Wild rootstock and mineral nutrients for the control of *Fusarium* spp in eggplant (*Solanum melongena* L.)

Portainjerto silvestre y nutrientes minerales para el control de *Fusarium* spp. en berenjena (*Solanum melongena* L.)

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Abstract

Vascular wilt caused by *Fusarium* spp. is a limiting disease for eggplant production in the Colombian Caribbean. Researchers evaluated nine repetitions of the wild rootstock *Solanum mammosum* L. grafted through the terminal splicing method with the commercial eggplant cultivar *Solanum melongena* L. Corpoica CO15 and compared these with controls, ungrafted *S. melongena* Corpoica CO15 and *S. mamosum*, under a completely randomized design, for determining these treatments resistance to wilting. In a second experiment, researchers evaluated three foliar nutrition alternatives: T1 = control (water spray), T2 = rich potassium source (K: 344 g L^{-1}); T3 = rich calcium source (Ca: 96 g L^{-1} of CaO); T4 = rich phosphorus source (P: 300 g L^{-1} of P2O5), under a randomized design with four repetitions. In both experiments, nine seedlings were planted per treatment in pots with 3.5 kg of silt loam soil and inoculated with *Fusarium* spp. FS02 strain in a mesh house. Inoculation was carried out on seedlings with 30 days of development (3-4 true leaves) through wounds at the base of the stem and roots, with 20 mL of suspension (5000 conidia mL^{-1}). Using wild *S. mammosum* rootstock and mineral nutrients through foliar application significantly reduced the incidence and severity of the disease.

Key words: Calcium; Phosphorus; Foliage disease index; Leaf nutrition; Potassium; Vascular wilting; Vegetable; vascular discoloration index

Resumen

La marchitez vascular causada por *Fusarium* spp. es una enfermedad limitante de la producción de berenjena en el Caribe colombiano. Se evaluó el portainjerto silvestre *Solanum mammosum* L., injertado por el método de empalme terminal, con el cultivar comercial de berenjena *Solanum melongena* L. Corpoica CO15, con nueve repeticiones, comparándolo con el desarrollo de los testigos *S. melongena* Corpoica CO15 y *S. mammosum* sin injertar, bajo un diseño completamente aleatorizado, determinándose la resistencia de estos tratamientos a la marchitez. En un segundo experimento se evaluaron tres alternativas de nutrición foliar T1 = testigo (aspersión de agua), T2 = fuente rica en potasio (K: 344 g L^{-1}); T3 = fuente rica en calcio (Ca: 96 g L^{-1} de CaO); T4 = fuente rica en fósforo (P: 300 g L^{-1} de P2O5), bajo un diseño completamente aleatorizado con cuatro repeticiones. En ambos experimentos se utilizaron nueve plántulas por unidad por tratamiento, sembradas en macetas con 3,5 kg de suelo franco limoso e inoculadas con *Fusarium* spp. cepa FS02, en casa de malla. La inoculación se realizó en plántulas de 30 días de desarrollo (de 3-4 hojas verdaderas), mediante heridas en la base del tallo y en las raíces de cada planta, con 20 mL de suspensión (5.000 conidias mL^{-1}). El uso del portainjerto silvestre *S. mammosum* y los nutrientes minerales mediante aplicación foliar, redujeron significativamente la incidencia y severidad de la enfermedad.

Palabras clave: Calcio; Fósforo; índice de la enfermedad en el follaje; índice de decoloración vascular; marchitez vascular; nutrición foliar; Potasio

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Introduction

In Colombia, the Caribbean region presents horticultural systems under a Family Agriculture scheme where eggplant (*Solanum melongena*) crops stand out (Martínez *et al*., 2020). The department of Córdoba is the fourth largest eggplant producer, with 2 991 tons year^{-1} (Agronet, 2022), where this crop is cultivated by small producers in plots between 1 000 and 2 500 square meters. Phytosanitary issues are a major limitation to production, especially vascular wilt MV caused by *Fusarium* spp. (Araméndiz *et al*., 2008; Santema, 2015). This fungus attacks several species of the Solanacea family, as reported by Cardona (2013) and Valenzuela (2014), and can cause losses between 70 % and 90 % of production (Cardona, 2013; Valenzuela, 2014).

Eggplant producers in the Colombian Caribbean cultivate genotypes susceptible to MV and resort to agrochemicals, which implies increased production expenses and a high environmental cost (Tamayo and Jaramillo, 2013; Villa *et al*., 2015). Alternatives for disease management must be preventive and environmentally friendly, such as using wild rootstock species of the *Solanum* genus and resistance inducers (Castellanos *et al*., 2015), planting in suppressive soils (Ogundeji *et al*., 2021), endophytic biocontrollers present in healthy seedlings (Farhat *et al*., 2022), or non-pathogenic *Fusarium* spp. strains able to inhibit soil pathogens (Mulero-Aparicio *et al*., 2019).

Grafting has been widely used worldwide in vegetables to prevent diseases caused by soil pathogens (Pradhan *et al*., 2017; Sánchez *et al*., 2015; Velasco, 2013). Among the wild Solanaceae species, *Solanum hirtum* Vahl and *Solanum torvum* Sw. have been successfully used (Fundación Hondureña de Investigación Agricultura [FHIA], 2005; Gisbert *et al*., 2011). Grafting the wild species *S. torvum* as an eggplant rootstock reduces the incidence of *Verticillium dahliae* Kleb (Bletsos, 2006).

Mortality caused by *Ralstonia solanacearum* was reduced in the *Solanum lycopersicum* L. and *Capsicum annuum* L. species using wild species rootstocks (Ad'Vincula *et al*., 2011; Sánchez *et al*., 2015). *Solanum mammosum* L. is another wild species reported as a rootstock (Acuña-Chinchila and Garro-Alfaro, 2018). Reports indicate higher yields than non-grafted seedlings and effective resistance against *Fusarium oxysporum* in tomatoes grafted onto *S. torvum* and *Solanum macrocarpon* L. (Awu *et al*., 2023).

Other documented strategies for managing eggplant MV are genetic resistance (Altinok *et al*., 2014) and biological control with *Trichoderma harzianum* (Hameed, 2011). Success with different *Trichoderma* species on soil pathogens is mainly attributed to their ability to colonize root surfaces and activate various antagonistic mechanisms such as nutrient competition, resistance induction, and antifungal metabolite production (Rai *et al*., 2020). The recent trend in biocontroller use is based on identifying environmentally friendly metabolites applicable to soil pathogen management (Rohit *et al*., 2022).

Resistance induction with chemicals or heavy metals is a non-polluting alternative currently implemented in disease management (Agrios, 2011); for example, phosphorus stimulates a plant's biochemical response to pathogens such as *Gaeumannomyces graminis* in barley and the potato scab, *Streptomyces scabies* (Castellanos *et al*., 2015). Mogollón and Castaño (2011) report the effectiveness of applying potassium phosphite to Dominico-Hartón plantain seedlings (*Musa balbisiana* AAB) for controlling *Mycosphaerella* spp., which decreases the severity of wheat stem rust, early blight in tomatoes, and stem rot in corn; while calcium reduces the severity of soil pathogens such as *Rhizoctia* spp., *Sclerotium* spp., and the nematode, *Ditylenchus dipsaci* (Agrios, 2011).

Induced resistance is an effective complement to genetic resistance and cultural crop-related practices, for which research progresses on various elicitors such as fatty acids for promoting resistance to Oomycetes (Dye and Bostock, 2021); some mycorrhizal fungi can increase the production of antioxidants such as reductalase, catalase, and phosphatase, improving photosynthetic performance and inducing resistance in the plant (Dey and Ghosh, 2022).

This research aimed to evaluate the incidence and severity of vascular wilt on the commercial eggplant variety Corpoica C015 grafted onto the wild species *Solanum mammosum* L. and the effectivity of inorganic fertilizers as resistance inducers to disease.

Materials and methods

Location and experimental material

Researchers carried out the study in a mesh house belonging to the experimental farm and Plant Pathology Laboratory of the Agricultural Sciences Faculty of the University of Córdoba, Colombia, at 14 meters above sea level, 8°44´ North latitude, and 75°53´ West longitude, related to the Greenwich Meridian. This area has an average annual rainfall of 1 200 mm, relative humidity of 84 %, and an average annual temperature of 28 ºC (IDEAM, 2022).

Researchers collected ripe fruits of the wild *S. mammosum*, extracted the seeds, and performed germination tests, which showed a germination rate of 86 %. Additionally, seeds of the commercial eggplant variety Corpoica C015 were available with a viability of over 90 %.

Experiment 1. Evaluation of the rootstock experimental design

Nine seedlings 30 days old were used per treatment, planted in 3.5 kg pots with silty loam soil composed of 21.3 % clay, 25 % sand, and 53.7 % silt, with a pH of 6.87 and previously sterilized with 5 % sodium hypochlorite.

Researchers evaluated three treatments: the commercial variety of ungrafted eggplant Corpoica C015 (T1), eggplant Corpoica C015 grafted onto the wild species *S. mammosum* (T2), and the wild species *S. mammosum* ungrafted (T3), under a completely randomized design with nine replications (each plant is considered a replicate). Plants were grafted when the eggplant seedlings and the wild species reached a stem diameter of approximately 4 mm at 15 cm above the neck of the plant, 60 days after sowing.

Treatments were inoculated with a strain of the pathogen *Fusarium* spp. FS02 (which causes MV in the eggplant) 30 days after grafting when the shoots had unfolded two leaves, by wounding the base of the stem and the roots of each plant, applying 20 mL of suspension with 5000 conidia mL^{-1}.

Experiment 2. Assessment of resistance inducers

Nine 30-day-old seedlings were used per treatment (each plant is considered a replicate), planted in 3.5 kg pots with silty loam soil composed of 21.3 % clay, 25 % sand, and 53.7 % silt, with a pH of 6.87 and previously sterilized with 5 % sodium hypochlorite.

Three foliar nutrition alternatives plus a control were assessed under a completely randomized design, with nine replications (Table 1). Treatments were applied at the time of transplant, with a second application 15 days later. One-liter treatment solutions were applied to each plant.

Table 1. Description of the mineral nutrition treatments applied to eggplant seedlings as resistance inducers to vascular wilt.

Treatments (g L^{-1})	ID	Dose (ml L^{-1})
T1= Distilled water, sprayed on the foliage	Witness	NA
T2= K (332.5) + free amino acids (6), drenched into the soil.	Rich source of potassium	62,5
T3= CaO (96) + N (58.8) + B (2.4) + free amino acids (54.9) drenched into the soil.	Rich source of calcium	1,6
T4= P2O5 (300) + N (200) + K (100) + CaO (0.15) + MgO (0.15) + S (1.17) +B (0.30) + Co (0.03) + Cu (0.15) + Fe (0.65) + Mn (0.32) + Zn (0.65), sprayed on the foliage.	Rich source of Phosphorus	2,5

ID: Treatment identification. NA: soil without inducer.

Two weeks after the treatments were applied, the seedlings were inoculated with *Fusarium* spp. strain FS02. Inoculation was carried out on 30-day-old seedlings (3-4 true leaves) using a manual scalpel disinfected with alcohol and passed over a burner flame; wounds were made at the base of the stem and roots of each plant, applying 20 mL of a suspension with 5000 conidia mL^{-1}.

Treatment effects

Researchers measured plant height (PH), number of leaves present (LP), and number of fallen leaves (FL) in both experiments for seven weeks after the application of the treatments. Researchers also determined the incidence of the disease as the percentage of seedlings with vascular wilt symptoms and the severity using destructive sampling, measuring the disease index (DI) as the product of the disease index on the foliage (DIF) by the vascular discoloration index (VDI) (Bletsos *et al.*, 2003). The DIF was rated using a scale of 1 to 6 according to the external symptoms, while the VDI was rated using a scale of 1 to 4 according to the internal symptoms in the plant; the scales were proposed by Bletsos *et al.* (2003).

Data analysis

Researchers performed a variance analysis on the variables plant height (PH), number of leaves present (PLN), number of fallen leaves (FLN), vascular discoloration index (VDI), leaf disease index (LDI), and disease index (DI) for experiment 1; all the variables met the assumption of variance homogeneity. The normality assumption was met for PH, VDI, and LDI. For PLN and LDI, the normality assumption was met by the square root transformation of the value plus the constant 0.5. The nonparametric Kruskal-Wallis test and range transformation were used for FLN, and orthogonal contrasts and correlation analysis were performed for all variables.

For experiment 2, a variance analysis and a 5 % Tukey multiple comparison test were performed for five variables (PH, PLN, FLN, LDI, VDI), which met the assumption of variance homogeneity. The normality assumption was met for the variables PH, PLN, and FLN. For LDI the normality assumption was met by the square root transformation of the value plus the constant 0.5, and for VDI the nonparametric Kruskal-Wallis test and rank transformation were used. The data was analyzed using the statistical software SAS version 9.2.

Results

Experiment 1. Rootstock Assessment

The height of the Corpoica C015 eggplant seedlings, alone and grafted, did not show significant differences in the contrasts tested (Table 2), revealing that height growth driven by the apical bud's activity occurred similarly in both species. However, the *S. melongena* seedlings showed the presence of the disease on the leaves to varying degrees.

On the other hand, when comparing the number of leaves present at the end of the trial (PLN), 42 days after inoculation, between ungrafted *S. melongena* Corpoica C015 (T1), specimens grafted with *S. mammosum* (T2) and ungrafted *S. mammosum* (T3), researchers found significant differences favoring grafted plants over ungrafted *S. melongena*. Meanwhile, grafted plants (T2) presented no significant differences from ungrafted wild genotypes (T3) (Table 2).

Table 2. Mean squares (MS) of orthogonal contrasts and contrast parameter estimators of the variables plant height (PH), number of leaves present (NLP), and number of fallen leaves of eggplant seedlings (FLN) evaluated under pressure of *Fusarium* sp. FS02 inoculum.

Treatment	PH	PLN	FLN
Solanum melongena (T1)	23,44 ± 1,12	3,22 ± 0,66	2,66 ± 0,50
S. melongena / *S. mammosum* (T2)	23,00 ± 1,24	4,00 ± 0,63	0,66 ± 0,51
Solanum mammosum (T3)	22,93 ± 0,94	4,00 ± 1,22	1,77 ± 0,66
Contrast		**MS**	
T1 vs T2, T3	1,26 ns	0,1104 *	680,35 **
T2 vs T3	0,02 ns	0,0410 ns	168,10 **
Parameter		**Estimator**	
T1 vs T2, T3	0,96 ± 0,92 ns	-1,55 ± 0,77*	2,89 ± 0,49**
T2 vs T3	0,07 ± 0,57 ns	0,00 ± 0,48 ns	-1,11 ± 0,30**

T1: *Solanum melongena*, T2: *Solanum melongena* grafted onto *Solanum mammosum;* T3: *Solanum mammosum*. * and **=significance of Snedecor's F test for MS and Student's test for contrast parameter estimators, at 5 % and 1 %, respectively; ns=not significant.

When comparing the number of fallen leaves (FLN) between the ungrafted *S. melongena* Corpoica C015 seedlings with plants grafted onto *S. mammosum* and lone *S. mammosum*, researchers found significant leaf fall differences in *S. melongena*, which might be caused by the commercial eggplant genotype's greater susceptibility to *Fusarium* spp. There was also a considerable contrast between the wild species *S. mammosum* grafted with *S. melongena* and ungrafted wild plants, suggesting grafted plants present lower rates of leaf senescence in response to biotic and abiotic factors in the environment.

Rootstock's effects on MV's incidence and severity.

When comparing the leaf disease index LDI of *S. melongena* Corpoica C015 seedlings with the wild genotype *S. mammosum* and the latter as a rootstock of *S. melongena*, researchers observed a higher LDI in the commercial eggplant material, which is consistent with a higher presence of the disease in this cultivar. Contrarily, contrasts between wild *S. mammosum* grafted with *S. melongena* and ungrafted *S. mammosum* were insignificant.

A similar result appeared in the vascular discoloration index (VDI), where *S. melongena* Corpoica C015 presented higher estimates, the same as with the DI (Table 3). The vascular wilt incidence in ungrafted *S. melongena* Corpoica C015 was 83 %, which dropped to 16 % when grafted with the wild species. The wild genotype *S. mammosum* did not show symptoms of the disease, indicating its resistance to the *Fusarium* spp. strain FS02.

Table 3. Means, orthogonal contrast estimates, and standard deviations of leaf disease index (LDI), vascular discoloration index (VDI), and disease index (DI) of eggplant seedlings evaluated under pressure of *Fusarium* sp. FS02 inoculum.

Treatment	LDI	VDI	DI
Solanum melongena (T1)	1,66 ± 0,50	2,00 ± 0,70	3,56 ± 1,87
S. melongena / *S. mammosum* (T2)	1,00 ± 0,00	1,17 ± 0,40	1,17 ± 0,40
Solanum mammosum (T3)	1,44 ± 0,52	1,44 ± 0,72	1,88 ± 1,05
T1 vs T2, T3	0,88 ± 0,38*	1,39 ± 0,56*	4,06 ± 1,14**
T2 vs T3	-0,44 ± 0,24 ns	-0,28 ± 0,35 ns	-0,72 ± 0,71 ns

T1: *Solanum melongena*, T2: *Solanum melongena* grafted onto *Solanum mammosum*, T3: *Solanum mammosum*. * and **=significance of Snedecor's F test at 5 % and 1 %, respectively; ns=not significant.

Correlation analysis between variables shows high degrees of positive and highly significant association between plant height, number of leaves per plant, and number of fallen leaves per plant. However, plant height did not correlate with the foliage disease index, vascular discoloration index, and disease index (Table 4).

The number of leaves present was negatively correlated with the number of fallen leaves, the 5 % foliage disease index, and the 7 % disease index, but no significant association was found with the vascular discoloration index, indicating that seedlings with external symptoms would not necessarily exhibit vascular discoloration.

On the other hand, the number of fallen leaves was positively correlated with the three disease indices, evidence that it is a good marker of the presence of the disease. In addition, the three disease indices were positively correlated with each other.

Table 4. Phenotypic correlations between the variables plant height (PH), number of leaves present (NLP) and number of fallen leaves (NLD), leaf disease index (LDI), vascular discoloration index (VDI), and disease index (DI) of eggplant seedlings evaluated under pressure of the *Fusarium* sp. FS02 inoculum.

Variables	PLN	FLN	IEF	IDV	IE
PH	**-0,63449**	**0,61626**	0,25825	0,23942 0,2598	0,34899
P>F	0,0009	0,0013	0,2231		0,0946
PLN	1	**-0,80740**	**-0,40653**	-0,24379	-0,38179
P>F		<,0001	0,0487	0,2510	0,0656
FLN		1	**0,66074**	**0,48532**	**0,65845**
P>F			0,0004	0,0162	0,0005
IEF			1	**0,43710**	**0,72553**
P>F				0,0327	<,0001
IDV				1	0,92399
P>F					<,0001
IE					1

Experiment 2. Evaluation of resistance inducers (RI)

The analysis of variance at 49 days after the treatments were applied showed highly significant differences between the treatments concerning the variables PH, PLN, FLN, and IEF (Table 5). The seedlings treated with the three nutrient formulations greatly surpassed the control in PH, PLN, FLN, and IEF; the highest PH was achieved with the calcium-rich source, which also provided nitrogen, boron, and free amino acids. On the other hand, the greatest leaf fall occurred in the control.

Table 5. Mean squares from the analysis of variance and treatment means for the variables plant height (PH), number of leaves present (NLP), number of fallen eggplant leaves (NLL), leaf disease index (FDI), and vascular discoloration index (VDI) of eggplant seedlings evaluated under *Fusarium* sp. FS02 inoculum pressure.

Mean Squares	GL	PH	PLN	FLN	IEF	IDV
CMT	3	86,06**	12,33**	12,30**	0,99**	21900*
CME	32	6,45	0,47	0,78	0,06	79,88
CV (%)		9,61	16,82	37,96	15,34	48,31
Treatments						
T1		22,40 c	2,33 b	4,00 a	2,44 a	2,33 a
T2		25,79 b	4,33 a	2,22 b	1,22 b	1,67 b
T3		29,43 a	4,67 a	1,78 b	1,11 b	1,44 b
T4		28,17ab	4,89 a	1,33 b	1,22 b	1,44 b

CMT: mean squares of treatments; SME: mean squares of the experimental error; CV: coefficient of variation; DF: degrees of freedom; ** and * significance of Snedecor's F test at 1 % and 5 %, respectively. T1 = control (water spray), T2: source rich in potassium (K); T3 = source rich in calcium (Ca); T4: source rich in phosphorus. PH = plant height, PLN: number of leaves present; FLN: number of fallen leaves, IEF: index of the disease in the foliage, IDV: index of vascular discoloration. Treatment means with the same letters do not differ significantly, according to the Tukey test at 5 %.

Effect of resistance inducers (RI) on incidence and severity

Treatments consisting of mineral nutrients applied to the soil (T2 and T3) or by foliar application (T4) significantly induced the resistance of eggplant seedlings to vascular wilt, expressed as Vascular Discoloration Index and Leaf Disease Index (Table 5). Vascular discoloration incidence when using RI inducers was 33 %, in contrast to 89 % in the control group. In addition, seedlings treated with RI developed mild symptoms in secondary roots, while the control group developed wilt symptoms with internal necrosis in the root system (Figure 1).

Figure 1. Seedlings in destructive sampling after *Fusarium* spp. FS02 inoculation, vascular bundle necrosis in the main roots.

Discussion

Experiment 1. Rootstock Assessment

The use of *S. mammosum* as a rootstock did not affect the development of the eggplant *S. melongena* Corpoica C015, matching results obtained in other investigations (Awu *et al.*, 2023; Bletsos, 2006); however, there are some studies where commercial Solanaceae species grafted with wild rootstock have affected the development of the crown (Bogoescu and Doltu, 2015). These results prove the rootstock's compatibility with Corpoica C015 and *S. mammosum* rootstock's resistance to MV.

Wild rootstock use is considered a viable alternative for managing vascular wilt caused by *Fusarium* spp. FS02, reducing the disease by more than 64 %, coinciding with Bletsos *et al.*, (2003), who lowered vascular wilt in *S. melongena* grafted on *S. torvum* by 72 %. According to Castro *et al.* (2012), the protection provided by rootstocks is usually attributed to chemical compound accumulation inducing resistance to cytological and histological changes in the tissues. This variety of defense mechanisms reflects the polygenic nature of the rootstock-graft interaction (Awu *et al.*, 2023).

This study's results suggest that *S. mammosum* has a potential use for grafted eggplant production since it reduces vascular wilt in terms of incidence and severity, and plant development is unaffected, coinciding with crop research on the Solanaceae family (Awu *et al.,* 2023; Bletsos, 2006; Bogoescu and Doltu, 2015; Lee, 2007; Ogundeji *et al.,* 2021).

Resistance Inductor Assessment

The calcium-rich source was the most effective of the resistance-inducing treatments, which demonstrates its relevance for the plant's defense against pathogens, having a structural function in cell walls and middle lamella (Spann and Schumann, 2010), and contributing to better development in the plant as greater height and functional leave quantity, reducing root damage and vascular wilt up to 56 %, results that match reports in other research (Altinok *et al*., 2014; Hameed, 2011).

Conclusions

Using wild *S. mammosum* as rootstock did not affect the development of the eggplant, *S. melongena* Corpoica C015, and significantly reduced vascular wilt incidence by 67 %.

There was a positive correlation between fallen leaves numbers and disease indices, demonstrating that these parameters are reliable for determining vascular wilt severity in eggplants.

Inorganic nutrient sources rich in potassium, calcium, and phosphorus influenced a greater resistance to vascular wilt, manifesting in lower fallen leave numbers and vascular discoloration index; disease incidence with resistance inducers was 33 % and 89 % in the control group.

Conflicts of interests

The authors declare no conflict of interest.

Authors' Contributions

María del Valle Rodríguez-Pinto, Rodrigo Orlando Campo-Arana, and Carlos Enrique Cardona-Ayala: Conceptualization, methodological design, data collection, data analysis, project administration, review, writing and editing.

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