

## Screening of tomato hybrids for resistance to Fusarium wilt

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**Abstract:** Few commercial genotypes of tomato are resistant to *Fusarium wilt* (*Fusarium oxysporum* f. sp. *lycopersici* [FOL]), especially to race 3. We developed five intraspecific hybrids of *Solanum lycopersicum* (FOX1 to FOX5) and assessed them for agronomic traits and resistance to FOL races 1, 2, 3, and a mixture of these races. FOX1 and FOX4 were resistant to all races and the race mixture but did not have the desired agronomic traits. Next, FOX1 and FOX4 were assessed as rootstocks of the cherry tomato Sweet Heaven. The grafting of Sweet Heaven on FOX4 did not reduce growth, gas exchange, yield, or fruit quality. When FOX1 was used as a rootstock, the fruits exhibited a reduction in firmness, fruit pulp pH, and total soluble solids by 14.3%, 1.2%, and 6.75%, respectively. Thus, the hybrid FOX4 can be used as a rootstock for Sweet Heaven to manage *Fusarium wilt*.

**Keywords:** *Cherry tomatoes*, *Fusarium oxysporum* f. sp. *lycopersici*, *grafting*.

### INTRODUCTION

*Fusarium wilt* caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen (FOL) is one of the most significant diseases affecting tomato (*Solanum lycopersicum* L.) worldwide, especially in the acidic sandy soils of tropical regions (Srinivas et al. 2019). The pathogen colonizes the xylem vessels, leading to leaf yellowing, wilting, and death of plants. The fungus has three physiological races (1, 2, and 3) based on their specific pathogenicity on tester plants and carry dominant race-specific resistance genes (Biju et al. 2017).

The management of FOL using chemical fungicides is limited by the low efficiency of the products and their health and environmental risks. The most sustainable strategy for *Fusarium wilt* management in infested areas involves the use of resistant cultivars. Globally, FOL races 1 and 2 can be managed by resistant cultivars carrying the resistance genes *I-1* and *I-2* (Ma et al. 2013). In Brazil, few cultivars harboring the gene for resistance to race 3 are available for growers, and areas infested by the pathogen have already been observed in the main tomato-producing regions (Reis et al. 2005, Barboza et al. 2013, Gonçalves et al. 2021).

Genes *I-3* and *I-7*, identified in accessions of *Solanum pennellii*, confer resistance to race 3 (McGrath et al. 1987). Thus, cultivars carrying one of these genes may be useful for managing the FOL race 3. The germplasm bank of the Tomato Genetic Resource Center (TGRC, University of California, Davis, CA, USA) stocks five accessions of *S. lycopersicum* carrying *I-3*. We hypothesized



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that these accessions have the potential to develop hybrids resistant to Fusarium wilt.

The hybrids of tomatoes resistant to soil-borne pathogens can be grown for fruit production. However, these hybrids must be productive in terms of both fruit yield and quality. Alternatively, resistant genotypes with undesirable traits can be used as rootstock.

The production of mini tomatoes, especially cherry tomatoes, has recently increased in Brazil. Nevertheless, this type of tomato has been grown in a protected environment with the use of a sterile substrate, owing to the lack of genotypes resistant to soilborne pathogens, including FOL. Rootstocks resistant to Fusarium wilt, for example, may make it possible to grow mini-tomatoes in open fields, thereby reducing production costs.

Grafting may increase the yield and enhance the fruit quality of tomatoes (Kyriacou et al. 2017). In the present study, tomato hybrids were developed by crossing lines carrying *I-3* with a commercial cultivar carrying *I-1* and *I-2*. These hybrids were assessed for resistance to FOL races 1, 2, and 3 and were studied as rootstocks for cherry tomatoes.

## MATERIAL AND METHODS

### Plant material and fungal isolates

Lines LA4025, LA4026, LA4065, LA4066, and LA4067 of *S. lycopersicum*, carrying *I-3* and donated by the TGRC (Davis, CA, USA), were used as male genitors for obtaining tomato hybrids. The female genitor was the commercial tomato cultivar Santa Clara<sup>®</sup>, which is resistant to the main soil-borne pathogens in Brazil, including *Verticillium dahliae* and FOL races 1 and 2, and tolerant to root-knot nematodes (*Meloidogyne* spp.). The isolates FUS-027 (race 1), FUS-023 (race 2), and FUS-374 (race 3) from FOL were provided by Embrapa Hortaliças, Brasília, Brazil (Gonçalves et al. 2021).

### Development of hybrids

Lines LA4025, LA4026, LA4065, LA4066, and LA4067 (male genitors) and Santa Clara<sup>®</sup> (female genitor) were raised for 28 d in a commercial substratum (Carolina Soil<sup>®</sup>, Santa Cruz do Sul, Brazil) in Styrofoam trays. Seven seedlings of each male parent and 16 seedlings of the female parent were cultivated in 10 L plastic pots filled with a mixture of soil, sand, and cattle manure (3:1:1; v:v:v) in a greenhouse.

The crossings were performed approximately 2 d before the opening of the flowers of the second cluster. The anthers of the female genitor were removed using tweezers, followed by the deposition of pollen from the male genitor. After artificial pollination, the flowers were wrapped in parchment paper for 48 h to avoid possible pollen contamination. This hybridization procedure was performed every 2 d until the opening of the eighth floral cluster.

The fruits from the hybridization were harvested when they reached an intense red color, followed by collection and storage of the seeds in a cold chamber (3-4 °C). The resulting hybrids were coded as FOX1, FOX2, FOX3, FOX4, and FOX5, respectively. For plant inoculation, the fungus was grown in potato dextrose medium for 14 d at 25 °C and shaking at 150 rpm. The spore suspension was calibrated to a concentration of  $1 \times 10^6$  spores mL<sup>-1</sup>.

### Selection of resistant hybrids

The hybrids FOX1, FOX2, FOX3, FOX4, and FOX5 and the cultivar Ponderosa, susceptible to the three races of FOL, were raised for 21 and 28 d in organo-mineral substrates in Styrofoam trays. The roots of the seedlings were washed in tap water, and a 2 cm cut was made in the apical portion of the roots to facilitate fungal infection. The roots of the seedlings were dipped in a spore suspension ( $1 \times 10^6$  spores mL<sup>-1</sup>) of the respective fungal races and immediately transplanted into pots under greenhouse conditions ( $25 \pm 2$  °C). The pots contained a mixture of soil and sand (1:1, v:v), previously autoclaved for 60 min at a pressure of 1.0 kgf cm<sup>-2</sup> and temperature of 120 °C. The cultivar Ponderosa and hybrids FOX1, FOX2, FOX3, FOX4, and FOX5 were inoculated with each of the three races of FOL, a mixture of the three races (containing  $1 \times 10^6$  spores mL<sup>-1</sup> of each race), and a negative control without the fungus. The treatments were arranged in a completely randomized design with six replicates.

Three weeks after inoculation, disease severity was assessed using a 1 to 5 rating scale (Santos 1997) as follows: 1 = plants without symptoms, 2 = plants without symptoms of wilt or leaf yellowing but with vascular browning, 3 = plants

with symptoms of vascular browning and wilt but without leaf yellowing, 4 = plants with severe wilting associated with foliar necrosis and chlorosis, and 5 = dead plants. The categorization of the resistance levels of the hybrids was based on the average severity scores, as follows: immune (I) = 1, highly resistant (HR) = 1.01–2.00, moderately resistant (MR) = 2.01–3.00, susceptible (S) = 3.01–4.00, and highly susceptible (HS) = 4.01–5.00 (Reis et al. 2004).

### Agronomic traits of resistant hybrids

The resistant hybrids were grown for 156 d (July to December 2017) in a greenhouse in Viçosa (lat 20° 45' 29" S, long 42° 50' 46" W, alt 685 m asl), state of Minas Gerais, Brazil under a regime of fertilization and irrigation via surface drip irrigation. The soil was free of FOL infestation. Plants were tutored with two stems of up to 1.80 m. The hybrids were distributed in five randomized blocks, with six plants per plot.

Gas exchange in the leaves was evaluated at 30, 60, and 90 d after transplanting (DAT) with an open system infrared gas analyzer (Model LI-6400XT, LI-COR, Lincoln, NE, USA). We measured CO<sub>2</sub> liquid assimilation (A, μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance (gs, mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), CO<sub>2</sub> concentration in the intercellular spaces (Ci, CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), transpiration (E, mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), water use efficiency (WUE = A/E), water use intrinsic efficiency (WUEi = A/g<sub>s</sub>), and the ratio of intercellular to ambient CO<sub>2</sub> concentrations (Ci/Ca). The third expanded leaves from top to bottom were assessed from 08:00 to 11:00 h, under irradiance of 1000 μmol photons m<sup>-2</sup>s<sup>-1</sup>, standard CO<sub>2</sub> conditions of 400 ppm, and a temperature of 25 °C. Data were obtained from five assessments of a randomly chosen plant within a useful plot.

Plant height and number of leaves (>10 cm) were assessed from 7 to 77 d after transplantation. The fruit setting rate was recorded on the second and fifth clusters of the main stem based on the ratio between the number of flowers and fruits per cluster. The fruit harvest was performed weekly from 87 to 156 DAT, for a total of 11 harvests. At each harvest, we assessed the number of fruits per cluster and per plant, average mass of fruits, mass of fruits per plant, color and diameter of fruits, and yield.

Fruit color was evaluated using a digital colorimeter (model CR-10; Konica Minolta, Tokyo, Japan) as L\*, a\*, b\*, chroma values, and hue angle. The longitudinal and transverse diameters of the fruits were recorded using a 150 mm digital caliper (model 205509; Jomarca, Guarulhos, Brazil). Total fruit acidity (pH), total soluble solids (TSS), total titratable acids (TTA, in % citric acid), firmness, and flavor (TSS/TTA) were measured every 15 d using 250 g of fruit from each plot. The pH, TSS, and TTA were analyzed according to Pregolato and Pregolato (1985). The firmness was recorded using a digital dynamometer (model DD-200; Instrutherm, São Paulo, Brazil).

### Grafting of cherry tomato onto FOL-resistant hybrids

A hybrid of the cherry tomato Sweet Heaven (SH) (Sakata Seed Sudamerica, Bragança Paulista, Brazil) was used as a scion. This genotype is resistant to *Stemphylium solani*, tomato mosaic virus (ToMV), and FOL race 1. The FOX1 and FOX4 hybrids, resistant to the three FOL races, were used as rootstocks. The treatments were a combination of rootstocks and scions (FOX1/SH and FOX4/SH), self-grafting of Sweet Heaven (SH/SH), and non-grafted Sweet Heaven (SH).

Thirty-day-old plants were grafted by whip grafting and kept in a growth chamber for 4 d in relative humidity > 90% and darkness. The plants were transferred to a greenhouse and were acclimatized over a period of 15 d during which they were kept under a regime of increasing exposition to light.

### Agronomic traits of the grafted genotypes

The grafted plants were grown in a greenhouse free of FOL, located in Viçosa (lat 20° 45' 29" S, long 42° 50' 46" W, alt 685 m asl), state of Minas Gerais, Brazil. Before transplanting the seedlings, 500 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, 250 kg ha<sup>-1</sup> K<sub>2</sub>O, and 200 kg ha<sup>-1</sup> N were applied to the soil. Micronutrients were not applied because of the high concentrations of these elements in the soil.

The plants were grown with a spacing of 1.0 × 0.5 m and tutored with two stems, up to 1.8 m height. The four treatments were distributed in five randomized blocks. Each plot contained six plants, with four central plants serving as the samples. The plants were grown for 156 d from July to December 2017. The fruits were harvested weekly from 87 to 156 DAT, for a total of 11 harvests. Plant height, number of leaves, fruit setting rate, leaf gas exchange, number of

fruits per bunch and per plant, average mass of fruits, mass of fruits per plant, color and diameter of fruits, and yield were assessed as previously described. Data were subjected to analysis of variance ( $p < 0.05$ ), and the means were compared using Tukey's test ( $p < 0.05$ ) using SISVAR software version 5.6 (Ferreira 2011).

## RESULTS AND DISCUSSION

### Selection of resistant hybrids

The response of plants to parasitism by FOL races varied among the hybrids 21 d after fungal inoculation. Plant growth was reduced by FOL, especially by races 2 and 3, and the race mixture. The cultivar Ponderosa showed typical symptoms of wilt and leaf yellowing, indicating that the isolates were virulent. This cultivar was highly susceptible to race 3 and the race mixture and was susceptible to race 2 (Table 1). None of the genotypes were susceptible or highly susceptible to race 1 (Table 1).

The FOX2, FOX3, and FOX5 hybrids were highly resistant to race 1. FOX2 and FOX3 were susceptible to races 2 and 3 and the race mixture. FOX 5 was moderately resistant to the race mixture and was susceptible only to race 3 (Table 1). Therefore, FOX2, FOX3, and FOX5 are not recommended for the management of FOL races 2 and 3.

No symptoms of stunting, wilt, or leaf yellowing were observed in FOX1 and FOX4 plants inoculated with the individual or a mixture of FOL races. FOX1 is highly resistant to race 3, immune to races 1 and 2, and the mixture of races. FOX4 was highly resistant to races 1–3 and was immune to the mixture of races (Table 1).

Genetic control is the most economical and efficient method for managing *Fusarium* wilt in tomatoes (Reis et al. 2005). Domestication and breeding of tomatoes has enabled their adaptation to diverse geographical locations and high yields. However, genes that are resistant to a wide range of pathogens and pests are not present in many modern cultivars. In Brazil, for instance, few commercial cultivars are resistant to FOL race 3.

Therefore, the maintenance of germplasm banks is critical as they serve as sources of accessions and wild lines carrying resistance genes (Long et al. 2013). In the present study, we used five genotypes from the TGRC - one of the most important germplasm banks of tomatoes in the world—and developed hybrids FOX1 and FOX4, which are resistant to FOL races 1, 2, and 3.

Lines LA4065 and LA4025, carrying *I-3*, were used as male genitors to generate hybrids FOX1 and FOX4. Gene *I-3* is potentially responsible for the resistance of the hybrids to the fungus and encodes an S-receptor-like kinase protein, which recognizes the effectors produced by FOL and activates the biochemical mechanisms of plant defense (Catanzariti et al. 2015).

**Table 1.** Reaction of tomato hybrids (FOX1 to FOX5) and the cultivar Ponderosa inoculated with individual and a mixture of *Fusarium oxysporum* f. sp. *lycopersici* races

Isolate		Genotype					
		Ponderosa	FOX1	FOX2	FOX3	FOX4	FOX5
FUS-027 (race 1)	Mean	2.33	1.00	1.33	1.33	1.20	1.33
	Reaction	MR	I	HR	HR	HR	HR
FUS-023 (race 2)	Mean	4.00	1.00	2.66	2.66	1.20	1.16
	Reaction	S	I	S	S	HR	HR
FUS-374 (race 3)	Mean	4.66	1.33	3.83	3.50	1.60	3.50
	Reaction	HS	HR	S	S	HR	S
Mixture of races	Mean	4.33	1.00	2.50	3.33	1.00	2.16
	Reaction	HS	I	S	S	I	MR
Absolute control	Mean	1.00	1.00	1.00	1.00	1.00	1.00
	Reaction	-	-	-	-	-	-

I, Immune; HR, Highly resistant; MR, Moderately resistant; S, Susceptible; HS, Highly susceptible. Disease severity (Santos 1997): 1, plants without symptoms; 2, plants without wilt symptoms of wilt or leaf yellowing but with vascular browning; 3, plants with vascular browning symptoms and wilt but without leaf yellowing; 4, plants with severe wilting associated with foliar necrosis and chlorosis; 5, dead plants.

### Agronomic traits of resistant hybrids

The height of plants and the number of leaves of FOX1 and FOX 4 were similar at 77 d, with a maximum height of 150 cm and 40 leaves with a size greater than 10 cm. The gas exchange of FOX1 and FOX4 was similar, and no abnormalities were detected (data not shown). However, some agronomic traits of these hybrids were considered unsatisfactory, especially yield, fruit quality, and fruit setting rate.

The mean yield of hybrids was 66.1 t ha<sup>-1</sup>, a value far below 120 t ha<sup>-1</sup>, which is commonly reached in the commercial production of tomato in a protected environment (Silva et al. 2013). The fruit setting rate of the hybrids was low (approximately 60% for FOX1 and <50% for FOX4). The pH, TSS, TTA, and flavor of the fruits of FOX1 and FOX4 were similar to those observed in fresh market tomatoes commercialized in Brazil (Brasil 2018). However, the fruits of the hybrids were smaller and lighter than the commercial standard for fresh-market tomatoes (data not shown). Thus, FOX1 and FOX4 did not have the productive potential or fruit quality that is required by producers and consumers. Alternatively, these hybrids could be used as rootstocks for highly productive and FOL-susceptible cultivars.

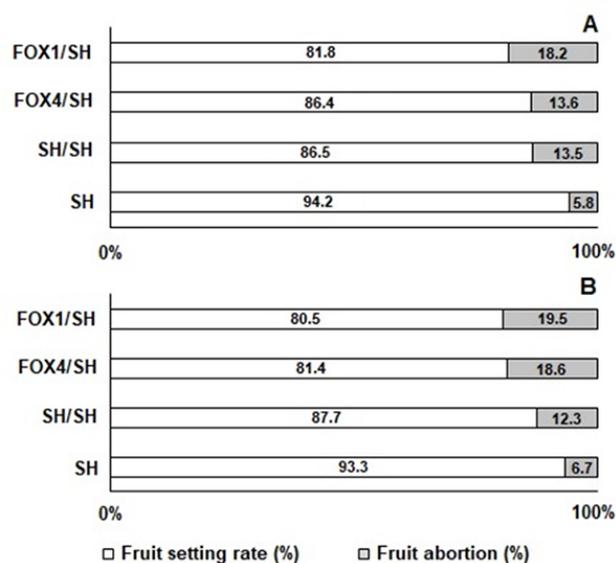
### Agronomic traits of grafted genotypes

Initial plant growth did not differ among the treatments, with final heights ranging from 140 cm to 160 cm. Growth of FOX4/SH plants was similar to that of non-grafted plants (SH) throughout the experiment. From 42 DAT, FOX1/SH plants grew less than SH plants did. The number of leaves was similar among all treatments, reaching approximately 40 (>10 cm) at 77 DAT. The stress caused by grafting can limit plant development, especially the disruption of vascular tissues during the technique (Kyriacou et al. 2017). When there is compatibility between the scion and rootstock, the re-establishment of vascular connections and carbon fixation during seedling acclimatization enables the plants to grow again. The type of grafting may influence eco-physiological aspects. Whip grafting, as performed in the present study, is widely used for tomatoes because it increases the CO<sub>2</sub> liquid assimilation rate and enhances water-use efficiency (Kumar et al. 2017, Zeist et al. 2017).

The fruit setting rate was higher than 95% in SH and varied from 80.5 to 87.7% in self-grafted plants (SH/SH) grafted onto FOX1 or FOX4 (Figure 1). The highest fruit abortion rate was observed in FOX1/SH and varied from 18.2 to 19.5%. In self-grafted plants, the fruit abortion rates varied from 12.5 to 13.5% (Figure 1). The leaf gas exchange was similar among all treatments (data not shown). The fruit setting rate is related to temperature, availability of water and nutrients, adequate hormonal levels for growth, and ovarian development (Zeist et al. 2017). The scion is mainly responsible for the yield and quality of the fruits. However, the vigor of the rootstock and compatibility between the scion and rootstock may influence the quantitative and qualitative aspects of fruit production (Krumbein and Schwarz 2013).

The fruits of Sweet Heaven in all treatments had oblong shapes (LD/TD > 1.00, Table 2). Thus, the use of FOX1 and FOX4 as rootstocks did not alter the shape of Sweet Heaven fruit. Fruits from self-grafted plants (SH/SH) had a greater longitudinal diameter than the other treatments and a greater transversal diameter than FOX4/SH (Table 2). Therefore, fruits from the self-grafted plants were heavier, although consumers preferred smaller cherry tomatoes.

The number of fruits per cluster and per plant was similar among treatments (Table 2). The combination FOX4/SH produced fewer fruits per plant than the self-grafted plants but did not differ from the non-grafted Sweet Heaven



**Figure 1.** Tomato fruit setting and abortion rates on the 3<sup>rd</sup> (A) and 5<sup>th</sup> (B) clusters of non-grafted Sweet Heaven (SH), self-grafted plants (SH/SH), or plants grafted onto the FOL-resistant hybrids FOX1 (FOX1/SH) and FOX4 (FOX4/SH). FOL, *Fusarium oxysporum* f. sp. *lycopersici*.

**Table 2.** Effect of grafting cherry tomato Sweet Heaven (SH) onto FOL-resistant hybrids FOX1 (FOX1/SH) and FOX4 (FOX4/SH), self-grafting (SH/SH) and absence of grafting (SH) on the longitudinal (LD, mm) and transverse diameter (TD, mm) of fruits, relationship between longitudinal and transverse diameter (LD/TD), number of fruits per plant (NFP), number of fruits per cluster (NFC), average mass of fruits (AMF, in g); mass of fruits per plant (MFP, in kg plant<sup>-1</sup>), and yield (Y, t ha<sup>-1</sup>)

Combination	LD	TD	LD/TD	NFP	NFC	AMF	MFP	Y
FOX1/SH	31.2 b	21.7 ab	1.44	477	31.8	8.2 b	3.9 ab	78.5 ab
FOX4/SH	30.3 b	20.8 b	1.45	444	29.6	7.5 b	3.4 b	67.3 b
SH/SH	32.8 a	22.4 a	1.47	482	32.1	9.9 a	4.7 a	94.8 a
SH	31.4 b	21.8 a	1.44	464	30.9	8.5 b	3.9 ab	78.9 ab
C.V.	2.2	2.3	1.2	9.9	9.9	8.2	14.3	14.3

Means within a column followed by different letters are significantly different according to Tukey's test ( $p < 0.05$ ). C.V. = coefficient of variation. FOL, *Fusarium oxysporum* f. sp. *lycopersici*

(Table 2). The yield of FOX1/SH was similar to that of non-grafted and self-grafted Sweet Heaven. These results indicate that FOX1 and FOX4 did not reduce the yield of Sweet Heaven.

Compared to non-grafted plants, the intensity of the red and yellow shades of FOX1/SH fruits was high, and the pH and TSS were low (data not shown). However, the fruits of Sweet Heaven grafted onto FOX4 did not show any alteration in color or quality compared to non-grafted plants (data not shown).

When evaluating the hypothesis that hybrids FOX1 and FOX4 could be used as rootstocks, we observed that the cherry tomato Sweet Heaven did not suffer alterations in growth, gas exchange, or quantitative and qualitative effects in fruits when grafted onto FOX4. The quality of Sweet Heaven fruits grafted onto FOX1 was slightly lower than expected, with a reduction in firmness, TSS, and pH. Such traits may limit the use of FOX1 as a rootstock for Sweet Heaven. Thus, grafting Sweet Heaven onto FOX4 may be an efficient strategy for maintaining crop productivity, even in areas infested with FOL. To increase the potential use of FOX1 and FOX4 among tomato growers, further studies should be conducted to evaluate the compatibility of these hybrids with other commercial cultivars and their host status to other soil-borne pathogens, including root-knot nematodes, *Verticillium* spp., and the *Ralstonia solanacearum* species complex.

FOX1 and FOX4 were resistant to *F. oxysporum* f. sp. *lycopersici* races 1, 2, and 3. However, they did not possess the productive potential or fruit quality required by producers and consumers. Grafting the cherry tomato Sweet Heaven onto FOX4 did not alter the growth, gas exchange, yield, or fruit quality. Therefore, hybrid FOX4 can be used as a rootstock for Sweet Heaven to manage Fusarium wilt disease.

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