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Article

Boosting Tomato Resilience in Tanzania: Grafting to Combat Bacterial Wilt and Abiotic Stress

Shem Elias Msabila [1,2,*], Thibault Nordey [1,3,4,*], Zablon Ernest [1], Nickson Mlowe [1], Ravishankar Manickam [5], Srinivasan Ramasamy [5] and Joël Huat [4,6]

[1] World Vegetable Center, Eastern and Southern Africa, Duluti, Arusha P.O. Box 10, Tanzania; zablon.ernest@worldveg.org (Z.E.); nickson.mlowe@worldveg.org (N.M.)
[2] Department of Plant and Soil Science, University of Delaware, 210 South College Ave., Newark, DE 19716, USA
[3] CIRAD (French Agricultural Research Centre for International Development), UPR HortSys, F-34398 Montpellier, France
[4] HortSys, University of Montpellier, CIRAD, F-34090 Montpellier, France; joel.huat@cirad.fr
[5] World Vegetable Center, Shanhua, P.O. Box 42, Tainan 74199, Taiwan; ravi.manickam@worldveg.org (R.M.); srini.ramasamy@worldveg.org (S.R.)
[6] CIRAD, UPR HortSys, F-97455 Saint-Pierre, Reunion, France
* Correspondence: selias@udel.edu (S.E.M.); thibault.nordey@orange.fr (T.N.)

Abstract: The grafting technique has successfully mitigated crop losses from diseases and stress in vegetable production; however, vegetable grafting in Tanzania is very limited. Field and greenhouse experiments conducted in Tanzania's mainland and islands compared the response of tomato determinate cv. 'Tanya' to production challenges when ungrafted and grafted onto five tomato rootstocks ('Hawaii 7796', 'Tengeru 1997', 'Tengeru 2010', 'R3034', and 'Shelter'), one eggplant variety ('EG 203'), and one wild *Solanum* species (*Solanum elaeagnifolium*). The visual symptoms of bacterial wilt varied significantly with location and season, ranging from 8 to 100%, attributed to varying bacterial wilt pressures and strains of *Ralstonia solanacearum* isolated (Phylotype I sequevars 17, 18, and 31). 'EG203' and 'Hawaii 7796' emerged as the most effective rootstocks, reducing wilting by 49.8 and 51.0% and improving yield by 57.2% and 27.7% on average across experiments conducted in three locations (Moshi, Pemba, and Unguja) over two seasons. Combining reduced water supply with grafting resulted in an average reduction in wilting of 76%, while also boosting yields by an average of 3.6 times in experiments conducted in Arusha over two seasons. Grafting onto 'Hawaii 7796' and 'Shelter' significantly improved 'Tanya' yields by 38.3% and 41.6% on average over two seasons, only under standard nutrient application rates. While certain rootstocks improved crop performance, yields across various sites and seasons were significantly hampered by pest pressure. These findings support grafting's potential to mitigate damage from common stresses, emphasizing the need for further research to identify suitable rootstocks for optimizing returns on investments in grafted plants in Tanzania.

Keywords: tomato grafting; abiotic stress; biotic stress; water deficit; *Ralstonia solanacearum*; Tanzania

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1. Introduction

Adopting a diverse and vibrant dietary regimen that includes a variety of vegetables represents a scientifically supported strategy, ensuring the intake of crucial nutrients, vitamins, minerals, and antioxidants, and thereby substantiating a proactive approach toward sustained health and well-being. Limited availability contributes to the ongoing deficiency in vegetable consumption within developing countries. Moreover, vegetable production presents an economic opportunity to alleviate unemployment and poverty in these nations [1]. However, this potential is hindered by financial risks associated with weather conditions, pest and disease damage, and fluctuating sales prices [2]. Among the

diseases, bacterial wilt caused by the pathogen *Ralstonia solanacearum* and root-knot nematodes (*Meloidogyne* spp.) alone or in combination limits tomato production. *R. solanacearum* is a soilborne plant pathogen that attacks the vascular system of plants. This results in the gradual withering, yellowing, and, eventually, death of the plant. A recent study reported a high diversity of *R. solanacearum* in Tanzania [3], resulting in significant losses for tomato crops. This pathogen has several hosts, including tomato plants, and the root-knot nematodes increase its access to its host plants. Root-knot nematodes (*Meloidogyne* spp.) are tiny, parasitic roundworms that infect the roots of plants through their feeding mechanisms. This leads to the formation of knots or galls on the root. These nematodes are a significant agricultural pest, affecting many crops, including tomato plants.

To combat crop damage caused by pests and diseases, vegetable growers are turning to the use of pesticides [4], which can have negative impacts on both their health [5] and that of consumers [6,7]. To achieve higher yields while minimizing chemical inputs, 'ecological intensification' principles recommend utilizing biological regulations in agroecosystems [8]. While using improved crop varieties is an effective method of sustainable production, it can also be time-consuming and costly, which hinders the development of suitable options [9].

Alternatively, vegetable grafting is becoming a popular technique for effectively merging the shoots (known as scions) of high-yield potential with the rootstocks that can withstand soilborne diseases and other environmental stresses [10]. While grafting is widely used in fruit and vegetable crops across Europe, Asia, and North America, it remains predominantly limited to fruit crops in sub-Saharan Africa (SSA). Numerous studies have highlighted the advantages of vegetable grafting in combating soilborne diseases and improving tolerance to abiotic stresses [11–13].

After analyzing 159 studies on tomato grafting, the authors of [14] discovered a concerning lack of research in SSA, with only three studies dedicated to this region. Most investigations on tomato grafting have been carried out in Europe, the Middle East, Asia, and, to a lesser extent, North America. This trend is also reflected in the top tomato-producing nations of 2018, according to the FAOSTAT database [10]. China (62 million tons), India (19 million tons), the European Union (17 million tons, with 6 million tons from Italy and 5 million tons from Spain making significant contributions), the United States (13 million tons), and Turkey (13 million tons) were the leaders in tomato production. Despite Africa's noteworthy 2018 tomato output (21 million tons), there is limited information on the merits of tomato grafting.

A current review [10] has highlighted the potential of vegetable grafting in SSA, specifically for tomato farmers who are struggling with soilborne pathogens such as bacterial wilt and root-knot nematodes, as well as abiotic challenges including water deficit, flooding, and poor soil fertility.

Grafting using resistant rootstocks to increase root resilience and endurance is a promising alternative. However, the practical implementation of grafted plants in SSA faces obstacles, such as the absence of appropriate rootstocks and insufficient evidence about their profitability in various production systems, such as open-field versus greenhouse cultivation.

Previous research on tomato grafting in sub-Saharan Africa has yielded conflicting results, making it difficult for farmers to make informed decisions [15–18]. To address this uncertainty, the 'GrAfrica' project funded by GIZ from 2017 to 2020 aimed to introduce grafted plantlets to help increase yields and income for small-scale tomato producers in Tanzania. The project included comprehensive training in tomato grafting for nursery operators and producers and experiments conducted in collaboration with national partners across diverse locations in Tanzania. The objectives of the experiments were to evaluate the response of a popular determinate tomato variety ('Tanya') when grown either ungrafted or grafted (i.e., onto rootstocks of tomatoes, eggplants, and wild species) in different 1. fertilization regimes, 2. soils infected by bacterial wilt, and 3. water deficit levels in soils infected by bacterial wilt. The findings of these experiments, presented in this article, offer

valuable and reliable information to guide future efforts toward successfully implementing tomato grafting practices in Tanzania.

2. Materials and Methods

2.1. Plant Material

Tomato seedlings were produced in a nursery covered with a plastic film as a roof and with insect-proof nets (60 mesh) on the sides. Seedling trays with a hole capacity of 66 were filled with a heat-sterilized mixture of forest soil, well-decomposed manure, and sand at 2:1:1 [17]. The seedlings were irrigated daily with a watering can and fertilized weekly with a solution comprising 0.4 g·L^{-1} each of nitrogen, phosphorus, and potassium. 'Tanya', an open-pollinated determinate tomato variety, was used both ungrafted as a control in all the experiments and grafted onto five tomato rootstocks ('Hawaii 7796', 'Tengeru 1997', 'Tengeru 2010', 'R3034', and 'Shelter'), one eggplant rootstock ('EG 203'), and one wild species (*Solanum elaeagnifolium*) rootstock (Table 1). Despite its high sensitivity to bacterial wilt, Tanya is widespread in Tanzania for its elongated fruits and long shelf-life. Three-week-old seedlings were used as scions at the two- to three-true leaf stages. Tomato, eggplant, and wild species seedlings used as rootstock were sown, respectively, two days, two weeks, and three weeks before the tomato scions to obtain a similar stem diameter for grafting [19].

Table 1. Tomato, eggplant, and a wild *Solanum* species were used in the experiments. N.B. Seeds of the wild *Solanum* species were collected from the fields of Arusha and characterized morphologically.

Varieties	Species	Type	Remarks	Use	Origin	Experiments
Tanya	*S. lycopersicum* L.	Open-pollinated, determinate	Extended shelf-life, highly sensitive to nematodes and bacterial wilt	Scion and ungrafted control	WorldVeg	1, 2, and 3
Tengeru 1997	*S. lycopersicum* L.	Open-pollinated, semi-indeterminate	Partial resistance to bacterial wilt	Rootstock	WorldVeg	2 and 3
Tengeru 2010	*S. lycopersicum* L.	Open-pollinated, semi-indeterminate	Partial resistance to bacterial wilt	Rootstock and ungrafted control	WorldVeg	2
Hawaii 7796	*S. lycopersicum* L.	Open-pollinated, determinate	Used as a rootstock in Asia to improve resistance to bacterial wilt	Rootstock	INRA	1 and 2
R3034	*S. melongena*	Open-pollinated, determinate	Used as a rootstock in Asia to improve resistance to bacterial wilt	Rootstock	WorldVeg	1
Shelter	*S. lycopersicum* L.	Hybrid	Commercial rootstock	Rootstock	Rijk Zwaan	1
EG 203	*S. melongena*	Open-pollinated	Used as a rootstock in Asia to improve resistance to bacterial wilt and flooding.	Rootstock	WorldVeg	1 and 2
Silverleaf nightshade	*S. elaeagnifolium*	Wild *Solanum* species widespread in Tanzania	Resistance to bacterial wilt is not known.	Rootstock	Wild species	1 and 2

The splicing technique described by [20] was used for grafting. Using sterilized blades, the stems of the scions and rootstocks were cut obliquely above the cotyledons at an angle of 30°. The surfaces of the cut scions and rootstocks were then gently aligned and held together using a transparent plastic silicon clip measuring 1.6 mm in diameter. Plants were grafted above the cotyledons to have a height advantage and prevent the scion from contacting the soil through adventitious roots, which would have forfeited the grafting efforts after transplanting. For recovery after grafting, the grafted plants were placed in a dark and shaded 'healing chamber' with high humidity (~90%) thanks to a shallow layer

of water on the polyethylene floor (for a complete description, see [20]). In the Pemba and Unguja locations, underground 'healing chambers' were used to lower the temperature and raise the relative humidity to increase the success rate of grafting [19]. After three days within the 'healing chambers', the doors were partially opened, allowing seedlings to acclimate to ambient humidity for two days. The seedlings were then taken out of the 'healing chambers' and placed in a nursery under shade nets for a recovery period of seven days, during which they were gradually exposed to sunlight. Care was taken when transplanting the grafted plants to ensure the graft union was above the soil surface to avoid the development of adventitious roots.

2.2. Experiments

Three sets of experiments were performed in four different locations in Tanzania (Arusha, Moshi, Pemba, and Unguja; see Figure 1) in two seasons from 2018 to 2019 to compare the response of 'Tanya' tomatoes when ungrafted and grafted to five tomato rootstocks ('Hawaii 7796', 'Tengeru 1997', 'Tengeru 2010', 'R3034', and 'Shelter'), one eggplant rootstock ('EG 203'), and one wild species rootstock (*Solanum elaeagnifolium*) (Table 1). All the rootstocks tested, except the wild species, were reportedly resistant to bacterial wilt. 'Tanya' tomatoes were used as scions and ungrafted controls in all the experiments, but to reflect local practices, the rootstocks tested at the experimental sites differed, as did planting density.

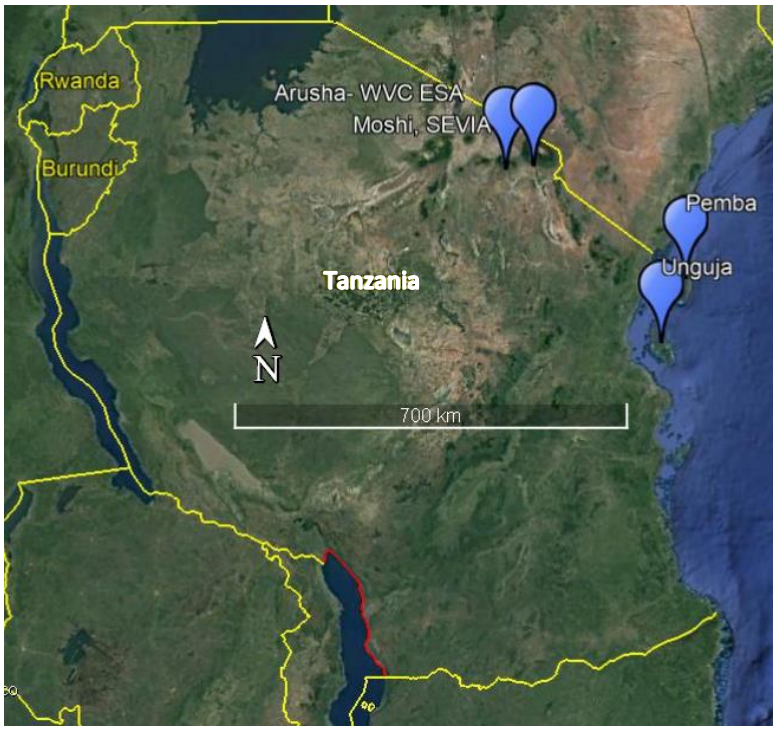


Figure 1. Location of experimental sites in Arusha, Moshi, Pemba, and Unguja, Tanzania.

We evaluated the effectiveness of grafting, as compared to an ungrafted control, on 'Tanya' tomatoes when paired with five different tomato rootstocks, namely 'Hawaii 7796', 'Tengeru 1997', 'Tengeru 2010', 'R3034', and 'Shelter'. One eggplant rootstock ('EG 203') and one wild species rootstock (*Solanum elaeagnifolium*) were also tested. It is worth mentioning that all rootstocks, except the wild species, were reportedly resistant to bacterial wilt. All experiments used 'Tanya' tomatoes as scions and as the ungrafted control. The rootstocks used varied across the experimental sites, and planting densities were adjusted to reflect local practices. Please refer to Figure 1 for a map of the experimental locations and Table 1 for additional information regarding the rootstocks tested.

Experimental sites with contrasted climate and bacterial wilt (*Ralstonia solanacearum*) pressures were selected to account for the different production challenges encountered in Tanzania. The northern sites, Arusha (latitude −3°22′22.82″, longitude 36°48′21.59″, altitude 1250 m asl) and Moshi (latitude −3°18′54.72″, longitude 37°15′8.28″, altitude 987 m asl), are located in the Tanzania highlands close to Mount Kilimanjaro and have an oceanic subtropical highland climate (Cwb), and a marine west coast (Cfb) climate, respectively, and loamy-clay soils. The sites in Pemba (latitude −5°4′3.72″, longitude 39°45′1.08″, altitude 13 m asl) and Unguja (latitude −6°6′24.48″, longitude 39°21′41.04″, altitude 46 m asl) are located on the eastern seaboard of Tanzania, more than 400 km away as the crow flies from the northern locations, and have a tropical monsoon (Am) and tropical savanna (Aw) climate, respectively, with clay to sandy-loam soils.

2.2.1. Experiment 1: Response of Ungrafted and Grafted Tomato Plants to Different Fertilization Regimes

In a field experiment conducted over two seasons at the World Vegetable Center for Eastern and Southern Africa near Arusha, the development and yields of ungrafted and grafted plants were assessed under different fertilization regimes. The experiment used a split-plot design with four replications and two fertilization rates (standard and low) on loamy to loamy-clay soils with good drainage and aeration. The standard fertilization rate consisted of 225 kg ha^{-1} of N, 90 kg ha^{-1} of P, and 250 kg ha^{-1} of K, designed to meet the NPK requirements of a tomato crop with a potential yield of 80 t ha^{-1} [21].

The soil was manually plowed to ensure optimal fertilization, and 5 t ha^{-1} of cow manure was incorporated during the last plowing. A week after transplanting, a side dressing of 17-17-17 NPK fertilizer (500 kg ha^{-1}) was added, followed by a side dressing of calcium ammonium nitrate (400 kg ha^{-1}) and muriate of potash (250 kg ha^{-1}) four weeks later for standard fertilization treatments. In comparison, plots with lower fertilization rates only received 5 t ha^{-1} of cow manure during plowing, equivalent to 15% of the NPK applied in the standard fertilization treatment. Adding 5 t ha^{-1} of cow manure during plowing is a good agricultural practice to slowly replenish nutrients in the soil. The subplot treatment included ungrafted 'Tanya' tomato plants (as controls) and tomato plants grafted onto 'Shelter', 'Hawaii 7796', and 'R3034' rootstocks. Each experimental unit (28 m^2) consisted of 66 plants (2.3 plants·m^{-2}) transplanted in bare soil across two raised beds (length 8 m, width 1 m, height 0.25 m) spaced 1.75 m apart, with 60 cm between plants in the same row. Irrigation with drip lines was scheduled every two days, with the quantity provided adjusted to meet plant requirements based on weather conditions and the stage of crop development. Chemical treatments were applied as needed to control insect pests and diseases using a knapsack sprayer (please refer to Table 2 for details).

One plant per experimental unit was randomly uprooted 10 weeks after transplanting (before the first harvest) to measure its leaf surface area and fresh weight (shoot and roots). The leaf surface area of each plant was assessed using a picture analysis method as described in previous studies [22,23]. Tomatoes were harvested thrice weekly at the mature green stage or more advanced ripening stages. They were then sorted based on their size (>7 cm length) and absence of defects (rot, discoloration, and cracking) to assess the marketable yields.

Air temperature, air relative humidity, and rainfall were measured at one-minute intervals and averaged every 30 min by a complete weather station (Vantage PRO2, Davis Instruments, Hayward, CA, USA) installed nearby.

Table 2. Pesticide treatments were applied during the field experiments on tomatoes in Tanzania. N.B. This information is not intended as a guideline or recommendation.

Experiments	Sites	Active Ingredients	Number of Applications in the First Season	Number of Applications in the Second Season	Targets
1	Arusha	Thiocyclam ($C_7H_{13}NO_4S_3$, Evisect, Arysta LifeScience, Noguères, France)	3	1	Whiteflies
		Lambda-cyhalothrin ($C_{23}H_{19}ClF_3NO_3$ Ninja, Posit. Inter. Limited, Daresalaam, Tanzania)	2	1	Whiteflies
		Emamectin benzoate ($C_{49}H_{75}NO_{13}$, Prove, Equatorial Africa Ltd., Daresalaam, Tanzania)	2	1	*Tuta absoluta*
		Metalaxyl and mancozeb (Ridomyl, Syngenta, Basel, Switzerland)	3	5	Early and late blight
		Copper (Mocrops, Daresalaam, Tanzania)	0	1	Bacterial spot
2	Pemba	Metalaxyl and mancozeb (Ridomyl, Syngenta, Basel, Switzerland)	4	4	Early and late blights
		Chlorothalonil (Daconil, Syngenta, Basel, Switzerland)	3	5	Powdery mildew
		Imidacloprid (Confidor, Bayer, Leverkusen, Germany)	4	4	Whiteflies
		Esfenvalerate (Sumi-alpha, Philagro, Pretoria, South Africa)	4	4	Whiteflies
		Azinphos-methyl (Gusathion, Bayer, Leverkusen, Germany)	1	2	Tomato fruit worms
		Methomyl (Lannate, Dupont, Tel Aviv, Israel)	1	2	Tomato fruit worms
		Emamectin benzoate ($C_{49}H_{75}NO_{13}$, Prove, Equatorial Africa, Daresalaam, Tanzania)	3	4	*Tuta absoluta*
	Unguja	Metalaxyl and mancozeb (Ridomyl, Syngenta, Basel, Switzerland)	5	4	Early and late blight
		Chlorothalonil (Daconil, Syngenta, Basel, Switzerland)	4	4	Powdery mildew
		Imidacloprid (Confidor, Bayer, Leverkusen, Germany)	4	2	Whiteflies
		Esfenvalerate (Sumi-alpha, Philagro, Tokyo, Japan)	4	2	Whiteflies
		Azinphos-methyl (Gusathion, Bayer, Leverkusen, Germany)	2	3	Tomato fruit worms
		Methomyl (Lannate, Dupont, Tel Aviv, Israel)	2	3	Tomato fruit worms
		Emamectin benzoate (Prove, Equatorial Africa, Daresalaam, Tanzania)	2	6	*Tuta absoluta*

Table 2. *Cont.*

Experiments	Sites	Active Ingredients	Number of Applications in the First Season	Number of Applications in the Second Season	Targets
	Moshi	Metalaxyl and mancozeb (Ridomyl, Syngenta, Basel, Switzerland)	3	7	Early and late blight
		Chlorothalonil (Daconil, Syngenta, Basel, Switzerland)	2	5	Powdery mildew
		Imidacloprid (Confidor, Bayer, Leverkusen, Germany)	5	3	Whiteflies
		Esfenvalerate (Sumi-alpha, Philagro, Tokyo, Japan)	5	3	Whiteflies
		Azinphos-methyl (Gusathion, Bayer, Leverkusen, Germany)	3	1	Tomato fruit worms
		Methomyl (Lannate, Dupont, Tel Aviv, Israel)	3	1	Tomato fruit worms
		Emamectin benzoate ($C_{49}H_{75}NO_{13}$, Prove, Equatorial Africa, Daresalaam, Tanzania)	8	2	*Tuta absoluta*
3	Arusha	Metalaxyl and mancozeb (Ridomyl, Syngenta, Basel, Switzerland)	1	2	Early and late blight
		Chlorothalonil (Daconil, Syngenta, Basel, Switzerland)	3	0	Powdery mildew
		Imidacloprid (Confidor, Bayer, Leverkusen, Germany)	3	4	Whiteflies
		Emamectin Benzoate ($C_{49}H_{75}NO_{13}$, Prove, Equatorial Africa, Daresalaam, Tanzania)	4	3	*Tuta absoluta*

2.2.2. Experiment 2: Response of Ungrafted and Grafted Tomato Plants to the Soil Infested with Bacterial Wilt Pathogen

After obtaining a positive streaming test, plots with repeated crop failures due to severe pressure from bacterial wilt were ideal for this study, which compared the symptoms and yields of grafted and ungrafted tomatoes. The trials were carried out over two seasons in Moshi, Pemba, and Unguja.

The Seeds of Expertise carried out the trials in Moshi for the Vegetable Sector of Africa (SEVIA) from May to September 2018 and from November 2018 to February 2019. A Latin square design with five treatments and five replications was used in both seasons. The treatments included ungrafted 'Tanya' as controls and 'Tanya' grafted onto 'Tanya' (self-grafted, to account for any grafting or healing effects), 'Hawaii 7796', 'EG 203', and onto a wild eggplant species (*S. elaeagnifolium*) rootstock. Each experimental unit (16 m^2) consisted of 20 plants (1.25 plants·m^{-2}) transplanted at the three- to four-leaf stage in two raised beds (length 400 cm, width 75 cm, height 25 cm) covered with a plastic mulch with 200 cm between-row spacing and 60 cm between plants in the same row.

The trials in Pemba and Unguja were conducted by the Tanzania Horticultural Association (TAHA) from November 2018 to January 2019 and June to November 2019. A complete randomized block design with three replications and five treatments was used in both seasons. The treatments included ungrafted 'Tanya' and 'Tengeru 2010' as controls and 'Tanya' grafted onto 'Tengeru 2010', 'Hawaii 7796', and 'Shelter' rootstocks. Each experimental unit (35 m^2) consisted of 50 plants transplanted (1.4 plants·m^{-2}) at the three-

to four-leaf stage into two raised beds (length 10 m, width 1 m, height 25 cm) covered with plastic mulch with 1.5 m between-row spacing and 40 cm between plants in the same row.

In Moshi, Pemba, and Unguja, irrigation was provided by drip lines every two days to meet plant requirements, and the same standard fertilization as that used in Experiment 1 was applied. Chemical pesticide treatments were applied based on scouting results using a knapsack sprayer to control insect pests and diseases (see details in Table 2).

The bacterial wilt incidence was assessed every week throughout the growing season. Ooze-out tests (streaming tests) were performed on wilted plants to confirm infection by *R. solanacearum*, and five samples from each site were sent to ANSES (the French Agency for Food, Environmental, and Occupational Health and Safety Laboratory) on Reunion Island at the end of the second season for the molecular typing of bacterial wilt strains.

Marketable yields were measured in the same way as in Experiment 1. Data on daily average temperature, air moisture content, and total rainfall were collected from the weather stations at the nearest airport (www.ncei.noaa.gov, accessed on 13 March 2020).

2.2.3. Experiment 3: Response of Ungrafted and Grafted Tomato Plants to Water Deficit Soil Infested by Bacterial Wilt

Experiment 3 was conducted at the World Vegetable Center for Eastern and Southern Africa in a greenhouse with repeated crop failures caused by bacterial wilt (*R. solanacearum*). The experiment aimed to compare the wilting symptoms and yields of ungrafted and grafted tomato plants under two irrigation regimes (standard and with a 50% reduction). The greenhouse was 120 m^2 (15 m × 8 m) and had a double door covered with a plastic film as a roof and insect-proof nets (60 mesh) on the sides. The experiment was conducted in two seasons, from December 2018 to March 2019 and March 2019 to July 2019.

The experiment used a split-plot design with three replications (blocks) in both seasons. The whole-plot treatment consisted of two irrigation regimes, the subplot treatments included the ungrafted 'Tanya' as a control and 'Tanya' grafted onto 'Tengeru 1997'. Each experimental unit (7.5 m^2) consisted of 12 plants (1.6 plants·m^{-2}) transplanted at the three- to four-true leaf stage to two raised beds (length 300 cm, width 75 cm, height 25 cm) covered with plastic mulch and spaced 1.25 m apart with 60 cm between plants in the same row.

The irrigation was carried out every two days with drip lines, and the quantity provided varied between treatments. Three tensiometers (Burdon, SDEC, 37310 Tauxigny, France) were used to monitor soil water potential and guide the amount of water added in plots to reach the target soil moisture level during irrigation. In plots with standard irrigation, the aim was to maintain a soil water potential between −0.03 and −0.02 MPa at 30 cm below ground to meet tomato plant water requirements [24]. In the plots with reduced irrigation, only half the amount of water was supplied compared to plots under a standard irrigation regime. This resulted in a soil water potential ranging from −0.08 to −0.02 MPa (please refer to Supplemental Figure S1 for more details). The fertilization process was the same as the standard fertilization treatment used in Experiment 1. Chemical treatments were applied using a knapsack sprayer, based on scouting, to control insect pests and diseases (please see Table 2 for more details). The bacterial wilt incidence was assessed as described in Experiment 2, and marketable yields were evaluated as described in Experiment 1. The temperature and air moisture content were recorded every 30 min using a data logger (HOBO Pro v2 U23-001, Onset Computer Corporation, Bourne, MA, USA). To avoid direct exposure to sunlight, the data loggers were suspended 1.8 m above the ground in the middle of the greenhouse under a perforated white cover with an open bottom section.

2.3. Molecular Typing of Bacterial Wilt Strains

At each experimental site, a 30 cm section above the plant root collar was gently cut from five wilted tomato plants at the end of the second season. After the samples were disinfected with 70% ethanol, they were packed in paper envelopes. They were sent to the ANSES (French Agency for Food, Environmental, and Occupational Health and

Safety) laboratory on Reunion Island for genetic identification. The isolation and genetic identification of bacterial wilt strains were performed following the method described by N'Guessan et al. [25]. Macerates of samples in distilled water were streaked on Kelman's triphenyl tetrazolium chloride (TZC) and modified Sequeira media, and incubated at 28 °C for two or three days. Bacterial colonies morphologically typical of *R. solanacearum* were purified and used for molecular typing. After applying multiplex-polymerase chain reactions (PCRs) to bacterial suspensions, the phylotype was assigned to each strain. The phylogeny of strains was characterized using a comparative analysis of the partial nucleotide sequences of all genes [26].

2.4. Statistical Analyses

The data from each experiment were analyzed separately. We checked all data for normality and homogeneity of variance using the Shapiro–Wilk and Bartlett tests, respectively. To identify significant differences between factors in the data concerning plant development in Experiment 1 (fresh weight and leaf surface area of plants) and yield in all three experiments, we used analysis of variance (ANOVA). Whenever we identified significant differences, we performed post hoc analyses using Fisher's least significant difference (LSD) test to compare treatments. However, in Experiments 2 and 3, the data did not follow a normal distribution. Therefore, we used the Kruskal–Wallis test to compare the percentage of wilted tomatoes. Furthermore, we performed post hoc analyses using Dunn's trial and the Holm method to adjust the *p*-value whenever we identified significant differences between treatments. Finally, we conducted all statistical analyses using R software version 4.0.0 with the agricolae package.

3. Results

3.1. The Response of Grafted Tomatoes to Different Fertilization Treatments

No wilt symptoms were observed in Experiment 1, which was conducted in Arusha to compare crop development and yields of ungrafted and grafted tomatoes with standard and low fertilization rates. The reduced plant vigor and yield during the second season resulted from the unsatisfactory control of early and late blight promoted by high rainfall (Table 3) despite an increase in fungicide treatments (Table 2). No significant differences were identified in the fresh mass of plants between fertilization treatments 10 weeks after transplanting in the first season (p-value = 0.204) and in the second season (p-value = 0.116) (Table 4). Although no significant differences were observed in the first season (p-value = 0.720), the leaf surface area measured in plants receiving the low fertilization treatment was smaller than with the standard treatment in the second season (p-value = 0.003). The reduction in fertilization significantly reduced yields in both seasons; the p-values were 0.019 and 0.018 in the first and second seasons, respectively. The yields of ungrafted 'Tanya' tomatoes decreased from 2.3 (standard fertilization) to 1.6 (low fertilization) $kg \cdot m^{-2}$ in the first season and from 1.2 to 0.6 $kg \cdot m^{-2}$ in the second season (Figure 2).

Ungrafted plants exhibited higher vigor levels, with greater fresh mass and leaf surface area than grafted plants, regardless of the fertilization treatment (Table 4). However, no significant differences were observed between the yields of ungrafted and grafted tomatoes when the fertilization rate was reduced, regardless of the rootstocks used and the season. Upon applying standard fertilization, higher yields were achieved from tomatoes grafted onto 'Hawaii 7796' (37% and 40% increases in the first and second seasons, respectively) and onto 'Shelter' (24% and 72% increases in the first and second seasons, respectively) in comparison to ungrafted tomatoes, but no differences were found in tomatoes grafted onto 'R3034'. Grafting onto 'Shelter' and 'Hawaii 7796' resulted in average yield increases of 38.3% and 24.4%, respectively, across both tested fertilization treatments.

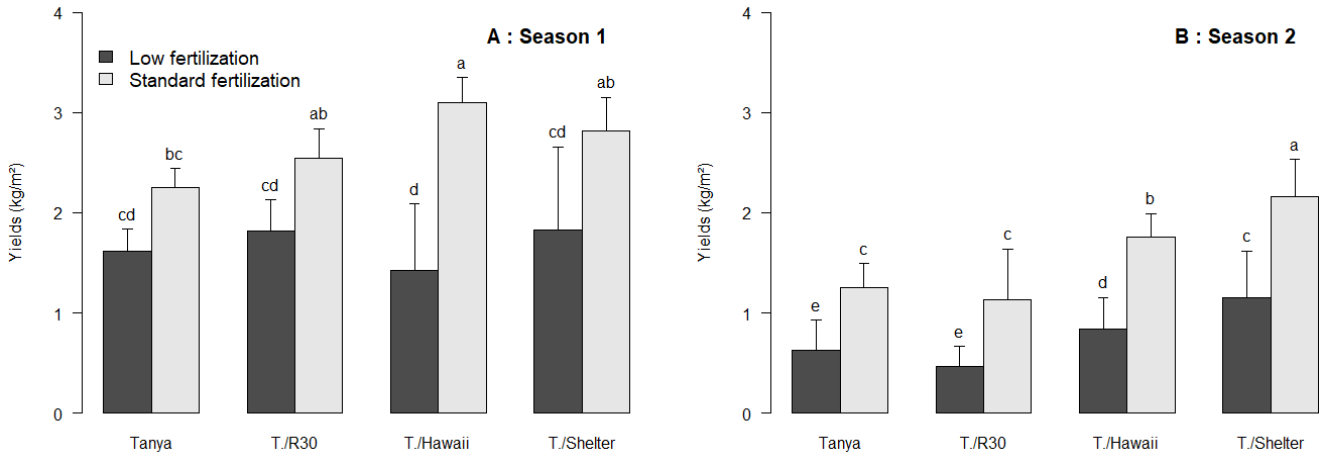


Figure 2. Comparison of marketable yields of grafted and ungrafted tomatoes under different fertilization treatments (standard or 15%) in two seasons. Data are the observed means of replications (±standard deviation, N = 4). Different lower-case letters mean significant differences based on Fisher's least significant difference (LSD) test (at $p = 0.05$).

Table 3. The average air temperature, rainfall, and average air relative humidity in trials conducted in the two seasons.

Experiment	Location	Season	Date of Transplanting	Date of the Last Harvest	Average Temperature (°C)	Rainfall (mm)	Average Relative Humidity (%)
1	Arusha (open field)	1	14 November 2018	15 February 2019	22.4	87.9	71.3
		2	8 March 2019	7 July 2019	20.1	287.4	82.3
2	Moshi (open field)	1	31 May 2018	11 September 2018	20.6	17.2	
		2	19 November 2018	19 February 2019	25.6	101.6	
2	Unguja (open field)	1	1 November 2018	31 January 2019	27.8	266.9	
		2	4 July 2019	15 October 2019	26.1	350.8	
2	Pemba (open field)	1	25 October 2018	25 January 2019	26.6	177.9	
		2	28 June 2019	15 Oct 2019	24.4	4.8	
3	Arusha (greenhouse)	1	3 December 2018	5 March 2019	28.1	0	64.5
		2	27 March 2019	18 June 2019	22.1	0	77.1

Table 4. Impacts of tomato grafting on plant vigor, i.e., plant fresh weight (aerial part and roots) and leaf surface area according to the fertilization treatment (standard or 15%), 10 weeks after transplanting. Data are the observed means of replications (N = 4), and values with the same letters are not significantly different based on Fisher's least significant difference (LSD) test ($p = 0.05$).

Season	Fertilization	Plants	Plant Fresh Weight (g)	Plant Leaf Surface (cm^2)
1	Low	Tanya	144.3 ± 18.6 ab	1108.2 ± 291.8 ab
		Tanya/Hawaii	101.1 ± 11.3 cd	993.3 ± 336.6 ab
		Tanya/R30	94.9 ± 11.1 d	1030.9 ± 355.3 ab
		Tanya/Shelter	124.6 ± 9.1 bc	848.8 ± 74.9 b
	Standard	Tanya	164.4 ± 32.0 a	1319.9 ± 57.5 a
		Tanya/Hawaii	100.0 ± 17.0 cd	972.3 ± 105.9 ab
		Tanya/R30	108.3 ± 14.0 cd	805.6 ± 82.5 b
		Tanya/Shelter	127.4 ± 22.9 bc	986.3 ± 317.2 ab

Table 4. *Cont.*

Season	Fertilization	Plants	Plant Fresh Weight (g)	Plant Leaf Surface (cm^2)
2	Low	Tanya	26.5 ± 9.5 bcd	1028.7 ± 23.7 ab
		Tanya/Hawaii	17.4 ± 1.1 e	861.5 ± 160.6 bcd
		Tanya/R30	18.6 ± 2.5 de	819.8 ± 73.0 cd
		Tanya/Shelter	21.9 ± 7.6 cde	1009.1 ± 36.6 abc
	Standard	Tanya	37.8 ± 7.8 a	1162.2 ± 179.3 a
		Tanya/Hawaii	28.9 ± 3.1 bc	940.1 ± 104.3 bcd
		Tanya/R30	24.3 ± 4.4 bcde	747.1 ± 112.7 d
		Tanya/Shelter	32.0 ± 8.3 ab	929.5 ± 86.9 bcd

3.2. Performances of Grafted Tomato in Soil Infested by Bacterial Wilt

The genetic analysis of wilted samples has confirmed that the wilting was caused by *R. solanacearum* in all locations. However, Phylotype I sequevar 31 strains were found in the experimental plots of Arusha and Moshi. In contrast, Phylotype I sequevar 17 strains were isolated in Pemba, and Phylotype I sequevar 18 strains were found in Unguja.

Rainfall and temperatures were higher in the coastal regions (Pemba and Unguja) compared to the northern highlands of Tanzania (Moshi and Arusha) (Table 3). In the coastal areas, the incidence of bacterial wilt was high throughout both seasons, and all ungrafted 'Tanya' and 'Tengeru 2010' tomatoes used as controls were wilted at the end of the experiments (Figure 3A–D), leading to a complete loss of yield (Table 4). Similarly, all plants grafted onto 'Tengeru 2010' were wilted at both sites by the end of the two seasons, resulting in almost zero yields (Table 4).

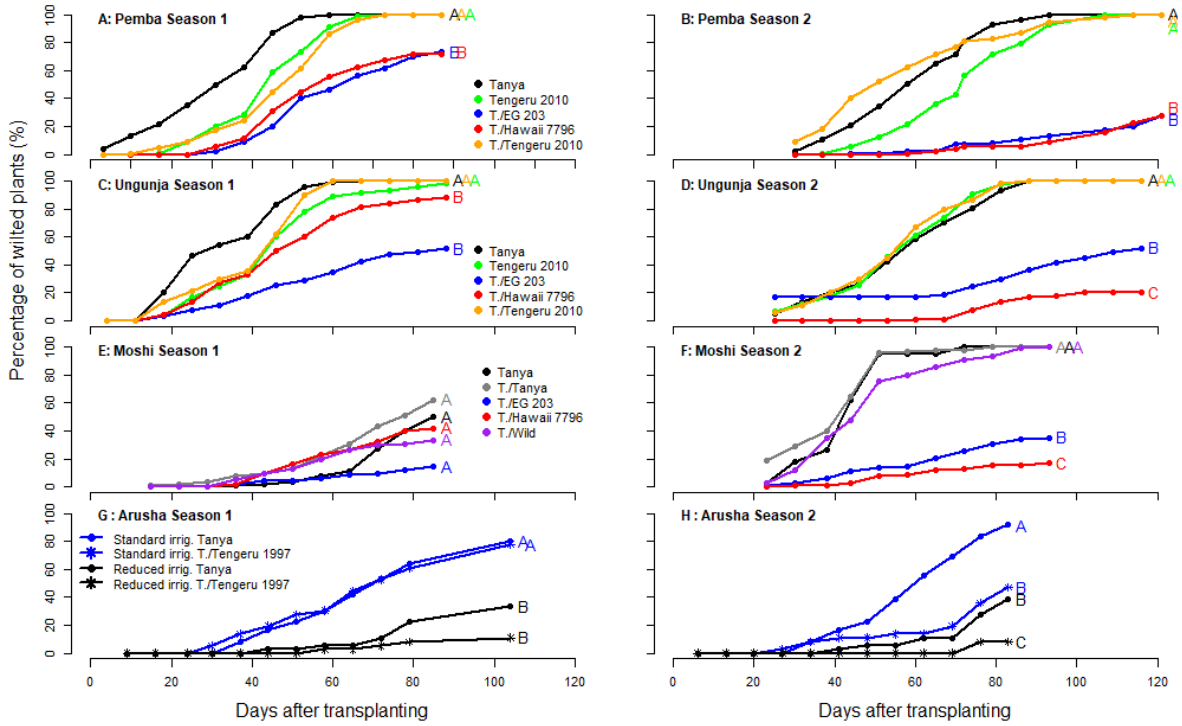


Figure 3. Bacterial wilt incidence (*R. solanacearum*) in the experiments conducted in two seasons in Pemba (**A**,**B**), Unguja (**C**,**D**), Moshi (**E**,**F**), and Arusha (**G**,**H**) with ungrafted 'Tanya' and 'Tengeru 2010' tomatoes and 'Tanya' grafted onto 'Tanya' (self-grafted), 'EG 203', 'Hawaii 7796', 'Tengeru 2010', 'Tengeru 1997', and a wild Solanaceous species (*S. elaeagnifolium*). Data are the observed means of replications (N = 5 in Moshi, N = 3 in Pemba, Unguja, and Arusha), and values with the same letters are not significantly different based on Dunn's test ($p = 0.05$).

Grafting tomatoes onto 'EG 203' slightly improved yields to 0.18 and 0.52 kg·m^{-2} in the first and second seasons by reducing plant wilting to 73% and 27% without significant differences. Grafting tomatoes onto 'Hawaii 7796' also reduced wilt to 71% and 28% in the first and second seasons, resulting in yields of 0.28 and 0.53 kg·m^{-2} (Table 5). Similar results were recorded in Unguja, with 51% and 52% of tomatoes grafted onto 'EG 203' exhibiting wilt symptoms at the end of the first and second seasons, respectively, resulting in yields of 0.28 and 0.53 kg·m^{-2}, as opposed to the 88% and 20% wilting of tomatoes grafted onto 'Hawaii 7996' with yields of 0.04 and 0.33 kg·m^{-2} (Table 5).

Table 5. Comparison of marketable yields of ungrafted 'Tanya' and 'Tengeru 2010' tomatoes and 'Tanya' tomatoes grafted onto tomato ('Tengeru 2010', 'Hawaii 7996') and eggplant ('EG 203') rootstocks at the different sites in Tanzania (Moshi, Pemba, Unguja, and Arusha) infested by bacterial wilt in two seasons. Data are the observed means of replications (N = 5 in Moshi and N = 3 in Pemba, Unguja, and Arusha), and values with the same letters are not significantly different based on the Fisher's least significant difference (LSD) test (p = 0.05).

Sites	Treatment	Plants	Marketable Yields (kg/m^2) Season 1	Marketable Yields (kg/m^2) Season 2
Pemba	Normal irrigation	Tanya	0.00 ± 0.00 c	0.00 ± 0.0 b
	Normal irrigation	Tengeru 2010	0.05 ± 0.06 abc	0.12 ± 0.11 b
	Normal irrigation	Tanya/EG 203	0.18 ± 0.04 a	0.52 ± 0.17 a
	Normal irrigation	Tanya/Hawaii 7796	0.10 ± 0.08 ab	0.55 ± 0.20 a
	Normal irrigation	Tanya/Tengeru 2010	0.01 ± 0.02 bc	0.04 ± 0.07 b
Unguja	Normal irrigation	Tanya	0.00 ± 0.00 b	0.01 ± 0.02 c
	Normal irrigation	Tengeru 2010	0.02 ± 0.04 b	0.07 ± 0.05 bc
	Normal irrigation	Tanya/EG 203	0.28 ± 0.02 a	0.53 ± 0.18 a
	Normal irrigation	Tanya/Hawaii 7796	0.04 ± 0.01 ab	0.33 ± 0.09 ab
	Normal irrigation	Tanya/Tengeru 2010	0.00 ± 0.00 b	0.01 ± 0.01 c
Moshi	Normal irrigation	Tanya	3.59 ± 1.85 a	0.00 ± 0.00 b
	Normal irrigation	Tanya/EG 203	3.59 ± 1.91 a	0.56 ± 0.11 a
	Normal irrigation	Tanya/Hawaii 7796	2.05 ± 1.13 a	0.85 ± 0.34 a
	Normal irrigation	Tanya/Tanya	2.46 ± 1.16 a	0.00 ± 0.00 b
	Normal irrigation	Tanya/Wild	2.32 ± 1.01 a	0.00 ± 0.01 b
Arusha	Normal irrigation	Tanya	0.49 ± 0.12 c	0.43 ± 0.05 c
	Normal irrigation	Tanya/Tengeru 1997	0.54 ± 0.07 c	0.55 ± 0.14 c
	Half-reduced irrigation	Tanya	1.02 ± 0.24 b	1.39 ± 0.36 b
	Half-reduced irrigation	Tanya/Tengeru 1997	1.22 ± 0.24 a	2.11 ± 0.47 a

In Moshi, the wilt incidence in tomatoes showed marked seasonal variations. At the end of the first season, 50% of ungrafted 'Tanya' tomatoes wilted, resulting in yields of 3.59 kg·m^{-2}. In contrast, by the end of the second season, 100% of ungrafted 'Tanya' tomatoes wilted, yielding zero yields (Figure 3F). Grafting tomatoes onto 'EG 203' reduced wilting to 14% and 35%, with 3.59 and 0.56 kg·m^{-2} yields in the first and second seasons, respectively. On the other hand, grafting tomatoes onto 'Hawaii 7996' resulted in 42% and 17% wilting and yields of 2.05 and 0.85 kg·m^{-2} in the first and second seasons, respectively. Self-grafting or using the wild eggplant (*S. elaeagnifolium*) rootstocks did not provide any advantage, as all plants wilted by the end of the second season with zero yields (Table 4). Taking into account results from all experimental sites and seasons, grafting onto 'EG203' and 'Hawaii 7796' increased yields by an average of 57.2% and 27.7%, respectively. It is noteworthy that these findings obscure significant variability between the sites, as the yields of grafted plants onto these rootstocks ranged from 0.1 to 3.59 kg·m^{-2}.

In Experiment 3, cutting the water supply by half reduced the wilting of ungrafted 'Tanya' tomatoes from 81% to 33% in the first season and from 92% to 39% in the second season (Figure 3G,H), increasing yields from 0.49 to 1.02 kg·m^{-2} in the first season and

from 0.43 to 1.39 kg·m^{-2} in the second season. Although no difference was observed in the first season, grafting 'Tanya' onto 'Tengeru 1997' reduced wilting from 92% to 47%, with standard irrigation increasing yields from 0.43 to 0.55 kg·m^{-2}. The advantages of using 'Tengeru 1997' as rootstock were clearer under the 50% irrigation treatment since wilting was reduced from 33% to 11% and 39% to 8% compared to ungrafted plants in the first and second seasons. This increased yields from 1.02 to 1.22 kg·m^{-2} and 1.39 to 2.11 kg·m^{-2} (Table 5). The combination of reduced water supply and grafting led to an average reduction in wilting of 76% and an average yield improvement of 3.6 times in experiments conducted over two seasons in Arusha.

4. Discussion

The results of the present study show that grafting tomatoes systematically leads to higher plant survival depending on the rootstock used for grafting but does not systematically lead to higher yields. The advantages of using grafted plants vary with the rootstocks, cultivation practices, and the degree of pressure and diversity from soilborne pathogens. Grafting presumably aids pathogen avoidance when the rootstock is resistant to the pathogen. That is why scion adventitious roots confer susceptibility to the grafted plant when they come into contact with the soil [27].

Though grafting can be advantageous, the mechanism is still unclear. Sometimes, the different strains of *R. solanacearum* of Phylotype I (sequevars 17, 18, and 31) isolated from the experimental sites emphasize the genetic diversity of the pathogen in Tanzania [3], which was previously reported to be limited to strains of Phylotype III [28]. King and coauthors [27] concluded in their review that there is a likelihood that new races or pathotypes will evolve, weakening the existing resistant rootstocks due to selection pressure brought by vast rootstock usage.

The field trials showed that grafting tomatoes onto 'Hawaii 7796', 'Tengeru 1997', and 'EG 203' improved resistance to bacterial wilt to a certain extent, whereas grafting tomatoes onto 'Tengeru 2010' and onto the wild species (*S. elaeagnifolium*) did not. Laboratory experiments conducted by Lebeau [26] showed no wilting of 'Hawaii 7796' and 'EG 203' after inoculation with a strain of Phylotype I (sequevars 13 and 18), although latent infection tests revealed that some plants did carry the pathogen. Rootstock functioning can be related to pathogen pressure in the soil; Herman and Treves [29] pinpointed the existence of fusarium pathogens in grafted cucumber stems but at low levels as compared to the control when commercially available rootstocks were used, meaning under low pathogen pressure, it is unlikely for the pathogen to overpower the semi-resistant rootstocks completely. However, they can still enter the xylem and colonize it. The improvements in yield obtained by using grafted plants in soils infected by bacterial wilt measured in the present study are, thus, far from those previously reported in studies conducted in the USA [30] and in Asia [31]. The difference between the results of the experiments performed on other continents can be attributed to differences in *R. solanacearum* strains and the combination of scions and rootstocks. The yield potential of tomatoes in this region may not be similar due to other pressures like leaf diseases such as early and late blights, which limits the production potential. Less wilting and higher yields with 'Tanya' tomatoes grafted onto 'Hawaii 7996' grown in soil infested by bacterial wilt have also been previously reported at one of the experimental sites (Arusha) used in the present study [32]. The lower performances of the grafted plants in the present study may be explained by a higher incidence of bacterial wilt in our study conditions, like the marked differences in wilting and yields between seasons in Moshi on grafted plants (Figure 3E,F). The all-year-round high incidence of bacterial wilt in the coastal areas (Pemba and Unguja), where the climate is hot and humid, limited yields to less than 0.55 kg·m^{-2} in all three experiments, regardless of the rootstock used. The impact of the soil moisture on bacterial wilt incidence was further confirmed by the results of Experiment 3, which showed that reducing irrigation by half improved yields thanks to reduced wilting (Figure 3G,H). This is consistent with the results of a previous laboratory study showing that tomato wilt incidence was lower in dry soil [33], and improved yields

were obtained from grafted tomatoes grown under a rain shelter [34]. The bacteria need a hot and wet environment for their multiplication and spread. Since the reduction in soil moisture reduces the spread of the pathogen, seedling mortality is reduced.

Rootstocks have the potential to alter yields negatively; Davis and coauthors [35] argue that there are optimal temperature and humidity ranges for the optimal performance of scion/rootstock combinations, and this may explain contrasting grafting benefits based on our geographical location situated with a different climatic condition from Asia or the US where these rootstocks had amazing results. In the present study, the maximum yields obtained from ungrafted 'Tanya' tomatoes in bacterial wilt-free soil with standard fertilization and irrigation did not exceed 2.2 $kg \cdot m^{-2}$. Although these yields are in agreement with those commonly observed for this variety when grown in the field [22,36], they are far below their potential yields (up to 5.0 $kg \cdot m^{-2}$) due to high pressure from insects and diseases during the experiments despite a large number of pesticide treatments (Table 2). This underlines the extent of crop losses caused by aerial pests, which limits the benefits of investing in grafted plants, and explains the reliance of Tanzanian tomato producers on pesticides [5].

Our results provide no evidence for significant improvement in yield to be achieved by grafting 'Tanya' tomatoes onto 'Hawaii 7796', 'R3034', or 'Shelter' with reduced fertilization. Rivard and Louws [37] advise using grafting only when there is disease pressure, meaning there can be a yield penalty. Previous studies reported improved plant nutrient uptake achieved by grafting tomatoes onto 'Maxifort' or 'Brigeor' rootstocks [38,39]. In agreement with these studies, higher yields were also reported for field-grown tomatoes (cv. 'Florida 47') grafted onto 'Multifort' or 'Beaufort' rootstocks with a nitrogen supply below the recommended rate [40]. The latter study confirmed higher yields from tomatoes (cv. 'Tribute') grafted onto 'Maxifort' with a low nitrogen supply. Still, contrasting results were obtained with the 'RST-106' rootstock, as improved yields were only obtained in one out of the two years of the experiment [41]. Plant responses to stress can be complex. Ullah and coauthors [42] summarized how plants could adopt different signaling pathways under environmental stress in conjunction with phytohormones like auxins. However, some response mechanisms were still unclear without a direct pattern due to the complexity of soil health, microbial diversity, and ecological functioning. In the present study, the absence of a significant difference between grafted and ungrafted tomatoes with reduced fertilization could be due to the choice of rootstocks used and/or to the high pressure of pests and diseases during the experiments that may have reduced the effect of grafting.

Overall, the results of the three experiments conducted in the present study provide no evidence for any advantage in using grafted plants even though the merits of some of the rootstocks tested ('EG 203' and 'Hawaii 7796') in increasing the tolerance of the plants to bacterial wilt and flooding have been widely demonstrated in Asia [20,31]. The lack of tangible results echoes observations of a meta-analysis indicating that among the 949 combinations of rootstock scions reviewed, only 37% produced higher yields than ungrafted tomatoes [14]. To benefit from tomato grafting onto suitable rootstocks, further efforts are required to identify rootstocks suitable for production in Tanzanian conditions, along with the appropriate management of other biotic and abiotic stresses that limit tomato production.

5. Conclusions

Several field experiments were conducted in various regions of Tanzania, including Arusha, Moshi, Pemba, and Unguja, to assess the response of the 'Tanya' determinate tomato variety. The experiments involved growing the tomatoes either ungrafted or grafted to rootstocks of tomato, eggplant, and wild species under different fertilization regimes in soils infected by bacterial wilt and water deficit levels in soils infected with bacterial wilt. The findings revealed that the rootstocks tested in the study, such as 'EG 203', 'Tengeru 1997', 'Tengeru 2010', 'Hawaii 7996', and *Solanum elaeagnifolium*, did not significantly improve tomato yields. However, some rootstocks were found to increase the plant's resistance to

bacterial wilt to some extent. Moreover, the research showed that grafting tomatoes onto 'Shelter', 'Hawaii 7796', and 'R3034' did not enhance yields under the 15% low fertilization treatment. As a result, further research is necessary to identify rootstocks resistant to the various strains of bacterial wilt present in Tanzania. Such research could help boost the tolerance of tomatoes to common production limitations, such as water deficit and poor soil fertility.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae10040338/s1: Figure S1: Comparison of the soil water potential at a depth of 30 cm below the soil surface under standard (from −0.03 to −0.02 MPa, in black) and 50% irrigation (reduced from −0.08 to −0.02 MPa, in light grey) supply in Experiment 3 in Arusha. Data are averages (N = 3).

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Data Availability Statement: The data presented in this study are available upon request from the corresponding author. They are not publicly available for a certain period and can later be accessed from https://worldveg.tind.io/ from 15 May 2024.

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