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Field evaluation of tomato varieties/breeding lines against tomato yellow leaf curl virus disease (TYLCV)

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ABSTRACT

Tomato Yellow Leaf Curl Virus (TYLCV) is currently the most devastating virus of cultivated tomatoes in tropical and subtropical regions, accounting for significant yield losses in cultivated tomato in Ghana. Severe population outbreaks of the whitefly vector (*Bemisia tabaci*), are usually associated with high incidence of the disease. Resistance breeding is the surest solution to TYLCV in developing viable seeds for increased tomato production in Ghana. The Wild tomato (*Solanum pimpinellifolium* L.) is a recognised crop Wild species (CWS) with resistance genes to different diseases including the TYLCV disease and possesses good fruit quality traits in Ghana. Three (3) cultivated tomato varieties and seven breeding lines developed from crosses between the Wild tomato and three hybrids, three backcrossed lines and the Wild tomato were evaluated with their parents against TYLCV disease under local field conditions. Field appraisal of whitefly populations, disease incidence and severity, agronomic and yield characteristics of the tomato varieties/breeding lines were undertaken to hasten selection of tolerant/resistant varieties or breeding lines in the breeding programme. Wild tomato (ISS_{AP} = 0.31 and ISS_{DP} = 0.76) and Woso (ISS_{AP}

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= 1.90 and ISS_{DP} = 2.27) recorded the least and highest average symptom severity on all plants (ISS_{AP}) and diseased plants only (ISS_{DP}); while the least and highest disease incidence was recorded by the Wild tomato (11.10%) and Roma (43.05%). Roma which recorded the highest population of whiteflies in the dry season also exhibited the highest symptom severity on all plants as well as diseased plants during the study period. There was a significant symptom relapse in Wild tomato and Woso x Wild in 6-8 WAT for both ISS_{AP} and $ISS_{DP.}$ Number of fruits per plant, ISS_{AP} and ISS_{DP} were positively and/or inversely correlated (r = 0.98, 0.93, -0.83) with average whitefly count, percent disease incidence and yield (t/ha).

Keywords: Backcross, Geminiviruses, varieties/ breeding lines, Ghana, Tomato, TYLCVD, resistance breeding

INTRODUCTION

Tomato yellow leaf curl virus (TYLCV) disease, caused by geminiviruses and transmitted by whitefly (*Bemisia tabaci* Genn.), has become a major problem in tomato cultivation globally, particularly in the tropics and subtropics (Czosnek & Laterrot, 1990, pp.1-6; Moriones & Navas-Castillos, 2000, pp.123-124; Moriones, Amo, Accotto, Noms, & Cavallarin, 1993, p. 953; Bellotti & Arias, 2001, pp.813-824). In Ghana, TYLCV disease is reported to be widespread, accounting for severe yield losses (Horna, Smale, & Falck-Zepeda, 2006, p. 23; Osei, Akromah, Shih, & Green, 2010, pp. 315-323). Three new distinct TYLCV-causing Begomoviruses were detected and reported from Akumadan and Kumasi, the major tomato producing communities in the country (Osei, Akromah, Shilh, & Green, 2008, p. 1585). Production losses vary from very dramatic to mild with devastating yields loss in the rainy season (Osei et al., 2010, pp. 315-323; Horna et al., 2006, p. 27). Whitefly populations and diseases in the dry season are usually severe during the dry season, especially with relatively higher incidence of *Bemisia tabaci* from cassava fields in the dry season (Appiah et al., 2012, pp.31-37). This variation in whitefly population has been attributed to differences in temperature and relative humidity (Triparthi & Varma, 2002, p.476).

Most commercial tomato varieties have been found to be completely susceptible to TYLCV, compelling breeders to screen Wild tomato accessions and some commercial varieties for potential resistance genes (Pilowsky & Cohen, 2000, pp. 351-353). Breeding for resistance to TYLCV in cultivated tomato varieties appears to be the most ideal control measure for the virus (Pico, Diez, & Nuez, 1999, p.1008; Osei et al., 2008, p.1585; Horna et al., 2006, p.31). Efforts have been initiated to introgress resistance genes from Solanum pimpinellifolium into some commercial varieties/breeding lines in Ghana (Quartey, 2010, p.173; Nunoo, 2010, pp.87-105). Field evaluation is essential to identify resistant plants after introgression of resistance gene from resistant plants. Field evaluation of resistance in some

tomato varieties/breeding lines has been widely used for primary appraisal of resistant lines (Osei et al., 2010, pp. 315-323; Lapidot & Friedmann, 2002, p.127). Under natural field conditions, spontaneous whitefly inoculation occurs, inducing severe TYLCV symptoms, especially during high whiteflies populations in the field. A number of breeding lines including three newly generated backcross lines (BC1) were developed and three of these breeding lines and their parents, together with their respective first backcross (BC1) generations and a local accession of the Wild tomato (*S. pimpinellifolium*) were evaluated in the field for their resistance to TYLCV disease. The objective of this study was to identify TYLCV resistant/tolerant varieties/breeding lines among 10 breeding lines of tomato.

MATERIALS AND METHODS

Study Area

The experiment was conducted at the research farm of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Kwabenya, Accra. The experimental field is located at latitude 05°40'N and longitude 0°13'W, at an elevation of 76 m above sea level within the Coastal Savannah Agro-Ecological Zone. The soil at the site is the Nyigbenya-Haatso series, which is a typically well-drained savannah Ochrosol (Ferric Acrisol) derived from quartzite schist (FAO/UNESCO, 1994, p.146). The maximum and minimum average temperatures for the study period were 30.7 and 23.2 oC respectively with a mean annual rainfall of 220 mm (Local Weather Station, 2013).

Experimental Material

Seeds of three tomato varieties, six breeding lines and a landrace (Wild) were raised in a nursery and seedlings transplanted to the field for evaluation in the field against tomato yellow leaf curl virus disease (TYLCVD) (Table 1).

Table 1
Identities and characteristics of tomato varieties/breeding lines used in the study

Varieties/Breeding Lines	Status	Pedigree	Growth habit
S. pimpinellifolium	Wild	-	Indeterminate
Wosowoso	Local	-	Determinate
Cherry Red	Exotic	-	Determinate
Roma	Exotic	-	Determinate
Hyb-1	Hybrid	Woso x Wild	Semi-indeterminate
Hyb-2	Hybrid	Roma x Wild	Semi-indeterminate
Hyb-3	Hybrid	C-Red x Wild	Semi-indeterminate
BC-1	Backcross	Woso x (Woso x Wild)	Semi-indeterminate
BC-2	Backcross	Roma x (Roma x Wild)	Semi-indeterminate
BC-3	Backcross	C-Red x (C-red x Wild)	Semi-indeterminate

Experimental Design and Field Management Practices

Seedlings were raised in trays filled with a mixture of topsoil, cow dung and coconut husk in the ratio 3:1:1 in a screen house. At 28 days after sowing (DAS) when 3-4 leaves were fully expanded, the seedlings were transplanted to the field. The Randomised Complete Block Design (RCBD) was used with four replications. A plot size of 3.2 m x 3.6 m with a planting distance of 80 cm x 60 cm was used for all varieties/breeding lines. Each plot contained 24 plants out of which ten (10) inner-rowed plants were randomly selected and tagged for data collection on all parameters studied. Agronomic practices such as watering, mulching and fertilisation were undertaken. Watering was done twice daily for the first two weeks after transplanting (2 WAT) and subsequently reduced to once daily using a watering can. The NPK (15-15-15) fertiliser was applied two weeks after transplanting at 250 kg ha-1 (Osei et al., 2010, pp. 315-323). Hand weeding was done frequently to control weeds. To avoid bias in data collection, pests and diseases were not controlled.

Data Collection

Whitefly Population Survey. A weekly count of whitefly populations was done on five broadly expanded leaves that were randomly selected on each of the 1 sample plants. Counting was started 2 WAT (after transplanting) and continued weekly for seven weeks. Leaves were gently turned with little or no disturbance of the whiteflies and their number on the adaxial side of each leaf were counted. This was done early morning (6:00am - 7:30am) before sunrise to avoid whiteflies from being too active when the sun rises.

Disease Incidence and Symptom Severity. Symptoms of TYLCV on the 10 sampled plants of each of the 10 tomato varieties/ breeding lines were observed and scored. A five-point scale adapted from Friedmann, Lapidot, Cohen and Pilowsky (1998, pp. 1004-1007) was used to score symptom severity at 2, 4, 6 and 8 weeks after transplanting (WAT). The scoring scales were: 0 = No visible symptoms, 1 = Slight yellowing of margins of apical leaflets, 2 = Moderate yellowing and slight curling of leaflet tips, 3 = Extensive leaf yellowing, curling and cupping with some reduction in leaf size, 4 = Very severe stunting of plant and leaf yellowing, pronounced cupping and curling of leaves (Plate 1 and 2). The disease incidence (DI) (number of symptomatic plants as per the number of plants on each plot) was also recorded at 2, 4, 6 and 8 WAT.

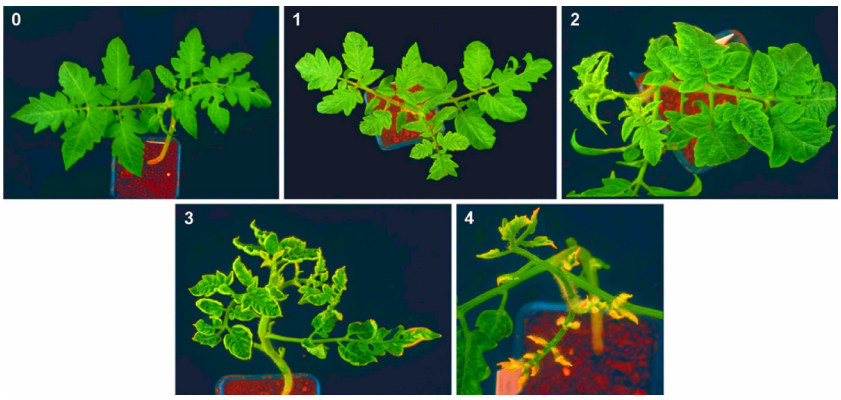

Plate 1. Tomato leaves showing varying degrees of symptom severity from TYLCV infection. Symptom scoring scale: 0-4 according to Friedmann et al. (1998)

Severity of the symptoms was estimated using the formula advanced by Njock and Ndip (2007).

(A) Index of severity of symptoms based on all plants (ISS_{AP}) = $(\sum_{S=0}^{4} (SXs) / \sum_{S=0}^{4} (Xs))$

Where S is severity class (0 – 4)
X is the number of plants giving the score S, and AP is all plants.

(B) Index of severity of symptoms based on diseased plants only.

$(ISS_{DP}) = (\sum_{S=1}^{4} (SXs) / (\sum_{S=1}^{4} (Xs))$

Where DP = diseased plants only

(C) Percent disease incidence was calculated as:

DI % = $100 ((\sum_{n=0}^{4} (SXs) / (\sum_{n=0}^{4} (Xs))$

Agronomic Evaluation and Yield Characteristics of 10 Tomato Varieties/ Breeding Lines. The following data was collected on tagged plants of the 10 varieties/breeding lines during the field trial using Descriptor List for tomato from the International Plant Genetic Resources Institute (IPGRI, 1991). Data was taken on number of days to 50% flowering; number of days to maturity; plant height; number and weight of fruits per plant and yield per hectare (t/ha) was estimated.

Statistical Analyses

Statistical analyses on all studied parameters were performed using GenStat statistical package software (Payne et al., 2007; ver. 12.0), Statgraphics (2010; Plus XV.I) and Microsoft Excel (ver. 2010). Mean of number of whiteflies, fruit number per plant, ISS_{AP} and ISS_{DP} were square root transformed [square root of (x + 0.5)] whereas means of percent disease incidence (DI) were arcsine transformed before

performing ANOVA. Pearson correlation analysis was performed on disease-related parameters of the varieties/breeding lines studied.

RESULTS

Whitefly Populations on 10 Tomato Varieties and Breeding Lines

The mean number of whiteflies counted in Roma, Wosowoso, Cherry Red and Woso x WW was relatively higher compared with Roma x RW and Wild tomato (Table 2). Average whitefly count showed significant differences ($p \leq 0.05$) among the 10 varieties/breeding lines (Figure 1). At 2 WAT, all tomato varieties/breeding lines had relatively high whitefly populations but decreased gradually by 3 WAT except Woso and Cherry Red. Whitefly numbers increased in 4 WAT for all varieties/breeding lines after which it fluctuated till 6 WAT. However, there was a reduction in whitefly counts at 7WAT with the lowest recorded for all the tomato varieties/breeding lines at 8 WAT. In general, whitefly preference for all the varieties/breeding lines was observed during the seven weeks of survey. The highest mean of whiteflies was found on Roma (49.08), whilst Wild tomato had the least (19.58). In the Roma variety, no significant differences were observed between mean whitefly counts from 2 WAT to 5 WAT and between 6 WAT and 7 WAT. However, a significant reduction was observed at 8 WAT. No significant differences were recorded between the average weekly whitefly counts over the whole survey period for Woso (Tab 2). There were relatively higher numbers of whiteflies in the early stages of the survey. Wild tomato recorded the lowest whitefly count throughout the study period.

Generally, fewer whitefly numbers were recorded in both the hybrid and backcross lines in comparison to the parental lines. The highest whitefly number was recorded at 2 WAT where significant differences were observed in the various varieties/breeding lines. At 3 WAT, Woso x Wild, Woso x WW, Cherry Red, Cherry Red x Wild, Cherry Red x CRW did not show any significant differences in whitefly numbers. There were however, significant differences in whitefly numbers at 2,4,5,7 and 8 WAT. Significant difference in weekly whitefly counts were observed in all varieties/breeding lines except Roma and Wosowoso. There were however, no significant difference between the weekly whitefly counts in Roma from 2 WAT-5 WAT and Wosowoso from 3 WAT-8 WAT (% CV: 11.76-15.75).

Table 2
Variation in average whitefly count with time on tomato varieties/breeding lines

Varieties/ Breeding Lines	Weekly Whitefly Counts							
	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT	Mean
Wild	19.58^{e}	19.23^{c}	19.90^{e}	21.00^{d}	21.38^{e}	20.03^{f}	14.65^{e}	19.39^{d}
Woso	43.75^{ab}	44.98^{a}	46.28^{a}	44.70^{a}	45.56^{a}	40.08^{a}	32.15^{a}	42.50^{a}
C-Red	30.33^{cd}	30.63^{b}	34.13^{bcd}	34.78^{b}	34.98^{bc}	33.08^{bc}	20.15^{c}	31.15^{b}
Roma	49.08^{a}	44.03^{a}	45.80^{a}	47.43^{a}	43.80^{ab}	37.75^{ab}	26.80^{b}	42.10^{a}
Hyb-1	35.80^{bcd}	31.03^{b}	30.88^{bcd}	29.68^{bc}	30.63^{cd}	29.60^{cd}	17.30^{cde}	29.27^{bc}
Hyb-2	28.23^{de}	27.35^{b}	29.23^{bcd}	29.98^{bc}	29.95^{cd}	28.50^{cd}	20.18^{c}	27.63^{bc}
Hyb-3	36.30^{bcd}	30.90^{b}	35.28^{bc}	34.25^{b}	28.5^{3cde}	27.55^{cde}	21.05^{c}	30.55^{b}
BC-1	40.88^{abc}	31.25^{b}	37.50^{ab}	31.90^{bc}	32.13^{cd}	28.00^{cde}	16.22^{de}	31.12^{b}
BC-2	28.75^{de}	25.23^{bc}	26.85^{d}	27.78^{c}	26.30^{de}	22.93^{ef}	15.80^{de}	24.80^{cd}
BC-3	31.23^{bcd}	28.55^{b}	27.38^{cd}	27.83^{c}	28.39^{cde}	25.18^{de}	19.55^{cd}	26.87^{bc}
CV%	15.75	14.05	14.48	12.71	13.72	11.76	13.58	15.75

Means in the same column and row followed by the same letter are not significantly different ($p \leq 0.05$)

Disease Incidence of TYLCV on 10 Tomato Varieties/Breeding Lines

Generally, disease incidence was observed on all the tomato varieties/breeding lines from 4-8WAT (Figure 1). Disease incidence for all the varieties/breeding lines increased during the evaluation period till 8WAT when the highest incidence was recorded. The Roma and Woso recorded the highest average percent disease incidences (43.1% and 30.5% respectively) whereas the lowest disease incidence (11%) was recorded by Wild tomato. All varieties/breeding lines recorded no incidence of disease at all or less than 5% at 2WAT and increased gradually to 8WAT except Roma (Figure 1) where 20% disease severity was recorded at 2 WAT.

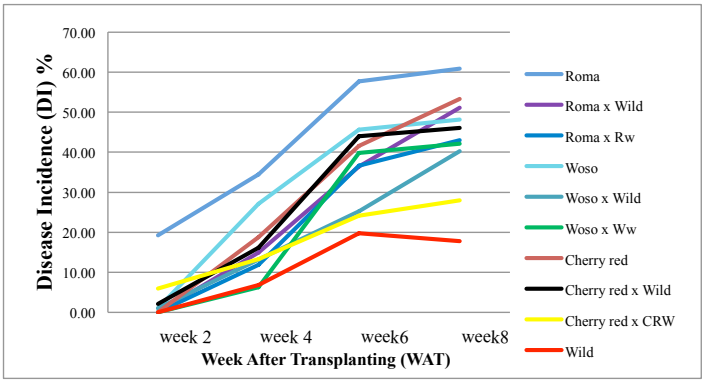

Figure 1. Average Disease Incidence on Tomato Varieties/Breeding Lines Across 8WAT

Symptom Severity of TYLCV on 10 Tomato Varieties/Breeding Lines

In general, symptom severity increased from 2-8WAT for both ISS_{AP} and ISS_{DP} in all the varieties/breeding lines. The Wosowoso gave the highest score in terms of ISS_{AP} and ISS_{DP} (1.9 and 2.27) respectively. Roma recorded the highest symptom severity for ISS_{AP} and ISS_{DP} over the entire study period (Table 3) whilst Wild tomato had the lowest ISS_{AP} and ISS_{DP} values. Wild tomato, Woso x Ww, Roma x Rw, Roma x Wild did not show any symptoms at all. Furthermore, all the backcross lines expressed only mild symptoms throughout the period except Roma x Wild which recorded 1.23 ISS_{AP} at 8WAT. All the F3 hybrid lines expressed mild symptoms (ISS_{DP}<2) during the study period, compared with the parental lines (Table 3). The lowest ISS_{AP} and diseased ISS_{DP} were observed in Wild tomato, and Woso x WW. There were, however, symptom reversion in Wild tomato and Woso x Wild at 6 and 8 WAT. Differences among the 10 varieties and breeding lines with respect to ISS_{AP} and ISS_{DP} were highly significant ($p \leq 0.05$).

Table 3
Variation in TYLCV symptom severity with time on 10 tomato varieties/breeding lines

Varieties/Breeding Lines	Disease Symptom Severity (%)									
	ISS_{AP}					ISS_{DP}				
	2WAT	4WAT	6WAT	8WAT	Mean	2WAT	4WAT	6WAT	8WAT	Mean
Wild	0.00^{b}	0.20^{cd}	0.58^{e}	0.48^{d}	0.31^{b}	0.00^{b}	0.63^{c}	1.26^{de}	1.15^{c}	0.76^{b}
Roma	0.53^{a}	1.33^{a}	2.68^{a}	3.08^{a}	1.42^{a}	1.25a	1.98^{a}	2.77^{a}	3.08^{a}	1.63^{a}
Wosowoso	0.03^{b}	1.05^{ab}	2.13^{ab}	2.48^{a}	1.90^{ab}	0.25^{b}	1.60^{ab}	2.21^{ab}	2.48^{a}	2.27^{a}
Cherry red	0.00^{b}	0.65^{abc}	1.15^{cd}	1.35^{b}	0.79^{ab}	0.00^{b}	1.44^{ab}	1.60^{cd}	1.49^{bc}	1.13^{ab}
Roma x Wild	0.00^{b}	0.45^{bcd}	1.08^{cde}	1.38^{b}	0.56^{ab}	0.00^{b}	1.20^{abc}	1.52^{cde}	1.53^{bc}	1.10^{ab}
Woso x Wild	0.03^{b}	0.35^{bcd}	0.75^{de}	1.10^{bc}	0.73^{ab}	0.25^{b}	1.15^{abc}	1.44^{cde}	1.56^{bc}	1.06^{ab}
Cherry red x Wild	0.08^{b}	0.40^{bcd}	1.50^{bc}	1.45^{b}	0.86^{ab}	0.25^{b}	0.94^{abc}	1.83^{bc}	1.70^{b}	1.18^{ab}
Roma x RW	0.00^{b}	0.20^{cd}	0.65^{e}	1.23^{bc}	0.48^{b}	0.00^{b}	0.75^{bc}	1.12^{e}	1.66^{bc}	0.78^{ab}
Woso x WW	0.00^{b}	0.18^{d}	0.95^{cde}	0.78^{cd}	0.52^{b}	0.00^{b}	0.56^{c}	1.33^{de}	1.22^{bc}	0.88^{b}
Cherry red x CRW	0.13^{b}	0.33^{cd}	0.75^{de}	0.85^{bcd}	0.51^{ab}	0.25^{b}	0.90^{abc}	1.41^{cde}	1.41^{bc}	0.99^{ab}
CV%	217.19	64.87	30.54	31.01	61.95	203.62	49.03	17.05	18.54	48.34

CV = coefficient of variation, ISSAP = index of symptoms severity based on all plants only, ISSDP = index of symptoms severity based on diseased plants only, WAT = weeks of transplanting

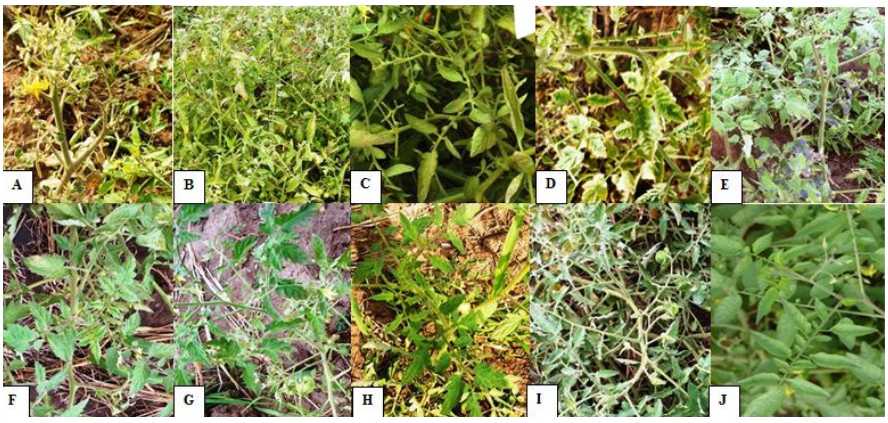

Plate 2. Variation in Leaf Symptoms of TYLCD among Ten Tomato Varieties/Breeding Lines in the Field. (A) Roma, (B) Roma x Wild, (C) Roma x Rw, (D) Wosowoso, (E) Woso x Wild, (F) Woso x Ww, (G) Cherry red, (H) Cherry Red x Wild, (I) Cherry Red x CRW, (J) Wild tomato

Agronomic Characteristics

Days to Flowering, Fruit Maturity, Plant Height (WAT) and Yield. Among the 10 varieties/breeding lines evaluated in the dry season, differences in the mean number of days to first flowering and 50% flowering were highly significant ($p \leq 0.05$) for Roma x RW, Woso, Cherry Red and Cherry Red x CRW. Differences in the mean number of days to first fruit maturity and 50% maturity were also highly significant ($p \leq 0.05$) for all varieties/breeding lines except Roma and Roma x RW (Table 4). In the dry season, Cherry red was the first to flower (41.25 days) but Cherry red x Wild was the first to attain fruit maturity (79.75 days after sowing seed at nursery) while Roma was the last to flower (47 days) and mature (98 days). Variations were observed in plant height recorded on the 10 tomato varieties/breeding lines. Generally, plant height increased with age for all the 10 varieties/breeding lines (Table 4). Average plant height at two, four and eight weeks after transplanting (WAT) represent average days to first, 50% flowering and 50% maturity respectively. Woso recorded the highest plant height at first flowering (30.6 cm), Cherry red for 50 % flowering (47.85 cm) and Cherry red x CRW for 50 % maturity (91.75 cm) (Table 4). The least plant height at first flowering, 50% flowering and 50% maturity were recorded in Wild tomato (20.4, 32.94 and 67.23 cm) respectively. The differences among mean plant heights of the varieties/breeding lines were highly significant ($p \leq 0.05$). Yield was determined by the number of fruits harvested per plant, average fruit weight per plant and total yield extrapolated as tonnes/ha (Table 4). In general, Roma x Rw breeding line attained the highest fruit yield in terms of all yield components. There were highly significant differences ($p \leq 0.05$) among the tomato varieties/breeding lines for average number of fruits per plant and average fruit weight per plant/(g) or average number of fruits per plant and yield (t/ha) (Table 4). The highest number

of fruits per plant, average fruit weight (g) and total fruit yield (t/ha) were achieved in Wild tomato (40.55), Woso (119.26 g) and Roma x Rw (48.49 t/ha) respectively at the end of the growing period. The least number of fruits per plant, average fruit weight (g) and total fruit yield (t/ha) were achieved in Roma (0.7), Wild tomato (11.10 g) and Roma (1.76 t/ha) respectively (Table 4).

Table 4
Days to flowering, fruit maturity, plant height 8 WAT and yield among the 10 varieties/breeding lines

Varieties/ Breeding Lines	Days to Flowering and Fruit Maturity				Plant Height of the Tomato Lines over 8WAT					Yield		
	DFFl	FpFl	DFF	FpFr	2 WAT	4 WAT	6 WAT	8 WAT	Mean	AFP	AFWtP (g)	FY (t/ha)
Wild	44.75^{abc}	50.75^{abc}	81.50^{ab}	90.00^{bc}	20.40^{d}	32.94^{c}	47.18^{b}	67.23^{d}	41.93^{d}	40.55^{a}	2.78^{c}	2.73 x 10^{-6e}
Roma	47.00^{a}	56.50^{a}	98.00^{a}	114.50^{a}	23.43^{cd}	34.38^{bc}	57.35^{ab}	69.58^{cd}	46.18^{cd}	0.70^{d}	13.72^{b}	13.50 x 10^{-6b}
Wosowoso	42.75^{bc}	55.50^{ab}	93.75^{ab}	103.00^{ab}	30.60^{a}	45.35^{a}	67.38^{a}	86.08^{abc}	57.35^{b}	6.15^{cd}	29.82^{a}	29.35 x 10^{-6a}
Cherry red	41.25^{c}	49.50^{bc}	88.00^{ab}	100.00^{ab}	29.50^{ab}	47.85^{a}	69.75^{a}	89.00^{ab}	59.03^{a}	17.65^{abc}	13.03^{b}	12.82 x 10^{-6b}
Roma x Wild	46.00^{ab}	55.50^{ab}	90.00^{ab}	98.25^{ab}	29.50^{ab}	41.40^{ab}	67.20^{a}	88.33^{ab}	56.61^{b}	24.63^{abc}	4.40^{dc}	4.33 x 10^{-6de}
Woso x Wild	44.50^{abc}	55.75^{ab}	93.50^{ab}	102.00^{ab}	30.00^{ab}	39.90^{abc}	67.53^{a}	84.90^{abc}	55.58^{b}	14.95^{bc}	7.85^{c}	7.72 x 10^{-6c}
Cherry red x Wild	43.50^{abc}	51.75^{abc}	79.75^{b}	88.50^{c}	29.10^{ab}	42.70^{a}	65.70^{a}	90.90^{ab}	57.10^{b}	27.64^{ab}	4.59^{de}	4.52 x 10^{-6de}
Roma x Rw	42.75^{bc}	51.25^{abc}	90.00^{ab}	107.50^{ab}	25.18^{bcd}	34.65^{bc}	55.88^{ab}	73.98^{bcd}	47.42^{c}	28.98^{abc}	8.36^{c}	8.23 x 10^{-6c}
Woso x Ww	43.50^{abc}	52.50^{abc}	87.25^{ab}	95.25^{bc}	26.20^{bcd}	41.33^{ab}	60.78^{ab}	79.15^{abcd}	51.86^{d}	15.52^{bc}	9.18^{c}	9.04 x 10^{-6c}
Cherry red x CRW	42.00^{bc}	49.00^{c}	83.00^{ab}	97.75^{ab}	23.08^{cd}	42.15^{ab}	62.55^{ab}	91.75^{a}	54.88^{c}	10.53^{cd}	7.25^{cd}	7.14 x 10^{-6cd}
CV (%)	3.51	4.42	7.06	6.95	17.68	16.74	19.55	16.49	42.18	53.10	76.05	76.05

Means in the same column followed by the same letter are not significantly different ($p \leq 0.05$). DFFl = days to first flowering; FpFl = days to 50% flowering; CV = coefficient of variation; DFFM = days to first fruiting; FpFr = days to 50% fruiting; AFP = average fruits per plant; AFWtP = average fruit weight per plant (g); FY = fruit yield (ton/ha).

Relationship between Disease Incidence, Symptom Severity, Plant Height and Whitefly Count among the 10 Tomato Varieties/Breeding Lines

Generally, all the traits measured showed very low correlation with fruit yield (t/ha) except fruit number per plant which exhibited low correlation with yield (t/ha) (Table 5). Disease severity for all plants (ISS_{AP}), disease severity for diseased plants (ISS_{DP}), average % disease incidence and average whitefly count showed very high negative correlation with fruit yield (t/ha). Percent disease incidence, disease severity for all plants (ISS_{AP}) and disease severity for diseased plants (ISS_{DP}) were moderately negatively correlated while average whitefly count showed negative correlation with number of fruits per plant. Furthermore, average whitefly count, percent disease incidence and disease severity (ISS_{AP}) showed very low positive correlation.

With regards to ISS_{DP}, average whitefly count showed high positive correlation (r = 0.89), percent disease incidence moderately correlating with ISS_{DP} (r = 0.67) while ISS_{AP} showed very high correlation (r = 0.98) with ISS_{DP}. Again, average whitefly count showed very high positive correlation (r = 0.93; r = 0.74) with disease severity (ISS_{AP}). Average whitefly count was highly correlated (r = 0.86) with average disease incidence.

Table 5
Correlation co-efficients of disease-related parameters on 10 tomato varieties/breeding lines

Traits	Average whitefly count	% disease incidence	ISS_{DP}	Fruit/ plant	Fruit yield (t/ha)
Average whitefly count					
Percent disease incidence	0.86**				
ISSAP	0.93***	0.74**			
ISSDP	0.89***	0.67*	0.98***		
Fruit/plant	-0.83**	-0.64*	-0.64*	-0.61*	
Fruit yield (t/ha)	-0.18	-0.18	-0.03	-0.03	0.36

* = significant ($P \leq 0.05$); ** = very significant ($P \leq 0.001$); *** = highly significant ($P \leq 0.0001$) computed using standard linear Pearson correlation

DISCUSSION

The whitefly life-cycle progresses from egg to adult emergence, governed mainly by temperature (Triparthi & Varma, 2002, p. 473-478). In warm climates, the life cycle takes approximately three weeks, but it may take up to two months under cool conditions (Triparthi & Varma, 2002, p. 473-478) with no adult emergence occurring when the temperature drops below 17°C (Czosnek, 2007, pp. 329-342). Generally, whitefly populations on the leaves of the tomato are more in the dry (hot) season than in the rainy (cool) seasons (Canto, Aranda, & Fereres, 2009, pp. 884-894). Thus, it is important to assess disease incidence severity in the dry season where abundance of whitefly populations ar expected as reported on cassava by Appiah et al. (2012, pp.31-37). The study revealed that whiteflies had high preference for Roma, Cherry red, Wosowoso and Woso x WW varieties/breeding lines. This has led to higher disease incidences and invariably higher disease severity for these varieties/breeding lines. Whitefly preference for specific varieties/breeding lines does not necessarily lead to incidence and severity of TYLCV disease as the feeding habits of whiteflies predispose the plant to several other infections other than TYLCV. However, the confirmation of TYLCV in these varieties/breeding lines earlier by TAS-ELISA and PCR (Segbefia, et al., 2015, pp. 17-24) shows that the disease was transmitted by whiteflies. This supports previous observations made by Brown, Costa and Laemmlen (1992, p. 426); Schuster, Mueller, Kring, & Priece (1990, pp. 1618-620) and Asare-Bediako, Wonkyi, van der Puije, Amenorpe and Osei

(2017, pp. 373-378) that direct crop damage occurs when whiteflies feed in plant phloem, remove plant sap, excrete honeydew, which promotes sooty moulds interfering with photosynthesis, and thus, reducing plant vigour. Additionally, higher whitefly numbers were recorded at the early growth stages of the plant and reduced gradually towards the end. This shows preference by whiteflies for younger leaves at two or four weeks after transplanting (WAT) than leaves of matured plants.

Furthermore, the Wild tomato had the lowest whitefly populations compared with other varieties/breeding lines throughout the study period. This may be attributed to resistance-related factors such as small leaf size, smell and /or other physical barriers. Bellotti & Arias (2001, pp. 813-824) reported that in tomato, non-preference of some varieties by whiteflies is due to physical barriers, such as waxy or thick cuticles or the presence of specialised trichomes that inhibit whiteflies from settling and feeding on leaves. The immediate manifestation of a pathogen infecting a plant is the expression of disease symptoms. The TYLCV-induced symptoms usually appear within 2–3 weeks after inoculation (Czosnek, 2007, p. 339). Incidence of TYLCVD on tomato plants is characterised by varied symptoms including upward/downward leaf curling, yellowing of young upper leaves, reduced leaf size, stunting of plants, reduced fruit yield (fruit size and number) and death of plants. Symptom expression, however, varies with viral strain, tomato varieties/ breeding lines, plant age at time of infection and 'enviro-climatic' conditions (Lapidot et al., 2000, pp. 31 7-321; Pico, Diez, & Nuez., 1998, pp. 259-271). In this study, the three backcrossed lines (Roma x RW, Woso x WW and Cherry Red x CRW) exhibited mild disease symptom severity throughout the study period. These were comparable with the Wild tomato (donor parent for TYLCV resistance genes), and showed significant improvements in levels recorded for the three adapted parents (Roma, Wosowoso and Cherry Red) used as recurrent parents in the backcrosses (Segbefia et al., 2015, pp. 17-24).

The resistance obtained using one screening approach may not be equivalent to that obtained using another approach. Thus, a comparison of the use of leaf discs and whole plants in screening for resistance indicated that although leaf disc assays were able to discriminate between immune and susceptible varieties/breeding lines, they were not able to "discriminate between sensitive and tolerant plants which support virus replication and cell-to-cell spread but not its long-distance movement" (Czosnek, et al., 1993, pp. 995-1005). In this study, all the varieties/breeding lines, exhibited a range of TYLCVD leaf symptoms including yellowing, curling and reduced leaf size in the field during the dry season. Based on symptomatology alone, there were no sign of resistance among the cultivated commercial varieties to TYLCVD. This is consistent with reports by Pilowsky and Cohen (2000, pp. 351-353); and Pico, Diez and Nuez (1999, pp. 1006-1012). Among the F3 hybrid lines, Roma x Wild

and Cherry Red x Wild proved to be slightly susceptible. On the other hand, the backcross lines had some level of resistance introgressed into them after the first generation of backcross. The TYLCV disease symptom development generally began two weeks after transplanting to the field. However, most varieties/breeding lines recorded no symptoms except Roma and Wosowoso. Symptom development and severity continued till eight (8) weeks after transplanting, where the highest severity was attained.

Symptom reversal was observed in Wild tomato and Woso x Wild in 6 and 8 WAT culminating in lower ISS_{AP} and ISS_{DP}. This may be a good indication of resistance in these lines, corroborating the report by Czosnek (2007, p.332) that symptoms in resistant plants tend to increase with time and then decrease, unlike those of susceptible plants which normally increase over time and then plateaus. This study has confirmed the successful transfer of resistant genes from the F_3 breeding lines (Roma x Wild, Woso x Wild and Cherry red x Wild) to the backcrossed lines (Roma x RW, Woso x WW and Cherry Red x CRW). All the breeding lines recorded low disease symptoms; conversely, the backcrosses recorded much lower or delayed symptoms, indicating that the transfer of resistant genes from the hybrid lines to the backcross lines was successful.

Plant height of the three F_3 hybrid lines was significantly higher than those of the backcrossed lines. This indicated the gene for tallness in the F_3 hybrid lines has not been transferred to the backcrosses. Differences in plant height among the 10 varieties/breeding lines were not significant ($p \leq 0.05$). All the commercial varieties showed determinate growth pattern whereas the F_3 hybrid lines and backcross lines showed semi-determinate growth pattern similar to the Wild tomato. Flowering and maturity were generally earlier in the backcrossed lines except Roma x RW comparable to the Wild tomato. Though, the F_3 hybrid lines are early maturing which is a desirable trait, they grow tall quickly which requires constant pruning and staking. The parental varieties which are determinate and comparatively shorter require no staking which is an indication that they have been improved upon over the years and are suitable for commercial cultivation. However, their fruits easily touch the ground at maturity making them susceptible to attack by pests. The backcross lines on the other hand, have two main advantages which are earliness to maturity and a longer harvesting period (semi-determinate), a trait desired by local farmers. Further improvement of these backcross lines would make them acceptable to local farmers.

The relevance of TYLCV resistance emanates from its effect on total yield and yield components, relative to uninfected controls (Lapidot et al., 1997, pp. 1425-1428; Lapidot, Weil, Cohen, Segev, & Gaba, 2007. pp. 143-148). In this study, the number of fruits per plant was relatively high in Roma x RW, Roma x Wild and Cherry red x Wild and total fruit yield (t/ha) was relatively high in Cherry red, Wosowoso and Roma x

Wild. These varieties/breeding lines would however perform better in the rainy season as there is a higher incidence of the TYLCV disease in the dry season, which drastically reduces total production with adverse consequences for farmers (Robinson & Kolavalli, 2010, pp.17-19). Similarly, Osei et al. (2010, pp. 315-323) reported that in the rainy season, Ghana is able to produce to meet domestic tomato demand. In terms of weight of fruits, Wosowoso recorded the highest while Cherry red had the lowest among the cultivated commercial varieties. Among the three F_3 breeding lines, Cherry red x Wild was the most prolific, producing the highest number of fruits. Woso x Wild produced the highest total fruit yield among the F_3 breeding lines.

The Wild tomato (*S. pimpinellifolium* L.) produced the highest number of fruits in this study. However, due to smaller fruit size, total fruit yield (t/ha) was low compared with the adapted varieties / breeding lines. In all cases, numbers of fruits recorded by the breeding lines were lower than Wild tomato. However, the total fruit yield (t/ha) of the breeding lines were higher than that of Wild tomato. This indicates that the backcrossing of the F3 breeding lines to the adapted varieties Cherry Red, Wosowoso and Roma resulted in increased fruit size compared to Wild tomato. Therefore, with the combination of desirable attributes such as high level of resistance to TYLCV disease in the field, early maturity, semi-determinate growth habit and large number of fruits, breeders could select Roma x RW, Woso x WW Tomato and Cherry Red x CRW for further improvement in fruit size (weight) towards high fruit yield and tolerance to TYLCVD. The obvious limitation of this study is that diagnosis based on symptom expression alone may be inadequate since other factors (mineral deficiencies) and attack by pests could play a major role in the overall appearance of plants in the field. It is however, relevant as a preliminary step in screening the varieties/breeding lines in the breeding programme against TYLCVD.

In an earlier work (Segbefia et al., 2015; pp. 17-24), TAS-ELISA detected TYLCV in Wosowoso, Woso x Wild, Woso x WW, Roma and Roma x Rw under field conditions. The PCR confirmed the presence of TYLCV in all varieties/breeding lines except Roma x RW. Detection of TYLCV in the symptomless Wild Tomato and the hybrids, Roma x Wild and Woso x Wild and all backcrosses indicate they are symptomless carriers of the virus.

CONCLUSION

Roma recorded the highest population of whiteflies in the dry season and also exhibited the highest symptom severity in all plants (ISS_{AP}) and diseased plants (ISS_{DP}) during the study period. There was symptom reversal in Wild tomato and Woso x Wild at 6-8 WAT for both ISS_{AP} and ISS_{DP} indicating their potential source of resistance. Average whitefly count showed very high positive correlation ($r = 0.93$) with disease severity (ISS_{AP}); while average whitefly count, average percent disease incidence, ISS_{AP} and ISS_{DP} correlated inversely ($r = -0.83$) with yield (t/ha). Roma x Wild, Woso x Wild,

Cherry Red x Wild, Roma x RW, Woso x WW and Cherry Red x CRW could be selected for future breeding work based on their superior resistance to the virus.

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