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Study on Evaluation of Forty-Two Pepper Lines Land Races for Resistance to Virus Diseases under Field Conditions

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Abstract:- Forty-two pepper line seeds obtained from the surveillance locations with the 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 at National Horticultural Research Institute Ibadan (NIHORT) 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 the 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 resistant Pepper lines were 40.48% (17 out of 42) which include RFK_2, RFK_3, RFK_7, RFK_8, RFK_{11}, RFK_{12}, RFK_{13} RFK_{15}, RFK_{16}, RMY_2, RMY_3, RMY_5, Ojikanrodo, Ose-isi1, Orumba, Essa-south, and Toba-rodo. 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 the infected pepper plant were subjected to Double antibody sandwich ELISA and CMV incidence was 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.*

I. INTRODUCTION

Fruit vegetable crops such as pepper tomato, eggplant, and watermelon are attacked by a large number of diseases. Control of these diseases through agronomical measures and chemical control leads to several disadvantages including the higher cost of production , environmental pollution, development of vector resistance to pesticides, and sometimes pesticides have a carcinogenic effect on the human being. Dhaliwal and Shama, (2016). Hence the development of disease-resistant variety is the only solution to overcome these problems most especially viral diseases. *Capsicum frutescens* is cultivated widely in Africa and it is considered a hot pepper and traditional spice. Nigeria is the current leading producer in Africa and ranked 7th in the world. The average yield per hectare stands at 9 tons per hectare, and the world average yield per hectare stands at 13.4tons ha^{-1}(FAO, 2005). This yield trend is associated with many problems such as pests and diseases and most especially viral diseases. Virus diseases annually reduce the yield and quality of all kinds 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 (Tomimilison, 1989; Zitter *et.al.*, 2005). Viruses could not just be identified based on symptoms, because symptoms could vary concerning 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 (Brunt *et al*, 1996). The genus *Potyvirus* (family *Potyviridae*) containing about 200 species accounts for almost 25% of known plant viruses. (Shukla *et al*,1994). Many potyviruses cause economically significant yield losses in pepper (*Capsicum spp*.) crops throughout the world (Pernezny *et al*,2003). *Potato virus Y* (PVY) is the most common *Potyvirus* infecting pepper (Glais *et al*,2002). Although this virus occurs worldwide, it mostly appears in warmer climates (Millsand Abdiil- Magid, 1987). In some areas, disease incidence may be as high as 100%, resulting in considerable crop loss (Green and Kim, 1991). *Pepper veinal mottle virus* (PVMV) is another member of *the Potyvirus* genus which infects the pepper plant and has been frequently reported in western Nigeria (Atiri and Dele, 1985: Arogundade e*t al*, 2012). Tobamoviruses including *Pepper mottle virus* (PeMV) 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 as responsible for significant economic losses on pepper across the world (Alonso *et, al.*,1991: Moyer,1999). Pepper crop is strongly affected by *the Cucumber mosaic virus* (CMV) from the genus *Cucumovirus (*Oke *et al*., 2009). These viruses are estimated to cause up to 50% losses in the potential production of pepper varieties (Francki *et al*, 1979). This study was carried out to develop high-yielding pepper lines of good qualities, resistance, and tolerance to virus diseases.

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II. MATERIALS AND METHODS

Forty-two pepper lines with desirable characteristics that were landraces obtained from farmers' fields during surveillance were raised in insect tight screen house at National Horticultural Research Institute Idi- ishin 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. The land was prepared by ploughing, harrowing, and manual bedding into 1.5m x 1.5m with 0.5m x 1.0m interrow 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 a total plots size of 14m x 40.5m. N:P: K 15:15:15 compound fertilizer was applied at the rate of 200kg per hectare in two equal doses using the rigging method; the first dose 3 weeks after transplanting (WAT) and the second dose at fruity set; weeding was 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 on the scale1-4, while symptoms severity scoring was done based on the extent of symptoms and damage observed on leaves on a 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); Allen *et.al*. (1983) formula:

$$\% \text{ Incidence of infected plants} = \frac{\text{Disease incidence} \times 100}{\text{Number of plants in the field}}$$

The percentage incidence and severity were rated as stated inTable1.

The symptoms were described and leaf samples were collected from the plants (WAT) and kept on $CaCl_2$ pellets in sample bottles.

These included both symptom and symptomless samples that were subjected to serological diagnosis.

III. 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 were purchased 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 10 seconds before the tubes were opened. This was used to test for the presence of the Cucumber mosaic virus (CMV), Pepper veinal 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 37^0C 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 were dispensed into each of the wells and then incubated overnight at 4^0C. The plates were then washed three times. 200 ul antivirus conjugate, diluted 50ul in 50ml of conjugate buffer was dispensed into each well and incubated at 37^0C 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 the 60-minute result was assessed by Visual observation and an 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.

IV. RESULTS

The observation from the field screened pepper lines showed that there was the 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 was quite similar to each other, but the percentage incidence and severity are relatively higher in 2018 than in 2017. The pepper lines were categorized into resistant (R), tolerant (T), moderately susceptible (MS), and susceptible (S). The resistant Pepper lines were 40.48% (17 out of 42) which include RFK_2, RFK_3, RFK_7, RFK_8, RFK_{11}, RFK_{12}, RFK_{13} RFK_{15}, RFK_{16}, RMY_2, RMY_3, RMY_5,Ojikanrodo, Ose-isi1, Orumba, Essa-south and Toba- rodo.. 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%) were highly susceptible Figure 1. The leaf samples collected from the infected pepper plant were subjected to Double antibody sandwich ELISA and CMV incidence was 88.33% cut across all the areas, PVY 50%, and mixed infection was also found CMV+PVY 33.3%.

V. DISCUSSION

The results of the investigation demonstrated that of all the 42 pepper lines screened for resistance to viral diseases 17of 42 are potential resistant lines to various kinds of viruses under field conditions. This favors the findings of Oke *et.al* (*2*009) Figure 1. The symptoms exhibited by the diseased plants in the fields resembled those reported elsewhere (Arogundade *et al*,2012). The high incidence observed in 2018 could be attributed to the high density of aphids and whiteflies that ravage the plots which could have enhanced the spread of disease (Alegbejo1987: Oke *et.al.*2009). The disease incidence varies from 0-63.88% and severity 1-3.00 in both seasons, this suggests that the lines under screening are resistant, tolerant, and

susceptible and may be influenced by the inherence genetic makeup of individual lines, the presence of an alternative host of the disease and viruliferous insect vectors population dynamics in, the environment (Atiri,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 threat in pepper growing areas. However, there is a need for further investigation to ascertain the resistance levels of the 17 lines that are resistant in this trial, which has been slated for further breeding work. Further screening of more local lines combined with good cultural practices, and effective IPM methods will enhance the pepper productivity.

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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

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

Source: Oke *et.al*.,2009, Alegbejo,2006

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,vc	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	Ms	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	RMY-2	Ms	15.00	13.05	1.67	1.50	R
16	RMY-1	Ms	50.09	62.11	2.50	3.00	HS
17	RFK-G	Ms ,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	RMY-3	No	0.00	0.00	1.00	1.00	R
23	RMY-4	Cu,ms,lc	41.10	39.42	2.00	3.00	MS
24	RMY-5	Mt	9.10	10.50	1.67	2.00	R
25	RMY-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	Ms	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	Osiele	Ms,ss	50.27	55.25	2.85	2.55	HS
30	Osenwaari	Ms,ld,mt	32.17	40.05	2.00	2.06	MS
31	Ojikanrodo	No	0.00	2.00	1.00	1.30	R
32	Oseisi	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	Ss ,ms,lc,	48.05	45.67	2.67	2.50	MS
38	Iwo 1	Ss,mt,ms	30.33	30..00	2.33	2.50	T
39	Ikwano	Ms,ss,mt	26.22	28.00	2.17	2.35	T
40	Kuto8	Ms,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	Ms,mt,cu	63.88	60.65	3.00	3.00	HS

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

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

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STATE	LGA	LOCATION	CMV	PVY	CMV+PVY
OGUN	ODEDA	Osiele	4 (6)	3(6)	2(6)
		Uso	4 (6)	2(6)	1(6)
		OSE-ISI 7	3 (6)	2(6)	2(6)
PLATEUE	JOS NORTH	RFK-G	6 (6)	2(6)	2(6)
		MNG-1	5 (6)	2(6)	1(6)
	RIYON	RMY-1	3 (6)	4(6)	2(6)
Total			25(30)	15(30)	10(30)
Viral prevalence across locations			83.33%	50%	33.3%

Table 3: ELISA result of the leaf samples from the experimental field

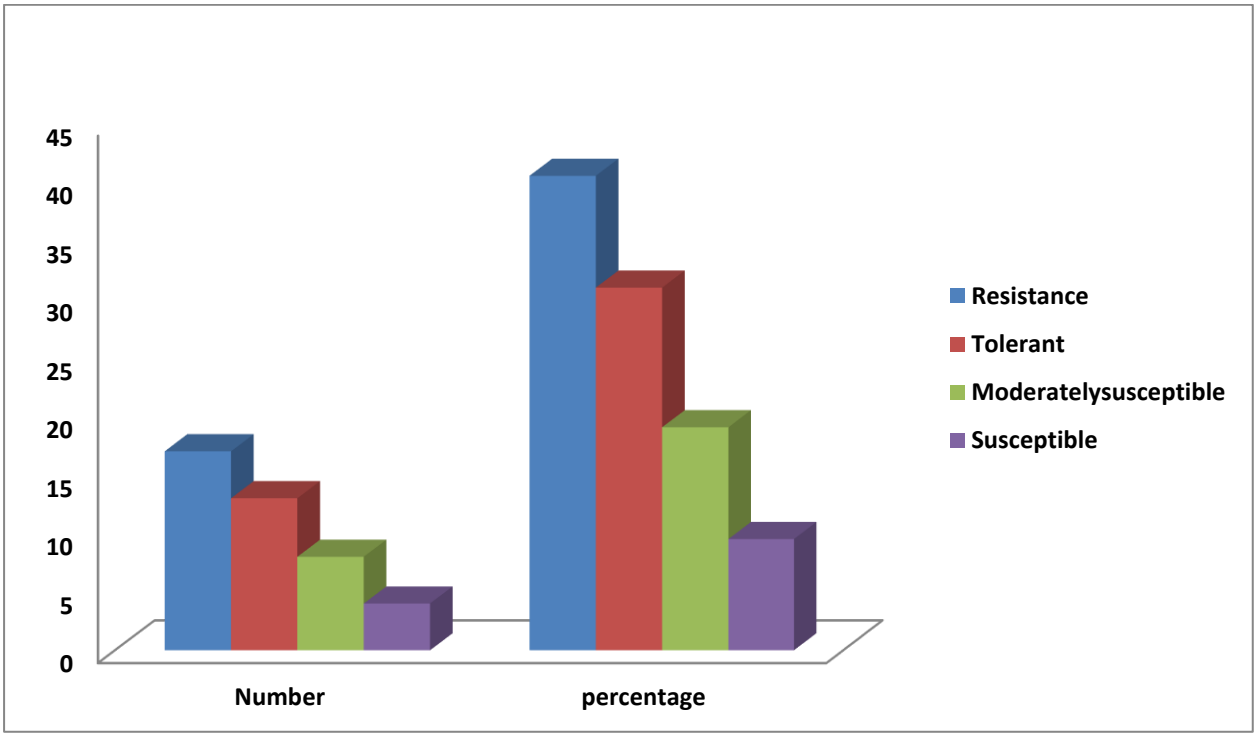

Fig. 1: Resistant , tolerant and susceptible grouping of tomato varieties