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*Full Length Research Paper*

# Phenotypic and molecular screening of some tomato germplasm for resistance to tomato yellow leaf curl virus disease in Ghana

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**Tomato yellow leaf curl virus (TYLCV) is a major tomato virus in Ghana and Africa as a whole. In this study, 30 accessions of *Solanum lycopersicum* L. with reported TYLCV-resistance from AVRDC were assessed for resistance to TYLCV in Ghana. Plants were grown in a field, a hot spot of the disease and the reactions of plants were evaluated based on the disease symptoms when they were 30, 45 and 60 days after transplanting. Molecular screening was also done to re-confirm the phenotypic evaluation. All the tomato accessions demonstrated various degrees of disease symptoms. Phenotypic evaluation was confirmed by amplification of TYLCV DNA fragment in all tested accessions. Based on the phenotypic and molecular evaluations, no accession provided complete resistance to TYLCV in Ghana. However, accessions with milder symptoms of TYLCV in the field were considered as tolerant. The high level of susceptibility to viral infection noted in the field was not observed in the molecular screening. The viral DNA was detected using six different primers and the primers indicated polymorphism. TYLCV was detected in 23 accessions using primer pair GhF and GhR. The results suggested that accessions that indicated symptoms of the disease on the field but had no TYLCV DNA amplification could be due to other viruses or virus strain. Accessions with reported resistance in other countries but collapsed in Ghana could be attributed to genotype – environment interactions and or the emergence of new mutants of the TYLCV in Ghana.**

**Key words:** Molecular screening, tomato, tomato yellow leaf curl virus, virus resistance, DNA, primer.

## INTRODUCTION

In Ghana, tomato is a very popular and important vegetable crop which is consumed on nearly a daily basis by every household (Horna et al., 2006). It is used in preparing soups and stews. The highest quality fruits and greatest yields are obtained in the dry season with supplementary water (Tweneboah, 1998). Currently, more money is spent on tomato cultivation than on any other vegetable (Wolff, 1999).

Despite tomato's importance in Ghana, local production is not able to meet the domestic demand and tomatoes are often imported, mainly from Burkina Faso. This is a drain on the country's economy. This situation is attributed to a number of constraints. Two such constraints are the pests and diseases that affect tomato production in Ghana. Among them, tomato yellow leaf curl virus (TYLCV) is of economic importance (Bhyan et al., 2007; Valizadeh et al., 2011).

TYLCV, a *Begomovirus* of the family Geminiviridae, is the most devastating virus of the tomato plant in tropical and subtropical regions including Ghana. The family Geminiviridae comprises plant viruses that have a circular, single-stranded DNA genome and geminate particles consisting of two incomplete icosahedra (Hull, 2002). Geminiviruses are classified into four genera based on the type of insect vector, host range, and genome organization (Rybicki et al., 2000; El-Din et al., 2005). The genus *Begomovirus* includes species with

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**Table 1.** Tomato accessions used for screening against tomato yellow leaf curl virus.

| Entries | Code | Resistance source | Origin |
|---|---|---|---|
| FLA 505 | A1 | LA 1969 (*L. chilense*) | J. Scott, Univ. Florida |
| FLA 456-4 | A2 | Tyking, LA2779 (*L. chilense*) | J. Scott, Univ. Florida |
| FLA 478-6-3-0 | A3 | LA1938, Tyking, Fiona | J. Scott, Univ. Florida |
| FLA 653-3-1-0 | A4 | LA2779 (*L. chilense*), Tyking | J. Scott, Univ. Florida |
| FLA 496-11-6-1-0 | A5 | LA1932 (*L. chilense*), Tyking | J. Scott, Univ. Florida |
| TLB 111 | A6 | H24 | AVRDC |
| TY52 | A7 | LA 1969 (*L. chilense*) | D. Zamir, Hebrew Univ. |
| 99S-C-39-20-11-24-17-0 | A8 | Unknown | Namdhari Seeds, India |
| H24 | A9 | *L. hirsutum* f.sp glabratum | G. Kallo, India |
| CLN2026D | A10 | Susceptible check | AVRDC |
| Pimpinellifolium | G11 | Unknown | CSIR-CRI |
| WSP2F1pt.3 | G12 | Unknown | CSIR-CRI |
| WS273.3 Large | G13 | Unknown | CSIR-CRI |
| WSP2F7 (3) pt.3 | G14 | Unknown | CSIR-CRI |
| 2641A | B16 | Unknown | AVRDC |
| Tomato Money Maker | B17 | Unknown | USA |
| Tomato Roma-Jam Vf | B18 | Unknown | Burkina Faso |
| Parona | B19 | Unknown | Local |
| Local Roma | B20 | Unknown | Local |
| Rando | B21 | Unknown | Local |
| Tomato Slumac | B22 | Unknown | Holland |
| Tomato Tima | B23 | Unknown | France |
| Tomato Red Cloud | B24 | Unknown | Holland |
| Tomato Rio Grande | B25 | Unknown | Holland |
| Petomech (Ghana/France) | B26 | Unknown | France |
| Tomato Roma VF | B27 | Unknown | USA |
| Petomech (Ghana/Burkina) | B28 | Unknown | Burkina Faso |
| Petomech (Ghana) | B29 | Unknown | Ghana |
| Tomato Ventura F | B30 | Unknown | USA |

monopartite or bipartite genomes such as TYLCV that are transmitted by the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Moriones and Navas Castillo, 2000; Cohen and Nitzany, 1966; Mazyad et al., 2007). *B. tabaci* has developed resistance against insecticides in recent years (Dittrich and Ernst, 1990) and therefore, a few viruliferous whiteflies may be enough for transmitting the virus to a large number of plants (Mazyad et al., 2007). Chemical control methods as well as integrated pest management (IPM) strategies employed for controlling the vector have not been successful in decreasing the incidence of TYLCV on tomato crop (Bhyan et al., 2007; Reynaud et al., 2003). Under these circumstances, breeding for resistance to TYLCV appears to be a promising and environmentally friendly approach for controlling the disease (Chague et al., 1997). Host plant resistance will therefore be an important component of an overall whitefly-transmitted geminivirus control strategy. Several techniques such as enzyme linked immunosorabent assay (ELISA), tissue-blotting immuno-binding assay (TBIA) and dot blotting immuno-binding assay (DBIA) as well as polymerase chain reaction (PCR) provide a sensitive and specific means for the detection and identification of whitefly transmitted geminiviruses (WTGV) in the infected plants and their vector whitefly *B. tabaci* (Cohen et al., 1989; Mehta et al., 1994; Fargette et al., 1996; Tsai et al., 2006; El-Din et al., 2005; Bhyan et al., 2007). The objective of this work was to screen available tomato germplasm through convectional and molecular approaches to identify stable sources for breeding against the TYLCV disease.

## MATERIALS AND METHODS

### Screening of germplasm for TYLCV resistance

Thirty tomato germplasm collected from Burkina Faso, USA, Holland, France and Green seeds (Taiwan), Ghana and Asian Vegetable Research Development Centre, Taiwan (Table 1) were screened at Afari (a hot spot of the disease) for resistance to TYLCV disease.

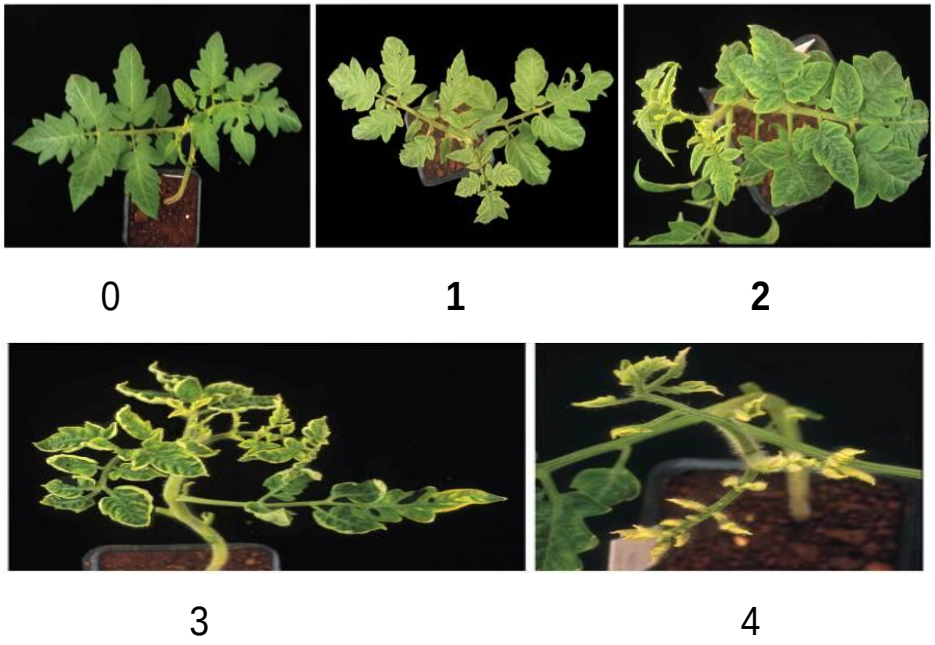



**Figure 1.** TYLCV Symptom Severity Scale. Severity scores were based on 0 - 4 scale developed by AVRDC where; 0- No symptoms, 1- Slight yellowing (mild symptom); 2- Leaf curling and yellowing (moderate symptom); 3- Yellowing, Curling and Cupping (severe symptom); 4- Severe stunting, curling and cupping; plant stops growth (very severe symptom). Source: Lapidot and Friedman (2002).

**Experimental design**

The tomato germplasm were planted on a plot size of 1863 $m^2$ (81 m × 23 m). A spacing of 90 cm × 70 cm in a randomized complete block design (RCBD) with three replications was used. They were planted in triple rows and each accession had 10 plants per row giving a total of 30 plants per accession. Standard agronomic practices such as weed control, fertilizer application (15-15-15) at 250 kg/ha and spraying of fungicides (Shavit F at a rate of 50 g/15 L) were used.

**Data collection**

Scoring for incidence and severity of TYLCV disease on the plants was done at 30, 45 and 60 days after transplanting using a symptom severity scale (Figure 1). Other characteristics on the fruits were taken. Data were taken on the number of fruits per plant and plot. A refractometer was used in taking the brix after harvesting.

**Viral DNA detection: Plant DNA extraction**

For molecular analysis, leaf samples were collected from 30 tomato accessions to confirm the phenotypic screening. DNA extraction was carried out using the DNA isolation method described by Egnin et al. (1998) with some modifications.

**Primer for PCR Amplification**

PCR amplification was done using six primer pairs. These included PAR1c496/PAL1v1978, PTYc1121/PTYv787, AC1048/AV494, GHF/GHR, KR/KF and MR/MF. The first three were degenerated primers; the last three were developed during this work (Table 2). The primer 3 program was used to develop the primers (Rozen and Skaletsky, 2000). The sequences in the last three are available in the NCBI. The primer pairs GHF/GHR, KR/KF and MR/MF have GenBank accession numbers EU350585, EU847739 and EU847740, respectively.

**PCR amplification of viral DNA**

PCR was performed in 10 µl volume using six primer pairs. The reaction mixture composition was 1.5 mM of 5x Buffer A with $MgCl_2$, 10 mM of dNTPs, 0.12 µM each of forward and reverse primer and 5 µl robust taq. Water was added to make a final volume.The same reaction mixtures were used. PCR amplification was carried out in a BIO-RAD Mycler™ Thermal cycler. For primer pairs, PAL1v1978/PAR1c496, GHF/GHR, KF/KR and MF/MR, DNA amplification parameters were 30 cycles of denaturation for 1 min at 94°C, 50°C for 1 min, 72°C for 3 min and final extension at 72°C for 3 min. Amplification products were maintained at 4°C prior to electrophoresis. The DNA amplification parameters for primer pair AC1048 and AV496 also had an initial denaturation of 92°C for 1 min followed by 35 cycles at 92°C for 1 min, annealing at 60°C for 20 s and extension at 72°C for 30 s. The primer pair PTYv/PTYc profile amplified at 94°C for 1 min, 58°C for 1 min, 72°C for 2 min and a final extension at 72°C for 10 min.

**Gel electrophoresis**

The PCR products were electrophrosed in a 1% agarose gel (7.5 µl ethidium bromide, 200 ml 1X TAE, 2.0 g agarose). 2 µl of loading dye was mixed with 10 µl of the sample and the mixture was loaded in a gel of 1 × TAE buffer. 4 kb of DNA ladder was used as a marker and water was used as a negative control. The samples

**Table 2.** Primer pairs used for PCR detection of TYLCV.

| Primer sequence | Source | Reference |
|---|---|---|
| PARc1496/PAL1v1978<br>5'GCA/TCT/GCA/GGC/CCA/CAT/YGT/CTT/YCC/NGT (30 mer)<br>5'AAT/ACT/GCA/GGG/CTT/ CT/RTA/CAT/RGG (27 mer) | Metabion International Company | Rojas et al. (1993) |
| AV494/AC1048 GCCCATGTATAGAAAGCCAAG<br>GGATTAGAGGCATGTGTACATG | Metabion International Company | Wyatt and Brown (1996) |
| PTYv787/PTYc1121 5-GTTCGATAATGAGCCCAG-3<br>5-ATGTAACAGAAACTCATG-3 | Metabion International Company | Zhou et al. (2008) |
| GHF/GHR F: GCCCGAAAGCTTCGTTGTT TTCCCGCT<br>R: ACGGATGGCCGCTTTGGGT ATTCG | Metabion International Company | Osei et al. (2008) |
| KF/KR F: GGACCCGGCGCACTATTTAT GTTGGC<br>R: ACCCCATTACCCCAATACCA | Metabion International Company | Osei et al. (2008) |
| MF/MR F: TGGCCGCGCCCTTCCTTTTGT<br>R: ACCAATGGCTCCCCAAAGCGT | Metabion International Company | Osei et al. (2008) |

were run in TAE buffer for 40 min at 120 V in a 150 ml BIO RAD electrophoretic apparatus. The gels were then observed by alpha imager software on a computer. Banding patterns were then observed and compared between individuals showing viral presence and absence.

## RESULTS

### Screening for TYLCV- *r*esistance under field conditions

#### *Incidence (number of plants infected)*

The results of the screening for TYLCV-resistance on the field are given in Table 3. Of the 30 accessions studied, there were highly significant differences in terms of percentage of infected plants at 30, 45 and 60 days after transplanting (DAT). At 30 days after transplanting, accessions A3, A5, G14, B16, B1, B20, B22, B24 and B26 had fewer numbers of infected plants. B23 however, recorded the highest percentage of plants that were infected at 30 DAT. More than half (>50%) of tomato accession were affected at 45 days after transplanting except accessions A3, A5, G14, B18, B20 and B22. 100% infection was recorded in accessions A1, A7, A10, B21, B23 and B29 at 60 days after transplanting.

#### *Symptom severity of the disease*

At 30 days after transplanting, B20, B22 and B26 were the only accessions with no symptoms. There were however, some accessions (A1, A6, A9, A10, G12, G14, G15 and B16) which showed mild symptoms. This changed to moderate and severe symptoms at 45 and 60 days after transplanting, respectively. Apart from tomato accessions B20, B26 which showed mild symptoms and G12, B24 which showed moderate symptoms of the disease respectively, the rest exhibited severe symptoms of the disease after 60 days of transplanting (Table 3).

### Fruit characteristics of tomato accessions studied

Accession B23 gave the highest average fruit weight (61.85 g). This was followed by B21 (46.54 g) and A7 (43.42 g). Accession G12 however recorded the lowest average fruit weight per fruit (8.9 g). In terms of the average number of fruits per plant, tomato accession G12 gave the highest number of fruits (37). This was followed by G14 (35) and G11 (33). Accession A1 however gave the lowest number of fruits per plant. The fruit shapes of the accessions studied were round, high round, plumshaped, heartshaped, flattened, slightly flattened and lengthened cylindrical. 23% of the tomato accessions had round and high round fruit shape with 20% plum shaped and heart shaped. 33% of the tomato accessions had flattened and lengthened cylindrical fruit shape and 6.7% have slightly flattened fruit. 63.3% of the tomato accessions had red fruit colour at maturity and the rest representing 36.7% had orange fruit colour at maturity. The highest brix (soluble solids) among the accessions was four and the least was two (Table 4).

**Table 3.** Reaction of different tomato accessions to TYLCV on field assays.

| Field code | Accession | % of TYLCV infected plants | | | Level of severity | | |
|---|---|---|---|---|---|---|---|
| | | 30 DAT* | 45 DAT | 60 DAT | 30 DAT | 45 DAT | 60 DAT |
| A1 | FLA 505 | 60.00±00$^{BCD}$ | 90.00±00$^{AB}$ | 100.00±00$^{A}$ | 1.00±00 | 3.00±00 | 3.00±00 |
| A2 | FLA 456-4 | 50.00±00$^{CDE}$ | 76.67±00$^{BCD}$ | 76.67±00$^{FGH}$ | 2.00±00 | 3.00±00 | 3.00±00 |
| A3 | FLA478-6-3-0 | 20.00±5.7$^{FG}$ | 50.00±5.7$^{EF}$ | 56.67±3.3$^{IJ}$ | 2.00±00 | 3.00±00 | 3.00±00 |
| A4 | FLA 653-3-1-0 | 60.00±00$^{BCD}$ | 70.00±00$^{CD}$ | 70.00±00$^{EFG}$ | 3.00±00 | 3.00±00 | 3.00±00 |
| A5 | FLA496-11-6-1-0 | 20.00±00$^{FG}$ | 40.00±00$^{F}$ | 73.33±3.3$^{GH}$ | 3.00±00 | 3.00±00 | 3.00±00 |
| A6 | TLB111 | 60.00±11.5$^{BCD}$ | 80.00±11.5$^{BC}$ | 60.00±00$^{I}$ | 1.00±00 | 3.00±00 | 3.00±00 |
| A7 | TY52 | 60.00±5.7$^{BCD}$ | 80.00±5.7$^{BC}$ | 100.00±00$^{A}$ | 2.00±00 | 3.00±00 | 3.00±00 |
| A8 | 99S-C-39-20-11-24 | 50.00±00$^{CDE}$ | 60.00±5.7$^{DE}$ | 86.67±3.3$^{CDE}$ | 2.00±00 | 3.00±00 | 3.00±00 |
| A9 | H24 | 50.00±00$^{CDE}$ | 70.00±5.7$^{CD}$ | 90.00±00$^{I}$ | 1.00±00 | 3.00±00 | 3.00±00 |
| A10 | CLN 2026D | 60.00±00$^{BCD}$ | 83.33±3.3$^{A}$ | 100.00±00$^{A}$ | 1.00±00 | 3.00±00 | 3.00±00 |
| G11 | Pimpinellifolium | 50.00±00$^{CDE}$ | 70.00±00$^{CD}$ | 93.3±3.3$^{ABC}$ | 2.00±00 | 3.00±00 | 3.00±00 |
| G12 | WSP2F1pt.3 | 50.00±00$^{FG}$ | 60.00±00$^{F}$ | 70.00±00$^{EFG}$ | 1.00±00 | 2.00±00 | 2.00±00 |
| G13 | WS273.3 Large | 40.00±5.7$^{DEF}$ | 70.00±5.7$^{CD}$ | 80.00±00$^{EFG}$ | 2.00±00 | 3.00±00 | 3.00±00 |
| G14 | WSP2F7(3)pt.3 | 20±00.5.7$^{FG}$ | 40.00±00$^{F}$ | 80.00±00$^{EFG}$ | 1.00±00 | 2.00±00 | 2.00±00 |
| G15 | WSP27F7(3)pt.3 | 60.00±00$^{BCD}$ | 70.00±00$^{BC}$ | 83.33±6.7$^{DEF}$ | 1.00±00 | 2.00±00 | 3.00±00 |
| B16 | 2641A | 30.00±5.78$^{EF}$ | 90.00±00$^{AB}$ | 90.00±00$^{BCD}$ | 1.00±00 | 3.00±00 | 3.00±00 |
| B17 | Tomato Money M. | 40.00±5.7$^{DEF}$ | 70.00±00$^{CD}$ | 76.67±3.3$^{FGH}$ | 3.00±00 | 3.00±00 | 3.00±00 |
| B18 | Tomato Roma-Jam | 30.00±00$^{EF}$ | 50.00±00$^{EF}$ | 60.00±00$^{I}$ | 1.00±00 | 2.00±00 | 3.00±00 |
| B19 | Parona | 80.00±00$^{AB}$ | 80.00±00$^{BC}$ | 96.67±00$^{AB}$ | 2.00±00 | 3.00±00 | 3.00±00 |
| B20 | Local Roma | 20.00±00$^{FG}$ | 50.00±00$^{AB}$ | 50.00±00$^{J}$ | 0.00±00 | 1.00±00 | 1.00±00 |
| B21 | Rando | 80.00±00$^{AB}$ | 80.00±00$^{BC}$ | 100.00±00$^{A}$ | 2.00±00 | 3.00±00 | 3.00±00 |
| B22 | Tomato Slumac | 33.3±3.3$^{G}$ | 50.00±00$^{EF}$ | 50.00± 00$^{J}$ | 0.00±00 | 3.00±00 | 3.00±00 |
| B23 | Tomato Tima | 90.00±00$^{A}$ | 100.00±00$^{A}$ | 100.00±00$^{A}$ | 3.00 ±00 | 3.00±00 | 3.00±00 |
| B24 | Tomato Red Cloud | 30.00±00$^{EF}$ | 70.00±00$^{CD}$ | 80.00±00$^{EFG}$ | 1.00±00 | 2.00±00 | 2.00±00 |
| B25 | Tomato Rio Grand | 60.00±00$^{BCD}$ | 70.00±00$^{CD}$ | 80.00±00$^{EFG}$ | 1.00±00 | 2.00±00 | 3.00±00 |
| B26 | Petomech-gh/Fr. | 40.00±00$^{DEF}$ | 70.00±00$^{CD}$ | 80.00±00$^{EFG}$ | 0.00±00 | 1.00±00 | 1.00±00 |
| B27 | Tomato Roma | 80.00±00$^{AB}$ | 90.00±00$^{AB}$ | 100.00±00$^{A}$ | 2.00±00 | 3.00±00 | 3.00±00 |
| B28 | Petomech-Bk. | 70.00±00$^{AB}$ | 70.00±00$^{CD}$ | 70.00±00$^{H}$ | 2.00±00 | 3.00±00 | 3.00±00 |
| B29 | Petomech-gh | 80.00±00$^{AB}$ | 100.00±00$^{A}$ | 100.00±00$^{A}$ | 2.00±00 | 3.00±00 | 3.00±00 |
| B30 | Tomato Ventura F | 60.00±00$^{BCD}$ | 70.00±00$^{CD}$ | 80.00±00$^{EFG}$ | 1.00±00 | 3.00±00 | 3.00±00 |
| df | 29.86 | 29.86 | 29.86 | 29.86 | 29.86 | 29.86 | 29.86 |
| P value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

0 = No symptom (NS); 1 = mild symptom (MS); 2 = moderate symptom (MoS); 3 = severe symptom (SS); 4 = very severe symptom (VSS) *DAT-Days after transplanting.

**Table 4.** Fruit and yield characteristics of tomato accessions.

| Field code | Accession | Mean fruit weight (g) | Average yield/plant No. of fruits per plant | Brix | Fruit shape | Fruit colour |
|---|---|---|---|---|---|---|
| A1 | FLA 505 | 19.44±0.13 | 3.00±0.58 | 3.00 ± 00 | Round | Red |
| A2 | FLA 456-4 | 26.98±0.59 | 16.00±1.53 | 4.00 ± 00 | Round | Red |
| A3 | FLA478-6-3-0 | 20.69±0.02 | 9.00±00 | 4.00 ± 00 | High round | Red |
| A4 | FLA 653-3-1-0 | 33.23±2.19 | 10.00±1.15 | 3.00 ± 00 | Round | Red |
| A5 | FLA496-11-6-1-0 | 28.18±0.12 | 20.00±1.73 | 2.00 ± 00 | High round | Orange |
| A6 | TLB111 | 22.47±0.20 | 15.00±0.58 | 3.00 ± 00 | Slightly flattened | Red |
| A7 | TY52 | 43.42±0.77 | 6.00±00 | 3.00 ± 00 | Plum shaped | Orange |
| A8 | 99S-C-39-20-11-24-17-0 | 29.43±0.01 | 10.00±2.31 | 2.00 ± 00 | Plum shaped | Orange |
| A9 | H24 | 12.62±0.03 | 5.67±0.33 | 3.00 ± 00 | Heart shaped | Red |
| A10 | CLN 2026D | 9.80±0.06 | 8.00±0.67 | 3.00 ± 00 | High round | Orange |
| G11 | Pimpinellifolium | 15.10±00 | 33.00±2.59 | 4.00 ± 00 | Round | Red |
| G12 | WSP2F1pt.3 | 8.9±0.06 | 37.00±1.15 | 4.00 ± 00 | Round | Red |
| G13 | WS273.3 Large | 33.1±2.08 | 20.00±0.58 | 4.00 ± 00 | High round | Red |
| G14 | WSP2F7(3)pt.3 | 25.9±0.58 | 35.00±1.15 | 4.00 ± 00 | Round | Red |
| G15 | WSP27F7(3)pt.3 | 19.00±1.20 | 14.00±00 | 4.00 ± 00 | Round | Red |
| B16 | 2641A | 19.24±00 | 7.00±1.15 | 4.00 ± 00 | Plum shaped | Orange |
| B17 | Tomato Money M | 36.47±0.20 | 22.00±1.15 | 4.00 ± 00 | Heart shaped | Red |
| B18 | Tomato Roma-Jam Vf | 10.00±1.15 | 11.00±0.58 | 2.00 ± 00 | Plum shaped | Red |
| B19 | Parona | 26.92±00 | 8.00±00 | 3.00 ± 00 | Slightly flattened | Red |
| B20 | Local Roma | 11.07±0.60 | 17.00±0.58 | 3.00 ± 00 | High round | Red |
| B21 | Rando | 46.54±00 | 4.00±00 | 3.00 ± 00 | Flattened | Orange |
| B22 | Tomato Slumac | 33.84±00 | 9.00±1.15 | 3.00 ± 00 | Lengthened cylindrical | Red |
| B23 | Tomato Tima | 61.85±0.49 | 18.00±00 | 2.00 ± 00 | Heart shaped | Orange |
| B24 | Tomato red cloud | 28.97±00 | 7.00±1.15 | 3.00 ± 00 | Heart shaped | Red |
| B25 | Tomato Rio grand | 12.54±0.06 | 4.00±0.58 | 3.00 ± 00 | Heart shaped | Red |
| B26 | Petomech (gh/France) | 24.68±0.33 | 13.00±00 | 3.00 ± 00 | High round | Orange |
| B27 | Tomato Roma | 17.73±00 | 4.00±00 | 3.00 ± 00 | Plum shaped | Red |
| B28 | Petomech (gh/Burkina) | 24.83±2.5 | 14.00±0.58 | 4.00 ± 00 | Heart shaped | Orange |
| B29 | Petomech (gh/France) | 24.68±0.33 | 13.00±00 | 3.00 ± 00 | High round | Orange |
| B30 | Tomato Ventura F | 16.48±0.56 | 4.00±0.58 | 3.00 ± 00 | Plum shaped | Orange |
| df | 29.86 | 29.86 | 29.86 | 29.86 | | |
| P value | <0.001 | <0.001 | <0.001 | <0.001 | | |

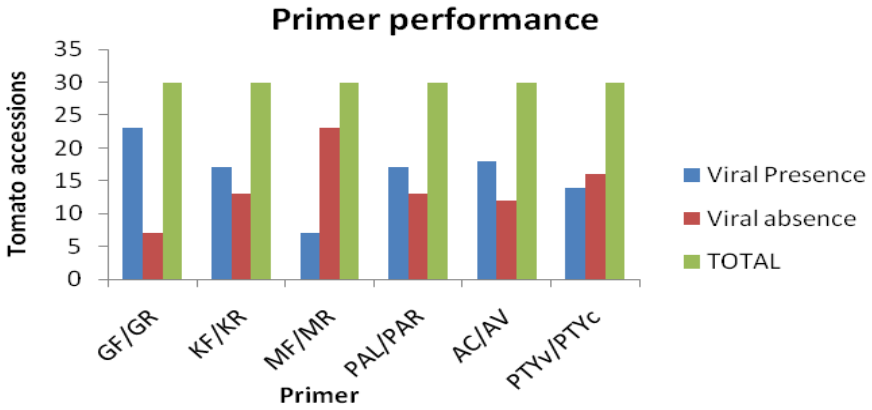



**Figure 2.** Primer performances in viral DNA amplification.

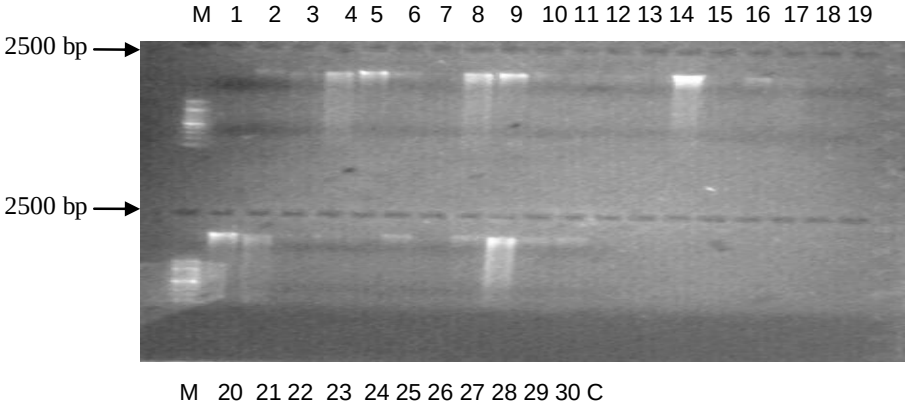



**Figure 3.** TYLCV amplified product on agarose gel using primer AC1048 and AV494.

Molecular *a*nalysis of *t*omato accessions for TYLCV resistance

### *Primer efficiency for PCR amplification*

The primers gave a high degree of polymorphism among the 30 tomato accessions. Figure 2 shows the efficiency of each primer in the amplification of the viral DNA between the accessions. The most consistent amplification of a viral DNA fragment was obtained with the primer pair GF and GR (Figure 3). It detected high number of viral DNA (23/30) than all the other primers used. However, primer pair MF and MR amplified low number of viral DNA (7/30).

### *PCR detection and amplification of viral DNA*

None of the six primer pairs used detected viral DNA in accession B24 (Table 5). All the primers amplified viral DNA in accessions A2, A5, A9 and G14. Five out of the six (5/6) primer pairs detected viral DNA in accessions B16, B20 and B27. Four out of the six (4/6) primer pairs detected viral DNA amplification for accessions A7, A8, B17, B21 and B28. DNA fragment of expected sizes 75, 2200 and 2500 bp were amplified using TYLCV specific primer pairs GHF/GHR, KF/KR and MF/MR, respectively. The other degenerate primers PAL1v1978/PAR1c496, AC1048/AV494 and PTYv787/PTYc1121 also gave the expected amplified viral DNA of 2500, 2500 and 2700 bp, respectively (Figures 3 and 4).

## DISCUSSION

### Screening for TYLCV- *r*esistant *p*lant under field conditions

Significant differences that emerged from tomato accessions on the incidence of the disease could possibly be attributed to the fact that the whiteflies had affinity for some particular accessions than others and resulted in some accessions being more susceptible to the virus than others. This probably made them feed and

**Table 5.** Scores of viral detection using six primer pairs.

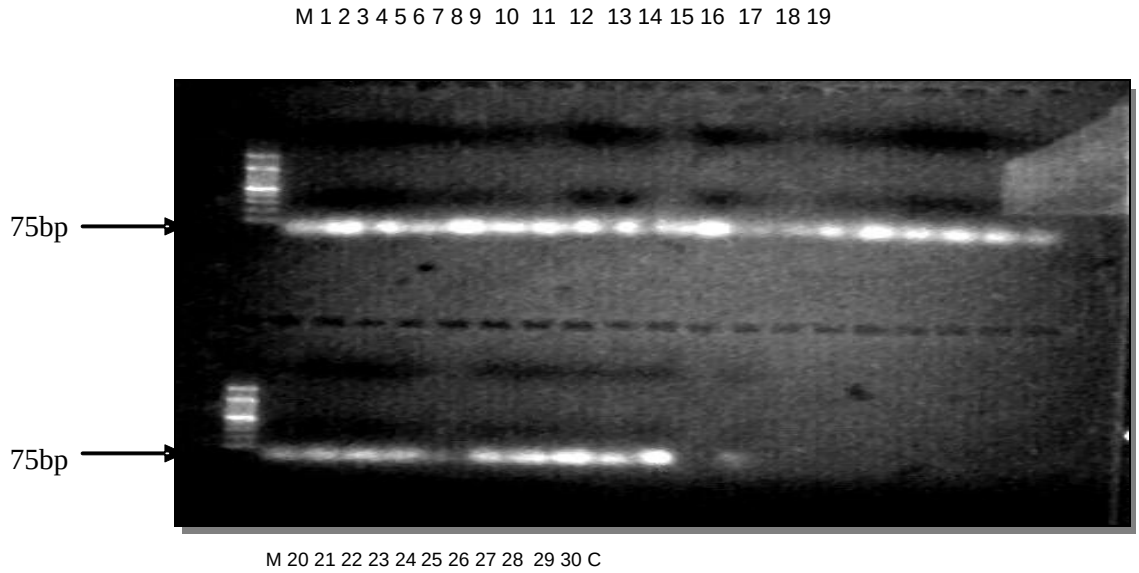
| Tomato lines | Primers | | | | | |
|---|---|---|---|---|---|---|
| | GHF/GHR | KR/KF | MF/MR | PAL/PAR | AC/AV | PTYv/PTYc |
| A1 | - | + | + | - | - | + |
| A2 | + | + | + | + | + | + |
| A3 | + | - | - | - | + | - |
| A4 | - | + | - | - | + | + |
| A5 | + | + | + | + | + | + |
| A6 | + | - | - | - | + | - |
| A7 | + | + | + | + | - | - |
| A8 | + | + | + | - | + | - |
| A9 | + | + | + | + | + | + |
| A10 | + | + | - | + | - | - |
| G11 | + | - | - | + | - | - |
| G12 | - | - | - | + | - | - |
| G13 | - | - | - | + | - | - |
| G14 | + | + | + | + | + | + |
| G15 | + | - | - | + | - | - |
| B16 | + | + | - | + | + | + |
| B17 | + | + | - | - | + | + |
| B18 | + | - | - | + | - | + |
| B19 | + | - | - | - | - | - |
| B20 | + | + | - | + | + | + |
| B21 | + | + | - | - | + | + |
| B22 | + | - | - | - | - | - |
| B23 | + | - | - | - | + | - |
| B24 | - | - | - | - | - | - |
| B25 | + | + | - | - | + | - |
| B26 | + | + | - | - | - | - |
| B27 | + | + | - | + | + | + |
| B28 | + | - | - | + | + | + |
| B29 | - | + | - | + | + | - |
| B30 | - | - | - | + | + | + |
| C | - | - | - | - | - | - |

+ = Presence of viral DNA; - = Absence of viral DNA.

transmit the virus following their longer stay on those plants hence the high levels of viral DNA into the plants. The accessions that had fewer number of plants infected could be explained by the late occurrence of TYLCV infection related to whitefly population variation. Moreover, this difference in reaction could be due to the virus strain, vector genotype or altered feeding conditions of the vector (Delatte et al., 2006; Navas-Castillo et al., 1999). The accessions that exhibited varying range of the disease symptoms lacked resistance. Most of them were found to be highly susceptible to TYLCV and only few of them showed very mild symptoms and considered as tolerant. Accessions that had in earlier studies been reported to be TYLCV tolerant (AVRDC, 2001) were found to be susceptible in this study. Accessions A1, A4, A6, A7, A10, G15, B19, B21, B23, B27, B28, and B29 showed a much faster development of disease symptoms at 30 days after transplanting than the rest and it could be due to the virus strain, vector genotype or altered feeding conditions of the vector (Delatte et al., 2006; Navas-Castillo et al., 1999). The tolerant accessions have been reported to be associated with the presence of exudates from trichome glands on the leaf surface, in which whiteflies become entrapped (Channarayappa and Shivashankar, 1992).

## Viral DNA detection in tomato accessions

After plants were assessed on the field for the development of disease symptoms, amplification of viral DNA by PCR was done. This was made to confirm the phenotypic screening. The primer pair GhF and GhR detected quite high number of viral DNA. This suggested




F**igure 4.** TYLCV amplified product on agarose gel using GhF and GhR Gh. Primers.

that the primer set was efficient and useful as a reliable marker for detecting or screening susceptible plants to TYLCV. Viral DNA was not detected in tomato accession B24 using the six primer pairs in this study. Meanwhile this accession B24 showed characteristic symptoms of the TYLCV disease in the field and could be putative. Mutations at the primer binding sites in the accession B24 could also account for that. It could also be as a result of other plant viruses other than the TYLCV attacking the plant on the field and indicating similar characteristic symptoms of TYLCV.

In general, based on both phenotypic and molecular evaluations, four categories of accessions were identified, accessions with severe symptoms and presence of viral DNA, accessions with moderate symptoms but relatively low levels of viral DNA, accessions with mild symptoms but high levels of viral DNA and accessions with mild symptoms and no viral DNA. There was actually no accession that showed no symptoms on the field as well as TYLCV DNA amplification. As such, no accession was considered as resistant in this study. Tolerance and resistance are relative terms, largely related to the rate of virus replication (Pilowsky and Cohen, 1990). The results obtained in this study also agreed to this.

## Conclusion

Complete resistance to the tomato yellow leaf curl virus disease in Ghana following field screening and molecular screening was not identified in any of the tomato accessions studied. The tomato accession, B24, showed the highest level of tolerance since it produced weak or mild symptoms and no viral DNA was detected with the six primer pairs used.

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