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Research Application Summary

Phenotypic and serological screening of okra genotypes against Okra mosaic virus infection under field conditions

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Abstract

Okra mosaic disease (OMD) caused by *Okra mosaic virus* (OkMV) is an important biotic constraint to okra (*Abelmoschus esculentus* L.Moench) production in West Africa. Management of OMD with insecticides is very difficult and ineffective, thus making the use of host resistance the most desirable approach. However, there is no information available on host resistance to OkMV. In 2015 rainy and dry seasons, field trials were conducted to screen 21 okra genotypes against OkMV infection in order to identify sources of resistance and or tolerance. In both seasons, field trials were laid out in a randomised complete block design with four replications. Field resistance was assessed using 1-5 visual scale based on disease symptoms, 1 denoting no disease symptom while 5 denoted very severe symptoms. Enzyme linked immunosorbent assay (ELISA) was used to confirm field resistance or infection. Genotypes GH3760, GH2052, GH5332, UCC6, GH5302, GH5793 and GH2063 exhibited mild symptoms during both rainy and dry seasons. ELISA detected OkMV in all the 21 genotypes in both major and minor season crops. The mean number of fruit per plant and the mean fruit yield (t ha^{-1}) differed significantly (P<0.05) among the okra genotypes in both major and minor cropping seasons. Genotype GH5332 with mild symptoms, had the highest fruit yield of 11.88 t ha^{-1}. Genotype GH6105 also had very high fruit yield (9.34 t ha^{-1}) but was very susceptible to OkMV infection. Both disease severity and yield were higher in the minor season than the major season in all the okra genotypes. It can therefore be concluded that genotype GH5332 exhibited partial resistance while genotype GH6102 was tolerant to OkMV. These two varieties are recommended for cultivation as truely resistance genotypes are sought.

Key words: *Abelmoschus esculentus*, Okra mosaic virus, okra mosaic disease, resistance

Résumé

La Maladie de la Mosaïque du Gombo (OMD) causée par le virus de la mosaïque du gombo (OkMV) est une importante contrainte biotique de la production du gombo (*Abelmoschus esculentus* L.Moench) en Afrique de l'Ouest. La gestion de l'OMD avec des insecticides est très difficile et inefficace. Cependant, il n'y a pas d'information sur la résistance des plantes OkMV. Au cours des saisons sèches et pluvieuses de 2015, les essais ont été menées pour évaluer la réaction de 21 génotypes gombo contre l'infection du OkMV afin d'identifier les sources de résistance et ou de tolérance. Pendant les deux saisons, les essais ont été installés en utilisant un bloc complet randomisé avec quatre répétitions. La résistance au champ a été évaluée sur une échelle visuelle de 1-5, basée sur les symptômes de la maladie (1 désigne aucun symptôme de la maladie alors que 5 désigne des symptômes très graves). Dosage immuno-enzymatique (ELISA) a été effectué pour confirmer la résistance ou l'infection. Les génotypes GH3760, GH2052, GH5332, UCC6, GH5302, GH5793 et GH2063 présentaient des symptômes légers pendant les deux saisons, pluvieuse et sèche. Le test sérologique ''ELISA'' a détecté le virus OkMV dans tous les 21 génotypes évaluées et ceci au cours des deux grandes et petites campagnes agricoles. Des différences significatives ont été observés entre les génotypes de gombo pour le nombre moyen de fruits par plante et le rendement moyen de fruits (t ha-1) pendant des deux campagnes agricoles (P<0.05). Le génotype GH5332 avec des symptômes légers, a eu le rendement en fruits le plus élevé de 11,88 t ha-1. Le génotype GH6105 avait aussi un rendement en fruits très élevé (9,34 t ha-1), mais était très susceptible au virus OkMV. La sévérité de la maladie et le rendement étaient tous deux plus élevés au cours de la petite saison qu'en grande saison et pour tous les génotypes de gombo évalués. On peut donc conclure que le génotype GH5332 présentait une résistance partielle tandis que le génotype GH6102 était tolérant au virus OkMV, les deux étant influencés par les conditions environnementales.

Mots clés: *Abelmoschus esculentus*, Virus de la mosaïque du gombo, Maladie de la mosaïque du gombo, résistance

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Background and literature summary

Okra, (*Abelmoschus esculentus* L. Moench) is a widely grown vegetable in the tropical, subtropical and warm temperate countries mainly for its immature edible green fruits, which are used as a vegetable both in green and dried state (Lamont, 1999; Saifullah and Rabbani, 2009). Okra is a source of carbohydrate, dietary fibre, fat, protein, calcium, iron, thiamine, riboflavin, nicotinamide and ascorbic acid (Tindall, 1986; Schippers, 2000). World okra production, was estimated at 4.8 million tons, as of 2007, with India leading the production by 70% followed by Nigeria (15%), Pakistan (2%), Ghana (2%), Egypt (1.7%) and Iraq (1.7%) (Gulsen *et al*., 2007). In Ghana, okra is widely grown in both rainy and dry seasons mainly by small holder farmers for whom it is a major source of income. Major areas of okra production in Ghana are Brong Ahafo, Ashanti, Northern, Volta, Greater Accra and Central regions (NARP, 1993).

Even though the West and Central African region including Ghana account for more than 75% of okra produced in Africa, the average productivity in the region is very low (2.5 t ha^{-1}) compared to East (6.2 t ha^{-1}) and North Africa (8.2 t ha^{-1}) (FAOSTAT, 2008). In Ghana, yield potential of 2000-3000 kg ha^{-1} has been reported for Okra (MoFA, 2007), depending on cultivar, harvesting frequency and period for harvesting (Cudjoe *et al*., 2005). However, actual yields of okra are usually low and have been decreasing over the years.

Viral diseases are important constraints in the production of okra worldwide (Asare-Bediako *et al*., 2014a, b; Ndunguru and Rajabu, 2004). Okra mosaic disease (OMD) caused by *Okra mosaic virus* (OkMV; genus *Tymovirus;* family *Tymoviridae*) (Givord and Koenig, 1974) is the most prevalent viral disease of okra in West Africa. It is the most commom viral disease of okra in Ghana with disease incidence of up to 100% recorded in some okra fields (Asare-Bediako *et al*., 2014b). Incidence of OMD has been reported in other West African countries including Ivory Coast (Givord *et al.,* 1972) and Nigeria (Givord and Koenig, 1974; Alegbejo, 2001; Fajinmi and Fajinmi, 2010). Typical symptoms of OkMV infection include mosaic, vein chlorosis and vein-banding and plant stunting (Givord and Koenig, 1974; Brunt *et al*., 1990; Swanson and Harrison, 1993). A yield loss of up to 100% due to OkMV infection has been reported (Atiri, 1984; Alegbejo, 2001). OkMV is transmitted in a nonpersistent manner by the coleoptera *Podagrica species* (flea beetles) (Brunt *et al*., 1990; Brunt *et al.,* 1996). The virus is also sap-transmissible (Givord and Koenig, 1974).

Managing OMD is therefore quite important in order to improve yields and production of okra. Most of the research on management of virus and its vector are oriented with chemical control. However, OkMV is very difficult to control with insecticides (Nono-Womdim, 2001). Breeding and planting of resistant varieties is the most effective way of managing viral diseases. However, there is no information available on host resistance to OkMV (Nono-Womdim, 2001). This study was therefore conducted to screen different genotypes of okra against OkMV infection under natural conditions in order to identify sources of resistance and or tolerance.

Study description

The experiment was conducted at the Teaching and Research farm of the School of Agriculture of the University of Cape Coast during the 2015 major and minor cropping seasons. The Research farm (5°10'N, 1.2°50'W) falls within the coastal savannah agro-ecological zone of the country with Acrisol soil type (Parker *et al*., 2010). It is an endemic site for OkMV disease.

Twenty-one genotypes of okra (both landraces and improved) were used in the study. They were obtained either from farmers or Plant Genetic Resource Research Institute (PGRR1) at Bunso. The okra genotypes were planted on a total land area of 1344 m^2 in Randomized Complete Block Design (RCBD) with four replications. Each replication was divided into 21 plots, with each plot measuring 3 m x 3 m. The okra genotypes were sown directly at two seeds per hole at a spacing of 0.6 m x 0.6 m and a planting depth of not more than 0.5 cm. NPK fertilizer (15:15:15) was applied at a rate of 250 kg ha^{-1}. Watering and weed control were done whenever it became necessary.

The 21 okra genotypes were evaluated at 2, 6 and 10 weeks after planting (WAP) for severity of OMD based on disease symptoms. Ten plants from each genotype were scored and the mean ordinal scores determined. Data were also taken on the number of fruits per plant, fruit weight and yield (t ha^{-1}). The plants were scored for severity of OMD based on a 0–5 scale adopted from Alegbejo *et al.* (1997) with modification as indicated in Table 1.

Incidence of okra mosaic disease (DI), based on visual symptoms, was determined as the proportion of infected plants per plot, expressed as a percentage of total number of plants observed, as described by Galanihe *et al.* (2004).

The presence of OkMV in the okra leaf samples collected were tested by standard double antibody sandwiched enzyme-linked immunosorbent assay (DAS ELISA) using paired wells in microtitre plates as described by Clark and Adams (1977) using antisera raised against OkMV (AC Diagnostics Inc. USA). The absorbance values at 405 nm were recorded using Anthos microplate reader (Biochrum Ltd, Cambridge, UK). Absorbance values of three (3) uninfected leaf samples were also measured. A test sample was deemed to be positive when absorbance was higher than 3 times the mean absorbances of the uninfected leaf samples (threshold value). Results revealed continuous variation among the okra genotypes in susceptibility to OkMV.

Results

Mean severity scores of OMD. Mean severity scores of OMD on the 21 okra genotypes at 2 WAP, 6 WAP and 10 WAP during the major and minor seasons are shown in Table 2. There were significant differences in the severity of OMD among the okra genotypes at all three growth stages of 2 WAP ($F = 1.86$; df = 60; $P = 0.033$), 6 WAP ($F = 3.27$; df = 60; $P = 0.027$) and 10 WAP ($F=1.93$; df = 60; $P < 0.001$) during the major season. Generally, disease severity increased consistently from 2 WAP to 10 WAP, with mean severity scores of 0.089, 1.449 and 2.156, respectively. At 10 WAP, GH3760 had the lowest mean severity score of 1.75, followed by GH2052, UCCC5, GH5793, UCCC1, GH2063, GH5302, GH5332, with mean severity scores of 1.944, 1.958, 1.972, 1.972, 2.00, 2.00, 2.028 and 2.056 respectively, indicating that they were mildly susceptible to the OMD. On the contrary, GH3734 had the highest severity score of 2.571, followed by GH6211, GH2057, UCCC3, GH5786, UCCC2, GH5321, GH4374, UCCC4 and GH6105 with mean severity scores of

Table 1. Visual scale for rating severity of okra mosaic disease in farmers' okra fields

Disease score	Description
0	Healthy, asymptomatic plant
1	Mild mosaic, mottle or chlorosis on leaves
2	Moderate chlorosis, mottle or mosaic without significant leaf distortion
3	Moderate chlorosis, mottle or mosaic with leaf malformation
4	Severe chlorosis, mottle or mosaic plus stunting or dwarfing of the whole plant
5	Score 4 plus drying and leaf drop

Table 2. Mean severity scores of OMD on 21 okra genotypes on the field during 2015 rainy and dry seasons

Genotypes	Mean severity scores of OMD in the rainy season			Mean severity scores of OMD in the dry season			OkMV detection by DAS-ELISA
	2WAP	6WAP	10WAP	2WAP	6WAP	10WAP	
UCCC 2	0.194 ^{ab}	1.861 ^{ab}	2.333^{abcd}	0.000^{ns}	1.417^{abc}	2.958^{bc}	++
UCCC 3	0.139 ^{ab}	1.611 ^{bcd}	2.361^{abcd}	0.042	1.667^{a}	2.958^{bc}	++
UCCC4	0.389 ^{a}	1.639 ^{bcd}	2.222 ^{abcd}	0.125	1.542^{a}	3.042^{bc}	++
GH2026	0.000 ^{b}	1.214 ^{de}	2.009 ^{cde}	0.000	0.896^{cde}	2.104^{e}	++
GH2052	0.000 ^{b}	1.194 ^{de}	1.944 ^{de}	0.000	0.292^{f}	1.833^{ef}	++
GH2057	0.000 ^{b}	1.071 ^{e}	2.382 ^{abc}	0.042	1.292^{abcd}	2.875^{bc}	++
GH2063	0.000 ^{b}	1.222 ^{de}	2.00 ^{cde}	0.000	0.667^{ef}	2.042^{ef}	++
GH3731	0.028 ^{b}	1.583 ^{bcd}	2.194 ^{abcd}	0.000	0.833d^{e}	2.292^{de}	++
GH3734	0.028 ^{b}	1.929 ^{ab}	2.571 ^{a}	0.083	1.583^{a}	3.708^{a}	++
GH3760	0.056 ^{b}	1.111 ^{e}	1.75 ^{e}	0.000	0.625^{ef}	1.750^{ef}	++
GH4374	0.000 ^{b}	1.500 ^{bcde}	2.25 ^{abcd}	0.000	1.304^{abcd}	3.188^{abc}	++
GH5302	0.000 ^{b}	1.111 ^{e}	2.028 ^{cde}	0.042	0.750^{ef}	1.917^{ef}	++
GH5321	0.361 ^{a}	2.111 ^{a}	2.306 ^{abcd}	0.042	1.458^{ab}	3.208^{abc}	++
GH5332	0.083 ^{b}	1.250 ^{de}	2.056^{bcde}	0.000	0.708^{ef}	1.833^{ef}	++
GH5786	0.000 ^{b}	1.333 ^{cde}	2.333 ^{abcd}	0.000	0.958^{bcde}	2.750^{cd}	++
GH5793	0.000 ^{b}	1.167 ^{e}	1.972 ^{cde}	0.000	0.667^{ef}	1.917^{ef}	++
UCCC5	0.167 ^{ab}	1.764 ^{abc}	1.958 ^{cde}	0.167	1.542^{a}	3.375^{ab}	++
GH6105	0.028 ^{b}	1.365 ^{cde}	2.163 ^{abcde}	0.000	0.833^{de}	2.083^{e}	++
GH6211	0.000 ^{b}	1.500 ^{bcde}	2.472 ^{ab}	0.000	1.333^{abcd}	3.208^{abc}	++
UCCC 6	0.000 ^{b}	1.250 ^{de}	1.972 ^{cde}	0.000	0.583^{ef}	1.475^{f}	++
UCCC 1	0.389 ^{a}	1.639 ^{bcd}	2.00 ^{cde}	0.125	1.708^{a}	3.750^{a}	++
Mean	0.089	1.449	2.156	0.032	1.079	2.584	
LSD	0.2801	0.4666	0.4272	=	0.5316	0.5672	

Means in the same column bearing identical letters are not significantly different by LSD test at $P < 0.05$

++ Presence of *Okra mosaic virus* (OkMV) in the okra genotypes during rainy and dry seasons

Severity scores: 0 implies no symptom while 5 denotes very severe symptom

2.472, 2.382, 2.361, 2.333, 2.333, 2.306, 2.25, 2.222, and 2.163, respectively, indicating that they were moderately susceptible to OkMV.

Unlike the major season, there was no significant differences in mean severity of OMD among the okra genotypes at 2 WAP in the minor season (F=1.57; df=60; P=0.092). However, highly significant differences in the mean severity of OMD among the okra genotypes were recorded at 6WAP (F=5.19; df=60; P<0.001) and 10WAP (F=12.18; df=60; P<0.001). Overall mean severity scores of 0.0317, 1.079 and 2.584 were recorded at 2, 6 and 10 WAP respectively. UCCC6 had the lowest mean severity (1.475) of OMD at 10WAP, but was not significantly different from GH5793 (1.917), GH5332 (1.833), GH5302 (1.917), GH 3760

(1.750), GH2063 (2.042) and GH2052 (1.833). UCCC1 had the highest mean severity (3.750) of OMD at 10WAP but was not significantly different from GH 6211 (3.208), UCCC5 (3.375), GH 5321 (3.208), GH 4374 (3.188) and GH 3734 (3.708).

ELISA serology conducted to confirm the field reactions of the okra genotypes to OkMV, detected the virus in all the 21 genotypes in both major and minor cropping seasons (Table 2).

Mean number of fruits and mean fruit yield. Both the mean number of fruits per plant and mean fruit yield (t ha^{-1}) recorded for the 21 okra genotypes differed significantly ($P<0.05$) (Table 3). In both major and minor cropping seasons, the mean number of fruits per plant and mean fruit yield (t ha^{-1}) recorded for genotype GH5332 were significantly higher than for the other 20 genotypes. The mean number of fruits per plant and mean fruit yield (t

Table 3. Mean number of fruits per plant and mean fruit yield (t ha^{-1}) of 21 okra genotypes

Genotype	Major season		Minor season	
	Mean number of fruits/plant	Yield (t $ha^{-1)}$	Mean number of fruit/plant	Yield (t ha^{-1})
UCCC2	4.75^{def}	2.49^{def}	4.62^{cde}	2.017^{cdef}
UCCC3	3.87^{ef}	2.57^{cdef}	3.67^{defgh}	1.996^{cdef}
UCCC4	4.20^{def}	2.23^{def}	3.67^{defgh}	1.535^{defg}
GH2026	7.00^{cde}	3.17^{cdef}	3.75^{defgh}	1.517^{defg}
GH2052	3.32^{ef}	1.55^{ef}	2.43^{efgh}	0.900^{fg}
GH2057	5.55^{cdef}	4.41^{cd}	4.33^{def}	3.000^{bc}
GH2063	1.90^{f}	0.86^{f}	1.75^{gh}	0.708^{g}
GH3731	2.53^{f}	1.39^{ef}	2.00^{fgh}	0.973^{fg}
GH3734	5.65^{cdef}	2.85^{cdef}	3.21^{defgh}	1.269^{efg}
GH3760	4.55^{def}	3.43^{cde}	3.83^{defgh}	2.383^{cde}
GH4374	3.92^{ef}	1.68^{ef}	2.71^{efgh}	1.042^{fg}
GH5302	4.25^{def}	1.58^{ef}	4.17^{def}	1.535^{defg}
GH5321	8.05^{cd}	4.96^{c}	5.46^{bcd}	2.516^{cd}
GH5332	20.12^{a}	11.88^{a}	12.33^{a}	6.108^{a}
GH5786	3.22^{ef}	1.43^{ef}	1.67^{h}	0.684^{g}
GH5793	3.77^{ef}	1.50^{ef}	2.33^{efgh}	0.884^{fg}
UCCC5	5.10^{def}	2.90^{cdef}	3.35^{defgh}	1.509^{defg}
GH6105	14.90^{b}	9.34^{b}	7.63^{b}	4.061^{b}
GH6211	3.25^{ef}	1.61^{ef}	3.13^{defgh}	1.291^{efg}
UCCC6	9.50^{c}	3.75^{cde}	6.83^{bc}	2.488^{cde}
UCCC1	4.70^{def}	2.36^{def}	4.11^{defg}	1.651^{defg}
Mean	5.91	3.23	4.14	1.908
LSD	3.977	2.394	2.387	1.2247

Means in a column with the same letter are not significantly different by LSD test at $P < 0.05$
Fruit yield was calculated as the cumulative of five harvesting done after 50% flowering

ha^{-1}) recorded for genotype GH6105 was significantly lower than for GH5332 but significantly higher than for the other 19 okra genotypes ($P<0.05$).

Generally, both the mean number of fruits per plant and mean fruit yield (t ha^{-1}) recorded in the major season were higher than those in the minor season (Table 3).

Discussion

The study has revealed that all the okra genotypes tested in both the rainy and dry seasons were infected by OkMV although they varied in disease severity. This finding is comparable to that of Udengwu and Dibua (2014) where all 15 okra cultivars screened under field conditions were susceptible to OMD and OLCD. Similarly, Nataraja *et al*. (2013) found that 23 cultivars of okra tested under field conditions were susceptible to Okra yellow vein mosaic and sucking pests.

The observed variation in disease severity could be due to different interaction effects between different genotypes and OkMV. Similar reasons also apply to the variations in the incidence and severity of Okra yellow leaf curl virus among tomato genotypes tested (Azizi *et al*., 2008; Abu *et al*., 2011) and to variation in the susceptibility of *Arabidopsis thaliana* accessions to *Turnip yellows virus* (Asare-Bediako, 2012).

Genotypes GH3760, GH2052, GH5332, UCC6, GH5302, GH5793 and GH2063 exhibited mild symptoms during both rainy and dry seasons. This suggests that they possess stable resistance across environments used in this study. On the other hand, genotypes UCCC5 and UCCC1 exhibited mild symptoms during the rainy season but became severe during the dry season. This indicates that their resistance was influenced by climatic conditions.

Among the okra genotypes which showed mild resistance to OkMV, GH5332 had the highest mean number of fruits per plot and mean fruit yield in tonnes per hectare. On the contrary, genotype GH6105, one of the susceptible had high fruit yield (see Table 3), indicating that it was tolerant to OkMV infection. Generally, the fruit yields recorded for the 21 okra genotypes in the major season declined during the minor season. Thus OkMV resistance /tolerance in GH5332 and GH6105 respectively, are not complete but can be influenced by environmental factors. Juergens *et al*. (2010) found similar results when they screened oilseed rape cultivars against *Turnip yellows virus* (TuYV). This type of resistance is controlled by a single major gene together with additional contributing genes (Dreyer *et al.*, 2001).

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