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Variation in the susceptibility of tomato (*Lycopersicon solanum* L.) genotypes to tomato yellow leaf curl virus (TYLCVD) infections at coastal savannah and forest zones of Ghana

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Abstract

Little information is available on the resistance level of tomato genotypes to *tomato yellow leaf curl virus disease* (TYLCVD) and vector. Thirty-six genotypes representing the current available and accessible tomato germplasm in Ghana were evaluated in RCBD with three reps, under Coastal savannah condition at the University of Cape Coast (UCC). Five genotypes (K005, K100, K213, K116 and K042) selected for mild severity (ms); two genotypes (K027, K202) selected for severe symptom (SS) and one genotype (LC) selected for moderate severity (MoS) were re-evaluated under RCBD in three reps at UCC and forest condition at "Asuansi". Genotypes K005, K100 and K213 were observed to maintain ms TYLCVD expression across locations but K116 and K042 had ms at UCC and MoS at Asuansi indicating respective stable and unstable host plant tolerance to TYLCVD across climatic zones. PCR test with two primer pairs for Asuansi samples revealed that PTYv787/PTYc1121 primer had amplified TYLCV DNA in all the eight genotypes. AV494/AC0148 primer had amplified TYLCV DNA in all the genotypes except in LV and K213, suggesting that TYLCVD expression in LV and K213 observed morphologically could come from different viral strains. The whitefly population was significantly affected by genotype by environment interaction indicating variation of whitefly population from location to location.

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Keywords: amplification; disease; DNA, infection; infestation; insect, PCR; tolerance; Tomato; viral; whitefly.

Introduction

Tomato, *Solanum lycopersicon* (L.) is a very important vegetable cultivated in 14% of the world (Bauchet and Causse 2012) by resource poor smallholder farmers. It is famous for fresh or processed consumption (Tshiala and Olwoch, 2010). Farmers **y**ield in Ghana (7.5tha^{-1}) is lower than potential researchers yield (15t ha^{-1}) (MoFA, 2011). The lower yield is mainly attributed to *tomato yellow leaf curl virus disease* (TYLCVD) caused by the *tomato yellow leaf curl virus* (TYLCV) (Osei et al., 2012). TYLCV is a single stranded DNA (ssDNA) in the genus Begomovirus, family Geminiviridae transmitted by the whitefly, *Bemisia tabaci* (Brown and Czosnek, 2002). TYLCVD reached a pandemic status because the virus is well adapted to wide range of hosts (Polston and Lapidot, 2007) including Solanaceae, Compositae, Leguminosae, Malvaceae and Plantaginaceae families (Azizi et al., 2008). The virus causes a decrease in leaf size, leaf curling upward, severe stunting, leaf distortion, interveinal chlorosis, flower abortion and stopping of marketable fruits production when early infection occurs (Al-ani et al., 2011), causing up to 100% yield losses in susceptible genotypes (Cohen and Antignus, 1994).

The spread of TYLCVD can be controlled by spraying the vector with recommended insecticides (Palumbo et al., 2001); the use of plastic mulch, growing plants in whitefly-proof screen and UV absorbing plastic and screen (Polston and Lapidot, 2007). Breeding host plant resistance to TYLCVD is more environmentally friendly approach to controlling the disease (Bhyan et al., 2007). Most research works on TYLCVD in Ghana are concentrated in the middle and northern sectors (Osei et al., 2008; 2012). Central region is an important tomato growing center in the southern sector where there was the need to evaluate host tomato genotypes resistant/tolerance to TYLCVD for effective and holistic management strategies.

Results

TYLCVD incidence of 36 tomato genotypes at coastal savannah zone

The TYLCVD incidence varies substantially among the 36 tomato genotypes (Table 1). The values ranged from 0 to 73.9%, 19.5 to 100% and 20.1 to 100% for the 30, 45 and 60 days after transplanting (DAT) respectively. The mean values at 30 DAT was significantly different among genotypes ($F_{35, 70} = 4.00$; $P < 0.05$). Host tomato plants K005, K100, K042 and K116 at 30 DAT had more incidence of the disease than others. All genotypes were significantly different in incidence

Table 1. Mean incidences of TYLCVD on 36 tomato genotypes 30, 45 and 60 days after transplanting (DAT) at coastal savannah zone.

Genotypes	Mean disease incidence (%) at various growth stages		
	30 DAT	45 DAT	60 DAT
K005	00.0 ^{m}	46.9 dfhi	46.9 dfg
K006	30.0 ^{defghijkl}	81.1 bc	81.1 bc
K011	30.0 ^{defghijkl}	68.1 bdfhi	68.1 bdfg
K027	55.4 ^{abcd}	72.3 bdfg	72.3 bdfg
K042	00.0 ^{m}	26.2 i	39.2 g
K045	08.9 ^{lm}	33.8 hi	42.7 fg
K050	13.1 jklm	68.9 bdfhi	68.9 bdfg
K083	73.1a	90.0 a	90.0 a
K084	46.9 abcdefg	55.4 bdfhi	55.4 bdfg
K087	59.2 abc	90 bdfhi	90.0 a
K088	38.9bcdefghijk	72.3 bdfg	72.3 bdfg
K098	60 ab	90.0 a	90.0 a
K100	00.0 m	26.6 i	26.6 g
K106	21.9 fghijklm	72.3 bdfg	72.3 bdfg
K116	00.0 m	38.9 fhi	46.9 dfg
K124	38.1 bcdefghijk	76.9 bde	76.9 bde
K138	38.1 bcdefghijk	81.1 bc	81.1 bc
K144	25.8 defghijklm	76.9 bde	76.9 bde
K146	59.2 abc	90.0 a	90.0 a
K186	42.7 bcdefghi	81.1 bc	81.1 bc
K188	38.9 bcdefghijk	47.3 dfhi	47.3 dfg
K190	08.9 lm	43.1 dfhi	43.1 fg
K191	17.7 fghijklm	60 bdfhi	55.8 bdfg
K194	21.9 efghijklm	72.3 bdfg	72.3 bdfg
K197	47.3 abcdef	60.0 bdfhi	60.0 bdfg
K200	38.9 bcdefghijk	60.0 bdfhi	60.0 bdfg
K202	51.1 abcde	90.0 a	90.0 a
K205A	16.9 hijklm	51.9 bdfhi	51.9 dfg
K205B	39.2 bcdefghij	68.1 bdfhi	68.1 bdfg
K213	00.0 m	30.0 hi	30.0 g
K214	43.1 bcdefgh	90.0 a	90.0 a
LV	42.7 bcdefgh	60.0 bdfhi	68.9 bdfg
P000	60.0 ab	73.1 bdfg	73.1 bdfg
P074	35.0 bcdefghijkl	64.2 bdfhi	64.2 bdfg
P077	51.1 abcde	55.4 bdfhi	55.4 bdfg
P085	38.9 bcdefghijk	68.1 bdfhi	68.1 bdfg
$Lsd_{(0.05)}$	27.5	32.0	32.0

Means in the same column bearing same letters are not significantly different from each other ($P < 0.05$) . Data was transformed using angular transformation.

Table 2. Mean incidence of TYLCVD on tomato plants at UCC and Asuansi experimental sites at 30, 45 and 60 days after transplanting (DAT).

Tomato Genotype	Incidence of TYLCV disease (%)					
	30 DAT		45 DAT		60 DAT	
	UCC	Asuansi	UCC	Asuansi	UCC	Asuansi
LV	35.0 efh	55.4 cd	51.1 cdfh	59 cd	46.9 bc	55.0 b
K005	17.7 i	39.2 efh	26.6 k	55 cdf	35.0 bceg	38.9 bce
K027	68.1 ab	81.1 a	90 ab	90 a	90.0 a	81.1 a
K042	35.0 efh	47.3 cdf	35 gh	59.2 cd	39.2 bce	30.0 deg
K100	17.7 i	43.1 efh	30.8 ij	55.8 cdf	30.8 deg	21.9 fg
K116	30.8 efh	38.9 efh	35 ghj	38.9 efh	43.1 bc	38.9 bce
K202	81.1 a	90.0 a	73.1 a	90 a	90.0 a	90.0 a
K213	21.9 gh	26.6 gh	30.8 ij	26.6 k	30.8 deg	17.7 h
Mean	38.4 b	52.7 a	46.5 b	59.3 a	50.7 a	46.7 a

Means in the same column bearing same letters are not significantly different from each other ($P < 0.05$). Data was transformed with angular transformation before ANOVA was carried out.

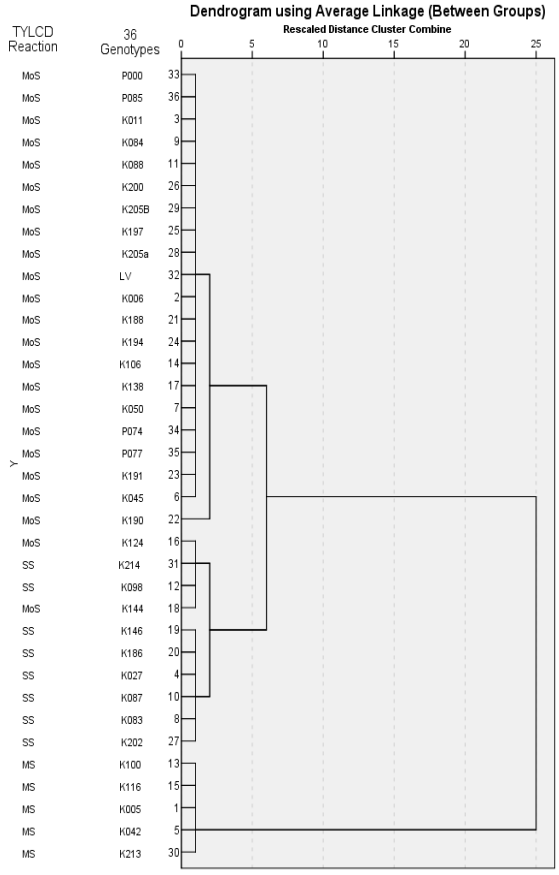

Fig 1. The dendrogram of 36 tomato genotypes based on average 30, 45 and 60 DAT respond to TYLCVD.

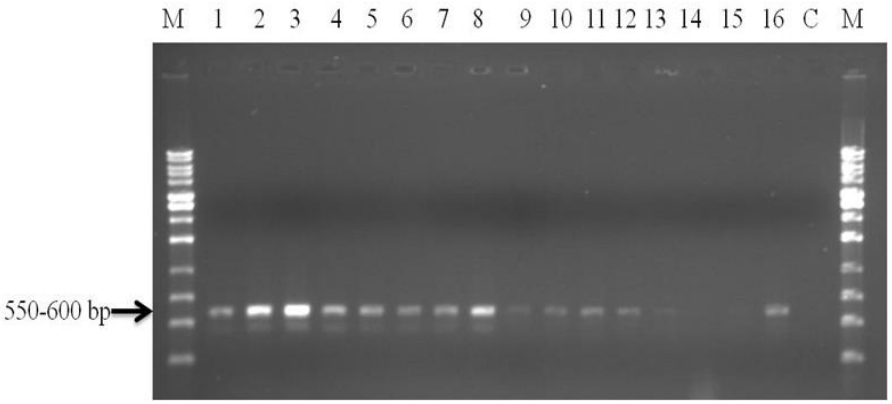

Fig 2. AV494/AC0148 primer pairs' amplicon of size between 550-600bp, for TYLCV in 8 tomato genotypes from UCC were represented by lanes 1-8 (1- K100, 2- K027, 3- K116, 4- K005, 5- K202, 6- LV, 7- K213 and 8- K042) and Asuansi were represented by corresponding samples in lanes 9-16. C denotes the negative control while M is 1 kb DNA Ladder.

of the disease from 45 DAP ($F_{35,\ 70}$ = 2.79; P < 0.05) to 60 DAT ($F_{35,\ 70}$ =3.00; P < 0.05).

Cluster analysis of TYLCVD severity of 36 tomato genotypes at coastal savannah zone

The clusters of 36 tomato genotypes based on average 30, 45 and 60 DAT respond to TYLCVD is shown in the dendrogram (Fig 1). Three clusters were observed at two (2) rescaled distance cluster combined. Cluster I contained 21 genotypes, II had 10 and III involves five (5). Cluster I genotypes (K205a, P066, K106, K200, K205b, K006, K138, K050, K197, K011, K190, K194, K084, LV, P077, K088, K188, K191, K045, P045, P074) had moderate symptom (MoS) of the TYLCVD followed by cluster II genotypes (K124, K144, K087, K146, K098, K027, K214, K186, K202, and K083) with variation from severe symptom (SS) to moderate severe (MoS) symptoms. Cluster III genotypes

Table 3. Mean severity scores of TYLCVD on tomato plants at UCC and Asuansi experimental sites at 30, 45 and 60 days after transplanting.

Tomato Genotype	Mean severity scores of TYLCV disease					
	30 DAT		45 DAT		60 DAT	
	UCC	Asuansi	UCC	Asuansi	UCC	Asuansi
LV	2.3 c	2.2 c	2.7 b	2.3 b	2.3 d	2.1 de
K005	1.4 d	1.2 d	1.6 c	1.4 c	1.4 h	1.4 h
K027	3.4 a	3.9 a	3.6 a	3.9 a	3.1 b	4.0 a
K042	1.3 d	2.3 c	1.5 c	2.3 b	1.6 fg	2.1 de
K100	1.1 d	1.4 d	1.2 c	1.3 c	1.3 h	1.4 h
K116	2.3 c	3.1 ab	2.5 b	3.3 a	2.4 bc	3.0 b
K202	3.3 a	3.8 a	3.5 a	3.8 a	3.1 b	4.0 a
K213	1.1 d	1.2 d	1.3 c	1.2 c	1.2 h	1.4 h
Mean	1.9	2.3	2.1	2,1	2.0	2.3

0=No symptom (NS) 1=Mild symptom (MS) 2=Moderate symptom (MoS) 3=Severe symptom (SS) 4= Very severe symptom (VSS). Means in the same column bearing same letters are not significantly different from each other ($P < 0.05$).

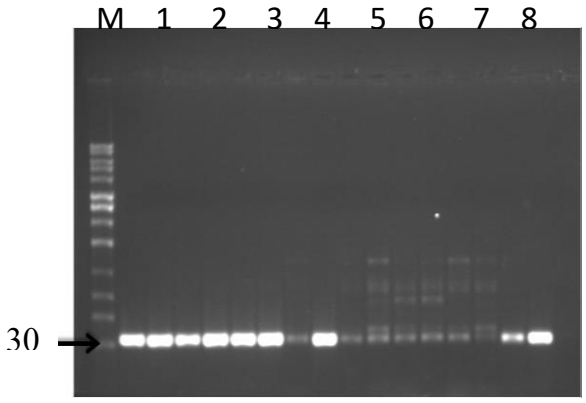

Fig 3. PTYv787/PTYc1121 primer pairs' amplicon of size 300 bp for TYLCV in 8 tomato genotypes from UCC represented by lanes 1-8 (1- K100, 2- K027, 3- K116, 4- K005, 5- K202, 6- LV, 7- K213 and 8- K042). C denotes the negative control while M is 1 kb DNA Ladder.

Table 4. Mean population of whitefly on tomato plants at UCC and Asuansi experimental sites.

Tomato Genotype	Incidence of TYLCVD (%) at indicated DAT and locations					
	30 DAT		45 DAT		60 DAT	
	UCC	Asuansi	UCC	Asuansi	UCC	Asuansi
LV	1.7 cdf	1.1 g	0.91 k	2.5 cfdh	0.7 h	2.8 e
K005	1.8 cdf	1.5 cdf	1.72 ij	3.3 abd	0.7 h	3.0 cd
K027	2.0 abd	1.6 cdf	3.86 a	3.0 abdf	0.9 fg	3.0 cd
K042	2.1 abd	1.6 cdf	1.94 efhj	3.7 ab	1.0 fg	3.3 cd
K100	2.7 a	1.2 e	1.84 ghj	4.2 a	1.1 fg	3.5 ab
K116	2.2 ab	1.3 e	1.48 ij	4.1 a	1.4 f	3.9 a
K202	1.6 cdf	1.6 cdf	0.84 k	3.4 ab	0.9 fg	3.3 cd
K213	1.8 cdf	1.7 cdf	1.4 ij	3.1 abdf	1.0 fg	3.2 ad
Mean	2.0	1.5	1.7	3.4	0.9	3.2

Means in the same column bearing same letters are not significantly different from each other ($P < 0.05$).

Table 5. Mean number of fruits per plant, mean fruit weight {kg) and mean yield (t ha^{--1}) of eight tomato genotypes planted at UCC and Asuansi.

Tomato genotype	Mean number of fruits/plant		Mean fruit weight per plant		Mean fruit yield (t ha^{-1})	
	UCC	Asuansi	UCC	Asuansi	UCC	Asuansi
LV	5.9 b	5.8 bc	3.1 b	2.4 c	6.2 b	4.8 c
K005	9.1 a	8.0 a	4.0 a	4.1 a	8.0 a	8.2 a
K027	4.3 bcd	4.7 cd	2.9 bc	1.4 d	5.8 bc	2.8 d
K042	8.3 a	5.8 bc	3.1 b	1.7 d	6.2 b	3.4 d
K100	7.9 a	8.0 a	4.4 a	4.2 a	8.8 a	8.4 a
K116	5.0 bcd	4.1 d	3.1 b	1.3 d	6.2 b	2.6 d
K202	3.7 d	3.4 d	0.5 e	0.5 e	1.0 e	1.0 e
K213	3.6 d	3.8 d	0.6 e	0.7 e	1.2 e	1.4 e
Mean	6.0 a	5.4 b	2.7	2.0	5.4 a	4.8 b

$Lsd_{(0.05)}$ Location: 0.5 (number of fruits), $Lsd_{(0.05)}$ Genotype*location interaction: 1.4 (number of fruits), Lsd for Location : 0.2 (fruit weight), Lsd for Genotype*location interaction: 0.5 (fruit weight), Lsd for Location: 0.4 (yield), Lsd for Genotype*location interaction: 1.0 (yield).

Table 6. Code, name and sources of 36 tomato genotype screened.

Codes	Genotype names	Source
K 116	Ashanti 2	Ghana (Ashanti Region)
K 045	Tomatose	Ghana (Volta Region)
K 042	Tomatose	Ghana (Volta Region)
K 100	Local 3	Ghana (Upper East)
K 074	Local 6	Ghana (Northern Region)
K 144	BK-Dotvert Yako	Burkina Faso (Burkina Faso)
K 124	Local 1	Ghana (Ashanti Region)
K 005	Petomec	Ghana (Eastern Region)
K 214	AVTO 9001	Taiwan(AVRDC)
K 138	BK-Koly zy	Burkina Faso
K 146	BK-Kong-L6	Burkina Faso
K 194	Magmet	Korea
K 087	5(K)	Ghana (SARI)
K 084	1R	Ghana (SARI)
K 188	Madiso	Korea
K 027	Local	Ghana (Volta Region)
K 098	Local 1	Ghana
K 088	Local1	Ghana (Upper East)
K205A	AVTO 1006	Taiwan (AVRDC)
K 197	REX	Ghana (Eastern Region)
P 077	Local 9	Ghana (Northern Region)
K 213	AVTO 9804	Taiwan (AVRDC
K 083	6(A)	Ghana (SARI)
K 050	Asante tomato	Ghana (Western Region)
K 011	Ntose	Ghana (Eastern Region)
K 106	Local 2	Ghana (Upper East)
P 085	21(B)	Ghana (SARI)
K200	2001 heat tolerant	Ghana (Eastern Region)
K 191	Dyune	Korea
K 186	Superdotaerang	Korea
K 190	Orange carl	Korea
K 006	Power Rano	Ghana (Eastern Region)
K 202	AVTO 0102	Taiwan (AVRDC)
P 009	Mmoboboye	Ghana (Eastern Region)
K 206	AVTO 1008	Taiwan (AVRDC)
L.V	Fadzebegye	Ghana (Central Region)

(K100, K116, K005, K042 and K213) with mild symptoms were re-evaluated at two different ecologies.

Incidence of TYLCVD at Asuansi in forest zone and University of Cape Coast (UCC) in a coastal savannah zone of Ghana

Incidence of TYLCVD at Asuansi located in the forest climatic zone and UCC in coastal savannah zones are presented in Table 2. Forest zone had higher TYLCVD incidence than savanna zone at both 30 and 45 DAT ($P < 0.05$) but similar values were observed at 60 DAT ($P > 0.05$). On average, more plants got infected with time at both locations. A significant genotype by environmental interaction was observed at all the growth stages ($P < 0.05$). At 30 DAT, the incidence of genotypes LV, K005 and K100 ($P < 0.05$) were significantly higher than coastal savanna zone but the two locations had similar incidences for genotypes K027, K042, K116 and K213 ($P > 0.05$). At 45 DAT, incidence of TYLCVD on genotypes K005, K042, K100 and K213 at both locations were significantly different ($P < 0.05$) whereas that of genotypes LV, K116 and K027 were not significantly different ($P > 0.05$).

Evaluation of severity of TYLCVD

The mean severity of TYLCVD on eight tomato genotypes at both forest and costal savannah zones at 30, 45 and 60 DAT were shown in Table 3. TYLCVD severity at forest zone was not significantly different from coastal savanna. The mean genotype by environment interaction at all growth stages for TYLCVD severity was significant ($P < 0.05$). Genotypes K005, K100 and K213 showed mild symptoms at both locations with mean severity scores ranged from 1.1 to 1.6. Genotype K042 showed mild symptoms at coastal savanna condition but moderate symptoms at forest condition, with the mean severity scores of 1.3, 1.5 and 1.6 and 2.1, 2.3 and 2.3 at 30, 45 and 60 DAT respectively. Genotype LV had moderate severity scores of 2.1-2.7 at both locations, for all growth stages. Genotype K116 showed moderate symptoms at coastal savanna condition but severe symptoms at forest condition for all growth stages. Also, genotypes K027 and K202 showed severe and very severe symptoms for the growth period (Table 3).

Mean whitefly infestation* of *eight tomato genotypes

Varying levels of whitefly populations were recorded at both Asuansi and UCC at different sampling dates (Table 4). Records at UCC were significantly higher than Asuansi at 30 DAT ($P < 0.05$). However, Asuansi had higher whitefly populations than UCC ($P < 0.05$) at 45 and 60 DAT. Records of eight tomato genotypes at both Asuansi and UCC showed significant genotype x location interaction effect ($P < 0.05$) at 30, 45 and 60 DAT. At 30 DAT, whitefly population on genotypes LV, K100 and K116 at UCC were significantly higher than at Asuansi. However, insect population on K005, K027, K042, K202 and K 213 at both UCC and Asuansi were not significantly different ($P > 0.05$). At 45 DAT, whitefly population at UCC was significantly different from Asuansi for all genotypes except on K027 ($P > 0.05$).

Mean number of fruits/plant, fruit weight per plant and fruit yield

The effect of different locations on the number of fruits per plant, fruit weight and fruit yields of eight tomato genotypes are shown in Table 5. Number of fruits per plant at the two locations showed significant differences between them ($F_{1, 27} = 6.5$; d.f. = 1; $P < 0.05$). Record from UCC (35.5) was significantly higher ($P < 0.05$) than Asuansi (28.7). A genotype x location interaction effect was significant ($F_{7, 21} = 1.6$; $P < 0.05$). The mean number of fruits per genotypes LV, K005, K027, K100, K116, K202 and K213 at both locations were not significantly different ($P > 0.05$) except K042 at UCC was observed to be significantly higher than Asuansi ($P < 0.05$).

The overall mean fruit weight was significantly different ($F_{7, 30} = 48$; d.f. = 1; $P < 0.05$) for the two locations. The mean fruit weight recorded at UCC (2.7) was significantly higher ($P < 0.05$) than that of Asuansi ($P < 0.05$). A significant genotype by environment interaction effect ($F_{7, 30} = 9.6$; $P < 0.05$) was observed. The mean fruit weight at UCC and Asuansi was similar for genotypes LV, K005, K100, K202 and K213 ($P > 0.05$) except for genotypes K027, K042 and K116 which were significantly higher at UCC than at Asuansi ($P < 0.05$).

Significantly higher fruit yield was recorded at UCC than Asuansi ($F_{7, 21} = 19.2$; $P \leq 0.05$) and these yields are much depended on environment ($F_{26, 163} = 2.11$; $P \leq 0.01$). The mean fruit yield recorded for K027, LV, K042 and K116 at UCC were significantly higher than at Asuansi ($P \leq 0.01$) while K005, K100, K202 and K213 had similar values for UCC and Asuansi.

PCR detection of tomato yellow leaf curl virus DNA in eight genotypes in the forest and coastal agro-ecological zones

The amplicon obtained from AV494/AC0148 primer pairs had a size between 550-600bp for TYLCV DNA as shown in Fig 2. Eight genotypes (K100, K027, K116, K005, K202, LV, K213 and K042) from UCC had amplification and six from eight genotypes (K100, K027, K116, K005, K202 and K042) were amplified from Asuansi samples. The lack of amplification in two genotypes signified their resistant to the virus. Generally, bands from UCC tomato samples were stronger than those from Asuansi. Secondly, all tomato samples from both UCC and Asuansi were amplified by primer pair PTYv787/PTYc1121 and size up to 300 bp (Fig 3.). This signifies that all genotypes were infected with the same strain of the virus. The UCC samples produced stronger bands, except genotypes K213, compared with the Asuansi samples except genotypes K213 and K042 (Fig 3.).

Discussion

Host plant resistance/tolerant to TYLCVD at coastal savannah zone

All the 36 tomato genotypes showed significant variation in the incidence and severity of the TYLCVD. The infection rate and development of TYLCVD varied from one genotype, growth stage and environment to another. These variations could be due to the differences in the genetic makeup of host plants, stage of infection of various viral strains, the favorability of the environment to virus and the whitefly vector present (Delatte et al., 2006; Azizi et al., 2008). This is congruent with whitefly populations at both Asuansi and UCC being highly influenced by genotype by environmental interaction. Favourable environmental factors significantly contributed to the development of vector population for faster infestation of 36 genotypes with the virus. Moreover, the variation in the incidence of TYLCV was also due to early attraction of vectors to some genotypes and the longer time used for the transmission of the TYLCV (Osei et al., 2012).

Variation in the incidence and severity of the disease could again be due to the age of plants at the time of infection probably due to increase in photosynthetic capacity, nutrient uptake or oxidative enzyme activity with age (Kessler and Baldwin, 2002). According to Pico et al. (1996) plants infected or inoculated at older age produce milder symptoms of disease which may be wrongly considered as manifestation of genetic resistance. This could partly explain why genotypes K005, K100, K042, K116 and K213 were cleat at 30 DAT, but began to show mild symptoms at 45 to 60 DAT.

Evaluation of eight tomato genotypes at coastal savannah and forest agro-ecological zones

Some genotypes either withstand the activities of the whitefly or recover from infectious virus activities (Smith, 2012). Those host plants that resist impact of the virus or its vector across locations with persistent mild symptoms observed were K005, K100 and K213 observed.

Quantitative traits of genotypes are influenced by different ecological zones (Al-ani et al., 2011*)*. Some genotypes did not show stable disease condition across locations. Such genotypes include K042 with mild symptoms at UCC but moderate symptoms at Asuansi. K116 with moderate symptoms at UCC but severe symptoms at Asuansi and genotypes K027 and K202 with severe symptoms at UCC but very severe symptoms at Asuansi, are said to be environmentally influenced. Highly environmentally influenced genotypes do not exhibit host plant resistance to the disease. These findings corroborated with Gibson et al., (2006) who stated that a resistant or tolerant variety could display poor resistance to a disease and a susceptible variety having more and worse disease severity at different environment. This could explain why some genotype performed better in the coastal zone reacted differently to TYLCV infection at the forest zone. Consequently, it has been reported (Obeng-Antwi et al., 2012), that it is better to evaluate different genotypes in different ecological zones to know their true genetic potential of disease resistance and environmental preferences.

During initial screening experiment at UCC, K100, K005, K042, K116 and K213 were clean of the disease at 30 DAT, but during evaluation trial at both locations, they showed

early (30 DAT) symptoms of TYLCVD at forest zone. It can therefore be deduced that disease pressure is high at forest than coastal agro-ecology and the early infection now observed could account for any yield difference. Even though the tomato genotypes K100, K005, K042, K116 and K213 experienced early infection during the evaluation experiment, genotypes K100 and K005 had higher yields than the average yield of 7.5 t ha^{-1} reported by MoFA (2011). It can therefore be deduced that even when disease pressure is high, K100 and K005 produced above average yields and could be said to possess a stable mild resistance to TYLCVD. The mild resistance means the disease has just started or started early but host conditions localized it and prevented it from spreading and having any significant impact on economic yield loss. Genotypes K100 and K005 could be advanced to farmers' field trial stage to enable breeders to compare the potential yield obtained at the research field with the actual yield on the farmers field before release. The commercial variety *Fadzebegye tires* (LV) which is an improved *S. pimpinellifolium* showed moderate symptoms and was therefore stable during the period of evaluation in terms of yield. This could explain the reason for its wide cultivation in the region.

The possible existence of different strains of TYLCV at coastal (UCC) and forest (Asuansi) experimental sites have resulted in different resistance reactions at the two locations. This was supported by the PCR test conducted with the two degenerate primer pairs. Primers AV494/AC0148 detected the virus in all the 8 genotypes from UCC farm but, could not detect the virus in genotype K213 from Asuansi farm (Fig 2.). This shows that UCC strains might have be different from Asuansi strains. Primers PTYv787/PTYc1121 was able to amplify the geminivirus in all the samples from both UCC and Asuansi, however, the DNA amplification generally resulted in stronger bands in the UCC samples, except genotypes K213, and weaker bands with the Asuansi samples except genotypes K213 and K042 (Fig 3.). K213 and K042 show host plant resistance to TYLCV than the remaining genotypes studied. Rotbi, et al. (2015) and Potter (2003), also attributed failure of primer amplification to viral concentration accumulated in those genotypes not being enough to encourage amplification. Again failure in the detection of viral DNA can be attributed to the absence of complementarity sequence at primer annealing sites though these primers have been shown by Rojas et al., (1993) and Osei et al., (2008) to be effective in the detection of begomoviruses. Variation in primer annealing sites can be a possible reason in terms of the primer's failure in detecting viral DNA (Hasan, 2013). This suggests K213 was more resistant / tolerant to both old and any new strain of TYLCV with high levels of variation with respect to primer annealing sites than the rest of the genotypes.

Materials and Methods

Plant materials

The 36 genotypes represent local and exotic tomatoes cultivars currently available and accessible in Ghana. The germplasm were collected from Research and Educational centers, seed vendors, markets and tomatoes farmers in Ghana. Table 6 showed codes, local names and some sources.

Experimental sites

The coastal savannah zone is the first experimental site located at UCC on latitude 5°10'N, longitude 1.2°50'W, with Acrisol soil type (Parker et al., 2010) and is a highly endemic site for TYLCVD. The site has a bi-modal rainfall pattern with 750 mm -1000 mm annually(Parker et al., 2010) and 23.2 - 33.2 ºC, with an annual mean of 27.6 ^{o}C (Owusu Sekyere et al., 2011). The second site is Asuansi Agricultural Station located in the forest zone. Asuansi has a mean rainfall of 980 mm, with double maxima (bimodal) distribution, with an average monthly temperature of 26.9 ºC. The soil is known to be rich in minerals especially potassium (MoFA, 2011). Both sites are located in the Central Region of Ghana.

Experimental design and field layout

The first experiment was conducted at UCC with 36 tomato genotypes representing the current available and accessible germplasm in Ghana. They were nursed and transplanted in a triangular pattern on 36 sub-plots ($8.5m^2$), spaced at 0.5m between plots in a block. Each genotype was planted in a randomized complete block design, with three reps on a field size of $918m^2$ (54m x 17m). Each sample plot had ten plants arranged in two rows of five.

The second experiment was conducted at UCC located in the coastal savannah climate and "Asuansi" an experimental station located in the forest climate. Five(5) genotypes (K100, K116, K005, K213 and K042) selected for mild severity (ms) and three (3) genotypes (K027, K202, LV) selected for severe symptom (SS) of TYLCVD expression were evaluated on a field $408m^2$ (48m x 8.5m) using RCBD with three replications. Each sample plot had 20 plants arranged in four rows of five.

Agronomic practices

Watering and weeding were done when necessary. N.P.K (15-15-15) fertilizer was applied at the rate of 250 kg ha^{-1} at three WAP. Fungicide (Mancozeb, 800 g/kg) was applied at a rate of 150 – 200 g per 100 L water to control blight and leaf spot diseases.

Data collection

Morphological evaluation of tomato genotypes against TYLCV infection

Morphological data were collected following standard tomatoes descriptor (IPGRI, 1996). The incidence and severity of TYLCVD and whitefly populations were scored at 30, 45 and 60 DAT. Disease incidence was determined as the proportion of infected plants per plot, expressed as a percentage of total number of plants observed (Javed et al., 2012). Disease severity was rated on individual plants using a visual scale of 0-4 (where 0 = No symptoms (healthy); 1 = Slight yellowing (mild symptom); 2 = Leaf curling and yellowing (moderate symptom); 3 = Yellowing, curling and cupping (severe symptom); and 4 = Severe stunting, curling and cupping (very severe symptom)) following standard operating procedures of AVRDC (Lapidot, 2002).

Molecular evaluation of tomato genotypes against TYLCV infection

The molecular assay was done to confirm the infection or otherwise of the (8) eight tomato genotypes by TYLCV at both coastal and forest zone ecological sites.

Collection of tomato leaf samples

Fresh leaf samples from all the eight tomato genotypes at both locations were taken at 30 days after transplanting, cleaned with 70% ethanol and kept on ice for DNA extraction with E.Z.N.A.® HP Plant DNA Mini Kit (Omega Bio-tek, Inc.) according to manufacturer's instruction. The DNA was eluted with 100 µL pre-warmed (65^{o}C) elution buffer, and then kept at -20 ^{o}C until needed for PCR.

Polymerase chain reaction (PCR)

The PCR cocktail in 20 µL reaction mix was performed with PTYv787/PTYc1121 and AC1048/AV494 pairs of degenerate primers, namely. PTYv787/PTYc1121 had a sequence (5' – 3') of F- GTTCGATAATGAGCCCAG /R-ATGTAACAGAAACTCATG and expected band size of ~300 (Zhou et al., 2008). AV494/AC1048 also had a sequence (5'–3') of F- GCCCATGTATAGAAAGCCAAG/ R:GGATTAGAGGCATGTGTACATG with expected band size between 550-600 bp (Wyatt and Brown, 1996).

The reaction mixture comprises of 50 ng DNA template, 5 pmoles each of forward and reverse primers, 2 U Taq DNA polymerase, 2.5 mM dNTP, 1.5 mM MgCl2, PCR buffer, and sterile distilled water. The reaction was performed in a pre-heated Applied Biosystems 2720 thermal cycler with the following reaction conditions: 94ºC for 4 minutes, 35 cycles of 94 ºC for 30 seconds with annealing temperature of 53 ºC for 1minute, 72ºC for 1 minute and final extension at 72ºC for 10 minutes. The PCR products were separated by electrophoresis in 1% agarose gel in 1 x TAE buffer at 100 V for 45 minutes. The gel was stained with ethidium bromide and viewed under UV light.

Data analysis

Significant levels of quantitative data were determined by ANOVA and the means were separated by Least Significant Difference (LSD) test at 95% confidence level. Data on disease incidence and whitefly population were transformed by angular and square root transformations respectively, in order to homogenize variances before ANOVA. The dendrogram was plotted following between-groups linkage cluster method based on squared Euclidean distance interval measure according to Nei (1973).

Conclusion

In the first trial under coastal savanna condition, mild symptoms were observed on genotypes K213, K005, K042, K100 and K116 at 60 DAT because the disease incidence started late, i.e. after 45 DAT, whilst moderate to severe symptoms were observed at 60 DAT for the rest of genotypes because the disease incidence started early, ie before 45 DAT. To be sure of host plant resistant to the TYLCVD, five genotypes with mild symptoms were re-evaluated with three checks under Coastal savannah and forest conditions using RCBD. The mild condition had changed for some to moderate severe and severe conditions. Genotypes K100 and K005 however showed mild and stable resistance to TYLCV infection across locations with high fruit yields. The forest conditions had higher disease pressure than the coastal savanna zone, as result yields were better at coastal environment than forest condition. PCR test with two primers for UCC samples revealed the presence of TYLCV DNA in all the eight genotypes, indicating that they were susceptible to TYLCVD. PCR test with two primers for Asuansi samples revealed that PTYv787/PTYc1121 primer revealed the presence of TYLCV DNA in all the eight genotypes but AV494/AC0148 primer could not amplify viral DNA in LV and K213 genotypes, suggesting that TYLCVD expression observed could come from infection by different strains. The whitefly population was affected by genotype by environment interaction effect (P < 0.05) at 30, 45 and 60 DAT, hence whitefly population expected to vary from location to location.

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