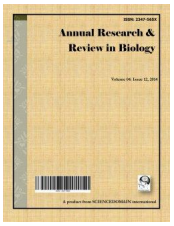

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Phenotypic and Molecular Screening of Okra (*Abelmoschus esculentus* L. Moench) Genotypes against Okra Leaf Curl Disease

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Okra leaf curl disease (OLCD) is a major constraint on okra (*Abelmoschus esculentus* L. Moench) production in West Africa**.** The most effective way of managing this disease is through breeding and planting of resistant varieties. In order to identify sources of resistance and or tolerance, 21 okra genotypes were screened against OLCD in field trials which were conducted from May to October, 2015 (rainy season) and November 2015 to March 2016 (dry season). Field resistance was assessed at 2, 6 and 10 weeks after sowing (WAS) based on disease symptoms, and then confirmed by PCR amplification of viral coat protein gene. Populations of whitefly *(Bemisia tabaci)*, the vector of begomoviruses associated with OLCD, as well as fruit yields were also assessed. Both PCR and field trials showed that all the okra genotypes were susceptible to the viral infection. The genotypes varied significantly ($P<0.05$) among them in terms of severity of OLCD, whitefly infestation, mean fruit yield (t ha^{-1}), and the average fruit weight per plant. Higher cumulative average population of whitefly and mean fruit yield (t ha^{-1}) were recorded in the dry season than in the rainy season. Genotypes GH5332 and GH6105 consistently showed mild symptoms of OLCD and also had very high fruit yields of 11.88 t ha^{-1} and 9.34 t ha^{-1} respectively in the rainy season,

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and 6.108 t ha^{-1} and 4.05 t ha^{-1} respectively in the dry season, far above the overall mean yields for all the okra genotypes. Both genotypes GH5332 and GH6105 should be evaluated multi-locationally at farmers' fields prior to their release as varieties or they should be incorporated into breeding lines.

Keywords: Okra leaf curl disease; Abelmoschus esculentus; field resistance; Bemisia tabaci; begomoviruses; cotton leaf curl Gezira virus.

1. INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench) is an important vegetable crop grown in both the tropical and sub-tropical regions of the world [1]. In Ghana, okra is grown in both wet and dry seasons mainly by resource-poor smallholder farmers, and hence a source of income to them, and is also a major source of foreign exchange in the country. It is a source of calories, protein, vitamins, calcium, potassium, iron and other mineral salts [2,3]. The tender green pods contain very high levels of antioxidants including β-carotene, xanthin and lutein which are of important medicinal values [4].

Okra leaf curl disease (OLCD) is a major constraint to okra production and is widespread in Africa [5,6]. Incidence of OLCD has been reported in several African countries including Ghana [7], Ivory Coast [8], Niger [9], Mali [10], Burkina Faso [6], Nigeria [11], Cameroon [12] and Sudan [13]. The disease has also been reported in the Middle East including Saudi Arabia [14], India [15] and Oman [16]. Affected plants show symptoms of wrinkled leaf, upward or downward curling of apical leaves, vein distortion and thickening, leaf yellowing and stunted growth [11]. The number of marketable fruits per plant, the fruit length, fruit diameter and fruit weight of the affected plant are also reduced significantly [6]. OLCD has been reported to cause yield losses of up to 100% depending on the date of planting, cultivar and locality [17]. The average economic losses due to OLCD have been estimated from 1950 to 11,100 United States Dollars for one hectare of crop, depending on the okra variety [6].

OLCD has been found associated with cotton leaf curl Gezira virus (CLCuGV), okra yellow crinklevirus (OYCrV) and hollyhock leaf crumple virus (HoLCrV) in Africa [13,18,19]. These begomoviruses of the family *Geminiviridae* are transmitted by the whitefly *Bemisia tabaci* Genn. [20]. Besides vectoring viral diseases, whitefly is also a serious pest that infests okra during all stages of the crop growth [21]. It sucks the cell sap from the leaves causing drying of the leaves and stunted growth [22].

Okra production in Ghana is currently challenged with severe OLCD characterised with leaf curl, vein thickening and plant stunting (Fig. 1). It is quite pertinent to manage the OLCD in order to improve yields of okra. Due to the availability of many potential alternative crop and weed hosts for the viruses and whitefly vector, management of OLCD by the smallholder farmers in West and Central Africa is very difficult [12]. It is also not desirable to manage the disease by controlling the whitefly vector with insecticides because of its high cost, environmental and health hazards [8]. *B. tabaci* vector has also developed resistance against insecticides in recent years [23]. The most effective way of managing this disease is through the use of resistant varieties. This study was therefore conducted to identify okra genotypes that are resistant or tolerant to OLCD.

Fig. 1. Okra plant with leaf curling and stunting symptoms
(Picture was taken by Elvis Asare-Bediako)

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at the Teaching and Research farm of the School of Agriculture, College of Agriculture and Natural Sciences of the University of Cape Coast during May to October 2015 (rainy season) and November 2015 to March 2016 (dry season). This site (5º10'N, 1.2º50'W) falls within the coastal savannah vegetation zone, with Acrisol soil type [24] and is a highly endemic site for OLCD. The area has a bi-modal rainy season from May to June and August to October with an annual rainfall ranging from 750 to 1000 mm [24] and temperatures ranging from 23.2-33.2ºC with an annual mean of 27.6ºC [25].

2.2 Plant Materials

Twenty-one genotypes of okra were used for the study (Table 1). These consist of fifteen accessions from Plant Genetic Resource Research Institute (PGRR1) at Bunso, Ghana and six farmers' varieties. The PGRRI's accessions were GH2026, GH2052, GH2027, GH2063, GH3731, GH3734, GH3760, GH4374, GH5302, GH5321, GH5332, GH5786, GH5793, GH6105 and GH6211. The farmers' accessions were UCCC1, UCCC2, UCCC3, UCCC4, UCCC5 and UCCC6.

2.3 Experimental Design and Field Layout

The experiments were laid out in a randomized complete block design (RCBD) with 21 treatments and four replications. The 21 okra genotypes represented the 21 treatments. A total land area of 1344 m^2 (84 m x 16 m) was ploughed and harrowed to render the soil loose. It was then divided into four blocks, spaced 1.0 m apart, and each block was further divided into 21 plots, spaced 1 m apart, and a plot size of 3 m x 3 m. Three seeds were sown per hill at a planting distance of 0.6 m x 0.6 m, and later thinned to two plants per hill when plantlets reached 3-4 leaves stage. Agronomic practices such as weeding and watering were done when necessary in order to ensure good crop establishment. NPK fertilizer (15:15:15) was also applied at a rate of 250 kg ha^{-1}.

2.4 Data Collection

Data was collected on disease incidence and severity, whitefly population, fruit weight and seed yield. In each case data was taken from 9 inner rows of each plot and the mean per plant determined. The 21 okra genotypes were evaluated at 2, 6 and 10 weeks after planting (WAP) for incidence and severity of OLCD based on disease symptoms. Disease incidence (DI) per plot was estimated as the percentage of plants in the plot displaying OLCD symptoms [26]. The severity of OLCD was assessed based on a visual scale of 0–4 (Table 1) which is essentially a modification of the 0 - 7 scale developed by Alegbejo et al. [27].

Whitefly infestation was assessed by counting individual adult insects on the five topmost fully expanded leaves per plant after 2, 6 and 10 WAP, according to Asare et al. [28]. Insect populations were taken from nine (9) plants per plot and the mean population per plant determined. The cumulative average number of adult whitefly (CANWF) per plant was then determined as the whitefly population that infested the crop during that experimental period [29]. Data were also taken on the number of fruits per plant, average fruit weight and yield (t ha^{-1}).

2.4.1 Polymerase chain reaction (PCR) detection of whitefly transmitted *Begomovirus*

Young leaves from the 21 okra genotypes were collected from both symptomatic and non-symptomatic plants at the experimental site. Total genomic DNA was isolated from the naturally infected okra leaf samples using the Cetyltrimethyl ammonium bromide method [30] with some modifications according to Asare et al. [28].

Table 1. Visual scale for rating severity of okra leaf curl disease

Disease score	Description
0	No symptom
1	Curling of few top leaves
2	Top leaves curled and slight stunting of plant
3	All leaves curled and slight stunting of plant
4	Severe curling of leaves, stunting of plant and proliferation of auxiliary branches

Polymerase chain reaction (PCR) amplification of viral DNA was performed using specific coat protein gene forward (CPF5'-TTA TGT CGA AGC GAG CTG CC-3') and reverse primers (CPR5'-TTT CAA TTC GTT ACA GAG TCA TA-3') resulting in amplicon of 250 bp fragment [15]. The primers were purchased from Metabion International AG (Germany,) and KAPA Taq ReadyMix (2X) containing KAPA Taq DNA Polymerase (0.5 U per 25 µL reaction), KAPA Taq Buffer, dNTPs (0.2 mM of each dNTP at 1X), MgCl2 (1.5 mM at 1X) and stabilizers were obtained from KAPA Biosystems, Germany). PCR reactions were performed in 50 µL total volume using 100 ng template DNA, 2.5 units of Taq DNA polymerase, 1x PCR buffer and 0.2 mM dNTps, 10 nM of each primer. The PCR was carried out in a Flexcycler^2 (Biometra GmbH, Germany) at 94ºC for 3 min (pre-heating), followed by 35 cycles at 94ºC (denaturation) for 1 min, 56ºC (annealing) for 1 min, 72ºC (extension) for 1 min, and 72ºC (final extension) for 7 min.

The PCR products were separated by electrophoresis in 2% agarose gels stained with ethidium bromide. The gels were then viewed under UV light-transilluminator (Biorad, UVItec Ltd., Cambridge, UK).

2.5 Data Analysis

Data on mean severity scores were used to calculate area under the disease progress curve (AUDPC) for each of the okra genotypes in Microsoft Excel according to Shaner and Finney [31]:

$$AUDPC = \sum_{i}^{n} [(Y_{i+1} + Y_i)/2] [X_{i+1} - X_i]$$

where:

Y_i – disease severity at the i^{th} observation
X_i – time (weeks) at the i^{th} observation
n –total number of observations

The AUDPC, which is a quantitative summary of disease intensity over a period 10 weeks, was used to measure disease resistance in each okra genotype. Data on disease incidence and cumulative average number of adult whitefly per plant were transformed with angular and square root transformations respectively in order to homogenize the variances before subjecting to analyses of variance (ANOVA). The other data (AUDPC, final severity scores, average fruit weight and yield) were subjected to ANOVA and the mean separated by the least significance difference (LSD) method at 5% level of probability. Pearson's correlation coefficients were calculated for the relationships between incidence and severity of OLCD, whitefly populations and fruit yield. All statistical analyses were performed using Gen Stat Discovery version 4 (VSN International).

3. RESULTS

3.1 Mean Incidence of Okra Leaf Curl Disease (OLCD)

Mean incidence of OLCD in the wet season trial varied among the okra genotypes and increased with increasing growth stage from 2 to 10 WAS (Table 2). Overall mean disease incidence of 0.23%, 50.8% and 57.2% were recorded at 2, 6 and 10 WAS respectively. An ANOVA did not show significant differences among the okra genotypes at 2 WAS (F=1.0; df= 60; *P*=0.476) but revealed significant differences among them at 6 WAS (F = 4.49; df = 60; *P*< 0.001) and 10 WAS (F = 4.99; df = 60; *P*< 0.001). At 10 WAS, genotype GH3760 had the lowest incidence of OLCD (24.5%) but it was not significantly different from GH2057, GH2063, GH5332, UCCC6, GH6105 and GH2052 with mean incidences of 41.6%, 41.6%, 36.5%, 35%, 33.7% and 29% respectively (Table 2).

Similarly, in the dry season, incidence of OLCD varied among the okra genotypes and increased with increasing growth stage from 2 to 10 WAS (Table 2). Overall mean disease incidences recorded at 2, 6 and 10 WAS were 0%, 30.8% and 57.8% respectively. None of the okra genotypes showed symptoms of OLCD at 2 WAS. ANOVA however showed significantly varying levels of incidences among the okra genotypes at 6 WAS and 10 WAS (*P*<0.05). At 10 WAS, genotype UCCC6 had the lowest incidence of OLCD (14.8 %) but it was not significantly different from GH5302, GH5332 and GH3760 with mean incidences of 20.9%, 26.4% and 29.3% respectively.

3.2 Mean Disease Severity Scores and Area under Disease Progress Curve (AUDPC)

Final severity scores of OLCD recorded at 10 WAS in the wet season varied significantly among the okra genotypes ($F_{20,60}$= 5.97; *P*<0.001). Genotype GH3760 had the lowest severity score of 0.278 which was not significantly different (*P*> 0.05) from GH6105, GH2052, UCCC6, GH5332, GH2063, GH5703, and GH2057 with mean severity scores of 0.338,

0.389, 0.417, 0.556, 0.556, 0.722 and 0.815 respectively.

Also in the dry season trial, the final severity scores of OLCD recorded for the okra genotypes varied significantly among them ($F_{20,60}$= 19.83; *P*<0.001) (Table 3).Genotype UCCC6 had the lowest severity score of 0.125 but it was not significantly different fromGH5302, GH5332, GH3760, GH2052, GH6105, GH3731, GH2026, GH5793,and GH2063 with mean severity scores of 0.167, 0.250, 0.333, 0.375, 0.458, 0.542, 0.604, 0.625 and 0.625 respectively (Table 3). Both seasonal effect and genotype x season interaction effect on the overall final severity score were significant (P<0.05) (Table 5). The overall final severity score recorded in the dry season (1.231) was significantly higher ($F_{20, 123}$= 5.57; *P*<0.001) than that of the wet season (1.075).

An ANOVA on the AUDPC calculated for the various okra genotypes during the wet season showed significant difference among them ($F_{20,60}$= 7.03; *P*<0.001). Genotype GH3760 had the lowest AUDPC but it was not significantly different from that of GH2026, GH2052, GH2057, GH2063, GH4374, GH5332, GH5793, GH6105 and UCCC6.Genotype UCCC2 had the highest AUDPC but was not significantly different from that of UCCC1, UCCC3, UCCC4, UCCC5, GH6211, GH5321, GH3734 and GH3731. Similarly, in the dry season, the ANOVA showed significant differences in AUDPC values amongst the okra genotypes ($F_{20, 60}$= 19.83; *P*<0.001). Genotype GH2052 had the lowest AUDPC but it was not significantly different from that of GH2026, GH2963, GH3731, GH3760, GH5302, GH5332, GH5793, GH6105, UCCC4 and UCCC6. The highest AUDPC was recorded for GH3734 but was not significantly different from GH4374, GH5321, UCCC1, UCCC2 and UCCC3.

A two-way ANOVA (Table 5) revealed that the overall mean AUDPC recorded in the major season across all the genotypes (5.01) was not significantly different ($F_{20,60}$= 7.86; *P*=0.06) from that of the dry season (4.09) but genotype x season interaction effect was significant (P<0.05).

Table 2. Mean incidences (%) of OLCD on 21 okra genotypes under field conditions during the two planting seasons

Geno type	Mean incidence of OLCD in the wet season (%)			Mean incidence of OLCD in the dry season (%)		
	2 WAS	6 WAS	10 WAS	2 WAS	6 WAS	10 WAS
GH2026	0^{ns}	43.2^{efgh}	53.4^{cdefg}	0.00	17.6^{efg}	38.6^{c}
GH2052	0	29.0^{gh}	29.0^{gh}	0.00	0.0^{h}	35.3^{cd}
GH2057	0	37.8^{efgh}	41.6^{efgh}	0.00	39.8^{bcd}	78.0^{a}
GH2063	0	40.0^{efgh}	41.6^{efgh}	0.00	20.9^{efg}	$42.6b^{c}$
GH3731	0	51.7^{cdefg}	62.1^{abcde}	0.00	8.8^{gh}	33.8^{cd}
GH3734	0	62.6^{abcde}	81.9^{ab}	0.00	52.3^{ab}	84.0^{a}
GH3760	0	22.4^{h}	24.5^{h}	0.00	12.0^{fgh}	29.3^{cde}
GH4374	0	38.3^{efgh}	50.9^{defg}	0.00	55.1^{ab}	90.0^{a}
GH5302	0	44.9^{defgh}	53.8^{cdef}	0.00	8.8^{gh}	20.9^{de}
GH5321	0	72.1^{abc}	72.1^{adcd}	0.00	55.1^{ab}	84.0^{a}
GH5332	0	22.1^{h}	36.5^{fgh}	0.00	6.0^{gh}	26.4^{cde}
GH5786	0	52.0^{bcdefg}	58.7^{bcdef}	0.00	33.8^{cde}	57.9^{b}
GH5793	0	38.4^{efgh}	53.4^{cdefg}	0.00	28.5^{def}	34.5^{cd}
GH6105	0	31.2^{gh}	33.7^{gh}	0.00	12.0^{fgh}	34.9^{cd}
GH6211	0	61.0^{abcdef}	71.4^{abcd}	0.00	45.4^{abcd}	84.0^{a}
UCCC1	0	80.3^{a}	85.1^{a}	0.00	58.7^{a}	90.0^{a}
UCCC2	4.87	78.1^{ab}	83.0^{ab}	0.00	47.8^{abc}	90.0^{a}
UCCC3	0	69.7^{abcd}	76.3^{abc}	0.00	49.9^{abc}	84.0^{a}
UCCC4	0	79.5^{a}	79.5^{ab}	0.00	40.1^{bcd}	78.0^{a}
UCCC6	0	35.0^{fgh}	35.0^{fgh}	0.00	14.8^{fgh}	14.8^{e}
Mean	0.23	50.8	57.2	0.00	30.8	57.8
LSD	3.005	26.14	24.86	*	17.12	17.67
P value	0.476	<0.001	<0.001	*	<0.001	<0.001

Means in the same column bearing identical letters are not significantly different (P>0.05), ns = not significant (P>0.05). Incidence data was arcsine transformed before ANOVA was done

Table 3. Mean severity scores of okra leaf curl disease (OLCD) and area under disease progress curve (AUDPC) recorded for 21 okra genotypes

Okra genotype	Wet season			Dry season		
	Final severity	AUDPC	Host resistance	Final severity	AUDPC	Host resistance
GH2026	0.897^{defg}	3.680^{defg}	MR	0.604^{e}	1.896^{f}	R
GH2052	0.389^{ghi}	1.833^{fg}	R	0.375^{e}	0.542^{f}	HR
GH2057	0.815^{efghi}	2.774^{efg}	R	1.583^{cd}	5.167^{de}	S
GH2063	0.556^{ghi}	3.333^{efg}	MR	0.625^{e}	1.708^{f}	R
GH3731	1.306^{bcde}	6.306^{abcd}	S	0.542^{e}	1.625^{f}	R
GH3734	1.741^{ab}	7.673^{ab}	HS	2.500^{a}	8.583^{a}	HS
GH3760	0.278^{i}	1.167^{g}	R	0.333^{e}	0.750^{f}	HR
GH4374	0.896^{defg}	3.854^{defg}	MR	2.329^{ab}	8.438^{a}	HS
GH5302	0.917^{defg}	4.528^{cdef}	MR	0.167^{e}	0.583^{f}	HR
GH5321	1.431^{abcd}	7.764^{a}	HS	1.958^{abc}	6.875^{abcd}	S
GH5332	0.556^{gi}	2.847^{efg}	R	0.250^{e}	0.833^{f}	R
GH5786	1.111^{cdef}	4.825^{bcde}	MR	1.375^{d}	5.042^{de}	S
GH5793	0.722^{fghi}	3.333^{efg}	R	0.625^{e}	2.292^{f}	R
GH6105	0.338^{hi}	1.940^{efg}	R	0.458^{e}	1.458^{f}	R
GH6211	1.667^{ab}	7.056^{abc}	HS	1.833^{bcd}	5.667^{cde}	S
UCCC1	1.722^{ab}	8.500^{a}	HS	2.500^{a}	7.583^{abc}	HS
UCCC2	1.556^{abc}	8.583^{a}	HS	2.167^{ab}	7.417^{abc}	HS
UCCC3	1.500^{abc}	6.778^{abc}	S	2.083^{abc}	7.750^{ab}	HS
UCCC4	1.556^{abc}	7.667^{ab}	HS	1.542^{cd}	4.625^{e}	MR
UCCC5	1.910^{a}	8.424^{a}	HS	1.875^{bcd}	6.375^{bcde}	S
UCCC6	0.417^{ghi}	2.250^{efg}	R	0.125^{e}	0.625^{f}	R
Mean	1.075	5.01		1.231	4.09	
LSD	0.5552	2.928		0.5434	1.991	
P value	<0.001	<0.001		<0.001	<0.001	

Means in the same column bearing different letters are significantly different (P<0.05).
Host resistance status was based on the AUDPC values: AUDPC (< 1) = Highly resistant, HR; AUDPC (1-3) = Resistant, R; AUDPC (3.1-5.0) = Moderately resistant, MR; AUDPC (5.1-7.0) = Susceptible, S; AUDPC (>7) = Highly susceptible, HS

3.3 *Begomovirus* Infection of Okra

Fig. 2 shows PCR amplification of the *begomovirus* with the CPF / CPR primers of DNA fragment size 250bp from all the maize genotypes (lanes 1-21) but no band for negative control (lane C), indicating that all the okra genotypes tested were infected with okra leaf curl *begomovirus*.

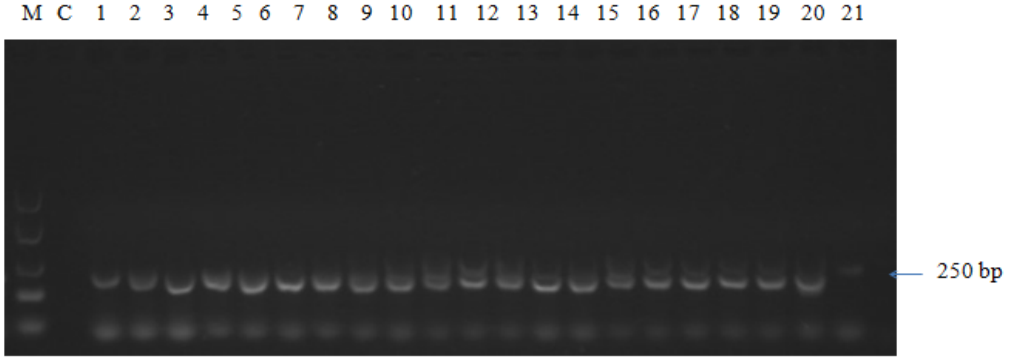

Fig. 2. PCR amplification of coat protein gene fragment of begomoviruses in 21 susceptible okra genotypes using CPF / CPR primer pair of amplicon size 250 bp
Lane M denotes 1 kb DNA ladder (Solis Biodyne), lane c is the negative control (distilled water), lanes 1-21 represents 21 okra genotypes

3.4 Cumulative Average number of Whitefly, Average Fruit Weight and mean Fruit Yield (t ha^{-1})

The cumulative average number of whitefly (CANWF), average fruit weight and mean fruit yield (t ha^{-1}) are shown in Table 4. An ANOVA showed significant difference among the okra genotypes with respect to the CANWF recorded during the wet season ($F_{20,60}$ = 2.83; P<0.001). Genotype UCCC1 had the lowest CANWF (1.5) which was not significantly different from that of UCCC5, UCCC4, UCCC3, GH6211, GH5793, GH5302, GH3760, GH3734, GH3731, and GH2052. Genotype GH4374 had the highest CANWF (7.7) but was not significantly different from GH2057, GH2063, GH5321, GH5786 and GH6105. Similarly, the CANWF recorded for the okra genotypes in the dry season were significantly different ($F_{20,60}$ = 2.41; P=0.005). Genotype UCCC6 had the highest value (217) but was not significantly different from that of GH2057, GH2063 and GH5332. On the other hand UCCC5 had the lowest CANWF (34) but it was not significantly different from UCCC4, UCCC3, UCCC1, GH6211, GH6105, GH5793, GH5786, GH5321, GH4374, GH3760, GH3734, GH3731, GH2052 and GH2026.

The results also indicated significant seasonal and genotype x season interaction effects on CANWF recorded (Table 5),

The okra genotypes also varied significantly (P<0.05) among them in respect of their average fruit weights in the wet and dry season trials (Table 4). Genotype GH2057 had highest average fruit weights of 28.61 g and 24.83 g during the wet and dry season trials respectively. UCCC6 on the other hand had the lowest average fruit weights of 13.87 g and 12.66 g during the wet and dry seasons respectively. There were significant (P<0.005) seasonal and genotype x season interaction effects on the average fruit weights obtained (Table 5).

The mean fruit yields (t ha^{-1}) recorded for the okra genotypes differed significantly (P < 0.05) among them during both the wet and the dry seasons (Table 4). The mean fruit yields for genotype GH5332 during the wet (11.88 t ha^{-1}) and dry seasons (6.108 t ha^{-1}) were significantly higher (P < 0.05) than the other 20 genotypes. The mean fruit yields recorded for genotype GH6105 during the wet season (9.34 t ha^{-1}) and dry season (4.061 t ha^{-1}) were significantly lower than GH5332 but significantly higher than the other 19 genotypes (P < 0.05), whereas genotype GH2063 recorded the lowest mean fruit yields of 0.86 t ha^{-1} and 0.708 t ha^{-1} in the wet and dry seasons respectively. A two-way ANOVA (Table 5) revealed that the overall mean fruit yield recorded in the wet season (3.23 t ha^{-1}) was significantly higher ($F_{20,123}$ = 39.65; P < 0.001) than the dry season (1.91 t ha^{-1}). Genotype x season interaction effect on mean fruit yield was also significant (P < 0.001) (Table 5).

3.5 Correlation between Insect Vector (whitefly), OLCD and Yield of Okra

The relationships between the cumulative average number of whitefly per plant, final incidence, final severity average fruit weight and mean fruit yields recorded in the wet and dry seasons are shown in Table 6. In the wet season, incidence of OLCD showed a highly significant positive correlation with disease severity (r = 0.911; P < 0.01) and negative but non-significant correlation with average fruit weight (r = -0.062; P > 0.05) and mean fruit yield (r = -0.172; P > 0.05). However, there was a significant negative correlation between disease incidence and mean fruit yield (r= -0. 242; P<0.05).

Similarly, in the dry season, final disease incidence correlated significantly positive with final disease severity (r = 0.911; P < 0.001) but did not significantly correlate with average fruit weight per plant (r = 0.064; P > 0.05), and mean fruit yield (r = -0.195; P > 0.05). There was however a significantly negative correlation between final disease severity and mean fruit yield (r = -0.4661; P<0.01). Also, in the dry season, there was significant negative correlation between cumulative average number of whitefly per plant and disease incidence (r = 0.291; P < 0.05) and disease severity (r = -0.331; P < 0.01) but non-significantly correlation with average fruit weight (r = -0.068; P > 0.05) and fruit yield (r = -0.037; P > 0.05).

4. DISCUSSION

In order to identify potential sources of natural resistance to OLCD, different okra genotypes in Ghana were evaluated based on symptom development under field conditions and PCR detection of *begomovirus* partial coat protein gene. All the okra genotypes were susceptible to the OLCD and exhibited a varying range of disease symptoms. This result agrees with that of Udengwu and Dibua [32] where all 15 okra cultivars screened under field conditions were susceptible to OMD and OLCD.

Table 4. Cumulative average number of whitefly per plant, mean fruit weight and mean fruit yield recorded for 21 okra genotypes during wet and dry planting seasons

Okra genotype	Cumulative average no. of whitefly per plant		Average fruit weight (g)		Mean fruit yield (t ha^{-1})	
	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season
GH2026	6.1^{abc}	90.0^{cdef}	18.89^{defg}	15.63^{cdefg}	2.49^{def}	2.02^{cdef}
GH2052	4.2^{bcdef}	61.2^{def}	23.55^{bc}	19.33^{bc}	2.57^{cdef}	1.99^{cdef}
GH2057	6.3^{abc}	150.7^{abcd}	19.29^{def}	15.15^{defg}	2.23^{def}	1.54^{defg}
GH2063	6.0^{abc}	129.2^{abcde}	16.00^{fghi}	14.41^{efg}	3.17^{cdef}	1.52^{defg}
GH3731	2.6^{def}	103.0^{bcdef}	14.76^{hi}	13.20^{fg}	1.55^{ef}	0.90^{fg}
GH3734	2.0^{def}	56.8^{def}	28.61^{a}	24.83^{a}	4.41^{cd}	3.00^{bc}
GH3760	4.5^{bcdef}	84.7^{cdef}	16.05^{fghi}	14.21^{efg}	0.86^{f}	0.71^{g}
GH4374	7.7^{a}	58.7^{def}	18.92^{defg}	17.17^{cde}	1.39^{ef}	0.97^{fg}
GH5302	3.6^{bcdef}	176.4^{abc}	17.00^{fghi}	13.99^{efg}	2.85^{cdef}	1.27^{efg}
GH5321	4.8^{abcde}	44.4^{ef}	26.84^{ab}	21.68^{ab}	3.43^{cde}	2.38^{cde}
GH5332	6.0^{abc}	191.2^{ab}	15.34^{ghi}	13.56^{efg}	1.68^{ef}	1.04^{fg}
GH5786	6.0^{abc}	99.0^{bcdef}	13.87^{i}	13.18^{fg}	1.58^{ef}	1.54^{defg}
GH5793	3.2^{cdef}	93.9^{cdef}	21.05^{cde}	15.97^{cdefg}	4.96^{c}	2.52^{cd}
GH6105	5.1^{abcd}	116.7^{bcdef}	21.37^{cde}	18.33^{bcd}	11.88^{a}	6.11^{a}
GH6211	2.6^{def}	68.9^{def}	16.29^{fghi}	14.47^{efg}	1.43^{ef}	0.68^{g}
UCCC1	1.5^{f}	38.0^{ef}	14.68^{hi}	12.83^{g}	1.50^{ef}	0.88^{fg}
UCCC2	2.5^{def}	76.1^{def}	20.76^{cde}	16.65^{cdef}	2.90^{cdef}	1.51^{defg}
UCCC3	2.7^{def}	67.2^{def}	22.58^{cd}	18.97^{bc}	9.34^{b}	4.06^{b}
UCCC4	2.1^{def}	46.9^{ef}	18.00^{efgh}	15.00^{defg}	1.61^{ef}	1.29^{efg}
UCCC5	1.7^{ef}	34.0^{f}	13.63^{i}	12.66^{g}	3.75^{cde}	2.49^{cde}
UCCC6	6.6^{ab}	217.0^{a}	17.81^{efgh}	14.26^{efg}	2.36^{def}	1.65^{defg}
Mean	4.2	95.4	18.82	15.97	3.23a	1.91b
LSD	3.199	94.12	3.722	3.750	2.394	1.225
P value	<0.001	0.005	<0.001	<0.001	<0.001	<0.001

Means in the same column bearing different letters are significantly different (P<0.05). Means in the same column bearing different letters are significantly different (P<0.05)

Difference in the cumulative average no. of whitefly per plant between wet and dry seasons was significant (LSD = 16.24; d.f. = 123, P < 0.001). Difference in the overall mean fruit yields between minor and major seasons was significant (LSD= 0.417; d.f. = 40; P < 0.001).

Table 5. Mean sum of squares for area under disease progress curve (AUDPC), final disease severity scores, cumulative average whitefly population and fruit yieldsof21okra genotypes

Variable	DF	AUDPC	Final disease severity	CANWF	Average fruit weight (g)	Fruit yield (t ha^{-1})
Genotype (G)	20	53.133**	3.399**	3547**	100.242**	30,944 **
Season (S)	1	35.407**	1.023*	349627**	341.138	73.949 **
G x S	20	164.730*	0.614**	5123*	3.348 ns	4.532**
Residual	123	554,20	0.184	2827	7.363	1.865

*DF = degree of freedom, * significant at P < 0.05, ** significant at P < 0.01, ns – not significant (P > 0.05)*

Mean incidence, severity and AUDPC of OLCD in the wet and dry season trials varied significantly among the okra genotypes. This finding is consistent with that of Tiendrébéogo et al. [6] who reported of higher OLCD incidence among okra accessions of the local cultivar than commercial cultivars. These variations could be due to different interaction effects between different host genotypes and that of viral pathogens and the biotypes of *B. tabaci* that were present.

Genotypes GH2026, GH2052, GH2063, GH3760, GH5302, GH5332, UCCC6 and GH6105 displayed mild symptoms with significantly lower AUDPCs values during both

Table 6. Correlation coefficients between insect vector (whitefly), OLCD and yield of okra for wet and dry seasons (2015-2016)

	$CANW_A$	$CANW_B$	DI_A	DI_B	DS_A	DS_B	AFW_A	AFW_B	Y_A
$CANW_A$	-								
$CANW_B$	-0.020	-							
$DI._A$	-0.109	-0.269*	-						
$DI._B$	-.324**	-0.291**	0.526**	-					
$DS._A$	-0.138	-0.306**	0.911**	0.540**					
$DS._B$	-0.316**	-0.331**	0.503**	0.911**	-				
AFW_A	-0.101	-0.142	-0.062	0.165	0.087	-			
AFW_B	-0.068	-0.036	-0.141	0.064	-0.008	0.859**	-		
YA	0.059	0.052	-0.172	-0.236*	-0.242*	0.422**	0.364**	-	
YB	-0.037	0.211	-0.160	-0.195	-0.187	-0.466**	0.510**	0.877**	

***Highly significant (P < 0.01), *Significant (P < 0.05), A-wet season, B-dry season, CANW-cumulative average number of whitefly, DI - disease incidence, DS = Disease severity, Y-Fruit yield, AFW-Average fruit weight*

rainy and dry seasons. This suggests that they possess partial resistance which is stable at varying environmental conditions, indicating a steady state pathogen-host-environment interplay, as reported by Anneke et al. [33]. On the contrary, genotypes GH2057, GH4374 and GH5786 exhibited mild symptoms with low AUDPC values in the rainy season symptoms but became severe with higher AUDPC values in the dry season (Table 4). This could be due to the interplay between the viral pathogen, host (the okra genotypes) and environment, as have been earlier reported [33,34]. This suggests that their mode of resistance was influenced by the different environmental conditions.

Significantly higher populations of the whitefly were recorded in the dry season than in the wet season. Whereas overall cumulative average number of whitefly per plant in the dry season was 95.4, that of the wet season was only 4.2. This could be due to the high temperatures and low relative humidity associated with dry seasons that are favourable for the multiplication of the whitefly. The results of this study are in supports of others [22,35,36] who reported that high temperature and low rainfall favour the rapid multiplication of the whitefly. The observed higher populations of whitefly in the dry season might have at least in part, accounted for the significantly negative correlation between the whitefly population and disease severity compared to the non-significant correlation observed in the wet season when there were lower whitefly populations. Association of whitefly with OLCD have been reported severally [19,20].

The population of whitefly which infested the okra genotypes at both wet and dry seasons varied significantly among them (see Table 4). This variation could be due to differences in the genetic makeup of the different genotypes and the biotype of whitefly that were present as have been reported by others [36,37].

The yield and yield components were observed to vary among the different okra genotypes during both wet and dry seasons. This finding is in line with that of Udengwu and Dibua [32] where all 23 okra cultivars, both protected and unprotected screened under field conditions against OLCD and OMD varied significantly among them in respect of their fruit yields during two different field trials. Also, in assessing the impact of OLCD on morphology and yield of okra, Tiendrébéogo et al. [6] observed variations in yield and yield components, where the number of marketable fruits per plant, the fruit length, fruit diameter and fruit weight were subject to reductions of 26–61%, 19–64%, 6–42% and 23–63%, respectively. The overall yield losses due to OLCD were reported [6] to be significantly higher in accessions of the local cultivar (26–55%) than in the commercial ones (4.4–9.6%). Similarly, in the present study, there were significant positive correlation between disease severity scores and mean fruit yield (Table 6), thus supporting the negative effect of OLCD on the yield of okra. The variations in yields could be due to different host-virus interactions [34] and age of plants at which plants were infected [38]. Also incidence and severity of OLCD correlated negatively with fruit yield of okra in both wet and dry seasons which indicates that at least, partly, fruit yield of okra is affected by OLCD.

Among the okra genotypes that showed resistance to okra leaf curl *begomovirus*, GH5332 and GH6105 consistently had the highest mean fruit yields in tonnes per hectare during both field trials. Fruit yields of 11.88 t ha^{-1}

and 9.34 t ha^{-1} recorded for GH5332 and GH6105 respectively during the wet season and their corresponding dry season yields of 6.108 t ha^{-1} and 4.05 t ha^{-1} are far higher than the West Africa and Central Africa's average yield of 2.5 t ha^{-1} reported by FAOSTAT [39]. Genotypes GH5332 and GH6105 could further be evaluated for subsequent release to farmers.

5. CONCLUSIONS

In screening 20 okra accessions against OLCD at both wet and dry seasons, all the genotypes were affected by the disease but at varying levels of incidence and amount of disease. Two (GH5332 and GH6105) out of eight genotypes which were resistant to the OLCD produced yields higher than the average yields of Ghana and West Africa. These genotypes could be evaluated further multi locationally, for subsequent release to farmers as varieties or they could be incorporated into breeding lines to produce OLCD-resistant varieties.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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