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Grafting Tomato Cultivars for Soil Borne Disease Suppression and Plant Growth and Yield Improvement

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Abstract

Soil borne fungal diseases are among the most damaging diseases of tomato in Tunisia. Among them, Fusarium wilt (FW) caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) races 1 and 2, Fusarium Crown and Root Rot (FCRR) incited by *F. oxysporum* f. sp. *radicis lycopersici*, and Verticillium wilt (VW) due to *Verticillium dahliae* (Vd) races 1 and 2 are of particular concern. In the current study, the grafting of three scion tomato cultivars (cvs. Kawthar, Amal and Malinche) onto the interspecific hybrid rootstock Maxifort was evaluated for diseases management and plant growth and yield improvement. Under artificial inoculation conditions, the present study demonstrates that the plant response to the tested pathogens (Vd races 1 and 2, FOL races 1 and 2 and FORL) used for inoculation differed according to the tomato cultivars used, the grafting treatment and their interactions. Overall, grafting was shown to be effective in significantly reducing disease severity, estimated via the relative vascular discoloration extent (RVDE), by 24%, and enhancing root and stem fresh weights and yield by 18%, 30% and 17%, respectively, compared to non-grafted controls. Under natural greenhouse conditions, disease severity was statistically comparable on grafted and non-grafted cvs. Kawthar and Malinche, plants. However, grafting cv. Amal plants have significantly reduced, by 61%, the RVDE as compared to non-grafted ones. Root fresh weight noted on Maxifort-grafted cvs. Kawthar, Amal and Malinche plants was significantly enhanced by 32, 59 and 55%, relative to non-grafted ones. Plants grafted onto Maxifort rootstock had produced 63% higher total yield than the non-grafted control. As assessed by comparative disease symptoms and plant growth and yield response, grafting tomato on the rootstock Maxifort have could be implemented in an integrated disease management with other soil disinfection methods for reducing soil borne populations in the soil.

Keywords: Cultivars; Grafting; Plant growth; Severity; Soil borne diseases; *Solanum lycopersicum*; Yield

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most cultivated and extensively consumed vegetable crops worldwide [1]. In Tunisia, it is one of the most economically important crops which is grown annually on an area of about 29000 ha with an average production of 1.2 millions of tons. Tomato is grown throughout the year under open fields, for season and late season crops, and under cold and geothermal greenhouses for off-season ones (early and extra-early seasons). Open field tomatoes production turned mainly into processed product while off season ones are used for fresh consumption or exported as fresh to European countries and contribute to the national economy of the country [2]. Tomato cold greenhouses are mostly located on the Eastern coast of Tunisia with a production beginning from December to June whereas heated greenhouses are concentrated in the regions of Gabes, Tozeur and Kebili, where the geothermal heating is available, producing tomatoes from November till the end of May [2]. However, even though tomato is a strategic crop in Tunisia, growers are still facing major challenges to have earlier production, high yield and good tomato quality, due to several abiotic (water and salt stress, workforce) and biotic (diseases and pests) constraints. For instance, soil borne fungal diseases are among the most damaging diseases that are particularly difficult to predict, detect, diagnose, and successfully control [3]. In Tunisia, the major tomato vascular soil borne diseases are Fusarium wilt (FW) caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) races 1 and 2, Fusarium Crown and Root Rot (FCRR) incited by *F. oxysporum* f. sp. *radicis lycopersici*, and Verticillium wilt (VW) due to *Verticillium dahliae* (Vd) races 1 and 2. These fungal diseases are severe and widespread in almost all tomato producing regions whatever the cultivars used, the climatic conditions, the soil type, the cultural practices, and the diseases control methods used. Furthermore, as farms are small, long-term rotation remains difficult and successive cropping of tomato in the same fields contributes to a continual increase of pathogen populations in the soil. As a result, these well-established soil borne diseases are still a restricting factor for tomato cultivation. [4-6]. Many control measures have been used to decrease the pressure of soil borne diseases such as the use of chemical fungicides, soil solarization and resistant varieties. However, even though their efficacy have been proved in many cases, these methods have some limitations related to their high costs, the water shortage and the emergence of new pathogen races [4]. Among the methods used in controlling the soil borne disease, grafting on resistant rootstocks has been highly effective, successfully practiced and becoming increasingly popular worldwide [7,8], in particular for tomato which is among the major vegetables used for grafting [6,9]. The introduction of excellent rootstocks possessing multiple disease resistance have greatly encouraged the extended use of grafted vegetables over the world. In Mediterranean regions, grafting is one of the most used alternatives to Methyl Bromide, which is often associated with soil solarization for tomato production [10,11]. In Morocco, for example, tomato grafting was initiated in 1996 and is now widely used at a commercial level in about 95% of tomato production

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in plastic houses, to control soil borne pathogens. In addition, it has also promoted tomato growth and increased yields, enhanced low temperature tolerance, extended growth periods, and improved fruit quality and quantity [10]. In Italy, 59 million vegetable plants are grafted. Grafting is used to reduce susceptibility against pests, root rots and wilts, and to increase yield [12]. In the other side of the world, in Lam Dong Province of Vietnam, which is the country's major tomato production area, 100% of commercial farmers are now using grafted seedlings to counter hostile soil borne diseases [13]. A wide range of crop varieties, wild relatives and intra- and interspecific hybrids have been tried and many of them have been shown to be of value as rootstocks for tomato in specific combinations and for defined environmental stresses [14]. In fact, improved resistances to many soil borne fungal, oomycete, bacterial, and nematode pathogens have been reported in grafted solanaceous [15]. For instance, Rivard and Louws [16] and Rivard et al. [17] found that grafting heirloom tomato onto vigorous rootstocks such as Beaufort and Maxifort effectively controlled bacterial wilt (caused by *Ralstonia solanacearum*), Fusarium wilt, Southern blight (caused by *Sclerotium rolfsii*), root-knot nematode (*Meloidogyne* spp.), and also increased plant vigor and yields. Giotis et al. [18] found that a more widespread use of grafting using tomato rootstocks (namely Beaufort, Maxifort, He-man, and R-5872) allowed significant reductions in the use of steam and chemical soil disinfection in glasshouse crops and was an effective strategy to control the most important soil borne fungal diseases caused by *Pyrenochaeta lycopersici*, *Verticillium* spp., and root-knot nematodes. In Tunisia, however, grafting has just recently been commonly used technique for soil borne diseases management and the use of grafted tomato plants has gained progressive popularity within tomato producers in some locations. In fact, despite the high additional cost, the efficacy of grafting tomato in reducing infection by soil borne pathogens has strongly encouraged farmers to use tomato plants grafted onto resistant rootstocks. For instance, in southern Tunisia, the use of multiple tomato rootstocks has efficiently controlled FCRR disease which incidence reached 90% on some soilless tomato cultivars [6]. Actually, Maxifort is one of the widely used rootstock for tomato grafting in many tomato producing regions. However, there is limited information on the performance of these grafted tomato plants in controlling soil borne fungal pathogens. Therefore, the objective of this research was to evaluate the efficacy of Maxifort-grafting tomato cultivars in reducing severity of main soil borne diseases in Tunisia (namely VW, FW, and FCRR) and in improving plant growth and fruit yield under controlled conditions and under natural field conditions.

Materials and Methods

Plant material

Three commercially available tomato cultivars (namely Malinche, Kawthar and Amal) were used in this study, as scions, while the tomato hybrid Maxifort (*Solanum lycopersicum* x *Solanum habrochaites*) was used as rootstock (Table 1). These tomato cultivars were used for fresh-market production under plastic greenhouses in the Tunisian Sahel regions. The interspecific rootstock hybrid cultivar, Maxifort, is among the most utilized rootstock for greenhouse production throughout Tunisia, Mediterranean countries, the United States, Canada, and Northern Europe [17,18]. The cleft grafting technique was carried out for all grafted plants. Grafted and non-grafted transplants were produced in commercial nursery facilities in Chott Mariem region and were allowed to grow for 10 to 14 days in the plastic greenhouse before being planted into greenhouse field plots or growth chamber pots.

Fungal material

Five isolates, belonging each to *V. dahliae* (Vd) races 1 and 2, *F. oxysporum* f.sp. *lycopersici* (FOL) races 1 and 2, and to *F. oxysporum* f. sp. *radicis-lycopersici* (FORL), were used in this study. These characterized isolates are held in the fungal culture collection of the Laboratory of Phytopathology of the Regional Research Center on Horticulture and Organic Agriculture at Chott-Mariem, Sousse, Tunisia. Vd and FOL isolates were originally recovered from wilted tomato plants showing severe wilting and vascular brown discoloration. For FORL isolate, these symptoms were associated with crown and root rots. All these isolates were cultured on Potato Dextrose Agar (PDA) medium supplemented with streptomycin sulphate (300 mg/L) and incubated at 25°C for 7-15 days before use. Inoculum production was initiated by suspending a mycelial plug (5 mm in diameter), cut from 5-day-old cultures into Potato Dextrose Broth (PDB) medium. After an incubation period of 5-7 days, under continuous shaking at 150 rpm, the conidial suspension was filtered through sterile Whatman No. 1 and the concentration was adjusted to 10^7 conidia/ml using a hemocytometer.

Tomato material	Resistance	Fruit form	Growth
Malinche (DeRuiter)	ToMV:0-2/TSWV/Ff:B,D/Fol:1,2/For/ Va:1/Vd:1	Oblong	Indeterminate
Kawthar (DeRuiter)	ToMV:0-2/Fol:1,2/Va:1/Vd:1	Round	Indeterminate
Amal (DeRuiter)	Aal/Fol:1,2/Va:1/Vd:1	Round	Indeterminate
Maxifort (DeRuiter)	ToMV:0-2/Fol:1,2/ For/Va:1/Vd:1	--	--

ToMV:0-2: *Tomato Mosaic Virus* races 0-2; TSWV: *Tomato Spotted Wilt* Virus; Ff: *Passalora fulvum* strains B, D; Fol:0,1: *Fusarium oxysporum* f. sp. *lycopersici* races 1,2; For: *Fusarium oxysporum* f.sp. *radicis-lycopersici*; Va:1: *Verticillium albo-atrum* race 1; Vd:1: *Verticillium dahliae* race 1; Aal: *Alternaria alternata* f. sp. *lycopersici*.

Table 1: Characteristics of the tomato cultivars and rootstock used in this study.

Growth chamber trial

The tomato cultivar Maxifort was tested in the present study, as rootstock for three tomato cultivars, in order to assess its ability to control VW, FW and FCRR diseases under growth chamber conditions. Grafted and non-grafted tomato plants were carefully uprooted and their roots were dipped, for 30 min, in the conidial suspension of each tested pathogen isolate. Inoculated plants were then potted in peat contained in 17 cm diameter-pot. Grafted and non-grafted tomato plants which roots were dipped in sterile distilled water (SDW) served as uninoculated controls. For each individual treatment (for each tomato cultivar, grafted or not, inoculated or not), seven plants were used and the hole experiment was repeated twice. All tomato plants were maintained in a growth chamber at 15-30°C during 60 days and regularly watered and fertilized with a standard nutrient solution according to Pharand et al. [19]. The experiment was carried out according to a factorial design with three cultivars (cv. Kawthar, cv. Amal and cv. Malinche), two levels of grafting (no grafting, or grafted onto the rootstock cv. Maxifort) and six levels of fungal treatment: uninoculated control, inoculated with Vd race 1, inoculated with Vd race 2, inoculated with FOL race 1, inoculated with FOL race 2, and inoculated with FORL. Assessment of disease severity was performed at the end of the experiment, 60 days post-inoculation (DPI), on tomato plants challenged with each tested pathogen (Vd, FOL, and FORL) via the relative vascular discoloration extent (RVDE) which is the percentage of stem height exhibiting vascular discoloration which was calculated as follow:

$$RVDE\ (\%) = (\text{vascular browning extent} / \text{plant height}) * 100$$

In addition, plant growth and production parameters were evaluated at the end of the trial via the plant height, and the root, stem and fruit fresh weights.

Greenhouse trial

Plastic greenhouse trial was conducted in 2017-2018 agriculture campaign at the experimental station of the Regional Research Centre on Horticulture and Organic Agriculture, located in Teboulba region (central-Est of Tunisia). Soil type consisted of sandy clay texture. The research trial was carried out in field site with a long history of tomato soil borne fungal diseases such as Verticillium and Fusarium wilts, Black dot and Rhizoctonia stem canker. Greenhouse planting occurred 30 d after grafting. Cultural management was consistent with typical commercial production in the region. Grafted and non-grafted plants were set into a 15 cm high, 75 cm wide raised bed plasticulture system with 75 cm row spacing and 40 cm in-row spacing. There were four beds in the greenhouse with two rows per bed. Plants were arranged according to a completely randomized design with 90 plants per individual treatment. The treatments were: cv. Kawthar non-grafted and grafted onto cv. Maxifort, cv. Amal non-grafted and grafted onto cv. Maxifort, cv. Malinche non-grafted and grafted onto cv. Maxifort. There were 8 rows in the greenhouse and each individual treatment was planted in one row. Black plastic mulch and drip irrigation were used, and a stake-and-weave cultural management was used to train the plants vertically. Grafted plants were set high enough in the planting hole to ensure that the graft union was sufficiently above the soil line. After planting, grafted plants were maintained to produce a double-stem plant with two main producing shoots. For the assessment of disease severity, ten randomly selected plants, with wilt symptoms, were dug from the row of each treatment. For each plant, stems were longitudinally cut and visually examined for the presence of vascular discoloration. The relative vascular discoloration extent (RVDE) was calculated as described above. For each wilted plant, stem segments were excised (1 cm), surface-sterilized in 10% NaOCl solution, rinsed with distilled water, and placed on PDA. The isolated pathogens were purified and identified. In addition, these ten selected plants served for the measurement of plant height and root fresh weight. Furthermore, for each harvest, tomato production was weighted per individual treatment and the total production was calculated at the end of the trial i.e., 150 days post planting (DPP).

Statistical analyses

For all parameters measured under growth chamber conditions, statistical analyses were performed following a factorial design with three factors: tomato cultivars (three cultivars, cv. Kawthar, cv. Amal and cv. Malinche), grafting (grafted or non-grafted plants onto the rootstock cv. Maxifort) and fungal treatments (uninoculated control, Vd race 1, Vd race 2, FOL 1, FOL 2 and FORL). Seven replicates were used per individual treatment. The greenhouse trial was arranged according to a completely randomized design with two fixed factors, tomato cultivars and grafting treatments. Means were separated using Duncan's Multiple Range test (at $p \leq 0.05$). Statistical analyses were performed using SPSS software version 16.

Results

Effect of grafting under control conditions (growth chamber trial)

Effect on disease severity: Tomato plants of cvs. Malinche, Kawthar and Amal grafted or not on the rootstock cv. Maxifort were inoculated with Vd races 1 and 2, FOL race 1, FOL race 2, and FORL, under growth chamber conditions. At 60 DPI, disease severity estimated via the RVDE varied significantly (at $p \leq 0.05$) depending on tomato cultivar used, fungal treatments tested and grafting; significant interactions were also noted between these factors, except for cultivar × grafting interaction. As illustrated in Figure 1, the response of grafted and non-grafted tomato cultivars to the tested pathogens was clearly different. In fact, on Maxifort-grafted and Vd race 2-inoculated cvs. Malinche, Kawthar and Amal plants, the RVDE was significantly reduced by 37, 30 and 2%, respectively, compared to non-grafted scions. However, on Vd race 1-inoculated cvs. Kawthar and Amal plants, this parameter was low and significantly comparable for grafted and non-grafted plants, but was significantly higher on grafted cv. Malinche plants compared to non-grafted ones. At 60 DPI, grafting tomato cvs. Malinche, Kawthar and Amal plants onto Maxifort rootstock resulted in complete suppression of the vascular discoloration noted on FOL race 1-inoculated plants. Maxifort-grafted and FOL race 2-inoculated cvs. Malinche and Amal plants showed 43 and 23% lower RVDE, even statistically insignificant, than that recorded on non-grafted scions. However, on grafted cv. Kawthar plants, this parameter was significantly 41% higher than that noted on non-grafted ones. Under artificial conditions, grafted and FORL-inoculated plants showed significantly similar but reduced disease severity by 100, 69 and 85% relative to non-grafted cvs. Malinche, Kawthar and Amal plants, respectively. Combined data of all fungal and grafting treatments tested showed that the longest RVDE was recorded on cvs. Kawthar and Amal, which is 40% higher than that recorded on cv. Malinche. Furthermore, for all tested cultivars and grafting treatments combined, RVDE was highest on Vd race 2-inoculated plants followed by that of FOL race 2-inoculated ones. For all cultivars combined and fungal treatments pooled, grafted plants significantly reduced the RVDE, by 24%, compared to non-grafted controls.

Effect on tomato growth parameters

Growth response significantly (at $p \leq 0.05$) differed in accord with tomato cultivars, pathogen inoculation and grafting treatment (Table 2). Significant interactions among tested factors (cultivar, grafting and inoculation) were observed.

Plant height: Plant height varied significantly (at $p \leq 0.05$) depending on tomato cultivars used and fungal treatments tested; significant interactions were also noted between the three tested factors except for cultivar × fungal treatments interaction. At 60 DPI with Vd race 2 and FOL race 1, only grafted cv. Amal plants showed plant height significantly higher, by 31 and 21%, respectively, compared to non-grafted plants. Grafting tomato scions onto cv. Maxifort did not affect significantly plant height noted on FORL-inoculated plants. As presented in Table 2, grafting cv. Amal onto cv. Maxifort did not significantly affect the height of FOL race 2-inoculated plants, however when cvs. Malinche and Kawthar were used as scions, this parameter was about 15 and 18%, respectively, lower than on non-grafted plants. Plant height noted on grafted- and Vd race 1-inoculated plants was increased by 21% when cv. Amal was used as scion, and was significantly reduced by 17 and 10%, when using cvs. Malinche and Kawthar. For non-inoculated control plants, grafting cv. Amal onto cv. Maxifort has improved by 22% plant height compared to non-grafted ones; while for cv. Malinche, grafted and non-grafted control plants showed statistically similar plant height. This parameter was reduced by grafting cv. Kawthar plants onto cv. Maxifort. For pooled data of all fungal treatments, plant height was statistically comparable on grafted or non-tomato cultivars and was significantly reduced only on non-grafted cv. Amal plants. When tomato cultivars and grafting treatments were pooled, the highest plant height was noted on FOL race 2- and

FORL-inoculated plants followed by non-inoculated control.

Root fresh weight: Root fresh weight, noted at 60 DPI, varied significantly (at $p \leq 0.05$) depending on fungal treatments tested, grafting, and the interactions between the three tested factors (fungal treatment, tomato cultivars and grafting). Data presented in Table 2 showed that for Vd race 2- and FORL-inoculated plants, root fresh weight was improved by, 59 and 25%, respectively, only when cv. Amal was Maxifort-grafted. On Vd race 1-inoculated plants, this parameter was comparable on grafted and non-grafted plants of all three tomato cultivars. Grafting FOL race 2-inoculated plants resulted in significantly 50% lower, 37% higher and statistically similar root fresh weights when cvs. Malinche, Kawthar and Amal were respectively used as scions. Maxifort-grafted and FOL race 1-inoculated cvs. Kawthar and Amal plants showed respectively 43 and 38% higher root fresh weight than non-grafted plants. On non-inoculated control plants, grafting have improved this parameter by 37 and 36%, on cvs. Kawthar and Amal, respectively, and significantly reduced it by 49% on grafted cv. Malinche plants. For pooled data of all fungal treatments, root fresh weight was statistically higher on grafted cv. Kawthar plants followed by grafted cv. Amal and non-grafted cv. Malinche plants. When tomato cultivars and grafting treatments were combined, the highest root weight was noted

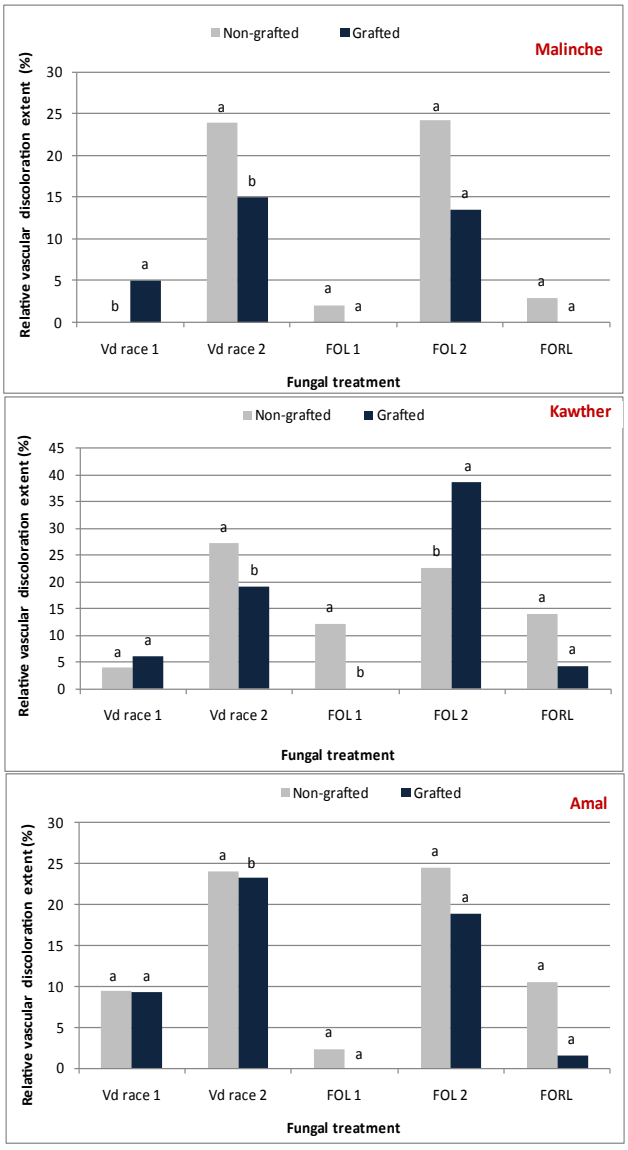

Figure 1: Relative vascular discoloration extent noted on Maxifort-grafted and non-grafted tomato cvs. Malinche, Kawthar and Amal plants at 60 days after artificial inoculation with fungal soilborne pathogens.

Note: Vd race 1: *Verticillium dahliae* race 1; Vd race 2: *Verticillium dahliae* race 2; FOL 1: *Fusarium oxysporum* f.sp. *lycopersici* race 1; FOL 2: *Fusarium oxysporum* f.sp. *lycopersici*; FORL: *Fusarium oxysporum* f.sp. radicis-*lycopersici*. For each pathogen, bars sharing the same letter are not significantly different according to Duncan test ($p \leq 0.05$).

The relative vascular discoloration extent which is the percentage of stem height exhibiting vascular discoloration is calculated as follow: (Vascular discoloration extent/plant height)*100.

LSD (Tomato cultivars × Grafting treatment × Fungal treatment)=6.34% at ($p \leq 0.05$).

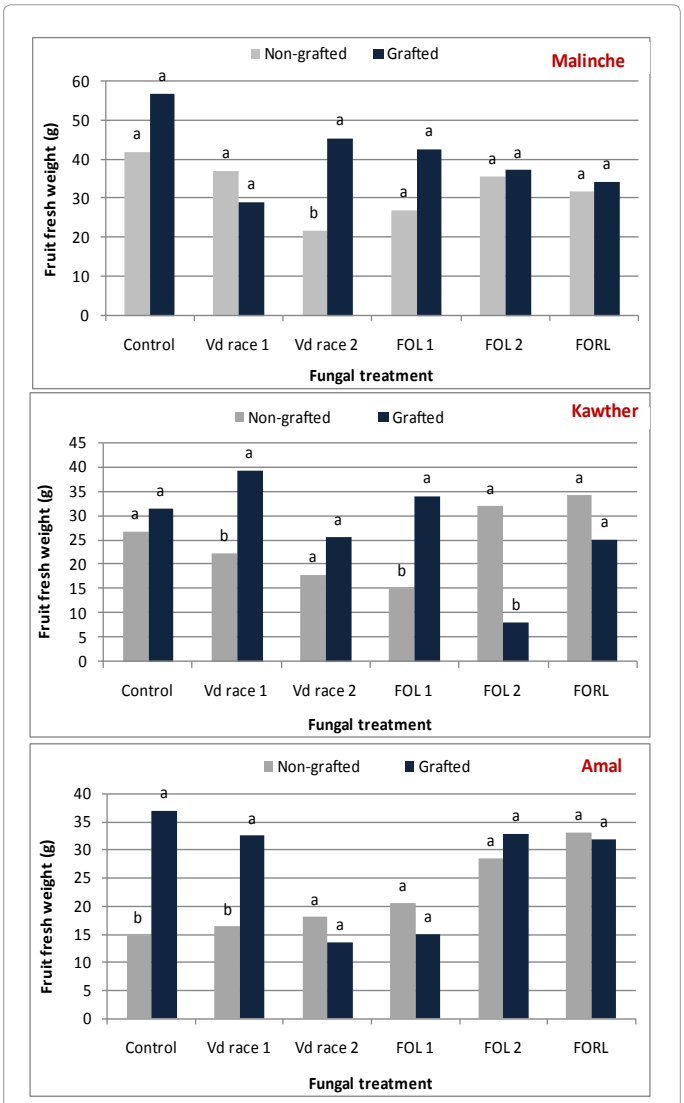

Figure 2: Fruit fresh weight noted on Maxifort-grafted and non-grafted tomato cvs. Malinche, Kawthar and Amal plants at 60 days after inoculation with fungal soilborne pathogens.

Note: Vd race 1: *Verticillium dahliae* race 1; Vd race 2: *Verticillium dahliae* race 2; FOL 1: *Fusarium oxysporum* f.sp. *lycopersici* race 1; FOL 2: *Fusarium oxysporum* f.sp. *lycopersici*; FORL: *Fusarium oxysporum* f.sp. radicis-*lycopersici*. For each pathogen, bars sharing the same letter are not significantly different according to Duncan test ($p \leq 0.05$).

LSD (Tomato cultivars × Grafting treatment × Fungal treatment)=13.65 g at ($p \leq 0.05$).

on FORL- and Vd race 2-inoculated plants while the lowest weight was recorded on Vd race 1-inoculated plants. For all tomato cultivars and fungal treatments combined, grafted plants showed statistically increased root weight, by 18%, over non-grafted ones.

Stem fresh weight: At 60 DPI, stem fresh weight noted on tomato plants was significantly (at $p \leq 0.05$) affected by fungal treatments tested and grafting; significant interactions were also noted between tested factors (fungal treatment, tomato cultivars, and grafting). Data presented in Table 2 showed that stem fresh weight was significantly similar or increased on Maxifort-grafted plants compared to non-grafted ones. Grafting cv. Malinche on cv. Maxifort resulted in an increase of stem weight by 41, 33, 44 and 36% recorded respectively on non-inoculated control, Vd race 2-, FOL race 2- and FORL-inoculated plants as compared to non-grafted cv. Malinche plants. On grafted cv. Kawthar plants, stem weight was improved by 35, 27, 23 and 23% respectively on FOL race 1-, FOL race 2-, FORL- and non-inoculated control, relative to non-grafted plants. Grafting cv. Amal plants has enhanced stem weight on non-inoculated plants and those inoculated with Vd races 1 and 2, FOL race 1, FOL race 2 and FORL by 47, 38, 43, 50, 20 and 23%, respectively, as compared to non-grafted ones. For pooled data of all fungal treatments, stem fresh weight was statistically higher on grafted cvs. Malinche, Kawthar and Amal plants. When tomato cultivars and grafting treatments were pooled, the lowest stem weights were noted on FOL race 1- and Vd race 2- inoculated plants. For all tomato cultivars and fungal treatments combined, grafted plants showed significant increase in stem weight, by 30%, compared to non-grafted ones.

Effect on fruit yield: Fruit yield, noted at 60 DPI, was significantly (at $p \leq 0.05$) affected by tomato cultivar used, fungal treatments tested and grafting; significant interactions (at $p \leq 0.05$) were noted between these factors, except for cultivar × grafting interaction. As presented in Figure 2, fruit yield noted on Maxifort-grafted and Vd race 1-inoculated plants was significantly increased by 44 and 50%, respectively, for cvs. Kawthar and Amal and was statistically comparable for cv. Malinche, when compared to inoculated and non-grafted scions. While fruit fresh weight noted on Vd race 2-inoculated cvs. Kawthar and Amal plants was statistically comparable for grafted and non-grafted plants, that noted on grafted cv. Malinche plants was 52% higher than that recorded on inoculated scion plants. As illustrated in Figure 2, grafting cv. Kawthar plants on the rootstock cv. Maxifort has increased tomato yield by 56% on FOL race 1-inoculated plants, and decreased it by 75% on FOL race 2-inoculated plants, as compared to non-grafted plants. Statistically similar fruit yield was recorded for grafted and non-grafted cvs. Malinche and Amal plants inoculated with FOL races 1 or 2. For all tomato cultivars, fresh fruit weight noted on FORL-inoculated plants was statistically similar for grafted and non-grafted plants. When grafted on cv. Maxifort, non-inoculated control cv. Amal plants showed higher tomato fruit yield, by 60%, than that noted on

Cultivar/Grafting/Treatment	Malinche		Kawther		Amal		Means per fungal treatment[x]
	Non-grafted	Grafted	Non-grafted	Grafted	Non-grafted	Grafted	
Plant height (cm)							
Control	55,57 ^{a*}	59,14 ^{a}	59.57 ^{a}	51.14 ^{b}	47.43 ^{b}	60.43 ^{a}	55.55 ^{B}
Vd race 1	57.00 ^{a}	47.57 ^{b}	55.43 ^{a}	50.00 ^{b}	38.14 ^{b}	48.57 ^{a}	49.45 ^{C}
Vd race 2	48.71 ^{a}	49.71 ^{a}	48.86 ^{a}	47.00 ^{a}	34.29 ^{b}	49.57 ^{a}	46.36 ^{C}
FOL 1	50.43 ^{a}	52.86 ^{a}	43.00 ^{a}	60.00 ^{a}	40.14 ^{b}	50.86 ^{a}	49.55 ^{C}
FOL 2	65.57 ^{a}	56.00 ^{b}	69.71 ^{a}	57.50 ^{b}	61.50 ^{a}	52.86 ^{a}	60.52 ^{A}
FORL	64.38 ^{a}	59.71 ^{a}	62.71 ^{a}	52.29 ^{a}	58.57 ^{a}	62.57 ^{a}	60.04 ^{A}
Means per cultivar per grafting treatment[y]	56.94 ^{A}	54.17 ^{A}	56.55 ^{A}	52.99 ^{A}	46.68 ^{B}	54.14 ^{A}	--
Root fresh weight (g)							
Control	9.57 ^{a}	4.86 ^{b}	5.86 ^{b}	9.29 ^{a}	4.29 ^{b}	6.71 ^{a}	6.76 ^{BC}
Vd race 1	4.43 ^{a}	6.29 ^{a}	6.71 ^{a}	5.57 ^{a}	4.43 ^{a}	6.86 ^{a}	5.71 ^{C}
Vd race 2	8.43 ^{a}	10.43 ^{a}	6.57 ^{a}	6.71 ^{a}	4.57 ^{b}	11.14 ^{a}	7.98 ^{AB}
FOL 1	5.29 ^{a}	5.43 ^{a}	5.43 ^{b}	9.50 ^{a}	5.86 ^{b}	9.43 ^{a}	6.82 ^{BC}
FOL 2	8.57 ^{a}	4.29 ^{b}	6.00 ^{b}	9.50 ^{a}	6.21 ^{a}	6.00 ^{a}	6.76 ^{BC}
FORL	8.88 ^{a}	9.14 ^{a}	7.00 ^{a}	9.43 ^{a}	6.29 ^{b}	8.43 ^{a}	8.19 ^{A}
Means per cultivar per grafting treatment[y]	7.53 ^{ABC}	6.74 ^{BC}	6.26 ^{CD}	8.33 ^{A}	5.27 ^{D}	8.10 ^{AB}	--
Stem fresh weight (g)							
Control	38.29 ^{b}	64.71 ^{a}	41.29 ^{b}	55.29 ^{a}	32.29 ^{b}	60.86 ^{a}	48.79 ^{A}
Vd race 1	62.29 ^{a}	52.43 ^{a}	44.14 ^{a}	49.43 ^{a}	34.43 ^{b}	55.14 ^{a}	49.64 ^{A}
Vd race 2	29.57 ^{b}	43.86 ^{a}	44.14 ^{a}	48.57 ^{a}	35.00 ^{b}	61.43 ^{a}	43.76 ^{B}
FOL 1	28.86 ^{a}	57.14 ^{a}	32.71 ^{b}	50.50 ^{a}	28.57 ^{b}	57.00 ^{a}	42.46 ^{B}
FOL 2	35.43 ^{b}	63.00 ^{a}	45.43 ^{b}	62.50 ^{a}	44.71 ^{a}	55.57 ^{a}	51.11 ^{A}
FORL	42.63 ^{b}	66.57 ^{a}	51.14 ^{b}	66.57 ^{a}	43.00 ^{b}	56.14 ^{a}	54.34 ^{A}
Means per cultivar per grafting treatment[y]	39.51 ^{BC}	57.95 ^{A}	43.14 ^{B}	55.48 ^{A}	36.33 ^{C}	57.69 ^{A}	--

Vd race 1: *Verticillium dahliae race 1;* Vd race 2: *Verticillium dahliae race 2;* FOL 1: *Fusarium oxysporum f. sp. lycopersici race 1;* FOL 2: *Fusarium oxysporum f. sp. lycopersici;* FORL: *Fusarium oxysporum f. sp. radicis-lycopersici;*

[*]For each tomato cultivar and within each line, values followed by the same letter are not significantly different according to Duncan test ($p \leq 0.05$).

[x]Means per fungal treatment for all cultivars and grafting treatments combined.

[y]Means per cultivar per grafting treatment for all fungal treatments combined.

LSD (Tomato cultivar×Grafting treatment×Fungal treatment)=8.56 cm ($p \leq 0.05$) (Plant height).

LSD (Tomato cultivar×Grafting treatment×Fungal treatment)=2.33 g ($p \leq 0.05$) (Root weight).

LSD (Tomato cultivar×Grafting treatment×Fungal treatment)=10.33 g ($p \leq 0.05$) (Stem weight).

Table 2: Effect of grafting three tomato scions onto the rootstock Maxifort on plant height, root and stem fresh weights noted at 60 days post inoculation under growth chamber conditions.

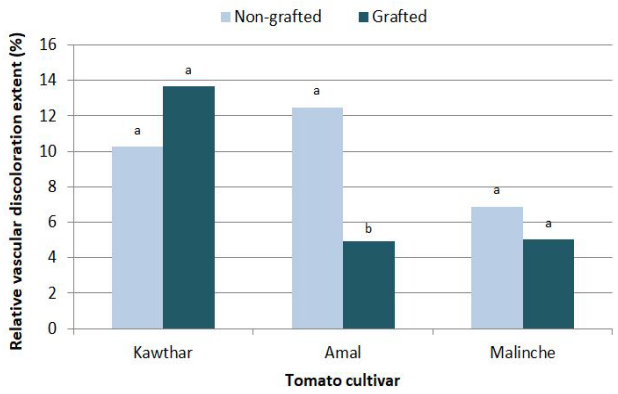

Figure 3: Relative vascular discoloration extent noted on Maxifort-grafted and non-grafted tomato cvs. Malinche, Kawthar and Amal plants at 150 days after plantation under natural greenhouse conditions.

Note: For each tomato cultivar, bars sharing the same letter are not significantly different according to Duncan test ($p \leq 0.05$). The relative vascular discoloration extent which is the percentage of stem height exhibiting vascular browning is calculated as follow: (vascular discoloration extent/plant height)*100.

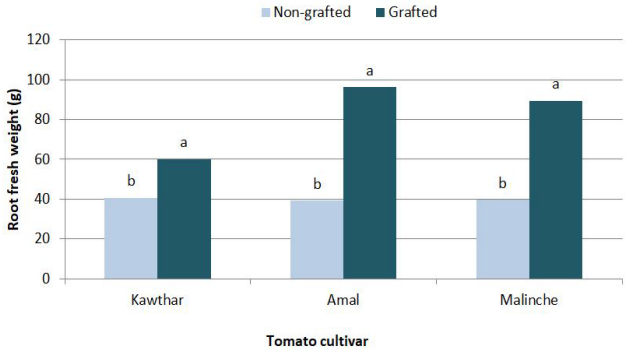

Figure 4: Root fresh weight noted on Maxifort-grafted and non-grafted tomato cvs. Malinche, Kawthar and Amal plants at 150 days after plantation under natural greenhouse conditions.

Note: For each tomato cultivar, bars sharing the same letter are not significantly different according to Duncan test ($p \leq 0.05$).

LSD (Tomato cultivars × Grafting treatment)=20.97 g at ($p \leq 0.05$).

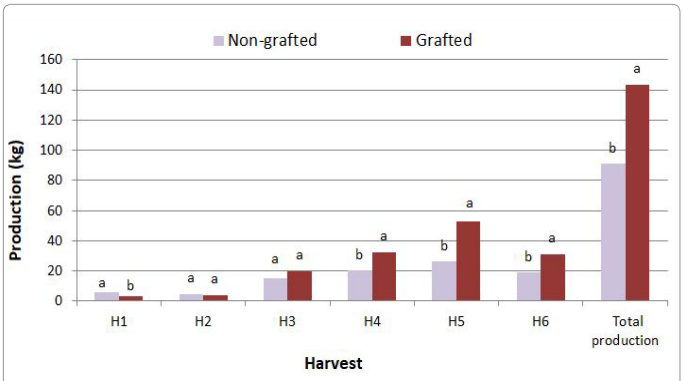

Figure 5: Tomato production noted on Maxifort-grafted and non-grafted tomato cvs. Malinche, Kawthar and Amal plants at the different harvests under natural greenhouse conditions.

Note: For each tomato harvest (H), bars sharing the same letter are not significantly different according to Duncan test ($p \leq 0.05$).

non-grafted ones. However, for the two other cultivars, cvs. Malinche and Kawthar, tomato yield noted on grafted and non-grafted plants was statistically comparable (at $p \leq 0.05$). Combined data of all fungal and grafting treatments tested showed that the highest fruit yield was recorded on cv. Malinche, which was 30 and 34% higher than that recorded on cvs. Kawthar and Amal, respectively. For all cultivars and grafting treatments combined, fruit yield was highest on non-inoculated control plants followed by that of FORL-inoculated plants, while Vd race 2-inoculated plants yielded the lowest fruit weight. For all cultivars combined and fungal treatments pooled, grafted plants yielded significantly higher fruit weight, by 17%, than non-grafted ones.

Effect of grafting under natural conditions (greenhouse trial)

Effect on disease severity: Under natural greenhouse conditions, disease severity, estimated via the RVDE, noted on tomato plants was low and did not exceed 14% (Figure 3). At 150 DPP, this severity varied significantly (at $p \leq 0.05$) depending on the tested tomato cultivars, where the least RVDE value, of about 6%, was recorded on cv. Malinche plants compared to ~ 9 and 12% noted on cvs. Amal and Kawthar, respectively. On cvs. Kawthar and Malinche, disease severity was statistically comparable on grafted and non-grafted plants. However, grafting of cv. Amal plants has significantly reduced, by 61%, the RVDE as compared to non-grafted ones (Figure 3).

Effect on growth parameters: Under natural conditions, all tomato cvs. Kawthar, Amal and Malinche plants, grafted or not, showed significantly similar plant height, noted at 150 DPP. Root fresh weight varied significantly (at $p \leq 0.05$) depending on tomato cultivars tested, grafting treatments and their interaction. As illustrated in Figure 4, root fresh weight noted on Maxifort-grafted cvs. Kawthar, Amal and Malinche plants was significantly enhanced by 32, 59 and 55%, respectively, relative to non-grafted ones. For pooled data of grafted and non-grafted plants, the root fresh weight noted on cvs. Amal and Malinche was significantly higher than that of cv. Kawthar plants. For all tomato cultivars combined, root fresh weight recorded on grafted plants was significantly improved, by 51%, as compared to non-grafted ones.

Effect on tomato yield: Tomato yield varied depending on the grafting treatment. As illustrated in Figure 5, during the first three harvests, tomato yield noted on Maxifort-grafted plants was significantly lower or comparable to that recorded on non-grafted ones. However, yield was higher in the Maxifort-grafted treatment as compared with the non-grafted control at the fourth harvest and remained significantly elevated through the last three harvests. By the end of the season, plants grafted onto Maxifort rootstock had produced 63% higher total yield than the non-grafted control (Figure 5).

Discussion

In Tunisia, farms devoted to protected tomato cultivation are generally small and the successive cropping of tomato in the same fields has contributed to a continual increase in the populations of various pathogens in the soil. The resultant inoculum upsurge from soil borne pathogens have led to increased disease pressure and consequently decreased the performance of commonly used resistant tomato cultivars. In such stressed conditions, grafting is used to reduce susceptibility against root rots and wilts, and to increase yield [12,20,21]. In fact, resistant hybrids with multiple resistance to several tomato pathogens such as *Verticillium*, *Fusarium* and *Pyrenochaeta lycopersici*, have been available for a long time [22]. Furthermore, grafting tomato is an increasingly adopted technique as it increased plant vigor and crop yield, even in the absence of disease pressure [16,23]. In the present study, we evaluated the ability of the commercial rootstock, cv. Maxifort, in reducing the severity of main tomato soil

borne diseases encountered in Tunisia and in increasing plant vigor and crop yield when three tomato cultivars, cvs. Kawthar, Amal and Malinche were used as scions.

Effect of grafting on disease severity

Under artificial inoculation conditions, our results showed that the tomato rootstock, Maxifort, conferred high level of resistance to FOL race 1 resulting in complete suppression of its relative growth in the tomato vascular tissue which ranged from 2.14 to 12.14%. These results are not surprising since the rootstock as well as all three tomato cultivars have resistance to FOL race 1. Similar results were mentioned by Rivard and Louws [16] who found that Maxifort offered complete control of Fusarium wilt when Heirloom tomatoes were used as scions. However, on FORL-inoculated plants, the RVDE noted has also been lowered to almost nil on all Maxifort-grafted plants showing that this rootstock have conferred resistance to the susceptible cultivars tested against this pathogen. It should be highlighted that, in the current study, disease severity noted on plants inoculated with FOL race 2, ranging from 22.14 to 24.57%, was significantly increased, by 41%, when cv. Kawthar plants were Maxifort-grafted. On the contrary, grafting cvs. Malinche and Amal led to reduced disease severity by 43 and 23%, respectively, even statistically insignificant. In fact, as mentioned by Gilardi et al. [24] the tomato rootstock Maxifort showed resistance to FORL and FOL race 1 while for FOL race 2 resistance or partial resistance was confirmed. Thus, from these ongoing results, it seems that cv. Maxifort as well as the tomato cultivars showed partial resistance to the tested FOL race 2 isolate tested. In addition, the combination Kawthar-Maxifort seems to be more susceptible to FOL race 2. In fact, the rootstock effect varies greatly depending on the scion cultivar used [7]. In the same sense, when evaluating the resistance of selected tomato rootstocks to FORL, Hibar et al. [6] found that the rootstock Beaufort F_1 was the best genotype capable of significantly improving the productivity and fruit quality of tomatoes cv. Durintha F_1; whereas, the rootstock He-Man F_1, seemed to be more suitable for tomato cv. Bochra F_1. Vitale et al. [25] demonstrates also that scion-rootstock combinations significantly influence tomato sensitivity to FORL, as assessed by comparative disease symptoms and plant growth response evaluation. Guan et al. [26] found also that the inconsistency in improved resistance and better yield with grafted plants might be attributed to differences in rootstock-scion combinations and growing conditions. Even though resistance to *V. dahliae* race 2 in tomato has not yet been recognized [27], the two races, Vd races 1 and 2, were included in this study as both are spread in most tomato producing regions of Tunisia. As opposed to race 1, none of the grafted or not plants showed high tolerance to race 2 of the pathogen. These results are in concordance with those of Giraldi et al. [24] and Paplomatas et al. [27] who mentioned that Maxifort harbor resistance to only *V. dahliae* race 1. Paplomatas et al. [27] showed also that none of the tested rootstocks exhibited high tolerance to Vd race 2, but that there was some variation in susceptibility among them. Similarly Giotis et al. [18] reported that grafting of tomato cultivars onto resistant rootstocks was shown to be an effective strategy to control the most important soil borne fungal diseases (*P. lycopersici* and *Verticillium* spp.) and root knot nematodes in soil based, protected tomato production systems. In the current investigation, disease severity, estimated via the relative vascular discoloration extent, was significantly reduced by 37, 30 and 2% when cvs. Malinche, Kawthar and Amal plants were respectively Maxifort-grafted and Vd race 2-inoculated. Interestingly, it seems that, when cv. Malinche was used as scion, the rootstock cv. Maxifort harbored the pathogen at a significant lower frequency than the other grafted tomato scions. These data suggest that the combination Maxifort-Malinche was effective in delaying the onset of disease development and slow down the rate of plant colonization compared with the non-grafted controls. Under natural greenhouse conditions, vascular wilt diseases noted on tomato plants were not severe since the highest RVDE did not exceed 14%. The least relative vascular discoloration extent, of about 6%, was recorded on cv. Malinche plants. Furthermore, isolation made from wilted plants on PDA showed that *F. oxysporum* was consistently isolated from stem segments. Verticillium wilt symptoms were not observed and *V. dahliae* was not isolated from infected root or stem tissue. This could be explained by the fact that higher soil populations may be required to cause damage on tomato [28] or that only populations of *V. dahliae* race 1 were present in the chosen greenhouse field.

Effect of grafting on plant growth and production

At 60 DPI under controlled conditions, the response of the tested plant material was different as assessed by plant growth evaluation. Overall, for all pathogens combined, grafting has improved growth parameters as compared to non-grafted controls. For instance, plant height, root and stem fresh weights noted on grafted cv. Amal plants were improved for at least four out of the six tested pathogens. For cv. Kawthar, root fresh weight was significantly enhanced for plants grafted and inoculated with FOL race 1 and FOL race 2, while an improvement of stem fresh weight was recorded for FOL race 1-, FOL race 2- and FORL-inoculated plants. For cv. Malinche, only stem fresh weight was enhanced on Vd race 2-, FOL race 2- and FORL-inoculated plants. Interestingly, fruit fresh weight was significantly improved by 52% on grafted and Vd race 2-inoculated cv. Malinche plants while increments of 44 and 50% were respectively noted on grafted cvs. kawthar and Amal plants inoculated with Vd race 1, relative to non-grafted controls. Under natural conditions, root fresh weight noted on Maxifort-grafted cvs. Kawthar, Amal and Malinche plants was significantly enhanced by 32, 59 and 55%, respectively, relative to non-grafted ones. Plants grafted onto Maxifort rootstock had produced 63% higher total yield than the non-grafted control. These results are consistent with many other studies reporting the enormous benefits from using grafted seedlings. For instance, Lee et al. [9] mentioned that these benefits include income increase by high yield and offseason growing, extension of the harvest period, efficient maintenance of popular cultivars against diseases. In this sens, Rivard and Louws [16] and Rivard et al. [17] found that grafting heirloom tomato onto vigorous rootstocks such as Maxifort effectively controlled Fusarium wilt and also improved plant vigor and yield. Furthermore, in Morroco, Besri [10] found that the total production of grafted tomato is significantly higher than non-grafted control. Buller et al. [29] reported also that grafting tomato, Cherokee Purple onto Maxifort rootstock had increased stem diameter and plant height throughout the growing season and cumulatively as compared with non-grafted plants. However, these researchers did not report any increases in yield, fruit size, or fruit quality as a result of grafting in their study. Similarly Giotis et al. [18] reported that grafting significantly increased fruit yields by between 27 and 156% in the standard fruit size tomato cultivars (Star fighter, Espero, and 72-224). Rivard et al. [17] showed also that tomato fruit yield was higher when resistant rootstocks were utilized, and that grafting was effective at maintaining crop productivity in soils infested with *S. rolfsii* and *M. incognita*. They explained that rootstocks may provide elevated yield through added vigor and plant growth.

Conclusion

From the present study, it is clear that the combination tomato scion-Maxifort rootstock affect the resistance of grafted plants to one

or more soil borne pathogens as well as their growth and production. Grafting with Maxifort under little soil borne disease pressure under natural condition would be advantageous as crop production could be significantly increased. In converse, when the soil borne populations are important (artificial inoculation), grafting onto Maxifort could decrease disease severity induced by the aggressive races of *V. dahliae* and *F. oxysporum* f. sp. *lycopersici* if a suitable scion is used. Consequently, grafting could be implemented in an integrated disease management with other soil disinfection methods such as solarization, biofumigation, composts, for reducing soil borne populations in the soil. In addition, grafting can be suitable for both conventional and organic tomato production. In fields with endemic populations of *V. dahliae* race 2, the combination Malinche- Maxifort seems to be most suitable both for disease control and crop promotion. On the contrary, the use of cv. Kawthar as scion is not preferable when the race 2 of *F. oxysporum* f. sp. *lycopersici* is widespread. The rootstock Maxifort could confer resistance to the three tested tomato cultivars against FORL. It is also to note that the important factor limiting the extensive adoption of grafted plants in Tunisia is the high cost of grafted seedlings. However, it is important to mention that in the present study, double-leader grafted plants were used, which could explain the increment in tomato production and which may substantially reduce the cost of the plant material. In addition, grafting an indeterminate tomato scion onto a vigorous rootstock makes it possible to extend the harvest period when environmental conditions are adequate. Further researches are needed to evaluate the appropriate combinations of new selected rootstocks with cultivars of commercial interest, so as to ensure not only suppression of soil borne diseases but also acceptable yields.

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