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Full Length Article

A Comparative Study Between Grafting and Mechanical Inoculation Mediated Transmission of *Tomato Yellow Leaf Curl Virus* in Tomato (*Solanum lycopersicon*) Cultivars Grown in Ghana

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Abstract

Global tomato production is seriously limited by the *Tomato Yellow Leaf Curl Virus* (TYLCV), leading to food insecurity globally. We studied the responses of six tomato cultivars to mechanical inoculation and grafting modes of TYLCV transmission in Ghana. Post mechanical inoculation and grafting mediated transmission resulted in upward leaf curl, stunted growth, and reduction in plant height, suggesting a successful transmission by both methods. However, disease symptom severity varied depending on the tomato cultivar as well as the mode of transmission. Transmission *via* grafting was more efficient in disease transfer, resulting in higher disease severity compared to those infected *via* mechanical inoculation. The presence of TYLCV decreased plant height and internode length regardless of the mode of transmission. TYLCV significantly decreased chlorophyll content over the controls but increased proline content than the controls. Correlation analysis revealed both positive and negative relationships between the methods of TYLCV transmission and the morphological traits of the plants studied. A strong positive correlation was observed in chlorophyll content by grafting, while plant height positively correlated with mechanical inoculation ($r = 0.973$). We conclude that the grafting mode of transmission is more effective than mechanical inoculation and could be used to study the propagation of phloem-limited TYLCV viruses in epidemiological studies for effective management.

Keywords: Chlorophyll; Grafting; Mechanical inoculation; Proline; *Tomato Yellow Leaf Curl Virus*; Tomato

Introduction

Tomato production is affected by various viruses, among which the most severe and common is the *Tomato Yellow Leaf Curl Virus* (TYLCV), posing a significant threat to tomato cultivation (Henghui and Wei 2025). It is a highly destructive pathogen causing significant economic losses (Azaryan *et al.* 2024). It has been reported that the *Tomato Yellow Leaf Curl Virus* (TYLCV) transmitted by *Bemisia tabaci* is one of the severest viral diseases that has plagued the tomato plant (Piedra-Aguilera *et al.* 2019), leading to low yields. In Ghana, it has been estimated that the average yield of the crop is considerably low (7.85 MT/ha) compared to 15 MT/ha per ha globally (Ghana Export-Import Bank Agri-Commodity Digest Newsletter 2024). Both biotic and abiotic factors, such as pests and diseases, have contributed to the low production of tomatoes in Ghana (Abdul-Rahaman and Gambrah 2023).

Symptoms of TYLCV disease consist of upward curling of leaflet margins, yellowing and reduction of leaflet area, stunted growth, and flower abortion, leading to fewer fruit production with reduced market value. Plant viruses are known to damage the chloroplast functioning within host cells, disintegrate the development of thylakoid lamellae, and induce the production of vesicles of the outer membrane, resulting in changes of chloroplast tissues (Liang *et al*. 2017). Chlorophyll breakdown as a result of viral infection leads to plant senescence, a phenomenon related to shorter fruit life and immature death of plants (Oda-Yamamizo *et al.*

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2016). This makes *Tomato Yellow Leaf Curl Disease* one of the most serious threats to global tomato production (Péréfarres *et al.* 2012; Rojas *et al.* 2018; El-Sappah and Rather 2021). It is estimated that in the tropical and subtropical regions, TYLCV disease can lead to total crop failure (100% yield loss) (Cathrin and Ghanim 2014).

The transmission of most plant viruses depends on vectors such as parasitic fungi, root nematodes, mites and insects (Ziegler-Graff and Brault 2008; Blanc *et al.* 2011). Tomato yellow leaf curl disease (TYLCD), is phloem-limited and is exclusively transmitted by the whitefly *Bemisia tabaci* in a persistent circulative non-propagative manner from phloem to phloem (Kil *et al*. 2016). Some viruses can also be mediated by the sap of virus-infected plants (mechanical transmission), vegetative propagation and grafting. According to El-Sappah and Rather (2021), inoculating tomato plants with TYLCV through grafting exposes test plants to high concentrations of viral inoculum, thus making it suitable for testing resistance or tolerance to varietal lines. Mechanical inoculation of viruses involving grinding up infected plant material and then physically applying it to the healthy plant plays a crucial role in understanding how plant viruses spread and how they can be controlled. Although, TYLCV is seedborne, it is not seed transmitted in tomato suggesting that seed-to-progeny transmission is not a general property of TYLCV (Perez-Padilla *et al*. 2019). In this study, we tested the infectivity of TYLCV using grafting and mechanical inoculation. The aim is to use these two modes of transmission techniques to determine host specificity of the virus.

Materials and Methods

Study area and planting materials

Six commercially grown tomato cultivars in Ghana namely, F1 Thales, F1 Anaya, Petomech, Tomato Rio Grande, Floradade and Local Tomato were used for this study. Viable seeds were obtained from Agriseed Limited (Ltd.) in Accra, Ghana. The infected plant materials with visible symptoms of leaf curling downwards and upwards were obtained from West African Centre for Crop Improvement (WACCI), University of Ghana agricultural fields (Fig. 1). Sap from donor plants were tested for the presence of TYLCV at the Plant Disease Research Centre-Biotechnology and Nuclear Agriculture Institute, Ghana Atomic Energy Commission, Kwabenya-Ghana using the Polymerase Chain Reaction method. The study was conducted twice between January 2023 and October 2023 in the screen house of the Department of Plant and Environmental Biology, University of Ghana (Latitude 5^0 39' 13.4" N; Longitude 0" 11' 07 7" W).

Soil preparation

The soil type used in this study was Haatso – Nyibgenya Series, obtained from the teaching garden in the Department of Plant and Environmental Biology, University of Ghana garden and sterilized at a temperature of 120°C and a pressure of 15 psi for 20 min. 30 g of soil was weighed and placed into polythene bags of dimension 20 x 30 cm. Seedlings were placed on a cement platform in the Screenhouse of the Department of Plant and Environmental Biology, University of Ghana at temperature of 33°C and 75% relative humidity.

Experimental design and mode of virus transmission

Fourteen (14) days old seedlings were used for both the mechanical inoculation and grafting to study the mode TYLCV transmission. A total of 90 tomato seedlings were used for the experimental set up under screen house conditions at 12 h/12 h (light/dark) photoperiod. 45 plants were each used for the mechanical and grafting experiments. The grafting experiment was done following the method described by Kashina *et al.* (2007). Susceptible TYLCV-infected tomato plants were used as scions. The scions were trimmed to make shallow incision on one side before they were gently inserted into the incision made on the understock stem of the tomato cultivars. The graft was secured using rubber strips and covered with a plastic bag as described by Spano *et al.* (2023). For mechanical inoculation, the method described by Hull (2009) was used. An inoculation buffer consisting of 500 ml of distilled water into which 20.214 g of $NaH_2PO_4.H_2O$ was added and stirred using magnetic stirrer. After thoroughly mixing, 3.394 g of Na_2HPO_4 was added to the solution and again stirred for 5 minutes. The solution was made up to a final volume of 1 L using sterile distilled water and pH adjusted to 7.4. Healthy uninfected leaves of tomato test plants were rubbed with sap obtained from leaf tissues of systemically susceptible TYLCV-infected tomato plants grounded in the prepared inoculation buffer and rubbed with celite. For the controls, healthy seedlings were neither grafted nor mechanically inoculated.

Growth Parameters Assessment

Viral symptom severity score

After the grafting and mechanical inoculation, TYLCV symptoms were scored both in mechanical inoculation and grafting experiments based on modified visual assessment using a scale of 1 to 6, where 0 is immune, 1 is absence of symptoms, 2 is mild infection, 3 is moderate infection, 4 is severe infection, 5 is most severe infection and 6 is highly susceptible as described by the International Institute of Tropical Agriculture (IITA) (Table 1).

Measurement of plant height, internode length and estimation of flowering time

Plant height was measured using a metre rule to measure the

Table 1: Symptom severity score used in assigning numerical value to the degree of infection

Viral Score	Rating Scale	Severity Range
0	Immune	0%
1	Absence of infection	1-10%
2	Mild infection	11-25%
3	Moderate infection	26-45%
4	Severe infection	46-60%
5	Most severe infection (Susceptible)	61-75%
6	Highly Susceptible (leaf distortion)	76-100%

Table 2: TYLCV symptom severity score on seedlings 77 days after inoculation and grafting

Cultivars	Mean symptom severity score ± SE		
	Control (healthy plants)	Mechanical Inoculation	Grafting
F1 Anaya	0.0 ± 0.0^{d}	5.0 ± 0.0^{ab}	6.0 ± 0.0^{a}
F1 Thales	0.0 ± 0.0^{d}	4.2 ± 0.5^{b}	4.5 ± 0.57^{b}
Floradade	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	3.0 ± 0.0^{c}
Local Tomato	0.0 ± 0.0^{d}	4.5 ± 0.58^{b}	5.2 ± 0.5^{ab}
Petomech	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	6.0 ± 0.0^{a}
Tomato Rio Grande	0.0 ± 0.0^{d}	5.0 ± 0.0^{ab}	5.5 ± 0.58^{a}

Means in columns with similar letters are not significantly ($P \geq 0.05$) different

Fig. 1: Infected tomato plant showing typical downward and upward leaf curling symptoms of *tomato yellow leaf Curl viral* disease

length of the shoot from ground level to the tip 77 days after inoculation (DAI). The internode length was also measured using a metre rule 77 DAI in both grafted and mechanically inoculated plants. The number of days for the appearance of the first flower was also recorded. State the number of replicates in each measurement.

Determination of chlorophyll content

Chlorophyll content was determined on three leaves obtained from both grafted and mechanically inoculated plants using the Spectra Absorbance Data (CCM-200 Chlorophyll Content Meter (Opti-Sciences, Hudson-USA) 100 DAI. The chlorophyll content was determined every week for four (4) consecutive weeks 100 days after the inoculation.

Determination of proline content

Proline content was determined using a modified method described by Bates *et al*. (1973). Two hundred and fifty (250 mg) of leaf samples taken from both grafted and mechanically inoculated plants were ground in 10 mL of 3 % sulphosalicyclic acid. The solution was then centrifuged at 33000 rpm for 10 min and 2 mL of supernatant was transferred into clean test tubes. Two (2 mL) of 6 *M* orthophosphoric acid, 2 mL of acid ninhydrin and 2 mL of glacial acetic acid were added to the supernatant and kept in a water bath for an h at 100°C. The resulting solution was then transferred into a separation funnel and 4 mL of toluene solution was subsequently added. After shaking carefully, the supernatant was collected and placed in an ultraviolet spectrophotometer and values read at 520 nm. Proline standards at different concentrations were prepared and proline content was calculated using the formula:

Proline content (μg/g Fw) = Absorbance/2 × 10/250×1000

Statistical analysis

The experiments in the screen house were carried out using Randomized Complete Block Design (RCBD) with five replicates. Analysis of variance was done using the Minitab Software (Version 17). Where ANOVA was significant, Tukey-pairwise comparison was used for mean separation. Pearson correlation coefficients were used to determine relationship between height and TYLCV transmission (Meena and Bahadur 2014).

Results

TYLCV disease symptom score

No virus symptoms were observed in any of the healthy tomato plants in grafted mode of virus transmission (Fig. 2a) as compared to disease plants under grafting method of virus transmission (Fig. 2b). Further in the mechanical inoculated method of virus transmission no virus symptom was produced in healthy plants (Fig. 3a). Suffice to say, plants also showed TYLCV symptoms in mechanical method of virus transmission (Fig. 3b), suggesting that the two methods allowed the transmission of the virus from plant to plant (Table 2). The degree of symptom severity was more pronounced in grafted plants (Fig. 3c) than mechanically inoculated virus plants. The days for symptom expression, however, varied between the two methods. For grafted plants, symptoms were first observed 35 days after grafting while for mechanical inoculation symptoms were observed 77 days after inoculation. For

Table 3: Plant height of six tomato cultivars subjected TYLCV infection using grafting and mechanical inoculation methods of virus transmission at 77 DAI

Cultivars	Mean plant height (cm) ± SE				
	Healthy	Mechanical Inoculation	% height reduction	Grafting	% height reduction
F1 Anaya	64.08 ± 5.50^{c}	120.30 ± 3.40^{a}	-87.7	64.92 ± 6.30^{c}	-1.3
F1 Thales	83.43 ± 9.39^{bc}	104.95 ± 1.79^{ab}	-25.7	143.28 ± 4.61^{a}	-71.7
Floradade	81.97 ± 5.60^{c}	74.53 ± 15.31^{c}	9.9	66.55 ± 7.75^{de}	18.8
Local Tomato	63.05 ± 3.75^{c}	67.13 ± 1.18^{c}	6.47	51.00 ± 6.20^{fg}	19.1
Petomech	77.85 ± 7.17^{c}	72.30 ± 26.4^{c}	7.12	59.55 ± 3.42^{ef}	23.57
Tomato Rio Grande	68.95 ± 4.05^{c}	80.22 ±10.52^{bc}	-16.34	44.40 ± 2.15^{g}	35.60

Means in columns with similar letters are not significantly ($P \geq 0.05$) different

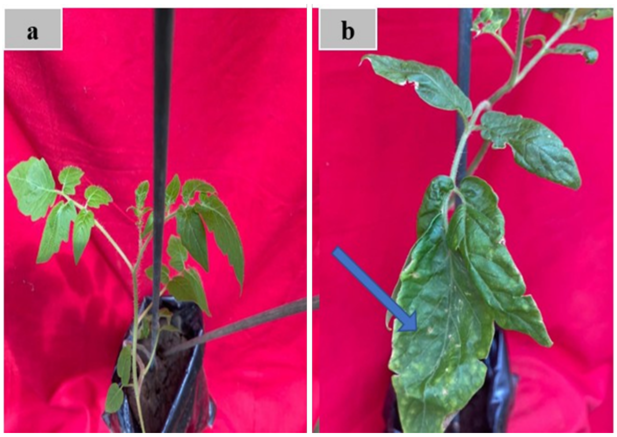

Fig. 2: *Tomato yellow leaf curl virus* symptoms on leaves of Petomech 35 DAI after grafting. (**a**) control (healthy) plant and (**b**) virus-infected plant under grafting

Fig. 3: *Tomato yellow leaf curl virus* symptoms on leaves of virus infected F1 Anaya at 77 DAI, (**a**) Healthy (control) plant (**b**) Virus-infected plant through mechanical inoculation and (**c**) Virus-infected plant through grafting

mechanical inoculation, symptom severity score ranged from no infection (0) in Floradade and Petomech to 5.0 in F1 Anaya and Tomato de Grande. Contrastingly, the grafting method allowed transmission in all the six varieties suggesting that it is more efficient in viral transmission than the mechanical inoculation method. The highest symptom severity score (6.0 ± 0.0) was observed in F1 Anaya and Petomech tomato cultivars while the least symptom severity score (3.0) was observed in Floradade. The control plants (healthy) did not show any symptoms of TYLCV disease. In both methods, significant ($P \leq 0.05$) differences in symptom severity between cultivars were observed. The symptom severity scores also showed that grafted plants were more infected with TYLCV than mechanical inoculation.

Plant height

Plant height of the tomato cultivars subjected to mechanical inoculation and grafting were measured to compare the effectiveness of the two modes of transmission of TYLCV 77 DAI in tomato plants (Table 2). The height of the plants varied significantly ($P \leq 0.05$) depending on the cultivar as well as the modes of transmission. For healthy plants (controls), the height of the plants ranged from 63.05 cm in Local tomato to 83.43 cm in F1 Thales cultivars which was slightly significant from the remaining cultivars. Of the two treatments, plants infected using mechanical inoculation grew taller than their respective counterparts infected *via* grafting except for F1 Thales which had the tallest plants (143.28 cm). The height of plants infected with TYLCV *via* mechanical inoculation ranged from 67.13 cm in Local tomato to 120.30 cm in F1 Anaya suggesting genotypic differences while for grafting, plant height after infection ranged from 44.40 cm in Tomato Rio Grande to 143.28 cm in FI Thales. The percentage height reduction after infection using both transmission modes did not show the expected growth reduction trend in all cultivars. In both mechanical inoculation and grafting, F1 Anaya and F1Thales grew taller after infection with the virus regardless of the mode of transmission. The same observation was made when Tomato Rio Grande plants were infected *via* mechanical inoculation. Consequently, the calculated height reduction in these two cultivars is negative ranging from -16.34% to -87.7% and -1.3% to -87.7% for plants infected *via* mechanical inoculation and grafting respectively (Table 3). Also, with the exception of F1 Anaya and F1 Thales, the percentage height reduction was comparatively higher when plants were infected *via* grafting than mechanical inoculation. In Floradade, the percentage height reduction in plants infected *via* grafting was twice that of those infected through

Table 4: Internode length of six tomato cultivars subjected to TYLCV infection using grafting and mechanical inoculation methods at developmental stage of 77 DAI

Cultivars	Mean internode length (cm) ± SE		
	Healthy	Mechanical Inoculation	Grafting
F1 Anaya	10.03 ± 1.40^{abc}	6.25 ± 1.02^{c}	4.40 ± 1.47^{d}
F1 Thales	13.80 ± 1.04^{a}	8.65 ± 0.55^{bc}	9.82 ± 2.92^{b}
Floradade	11.35 ± 0.73^{ab}	6.58 ± 0.85^{c}	5.58 ± 0.29^{cd}
Local Tomato	8.25 ± 0.64^{bc}	6.55 ± 0.72^{c}	3.58 ± 0.99^{d}
Petomech	10.00 ± 0.16^{bc}	6.70 ±1.85^{c}	3.38 ± 0.76^{d}
Tomato Rio Grande	9.68 ± 3.16^{bc}	9.38 ± 2.87^{bc}	3.53 ± 0.58^{d}

Means in columns with similar letters are not significantly ($P \geq 0.05$) different

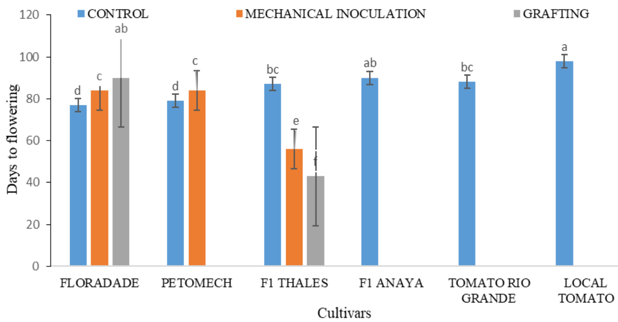

Fig. 4: Effect of post-grafting and mechanical inoculation modes of transmission of TYLCV on days to flowering. Bars with similar letters are not statistically significant ($P \geq 0.05$)

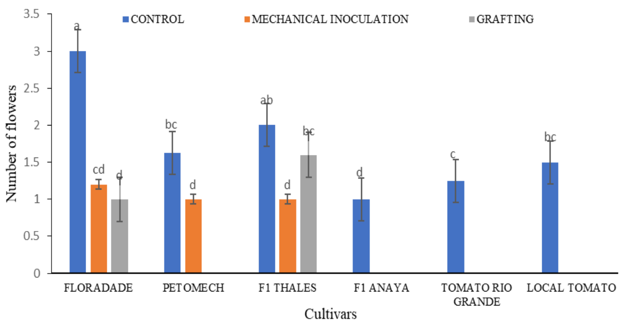

Fig. 5: Effect of post-grafting and mechanical inoculation modes of transmission of TYLCV on the number of flowers. Bars with similar letters are not statistically significant ($P \geq 0.05$)

mechanical inoculation while in both Local tomato and Petomech, the percentage height reduction in grafting was three times those of mechanical inoculation.

Internode length

Table 4 shows the effect of TYLCV disease after infection through mechanical inoculation and grafting methods on the length of internode in the five tomato cultivars. Unlike the plant height, the effect of the virus on the internodes showed the expected trend with the controls (healthy plants) showing longer internodes than the virus infected plants. The severest effect of the virus was observed in Petomech shown by internode length of 3.38 cm when plants were infected *via* grafting. For mechanical inoculation, the severest effect of the virus was observed in Local tomato cultivar shortening the internode from 8.25 cm in the controls to 6.55 cm. Independent of the mode of transmission, the viral infection in F1 Anaya, F1 Thales, and Floradade differed significantly ($P \leq 0.05$) from the remaining cultivars (Table 4). Generally, the effect of the virus on shortening of internodes in plants infected via grafting was severe than those infected by mechanical inoculation.

Days to flowering

The effect of TYLCV infection on flowering in six tomato cultivars is shown in Fig. 4. All the controlled healthy plants flowered while three of the infected cultivars (F1 Anaya, Rio De Grande and Local tomato did not flower during the study period. Of the three remaining cultivars, Petomech plants infected via grafting did not also flower. The days to flowering varied depending on the cultivar as well as the mode of the transmission of the virus. The days to flowering was delayed in Floradade (84 days) and Petomech (85 days) plants infected via mechanical inoculation compared to the controls (76 days) while in F1 Thales, flowering was earlier (52 days) when plants were infected either via mechanical inoculation or grafting.

Number of flowers

All controlled healthy plants produced flowers while three of the virus infected plants (F Anaya, Tomato Rio Grande and Local tomato) did not produce flowers. Of those cultivars which produced flowers, the controls significantly ($P \leq 0.05$) produced more flowers than the infected plants. In Petomech, grafted mediated transmission of TYLCV did not produce flowers throughout the study period. The number of flowers produced by the virus infected plants varied depending on the cultivar. In Floradade, the number of flowers produced by mechanical inoculation (1.25) was comparatively higher than grafted mediated (1.8) transmission while in F1 Thales, grafted mediated transmission significantly produced more flowers per plant than mechanical inoculation (Fig. 5).

Chlorophyll content

The effect of the virus on chlorophyll content after transmission was studied using Spectra Absorbance Data and the results are graphically presented in Fig. 6. The chlorophyll content in all the healthy controlled plants was more than 10 mg/m^2 which was significantly ($P \leq 0.05$) higher than the infected plants. Also, in most cultivars, it was more than double the infected plants. Local tomato and Floradade controls had the highest chlorophyll content among all the cultivars. Of the infected plants, mechanical inoculation mediated transmission was higher than grafting. In Floradade and F1 Thales the effect of the virus *via*

Table 5: Correlation between plant attributes measured and the mode of TYLCV transmission into six tomato cultivars used in this study

		PHC	PHMI	PHG	ILC	ILMI	ILG	VSC	VSMI	VSG	CCC	CCMI	CCG	DFC	DFMI
PHMI	R	-0.018													
	P	0.973													
PHG	R	0.63	0.499												
	P	0.18	0.314												
ILC	R	0.569	0.594	0.813											
	P	0.238	0.214	0.049											
ILMI	R	0.163	-0.064	0.198	0.454										
	P	0.757	0.904	0.707	0.366										
ILG	R	0.765	0.477	0.974	0.832	0.145									
	P	0.076	0.338	0.001	0.04	0.785									
VSC	R	*	*	*	*	*	*								
	P	*	*	*	*	*	*								
VSMI	R	-0.492	0.472	0.347	0.252	0.201	0.142	*							
	P	0.322	0.345	0.501	0.63	0.703	0.789	*							
VSG	R	-0.658	0.144	-0.259	-0.314	0.318	-0.412	*	0.533						
	P	0.156	0.786	0.621	0.544	0.539	0.417	*	0.276						
CCC	R	-0.605	-0.703	-0.848	-0.727	-0.128	-0.875	*	-0.15	0.081					
	P	0.203	0.119	0.033	0.102	0.809	0.023	*	0.776	0.878					
CCMI	R	0.647	-0.73	-0.079	-0.077	0.249	0.05	*	-0.775	-0.463	0.139				
	P	0.165	0.1	0.882	0.884	0.634	0.924	*	0.07	0.355	0.793				
CCG	R	0.717	0.018	0.582	0.573	-0.136	0.676	*	-0.22	-0.919	-0.298	0.331			
	P	0.109	0.973	0.225	0.235	0.797	0.14	*	0.675	0.01	0.566	0.521			
DFC	R	-0.556	0.553	0.271	0.187	0.187	0.072	*	0.973	0.674	-0.196	-0.836	-0.394		
	P	0.252	0.255	0.604	0.723	0.723	0.892	*	0.001	0.142	0.71	0.038	0.44		
DFMI	R	0.853	-0.269	0.171	0.126	-0.069	0.362	*	-0.857	-0.622	-0.307	0.802	0.475	-0.859	
	P	0.031	0.606	0.746	0.811	0.897	0.481	*	0.029	0.188	0.554	0.055	0.341	0.028	
DFG	R	0.783	-0.025	0.503	0.484	-0.226	0.636	*	-0.408	-0.963	-0.306	0.432	0.976	-0.554	0.635
	P	0.066	0.962	0.309	0.331	0.667	0.175	*	0.421	0.002	0.556	0.393	0.001	0.254	0.176

*Identical values; R – Correlation value; P – *P* value

Characters; PHC - Plant height control (cm), PHMI - Plant height mechanical inoculation (cm), PHG - Plant height grafting (cm), ILC - Internode length control (cm), ILMI - Internode length mechanical Inoculation (cm), ILG - Internode length grafting (cm), VSC - Viral score control, VSMI - Viral score mechanical inoculation, VSG - Viral score grafting, CCC - Chlorophyll content control (mg/m^2), CCMI - Chlorophyll content mechanical inoculation (mg/m^2), CCG - Chlorophyll content grafting (mg/m^2), DFC - Days to flowering control, DFMI - Days to flowering mechanical inoculation and DFG - Days to flowering grafting

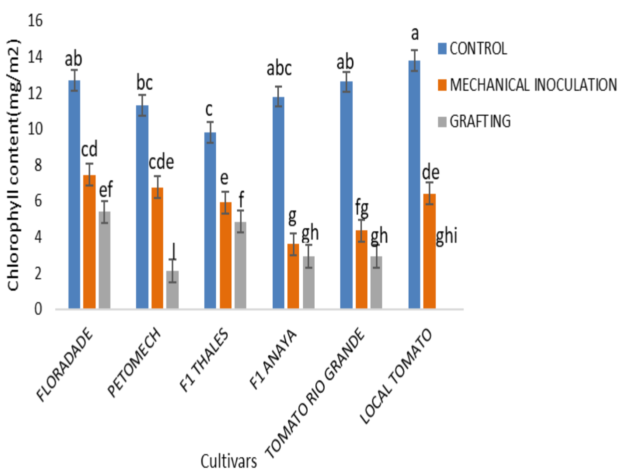

Fig. 6: Chlorophyll content of tomato cultivars infected with mechanical inoculation and grafted mode of transmission of TYLCV. Bars with similar letters are not statistically significant ($P \geq 0.05$)

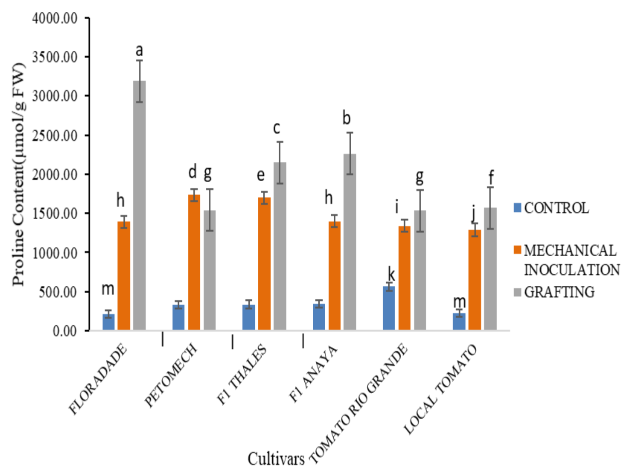

Fig. 7: Effect of TYLCV disease on proline content transmitted mechanically inoculated or grafting on tomato cultivars. Bars with similar letters are not statistically significant ($P \geq 0.05$)

mechanical inoculation was highly significant than the grafted method. For the remaining cultivars the differences between mechanical inoculation and the grafted method are slightly significant. The chlorophyll contents of TYLCV infected local tomato *via* grafting were below a detectable threshold.

Proline content

The proline content in all the healthy plants was significantly lower than the infected plants regardless of the mode of transmission; it was below 500 μmoL/gFW except in Tomato Rio Grande (500 μmoL/gFW). Contrastingly, the proline content of the TYLCV infected plants *via* grafting was significantly higher than the mechanical inoculation method ranging from 1500 μmoL/gFW in Tomato Rio Grande to 3250 μmoL/gFW in Floradade. For the mechanical inoculated method, the proline content ranged from 1350 μmoL/gFW in local tomato to 1750 μmoL/gFW in Petomech (Fig. 7).

Correlation analysis was done to determine the extent of relationship between the morphological traits of the plants and the effect of the virus transmitted *via* mechanical inoculation or grafting and the results are tabulated in Table 5. There were both positive and negative correlation between control, mechanical inoculation and grafting under plant height, internode length, viral score, chlorophyll content and days to flowering. A strong positive correlation was seen in the interaction between internode length through mechanical inoculation (ILMI) and plant height through mechanical inoculation (PHMI) (r = 0.904). This strong correlation was mirrored in chlorophyll content through grafting (CCG) and plant height through mechanical inoculation (PHMI) (r = 0.973). In addition, days to flowering obtained by grafting (DFG) and plant height through mechanical inoculation (PHMI) equally showed a positive correlation (r = 0.962). Interestingly, chlorophyll content through grafting (CCG) negatively correlated with viral symptom score through grafting (VSG) (r = -0.919). Similar observation was seen in days to flowering through grafting (DFG) and chlorophyll content through grafting (CCG) (r = -0976).

Plant height under mechanical inoculation (PHMI) showed a moderate positive correlation towards plant height through grafting (r = 0.499), internode length-control (r = 0.594), internode length through grafting (r = 0.477), viral score-mechanical inoculation (r = 0.472) and days to flowering-control (r = 0.553). Likewise, a moderate positive relationship was observed between plant height, chlorophyll content (r = 0.582) and days to flowering (r = 0.503) under grafting (Table 5). Plant height-control (PHC) also showed a moderate positive relationship towards plant height-grafting whereas plant height grafting gave a strong negative correlation with plant height control (r = -0.658). A similar correlation was observed between internode length-control (ILC), internode length-mechanical inoculation (ILMI) (r = 0.454) and chlorophyll content-grafting (CCG) (r = 0.573), viral score grafting (VSG) and mechanical inoculation (r = 0.533), chlorophyll content-mechanical inoculation (CCMI) and days to flowering-grafting (r = 0.432), chlorophyll content-grafting (CCG) and days to flowering-mechanical inoculation (DFMI) (r = 0.475).

Plant height under control (PHC) showed a moderate negative correlation towards viral score-mechanical inoculation (VSMI) (-0.492) and days to flowering-control (-0.556) and a very weak positive and negative correlation towards internode length (r = 0.163) and plant height (-0.018) under mechanical inoculation, respectively.

Discussion

Tomato Yellow Leaf Curl Virus (TYLCV) is one of the most devastating viral diseases in tomato, posing a serious threat to food security globally. It is vectored by *Bemissia tabaci* and it is symptomatically expressed as upward leaf curling, cupping with or without marginal chlorosis, smaller leaflets and stunted growth, thereby leading to reduced yield. In the present study, the leaves of tomato plants infected with TYLCV *via* mechanical inoculation or grafting showed these symptoms, indicating successful transmission by both methods. However, days to transmission and disease symptom severity varied depending on the mode of transmission. Grafting mediated transmission comparatively showed earlier symptom expression and higher symptoms severity than mechanical inoculation. Several authors have reported that grafting-mediated TYLCV transmission is more efficient than mechanical inoculation. Lee *et al.* (2017) reported that single leaflet grafting in tomato is an efficient method for transmitting Tomato Chlorosis Virus (ToCV) and TYLCV, providing a reliable inoculation technique for research. Tomato yellow leaf curl disease is phloem limited (Cathrin and Ghanim 2014) and its efficient transmission *via* grafting may be attributed to continuous exposure of a recipient plant to a high level of inoculum once the vascular system between the scion (recipient) and the rootstock (source) is connected. Consequently, grafting has been used to transmit several viral pathogens, as mechanical inoculation does not readily transmit them (Mirzayeva and Huseynova 2024). Thus, grafting has been applied for the inoculation of various viral pathogens that are not easily transmissible by mechanical inoculation (Lee *et al*. 2017).

The severity of disease symptoms can represent the degree of plant tolerance and/or tolerance to a disease. We observed that the six tomato cultivars used in the study differed in their symptom severity suggesting that their tolerance and/or susceptibility to the TYLCV viruses also differed. Floradade showed the least disease severity suggesting that it was tolerant, while F1 Anaya and Petomech exhibited high symptom severity score suggesting that they are highly susceptible to the virus. The observed differences could be attributed to cultivar specificity as well as the inherent genetic constitution of the individual tomato cultivars. Domesticated tomato *Solanum lycopersicum,* is highly susceptible to the TYLCV causing total crop failure in several cultivars, thus there is the need to breed for highly tolerant cultivars by introgression of tolerant genes into susceptible cultivars. Recent breeding efforts have released several commercially available TYLCV-resistant cultivars (Dhaliwal *et al.* 2020). However, these resistance cultivars are not immune to the virus and accumulate TYLCV, which make them exhibit mild symptoms or no symptoms at all following TYLCV infection (HEl-Sappah *et al*. 2022).

The successful transmission of the TYLCV virus *via* grafting subsequently led to significantly impaired growth by decreasing the plant height and internode length. Earlier studies by Vitti *et al.* (2016) and Villalón *et al*. (2018) also revealed similar reduction in plant height by viruses in *Trichoderma harzianum*. The transmitted viruses imposed biotic stress on the plants resulting in decreased growth and development due to inhibition of cell division and differentiation in the shoot apical meristem (Bradamante *et al*. 2021). Choe *et al*. (2021) hypothesized that TYLCV - induced symptoms leading to impairment of plant growth and development might be directly associated with the suppression of cellulose synthesis genes.

Post viral transmission of TYLCV significantly reduced the chlorophyll content in all the six cultivars independent of the mode of transmission, although the reduction was comparatively higher in mechanical inoculation than grafting. Bellah *et al.* (2023) have reported that plant viruses destroy the structure and function of host tissues and organelles, prevent the normal physiological development of the host, causes a rise in the activity of chlorophyll-degrading enzymes and decreases chlorophyll content. The reduction in chlorophyll content reduces the photosynthetic efficiency of the virus infected host plants. Infected tomato plants with Tomato yellow mosaic virus (TYMV) have been found to cause a reduction in photosynthetic rate and an increase in respiration (Montasser *et al.* 2012).

Proline is known to protect the plant under stress conditions as it maintains the integrity of the cell membrane by balancing the change in the turgor pressure due to pathogenic infection (Singh *et al*. 2021). We therefore studied the effect of the TYLCV on the proline content of the infected plant. The proline content was significantly higher in the infected plants regardless of the mode of transmission than the controls. These findings were similar to those of Bassiouny *et al.* (2015) who reported that tomato plants infected with TYLCV led to a significant increase in proline content. Proline is a stress marker in plants, playing a role in scavenging hydroxyl radicals and helping plants resist stress. TYLCV infection triggers this increases as part of the plant's defense response to the viral stress (Mishra *et al.* 2022).

Correlation studies revealed that both grafting and mechanical inoculation modes of virus transmission improved plant attributes through the strong positive correlation observed in this study. It appears that the exact relationship between modes of virus transmission and plants attributes (measurements) seen in this study may be obscure and yet to be verified. Nevertheless, individual varietal responses may account for the differences seen in the mechanism of viral transmission and plant attributes observed in this study. Further, chlorophyll content through grafting seems to expose tomatoes cultivars to high viral load which negatively impacted chlorophyll content. In related research, chlorophyll contents were found to be negatively correlated to viral load in Brassica after *Turnip mosaic virus* infection (De-Ping Guo 2005). Plant viruses have a pronounced effect on plant height because viruses impair meristematic activity leading to stunted growth. Iftikhar *et al.* (2021) reported related findings in tomatoes where strong negative correlation was found to exist between tomato leaf curl virus disease incidence and plant height.

Conclusion

Both grafting and mechanical inoculation allowed successful transmission of *Tomato Yellow Leaf Curl Virus* (TYLCV) to non-infected plants. Nevertheless, virus transfer to healthy plants was more efficient in grafting than mechanical inoculation. The successful transmission *via* grafting could be used for propagation and management of the virus. The incidence of the disease varied depending on the tomato cultivar as well as the mode of transmission. The high symptom severity resulted in reduced chlorophyll content which may have adverse effect on photosynthetic efficiency and yield. Floradade with low disease incidence could be used in tomato breeding programmes to breed for TYLCV tolerance varieties to benefit farmers.

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Authors' Contributions

Conceptualization: KOA, LBG and ASA. Methodology: ASA, KOA and LBG. Investigation: KOA, LBG and ASA. Original Draft: LBG and KOA. Formal analysis: LBG, ASA, WN and KOA. Writing, review and editing: LBG, KOA, ASA, WN, SA and KD.

Conflict of Interest

The author(s) declare(s) that they have no competing interests.

Data Availability

The reported data can be made available upon requesting to the corresponding author.

Ethics Approval

Not applicable to this study.

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