Table 2: Incidence and severity of different pepper cultivars against viral diseases under natural field conditions

| S/N | cultivars | symptoms | % incidence 2017 | % incidence 2018 | severity scoring 2017 | severity scoring 2018 | Remark |
|-----|-----------|----------|------------------|------------------|-----------------------|-----------------------|--------|
| 1   | RFK-1     | Ss,mt    | 15.87            | 12.45            | 1.67                  | 2.01                  | T      |
| 2   | RFK-2     | Mt       | 4.77             | 7.70             | 1.67                  | 1.50                  | R      |
| 3   | RFK-3     | Cu       | 7.40             | 6.67             | 1.33                  | 2.05                  | R      |
| 4   | RFK-4     | Cu,v     | 16.67            | 20.41            | 1.67                  | 2.15                  | T      |
| 5   | RFK-5     | Mt,cu,v  | 18.83            | 15.44            | 2.00                  | 2.60                  | T      |
| 6   | RFK-6     | Cu,lc    | 17.77            | 18.52            | 1.67                  | 2.50                  | T      |
| 7   | RFK-7     | Cu       | 15.00            | 15.00            | 1.67                  | 2.00                  | R      |
| 8   | RFK-14    | Cu,lc    | 16.70            | 16.70            | 1.67                  | 2.00                  | T      |
| 9   | RFK-13    | Ma       | 5.57             | 0.00             | 1.33                  | 1.00                  | R      |
| 10  | RFK-12    | Vy       | 3.70             | 3.70             | 1.67                  | 1.67                  | R      |
| 11  | RFK-11    | cu       | 10.00            | 11.25            | 1.33                  | 1.50                  | R      |
| 12  | RFK-10    | Cu,mt,lc | 39.03            | 43.22            | 2.33                  | 3.00                  | MS     |
| 13  | RFK-9     | Cu,lc    | 20.47            | 23.45            | 1.67                  | 2.50                  | T      |
| 14  | RFK-8     | Ms,lc    | 11.10            | 12.20            | 1.33                  | 1.50                  | R      |
| 15  | RMV-2     | Ma       | 15.00            | 13.05            | 1.67                  | 1.50                  | R      |
| 16  | RMV-1     | Ma       | 50.09            | 62.11            | 2.50                  | 3.00                  | HS     |
| 17  | RFK-G     | Ma,lc    | 59.23            | 58.45            | 2.67                  | 3.00                  | HS     |
| 18  | RFK-18    | Vc,ms,lc | 40.73            | 49.15            | 2.00                  | 2.50                  | MS     |
| 19  | RFK-17    | Ms,lc    | 41.67            | 46.45            | 2.33                  | 2.60                  | MS     |
| 20  | RFK-16    | No       | 0.00             | 0.00             | 1.00                  | 1.00                  | R      |
| 21  | RFK-15    | No       | 0.00             | 0.00             | 1.00                  | 1.00                  | R      |
| 22  | RMV-3     | No       | 0.00             | 0.00             | 1.00                  | 1.00                  | R      |



|    |            |          |       |       |      |      |    |
|----|------------|----------|-------|-------|------|------|----|
| 23 | RMV-4      | Cu,ms,lc | 41.10 | 39.42 | 2.00 | 3.00 | MS |
| 24 | RMV-5      | Mt       | 9.10  | 10.50 | 1.67 | 2.00 | R  |
| 25 | RMV-6      | Vc,ms    | 25.76 | 22.33 | 1.67 | 2.07 | T  |
| 26 | MNG-1      | Cu,ms    | 44.43 | 43.45 | 2.00 | 2.02 | MS |
| 27 | MNG-2      | Ma       | 28.43 | 30.00 | 1.33 | 1.35 | T  |
| 28 | MNG-3      | Lb,ms,lc | 25.90 | 29.40 | 2.33 | 2.03 | T  |
| 29 | Osielc     | Ma,ss    | 50.27 | 55.25 | 2.85 | 2.55 | HS |
| 30 | Osenwaari  | Ma,ld,mt | 32.17 | 40.05 | 2.00 | 2.06 | MS |
| 31 | Ojikanrodo | No       | 0.00  | 2.00  | 1.00 | 1.30 | R  |
| 32 | Osiisi     | Lc,mt    | 12.25 | 5.52  | 1.67 | 1.57 | R  |
| 33 | Orunba     | No       | 0.00  | 0.00  | 1.00 | 1.00 | R  |
| 34 | Ezza south | Mt       | 3.70  | 0.00  | 1.17 | 1.00 | R  |
| 35 | Toba rodo  | Mt,cu    | 11.12 | 5.52  | 1.50 | 1.67 | R  |
| 36 | Iwo2       | Mt,lc,cu | 26.68 | 27.45 | 2.17 | 2.50 | T  |
| 37 | Uso        | So,ms,lc | 48.05 | 45.67 | 2.67 | 2.50 | MS |
| 38 | Iwo 1      | So,mt,ms | 30.33 | 30.00 | 2.33 | 2.50 | T  |
| 39 | Ikwano     | Ma,ss,mt | 26.22 | 28.00 | 2.17 | 2.35 | T  |
| 40 | Kuto8      | Ma,lc,mt | 24.72 | 25.00 | 2.50 | 2.75 | T  |
| 41 | Ose isi3   | D,lc,cu  | 35.58 | 40.45 | 2.83 | 3.00 | MS |
| 42 | Ose isi7   | Ma,mt,cu | 63.88 | 60.65 | 3.00 | 3.00 | HS |

Footnote: mottling mt, mosaic ms, leaf bunchy lb, vein yellowing vy, leaf curl lc, vein clearing vc, shoe strings ss, no symptoms no, leaf cupping cu.

Table3: ELISA result of the leaf samples from the experimental field

| STATE                             | LGA       | LOCATION  | CMV    | PVY | CMV+PVY |
|-----------------------------------|-----------|-----------|--------|-----|---------|
| OGUN                              | ODEDA     | Osielc    | 2      |     |         |
|                                   |           | TOBA      | 2      |     | 1       |
|                                   |           | OSE-ISI 3 | 1      | 2   |         |
| PLATEUE                           | JOS NORTH | RFK-G     |        | 2   |         |
|                                   |           | MNY-1     | 1      | 2   | 1       |
|                                   | RIVON     | RMV-4     | 2      |     |         |
|                                   |           |           | 8      | 6   | 2       |
| Viral prevalence across locations |           |           | 83.33% | 50% | 33.3%   |