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Grafting for sustainable management of *Fusarium* wilt disease in tomato production in Ghana

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ABSTRACT

Fusarium wilt disease limits tomato production, especially in Ghana. In managing the *Fusarium* wilt disease, two rootstocks (*Solanum torvum* and *Solanum macrocarpon*) were used in grafting experiments. Plant growth, yield, disease severity and incidence of both grafted plants, and non-grafted plants were evaluated in a pot experiment and also under a naturally infected open field condition at Berekum. During the early stage (14 days after inoculation) under artificial inoculation conditions, grafted plants exhibited higher photosynthetic rates (10.41 $\mu mol^{-2}s^{-1}$) compared to the non-grafted plants (8.36 $\mu mol^{-2}s^{-1}$). Under naturally infested field conditions, chlorophyll content and photosynthetic rate of non-grafted plants decreased. *Solanum lycopersicum* grafted onto *S. macrocarpon* and *S. torvum* were moderately susceptible (20%–40%) to *Fusarium oxysporum*. However, the non-grafted plants were highly susceptible (50%–100%). Yield from the pot experiment for *S. lycopersicum* grafted onto *S. macrocarpon* was significantly higher (453.1 g/plant), compared to *S. lycopersicum* grafted onto *S. torvum* (350.3 g/plant) and the non-grafted plant (205 g/plant). However, in naturally infected field, the grafted plants increased in fruit yield compared to the non-grafted tomato plants. *Solanum macrocarpon* and *S. torvum* as rootstocks offered resistance against *F. oxysporum* and showed significantly lower disease progression, than the non-grafted plants ($P < 0.05$). This study revealed that grafting is an effective tool for the management of *Fusarium* wilt disease and for tomato growth and yield improvement.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is a high valued crop in Ghana and the most consumed vegetable by people from diverse backgrounds. Tomato production is a significant agricultural activity in Ghana that contributes to poverty alleviation, through creation of jobs, increase food security and generation of income. In Ghana tomato production is predominantly a small-scale activity and seasonal with a few large-scale irrigation sites [1]. The average yield in the country is estimated at 395, 755 tonnes on 92,045 Ha of land which exceeds the annual consumption (400,900 tonnes) of the country [2]. Tomato production in Ghana has not been able to reach its full capacity to attain yields that can meet the demand of the country [3]. Consequently, 8753 tonnes of tomatoes are imported from neighbouring countries (Burkina Faso and Togo) into the country to make up for the market and consumer demand. This is due several constraints could be attributed to production and post-harvest; pest, diseases, limited availability of improved seeds and inputs, unavailable source of water for irrigation, access to credit, inability to get extension services, abiotic stress and other post-harvest factors [4].

Amongst these constraints, soil-borne fungal diseases are the most destructive diseases faced in Ghana. *Fusarium* wilt disease of tomato is a highly destructive pathogen, difficult to diagnose and control. Control measures including, long-term rotation, soil solarization, use of synthetic chemicals and regulated deficit irrigation and use of resistant varieties have been employed by farmers [5]. However, these control methods have some limitations and this makes farmers still seek for other approaches which are efficient and cost effective to control *Fusarium* wilt disease. In Ghana, *Fusarium* wilt incidence recorded in three regions ranged between 24% and 46% [6]. A survey conducted in four farming communities in the central region of Ghana identified three different *Fusarium* spp. causing wilt on their farms [7]. *Fusarium oxysporum* is phylogenetically diversity and serves as a model organisms for biological and evolutionary research [8]. The various strains are classified into forma specialis (f. sp.) based on parasitism on specific hosts.

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Further, strains of *F. oxysporum* could be saprophytic or non-pathogenic [9]. *Fusarium oxysporum* enters the epidermis of the root, and spreads through the vascular tissue, inhabits the plant xylem vessels, which become blocked, leaving the plant to become water stressed, the plant then wilts ultimately [10]. The dormant chlamydospore of Fusarium in infested soil can survive for long periods or indefinitely in the absence of host [11,12]. However, in Ghana, there is scarce information on the identities of species causing diseases on tomato.

The indiscriminate use of fungicides to control *Fusarium* wilt has led to the development of more virulent strains of this pathogen [13]. Grafting is therefore being encouraged in place of soil fumigant usage because of environmental concerns [14]. A previous study conducted using wild *Solanum* resistant species (*S. torvum*, *S. xanthocarpum*, *S. khasianum* and *S. surathense*) as graft rootstocks, gave positive results for managing tomato bacterial wilt diseases. *Solanum torvum* grafted onto *Pusa shyamala* had the lowest disease infection (12.2%) and the highest graft compatibility of 81.85% [15]. In another study conducted by Agyeman [16] graft success percentages of 93% and 94% were observed for *S. lycopersicum* grafted onto *S. macrocarpon* and *S. aethiopicum* respectively. The use of Maxifort rootstock to control *Fusarium* wilt in heirloom tomato provided low levels of disease severity compared to the non-grafted plants (75%) [17].

The objectives of this study were to: evaluate the potential of grafted tomato plants onto *S. torvum* and *S. macrocarpon* for enhanced agronomic performance, improved yield, and reduced disease incidence and severity in pot and field conditions to *Fusarium* wilt disease.

2. Materials and methods

2.1. Isolation of fungal isolate

Fusarium oxysporum isolate FO11 was isolated from the stem of an infected tomato plant grown in a *Fusarium* infested field in Berekum. The stem of the tomato plant was cut at the advancing edge or border of the diseased and healthy portion of the plant at a size of 3 mm–6 mm. The cut tissues were surfaced sterilized in 1% Sodium hypochlorite for 1 min. The cut tissue was then plated onto water agar and incubated at 26 °C ± 1 °C. Three days after incubation, all plated samples with mycelia growth were cut and placed onto a plate containing Potato Dextrose Agar (PDA). Penicillin was added to the sterile PDA to minimize the chance of bacterial growth and incubated at 26 °C ± 1 °C. The plates were observed periodically. The preferred fungal colonies were sub-cultured to obtain a pure culture and stored on a PDA slant at 4 °C for future use.

2.2. Grafting

2.2.1. Planting material

Two *Solanum* species (*S. macrocarpon* and *S. torvum*) were selected as rootstock based on their ability to tolerate drought, flooding, salinity and soil-borne diseases. *Solanum lycopersicum* variety Petomech was selected as the scion because farmers commonly used it. Seeds of *S. torvum* were sown 3 weeks earlier than *S. macrocarpon*. Seeds of Petomech (the scion) were sown two weeks after sowing *S. macrocarpon*. This was done to obtain similar stem diameters during grafting because both rootstocks and scions have uneven emergence and seedling growth. Petomech used as a control in the study, was sown two weeks after sowing the rootstocks to maintain a uniform age of all plants for the experiment because the grafted plants will undergo a period of acclimatization. All seeds nursed were kept under good nursery management practices.

2.2.2. Grafting procedure

Grafting was carried when all seedlings had initiated their third true leaves. Seedlings were well watered in the late afternoon, a day before grafting. The working bench, blade, and grafting clips were sterilized with 5% sodium hypochlorite. Petomech was grafted onto the rootstocks: *S. torvum* (P/ST) and *S. macrocarpon* (P/SM). Grafting was done using the cleft method as described by Lee et al. [18] (Fig. 1.). Subsequently, the grafted plants were then placed in a chamber. The healing chamber measured 1.4 × 1.0 × 0.4 m and was made of Wawa wood, plastics and iron rods. The base of the chamber was made with a wooden slab, and the inner portion was lined with a black plastic sheet. The apical portion was covered with a double layer of plastic sheet (one black and a transparent sheet) to control light and humidity in the chamber.

2.2.3. Healing of grafted plant

The grafted plants were healed in complete darkness in a healing chamber under ambient temperatures and 98% relative humidity for 2 days. The humidity level was achieved at 98% by misting the chamber with water and covered with a black plastic sheet to prevent light from entering. From day three onwards, the black plastic sheet was removed, leaving the transparent sheet for partial entry of light. Besides this, the chamber was opened partially at noon for 30 min to allow ventilation and to reduce the build-up of heat in the chamber. This was repeated for four days before opening the chamber fully under a shade structure. Grafts that showed mouldy growth or appeared weak or toppled were discarded to prevent contamination during healing. The widest temperature range recorded over seven days during the wet season in the wooden chamber was 24.0°C–29.5 °C and a corresponding humidity range of 78.5–100%. On the other hand, the environmental conditions outside the wooden chamber (in the shed house) gave a temperature range of 24.1°C–30.4 °C and 67.3%–90.7% of humidity. During the dry season temperature range recorded in the wooden chamber was 23.9°C–30.5 °C and a corresponding humidity of 76.7%–100.0%. The external environment recorded a 24.1°C–30.2 °C temperature range and a humidity of 65.4%–88.5%.

2.2.4. Acclimatization of successful grafts

The plants were acclimatized by exposing them to sunlight and ambient temperature daily for seven days. The acclimatization was done gradually; the plants were exposed to ambient conditions in the chamber for 30 min s on the 1st and 2nd days, 1 h on day 3, 4 h on day 4, 7 h on days 5 and 6, and a full day on days 7 and 8. The plants were kept hydrated by applying water to just the base of the rootstock. At this stage, side shoots on the rootstocks were removed whiles those on the scion were kept to allow for new growth. The media was regularly checked and fertigated with NPK 19:19:19 at 3 g/L by capillary action when necessary. As part of the hardening process, the grafts were brought out of the chamber for 1, 3, and 6 h from the 10th to the 14th day. Grafted plants were transferred into plastic pots and carried to the experimental fields.

2.3. Potting and field experiments

The research trials were carried out at two sites. A plastic pot experiment was conducted at the University of Ghana school farms located in the Greater Accra region of Ghana (GPS coordinate: Lat. 5° 3′ 34″N and Long. 0° 11′ 31″W). The second experiment was conducted on a farmer's field with a history of *Fusarium* wilt infestation located at Berekum, Bono region of Ghana (GPS coordinate: lat. 7° 30′ 16″N and long. 2° 46′ 37″W)

2.3.1. Fungal inoculum

A pure culture with morphological characteristics of *Fusarium oxysporum* f. sp. *lycopersici* was plated on PDA and incubated at 25–27 °C in light/dark conditions for two weeks. The mycelia were scraped from the Petri dishes into a blender and topped with distilled water to a 1000 ml

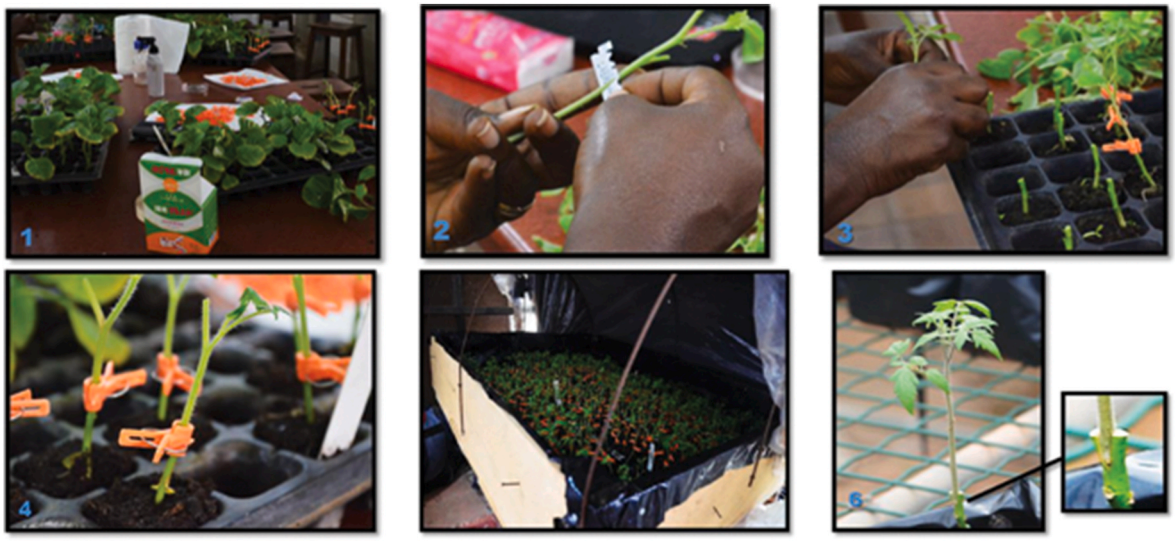

Fig. 1. Grafting process using the cleft grafting method. **1**- Roots stocks; **2**- Severing the scion below the cotyledon into a wedge form after defoliation of leaves; **3**- making a vertical slit in the middle of the rootstock (~4 mm deep) after it has been severed; **4**- A wedge-shaped scion gently inserted into the vertical slit on the rootstock with a supporting clip; **5-** grafted plants placed in a healing chamber; **6-** A grafted plant showing a successful union between the scion and the rootstock.

mark. The concentration of the spore suspension was adjusted to 4.4 × 10^6 spores/5 ml using a hemocytometer under an optical microscope. Each selected seedling was inoculated with the conidial suspension at 4.4 × 10^6 spores/5 ml of distilled water. Control seedlings were inoculated with only sterile distilled water.

2.3.2. Pot experiment

Grafted and non-grafted plants were placed in plastic bags (9 cm × 14 cm) filled with sandy-clay soil. Before filling the bags, the soil was heat-sterilized using hot water (at boiling point). A 3 × 2 factorial treatment structure was used. Factor one consisted of three rootstocks (ungrafted *S. lycopersicum* (control), *S. lycopersicum* grafted onto *S. torvum* and *S. lycopersicum* grafted onto *S. torvum*), factor 2 consisted of inoculum (4.4 × 10^6 spores suspension of *F. oxysporum* and sterile distilled water as control). The treatments used were as follows; PIo = Control- Petomech ungrafted + distilled water, PIi = Petomech ungrafted +4.4 × 10^6 spores suspension *F. oxysporum*, P/SMIo = Petomech grafted onto *Solanum macrocarpon* + distilled water, P/SMIi = Petomech grafted onto *Solanum macrocarpon* + 4.4 × 10^6 spores suspension *F. oxysporum,* P/STIo = Petomech grafted onto *Solanum torvum* + distilled water, P/STIi = Petomech grafted onto *Solanum torvum* + 4.4 × 10^6 spores suspension *F. oxysporum*. They were laid in a Completely Randomized Design with three replicates. There were 18 plots, each containing 12 plants, three (3) replicates and six treatments in each replicate. The planting distance of the potted plants was 0.70 m × 0.50 m. Distance between plots was 0.5 m and 1 m between blocks.

2.3.3. Field experiment

The design used for the field experiment was a randomized complete block design (RCBD) with four replicates. The treatments were: Petomech grafted onto *S. macrocarpon* (P/SM), Petomech grafted onto *S. torvum* (P/ST) and Petomech non-graft as control (P). Each of the three treatments was replicated four times with twenty-five (25) seedlings in an experimental unit.

2.4. Data collection

Data was collected on percentage graft take (%), disease incidence and severity, plant height, stem girth, number of leaves per plant, chlorophyll content, photosynthetic rate, yield per plant (number of fruit per plant, fruit weight), fruit firmness, total soluble solids (TSS), pericarp thickness, number of locules, pH of fruits, fresh and dry shoot and root weights.

2.4.1. Chlorophyll content

Chlorophyll content was measured using a Chlorophyll meter (Apogee Instruments, USA) on six record plants for each treatment. Four weeks after the tomato plants were transplanted, the chlorophyll content was taken every two weeks for three more times.

2.4.2. Photosynthetic rate

A CIRAS 3 portable gas analyser equipped with an automated broad leaf cuvette (PP systems, USA)was used to measure the tomato plants' photosynthetic rates. The following parameters were measured: change in carbon dioxide (Ci) concentration (μmol mol^{-1}), stomatal conductance (mmol H_2O m^{-2} s^{-1}).

2.4.3. Fruit quality attributes

Fruit firmness of the tomato fruits was measured using a hand penetrometer with an 11 mm plunger pressed against the flesh until it reached a marked fixed depth on the piston. Total soluble solids (TSS) were determined using a digital hand refractometer (Hanna© refractometer 96801) at room temperature. The number of locules and pericarp thickness involved samples of the tomato fruits transversely cut open, and the number of locules in one half of the fruit counted. Using the same cut fruit, a vernier caliper was used to measure the pericarp thickness of the fruit. The pH of the tomato fruit juice was determined using a 3330 pH meter, which was buffered at a pH of 7.

2.5. Statistical analysis

Analysis of variance (ANOVA) for the data collected was carried out using Genstat statistical package (v.12). Where there were significant differences, the Least Significant Difference test (LSD) was used to determine differences among the means for the various parameters studied. Significance was defined at $P < 0.05$; where necessary, data were transformed for normality before ANOVA was conducted.

3. Results

3.1. Graft-take success

Petomech plants grafted onto *S. torvum* (P/ST) had the highest graft-take during the dry season (72%) and the rainy season (91%). However, Petomech grafted onto *S. macrocarpon* (P/SM) had much lower graft-take during the dry season (68%) and the rainy season (83%).

3.2. Effect of grafting and Fusarium oxysporum inoculum on the vegetative growth of tomato plant (Pot experiment)

3.2.1. Plant height

There was a significant interactive effect ($P < 0.05$) between the inoculum and the graft combinations on plant height at week 2 after inoculation (Tables 1 and 2). *Solanum lycopersicum* (Petomech) grafted onto *S. torvum* with *Fusarium* inoculum (P/ST x I) produced the tallest plants (49.9 cm), followed by Petomech grafted onto *S. macrocarpon* rootstock inoculated with *Fusarium* (P/SM x I) (46.1 cm) and the shortest plants were Petomech non-grafted (control) with *Fusarium* inoculum (P x I) (42.3 cm). However, no significant interactive effects in plant height were found among the various treatment combinations at 4 weeks after inoculation (Table 1). The height of P/ST (53.09 cm) was significantly increased compared to the other rootstocks.

Table 1
Rootstocks and *Fusarium oxysporum* inoculum and their interaction on the growth and yield of tomato plants in the pot and field experiment.

Parameters	Rootstock (R)		Inoculum (I)		R x I	
	F	P-value	F	P-value	F	P-value
Pot Experiment						
Plant height 2WAI	15.85	<.001	1.67	0.226	5.25	0.028
Plant height 4WAI	7.01	0.012	5.13	0.047	2.20	0.161
Plant girth 2WAI	8.69	0.006	0.10	0.756	5.45	0.025
Plant girth 4WAI	6.62	0.015	0.40	0.540	4.54	0.040
Chlorophyll content 2WAI	16.52	<.001	0.61	0.454	4.89	0.033
Chlorophyll Content 4WAI	19.11	<.001	7.62	0.020	70.35	<.001
Photosynthetic rate 2WAI	19.92	<.001	20.49	0.001	30.78	<.001
Photosynthetic rate 4WAI	7.37	0.011	1.75	0.216	0.71	0.516
Total Soluble Solid (TSS)	0.04	0.956	1.18	0.302	3.90	0.056
Fruit Firmness	1.00	0.403	0.05	0.836	0.68	0.531
Pericarp Thickness	19.41	<.001	1.87	0.202	1.97	0.190
pH	12.75	0.002	3.84	0.078	1.70	0.231
Fruit diameter	70.23	<.001	23.57	<.001	4.03	0.052
Fruit length	128.77	<.001	29.46	<.001	2.30	0.151
Fruit per plant	1.13	0.361	0.67	0.432	3.32	0.078
Yield per plant	92.58	<.001	0.01	0.940	10.42	0.004
Field Experiment						
Plant height 3WAT	12.07	0.008				
Plant height 6WAT	1.54	<.001				
Plant girth 3WAT	0.33	0.731				
Plant girth 6WAT	1.53	0.290				
Chloropyll Content 3WAI	66.74	<.001				
Chloropyll Content 6WAI	425	<.001				
Photosysnthetic rate 3WAT	3.65	0.092				
Photosynthetic rate 6WAT	13.79	0.006				
Total Soluble Solid (TSS)	1.44	0.308				
Fruit Firmness	17.99	0.003				
Pericarp Thickness	2.04	0.211				
pH	2.62	0.152				
Fruit diameter	9.83	0.013				
Fruit length	15.75	0.004				
Fruit per plant	3.02	0.43				
Yield per plant	55.00	<.001				

3.2.2. Stem diameter

There was a significant interactive effect of the inoculum and the rootstock on the stem diameter of the plants (Table 2). The girth of P/ST x I at week 2 (10.19 mm) and week 4 (13.0 mm) was significantly thicker than the girth of P/SM x I at week 2 (9.06 mm) and week 4 (9.70 mm) after inoculation. P x I had the thinnest girth at week 2 (9.20 mm) and week 4 (9.93 mm) after inoculation. The girth of P/SM x I was similar to that of P x I.

3.2.3. Chlorophyll content

The chlorophyll contents for all treatments showed a significant interaction ($P < 0.05$) between the *F. oxysporum* inoculum and the rootstocks (Table 2). P/SM x I had the highest chlorophyll contents, 57.22 μmol m^{-2} and 49.49 μmol m^{-2} at 2 and 4 weeks after inoculation, respectively. However, P x I had the least chlorophyll content of 37.6 μmol m^{-2} and 25.18 μmol m^{-2} at 2 and 4 weeks after inoculation, respectively.

3.2.4. Photosynthetic rate

A significant interactive effect ($P < 0.05$) for net photosynthetic rate between *F. oxysporum* inoculum and rootstock combinations at 2 weeks after inoculation (Table 2). P/ST x I had the highest photosynthetic rate of 9.59 $\mu mol^{-2}s^{-1}$, P/SM 7.83 $\mu mol^{-2}s^{-1}$ and P x I had the least photosynthetic rate of 7.64 $\mu mol^{-2}s^{-1}$. There was a steady increase in the photosynthetic rate for both P/ST (10.14 $\mu mol^{-2}s^{-1}$) and P/SM x I (9.04 $\mu mol^{-2}s^{-1}$) compared to the non-grafted plant (7.90 $\mu mol^{-2}s^{-1}$) at week 4 after inoculation.

3.3. The influence of graft combinations and Fusarium oxysporum inoculum on fruit quality and yield of Petomech tomato plants (Pot experiment)

3.3.1. Fruit quality

There were no significant interactive effects ($P < 0.05$) between *F. oxysporum* inoculum and the rootstocks; P, P/SM, and P/ST on fruit qualities, which are total soluble solids, fruit firmness, pericarp thickness, power of hydrogen, fruit girth, fruit length and number of fruits per plant (Table 3). The fruit pericarp thickness, girth and size increased significantly in the grafted plants compared to the non-grafted plants. Also, small size fruits (girth and length) were obtained in the FOL-infected plants, but they were significantly ($P < 0.05$) bigger in the disease-free plants. The fruit number/plant was similar among all the treatments.

3.3.2. Fruit yield

There was a significant interaction ($P < 0.05$) in fruit yield per plant between the rootstocks and the *F. oxysporum* inoculum (Table 3). P/SM with *F. oxysporum* recorded the highest fruit yield (465.9 g/plant), while the non-grafted Petomech inoculated with *F. oxysporum* (P x I) produced the least fruit yield (157.7 g/plant). P/ST with or without *F. oxysporum* recorded an intermediate fruit yield of 383.2 and 317.3 g/plant, respectively.

3.4. Disease incidence and severity of grafting combinations and non-grafted plants after inoculation (Pot experiment)

At week 2, 100% of the plant population of non-grafted plants were diseased (Fig. 2A), with a sharp increase in disease severity (Fig. 2B). Diseased incidence and severity by of P/SM were very low compared to P/ST which was moderately resistant. However, incidence and severity increased steadily with time.

Table 2
Influence of graft combinations and *Fusarium oxysporum* inoculum on vegetative growth, chlorophyll content, and photosynthetic rates of tomato plants at 2 weeks and 4 weeks after inoculation (**Pot Experiment**).

Scion/Rootstock	Plant Height (cm)		Plant Girth (mm)		Chlorophyll content (μmol m^{-2})		Photosynthetic rate (μmol $^{-2}s^{-1}$)	
	2WAI	4WAI	2WAI	4WAI	2WAI	4WAI	2WAI	4WAI
P (Control)	43.25 ± 0.76 a	49.57 ± 0.45 a	8.86 ± 0.95 a	10.21 ± 0.40 a	40.78 ± 2.00 a	33.79 ± 3.51 a	7.90 ± 0.28 a	7.03 ± 0.56 a
P/SM	45.58 ± 0.42 b	50.18 ± 0.47 a	9.64 ± 0.79 b	10.65 ± 0.51 a	53.83 ± 2.34 b	42.17 ± 3.51 c	11.32 ± 1.65 b	9.04 ± 0.67 b
P/ST	48.08 ± 0.95 c	53.09 ± 1.25 b	10.06 ± 0.20 b	12.22 ± 0.50 b	45.66 ± 1.70 a	38.95 ± 1.70 b	8.93 ± 0.40 a	10.14 ± 0.55 b
Inoculum (I)								
Non-Inoculated	45.18 ± 0.46 a	50.0 ± 0.42 a	9.56 ± 0.31 a	11.18 ± 0.38 a	47.49 ± 1.69 a	39.85 ± 1.50 b	10.41 ± 1.15 b	9.18 ± 0.66 a
FOL Inoculated	46.09 ± 1.18 a	51.9 ± 1.01 b	9.48 ± 0.25 a	10.88 ± 0.55 a	46.02 ± 3.07 a	36.76 ± 3.59 a	8.36 ± 0.39 a	8.29 ± 0.63 a
Interaction (R x I)								
P/SM x U	45.05 ± 0.40 b	49.72 ± 0.48 a	10.22 ± 0.46 c	11.60 ± 0.57 c	50.44 ± 3.12 bc	34.86 ± 2.52 b	14.81 ± 0.94 c	10.04 ± 0.71 a
P/SM x I	46.10 ± 0.65 b	50.63 ± 0.81 a	9.06 ± 0.52 ab	9.70 ± 0.22 a	57.22 ± 2.47 c	49.49 ± 1.38 d	7.83 ± 0.38 a	8.03 ± 0.96 a
P/ST x U	46.27 ± 0.58 b	50.96 ± 1.07 a	9.92 ± 0.28 bc	11.44 ± 0.73 bc	48.06 ± 2.54 b	42.28 ± 1.13 c	8.27 ± 0.36 ab	10.26 ± 0.92 a
P/ST x I	49.89 ± 0.98 c	55.23 ± 1.46 a	10.19 ± 0.31 c	13.01 ± 0.33 c	43.26 ± 0.31 ab	35.63 ± 1.48 b	9.59 ± 0.50 b	10.01 ± 0.77 a
P x U	44.22 ± 0.96 ab	49.37 ± 0.30 a	8.52 ± 0.26 a	10.50 ± 0.71 ab	43.96 ± 2.68 ab	42.40 ± 0.81 c	8.15 ± 0.11 ab	7.23 ± 0.98 a
P x I	42.27 ± 0.99 a	49.77 ± 0.93 a	9.20 ± 0.18 ab	9.93 ± 0.44 ab	37.60 ± 1.67 a	25.18 ± 1.25 a	7.64 ± 0.57 a	6.83 ± 0.74 a

Means (± SEM) followed by the same letter in a column do not differ statistically from each other by the Fisher's Protected LSD test at 5% probability. WAI indicates weeks after inoculation, P indicates Petomech non-grafted, P/ST= Petomech grafted onto *Solanum macrocarpon*, P/ST indicates Petomech grated onto *Solanum torvum*, R= Rootstock, I = Inoculated plants, and U = non-inoculated plants.

Table 3
Influence of graft combinations and *Fusarium oxysporum* inoculum on fruit quality parameters and yield of grafted (Petomech) tomato plants.

Scion/Rootstock	TSS (%)	FF (Lbs)	PT (mm)	Ph	FG (mm)	FL (mm)	NF/Plant	Yield/Plant (g)
P (Control)	4.99 ± 0.27 a	2.67 ± 0.14 a	5.85 ± 0.19 a	3.87 ± 0.04 a	34.91 ± 1.27 a	40.63 ± 1.49 a	6 ± 0.45 a	205.1 ± 25.2 a
P/SM	5.00 ± 0.11 a	2.68 ± 0.17 a	6.70 ± 0.16 b	4.00 ± 0.02 b	44.32 ± 1.47 b	53.78 ± 1.34 b	6 ± 0.31 a	453.1 ± 10.6 c
P/ST	4.94 ± 0.15 a	3.02 ± 0.21 a	7.43 ± 0.24 c	4.03 ± 0.03 b	44.73 ± 0.59 b	53.27 ± 0.643 b	7 ± 0.28 a	350.3 ± 18.5 b
Inoculum (I)								
Non-Inoculated	5.07 ± 0.16 a	2.81 ± 0.17 a	6.52 ± 0.27 a	3.94 ± 0.03 a	43.18 ± 1.56 b	51.28 ± 2.17 b	6 ± 0.26 a	336.8 ± 28.3 a
FOL Inoculated	4.89 ± 0.13 a	2.76 ± 0.13 a	6.80 ± 0.28 a	3.99 ± 0.04 a	39.46 ± 0.19 a	47.17 ± 2.17 a	6 ± 0.32 a	335.6 ± 47.3 a
Interaction (R x I)								
P/SM x U	4.83 ± 0.10 a	2.58 ± 0.26 a	6.83 ± 0.26 a	3.99 ± 0.03 a	46.98 ± 1.44 a	56.60 ± 0.71 a	6 ± 0.40 a	440.4 ± 9.2 de
P/SM x I	5.17 ± 0.13 a	2.77 ± 0.26 a	6.57 ± 0.22 a	4.00 ± 0.04 a	41.65 ± 1.28 a	50.95 ± 0.71 a	7 ± 0.54 a	465.9 ± 17.8 e
P/ST x U	4.98 ± 0.30 a	2.98 ± 0.46 a	7.06 ± 0.39 a	3.97 ± 0.01 a	45.05 ± 0.89 a	54.20 ± 0.06 a	6 ± 0.58 a	317.3 ± 13.7 c
P/ST x I	4.91 ± 0.12 a	3.05 ± 012 a	7.81 ± 0.07 a	4.10 ± 0.04 a	44.41 ± 0.90 a	52.34 ± 0.94 a	7 ± 0.10 a	383.2 ± 21.7 d
P x U	5.41 ± 0.35 a	2.88 ± 0.35 a	5.67 ± 0.35 a	3.85 ± 0.03 a	37.49 ± 1.08 a	43.05 ± 1.89 a	7 ± 0.48 a	252.5 ± 15.8 b
P x I	4.58 ± 0.29 a	2.46 ± 0.20 a	6.03 ± 0.17 a	3.88 ± 0.07 a	32.33 ± 0.84 a	38.21 ± 1.31 a	5 ± 0.22 a	157.7 ± 26.2 a

Means (± SEM) followed by the same letter in a column do not differ statistically from each other by the Fisher's Protected LSD test at 5% probability. P indicates non-grafted Petomech, P/ST= Petomech grafted onto *Solanum macrocarpon*, P/ST indicates Petomech grated onto *Solanum torvum*, R= Rootstock, I= Inoculated plants, U = non-inoculated plants and TSS = Total Soluble Solids; FF = Fruit Firmness; PT = Pericarp thickness; pH = Power of Hydrogen; FG =Fruit Girth; FL = Fruit Length; NF = number of fruits.

3.5. Influence of graft combinations on vegetative growth and photosynthetic rate of tomato plants in a field naturally infected by F. oxysporium

3.5.1. Mean plant height, plant girth, chlorophyll content and photosynthetic rate

A significant increase ($P \leq 0.05$) in plant height and chlorophyll contents were observed at 3 weeks after transplanting for the grafted plants, relative to the non-grafted plants (Tables 1 and 4). P/ST had the tallest plant (41.5 cm), and P/SM had the highest chlorophyll content (64.93 μmol/cm^3). The stem girth of P/ST was thicker (9.48 mm) and also had a significantly higher photosynthetic rate (5.51 μmol$^{-2}s^{-1}$) at week 6 after transplanting (Table 4).

3.5.2. Fruit yield

Petomech grafted onto *S. torvum* and *S. macrocarpon* showed significantly ($P \leq 0.05$) high fruit yield (153.0 and 148.7 g/plant, respectively) compared to the non-grafted plants (51.4 g/plant) (Table 5). Although there was about 2.9% yield increase for P/ST than P/SM, the difference was not statistically significant. P/SM had fruits with bigger girth and length (42.6 and 49.12 mm) followed by P/ST (41.08 and 46.64 mm), while the non-grafted plants produced smaller fruits (35.84 mm girth and 43.11 mm length) (Table 5).

3.5.3. Fruit qualities

Grafting did not significantly ($P \leq 0.05$) influence the brix of the fruit, pericarp thickness and pH. Fruit firmness was significantly higher for both grafting combination P/ST and P/SM (4.12 KgN^{-1} and 4.07 KgN^{-1}) in comparison to non-grafted plants (2.80 KgN^{-1}) (Table 6).

3.6. The influence of graft combinations on disease incidence and severity in a field naturally infected by F. oxysporium

3.6.1. Disease incidence

At weeks 2, 3 and 4 after transplanting, P/SM recorded a significantly ($P < 0.05$) lower population of diseased plants, followed by P/ST. In contrast, the non-grafted plants had 60% of the plant population showing *Fusarium* wilt symptoms at week 2 and 100% at week 3. There was a significant difference ($P < 0.05$) in *Fusarium* wilt incidence between the grafted and non-grafted plants at weeks 2, 3, and 4 after transplanting. However, at weeks 5 and 6 after transplant, there was no significant difference in *Fusarium* wilt incidence among all treatments (Fig. 3A).

3.6.2. Disease severity

The grafted plants significantly ($P < 0.05$) reduced disease severity compared to non-grafted plants. On the severity rating scale of 1–6, the highest disease severity of 5.45 was observed in the non-grafted tomato plants (P) at week 6 after transplanting, followed by P/ST with a disease

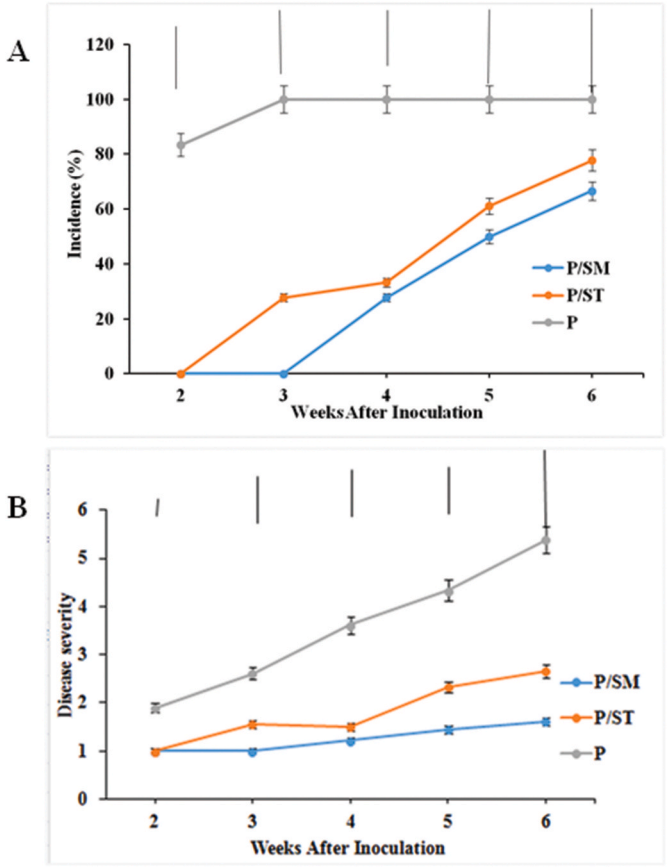

Fig. 2. Disease incidence (A) and Disease severity (B) of grafted and non-grafted tomato plants at 2, 3, 4, 5 and 6 weeks after inoculation in pots with *Fusarium oxysporum* [vertical bars represent LSD ($p < 0.05$)].

severity rating of 3.5. The lowest disease severity rating of 3.2 was observed in P/SM at week 6 after transplanting (Fig. 3B).

4. Discussion

Vegetable grafting has proved to be an effective tool in improving plant growth and yield under biotic and abiotic stress conditions [19]. However, successful grafting can be challenged by incompatibility between scion and rootstock traits, grafting technique and environmental conditions [19,20]. For the environment, a high relative humidity (RH) (90%) for the first 2 or 3 days, followed by a lower RH (70%) at 23 °C during healing and acclimatization, is recommended to promote graft-take and quality of grafted tomato seedlings [18]. In the current study, graft-take or union was not dependent on the taxonomic affinity of the rootstocks used as there were no significant differences in success rate among the two rootstocks [72–91% for Petomech grafted onto *S. torvum* (P/ST) and 68–83% for Petomech grafted onto *S. macrocarpon* (P/SM)]. In contrast, grafting success during the wet season (83–91%) was higher compared to the dry season (68–72%). Although RH was regulated inside the healing and acclimatization chambers, temperatures were high and fluctuated rapidly during the dry season but were fairly stable during the rainy season. The temperature fluctuations might have affected the graft success rate, highlighting the need to control temperatures inside the chambers in further studies.

This study demonstrated that grafting tomato onto *S. macrocarpon* and *S. torvum* rootstocks offered moderate to higher protection against *Fusarium* wilt than non-grafted tomato plants. Both rootstocks delayed the onset of *Fusarium* wilt disease from week one to the sixth week after transplanting. They also lowered the disease incidence and severity in the naturally infected and artificially inoculated soil. These characteristics of *S. macrocarpon* and *S. torvum* highlighted the potential use of these rootstocks as valuable sources of resistance/tolerance against *Fusarium* wilt in tomato crop improvement. Although the exact cause of resistance in the rootstocks is not fully known, several possible explanations for defense include the possession of one or more r-genes [21] and/or the production of antimicrobial compounds such as phytoanticipins and phytoalexins in response to pathogen attack [22]. The results of this study are congruent with earlier findings. For example, some accessions of *S. torvum* obtained from Java Island were found highly resistant to *Fusarium* wilt and *Ralstonia* wilt in cultivated eggplant and tomato [23]. Similarly, *S. macrocarpon* was found resistant to *Fusarium* wilt of aubergine (caused by *F. oxysporum* f. sp. *melongenae*) [24] and tolerant to root-knot nematode disease of tomato [25].

Table 5
Influence of tomato graft combinations on number of fruits per plant, yield, fruit girth and fruit length in a field naturally infected with *Fusarium oxysporum*.

Scion/ Rootstock	Number of fruits/plant	Yield/plant	Fruit girth (mm)	Fruit length (mm)
P (Control)	7 ± 1.21 a	51.4 ± 13.50 a	35.84 ± 1.01 a	43.11 ± 1.74 a
P/SM	5 ± 1.38 a	148.7 ± 22.60 b	42.67 ± 1.07 b	49.12 ± 1.32b
P/ST	7 ± 0.85 a	153.0 ± 6.35 b	41.08 ± 2.51 b	46.64 ± 1.37b

Means (± SEM) followed by the same letter in a column do not differ statistically from each other by the Fisher's Protected LSD test at 5% probability. P indicates non-grafted Petomech, P/ST= Petomech grafted onto *Solanum macrocarpon*, P/ST= Petomech grated onto *Solanum torvum*.

Table 6
Influence of grafting on brix level, fruit firmness, pericarp thickness and pH.

Scion/ Rootstock	Brix	Fruit firmness (KgN^{-1})	Pericarp thickness (mm)	pH
P	5.76 ± 0.68 a	2.80 ± 0.64 a	5.39 ± 0.41 a	3.94 ± 0.08 a
P/SM	6.37 ± 0.74 a	4.07 ± 0.36 b	4.86 ± 0.27 a	4.13 ± 0.03 a
P/ST	5.99 ± 0.60 a	4.12 ± 0.05 b	5.12 ± 0.376a	3.99 ± 0.57 a

Means followed by the same letter in a column do not differ statistically from each other by the Fisher's Protected LSD test at 5% probability. P indicates non-grafted Petomech, P/ST= Petomech grafted onto *Solanum macrocarpon*, P/ST= Petomech grated onto *Solanum torvum*.

Table 4
Influence of tomato graft combinations on the height, girth, chlorophyll and photosynthetic rates in a field naturally infected with *Fusarium oxysporum*.

Scion/Rootstock	Plant height (cm)		Plant girth(mm)		Chlorophyll content (μmol/cm^3)		Photosynthetic rate ($\mu mol^{-2}s^{-1}$)	
	3 WAT	6 WAT	3 WAT	6 WAT	3 WAT	6WAT	3WAT	6WAT
P (Control)	33.6 ± 3.76 a	49.4 ± 2.91 a	5.85 ± 0.83 a	8.41 ± 1.04 a	53.85 ± 2.84 a	41.67 ± 1.82 a	12.45 ± 0.89 a	3.40 ± 1.09 a
P/SM	38.0 ± 0.86 b	46.4 ± 1.15 a	6.00 ± 0.29 a	9.00 ± 0.54 a	64.93 ± 2.84 b	57.39 ± 1.22 a	15.68 ± 1.94 a	4.16 ± 1.11 a
P/ST	41.5 ± 2.28 b	46.3 ± 4.02 a	5.62 ± 0.34 a	9.48 ± 0.63 a	64.92 ± 6.07 b	53.19 ± 2.89 a	13.30 ± 1.17 a	5.51 ± 2.07 b

Means (± SEM) followed by the same letter in a column do not differ statistically from each other by the Fisher's Protected LSD test at 5% probability. P indicates non-grafted Petomech, P/ST= Petomech grafted onto *Solanum macrocarpon*, P/ST= Petomech grated onto *Solanum torvum*. WAT =Weeks After Transplanting.

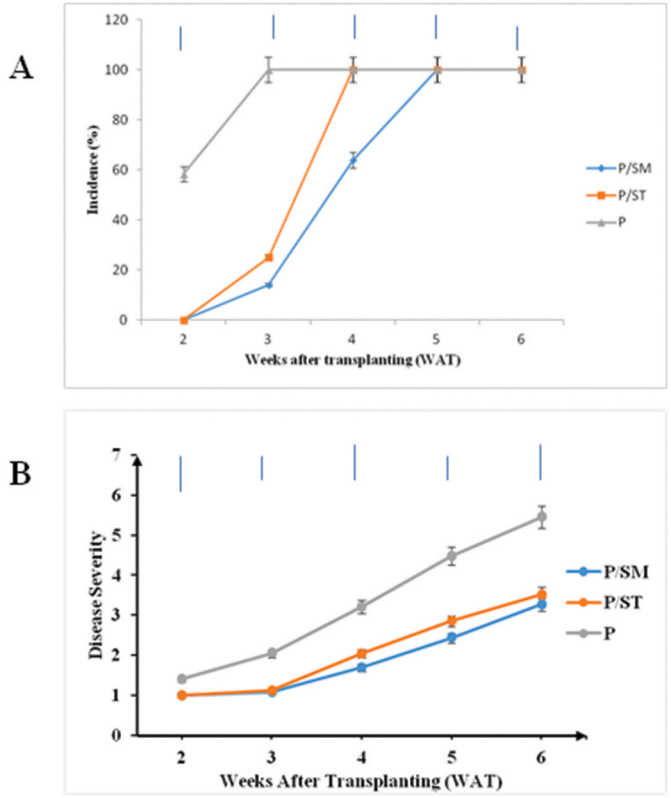

Fig. 3. Disease incidence (A) and Disease severity (B) of grafted and non-grafted tomato plant in a natural infected *Fusarium* field at 2, 3, 4, 5 and 6 weeks after transplanting [vertical bars represent LSD ($p < 0.05$)].

Besides providing protection, the interaction of the grafted plant's rootstock with/without the pathogen resulted in either equivalent or improved growth in most cases when compared to the non-grated plants. The grafted plants had robust stems that were tall and thick. Their leaves had the highest chlorophyll content in the naturally infected field. However, when inoculated with the pathogen in the pot experiment, P/SM recorded the highest chlorophyll content relative to P/ST. It is worth noting that the vigorous growth of P/SM plants resulted in the highest fruit yield in the pot experiment and provided a similar effect as P/ST in the field experiment. Thus, *S. macrocarpon* was more promising than *S. torvum* in enhancing scion growth and yield performance. The differences in the rootstocks, including their species origins, disease resistance, nutrient absorption capacities, and the experimental conditions used, may have contributed to the observed differences in the scion's growth and yield. We observed that the infection rate and severity of *Fusarium* wilt on *S. macrocarpon* were generally lower than on *S. torvum*. The data confirmed that plant resistance responses are associated with plant fitness and greater fruit production [20,21,26]. It also emphasized grafting as an important strategy for combating soilborne diseases.

The fruit qualities of the grafted tomato plants were generally similar to the non-grafted plants. This observation could be due to the dependence of fruit quality attributes on the scion rather than the rootstocks. A similar trend was reported by Agyeman et al. [27] and Jabnoun-Khiareddine et al. [28] when tomato scion was grafted onto several *Solanum* plants under root-knot nematode and *Fusarium* wilt (*Fusarium oxysporum* f. sp. *radicis-lycopersici*) disease conditions. In contrast, other studies indicated that wild tomato (*S. pimpinellifolium*) and *S. lycopersicum* 'Moneymaker' utilized as rootstocks influenced fruit sugar content and flavor [29].

5. Conclusion

Grafting tomato onto *S. macrocarpon* has the potential to significantly improve crop production and reduce *Fusarium* wilt incidence and severity.

Declaration of competing interest

The author(s) declare none.

Data availability

Analyzed data is reflected in Tables and Figures in the manuscript

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