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ORIGINAL PAPER

A comparison of wild-type, old and modern tomato cultivars in the interaction with the arbuscular mycorrhizal fungus *Glomus mosseae* and the tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici*

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Abstract The effect of the arbuscular mycorrhizal symbiosis (AM) varies in plant cultivars. In the present study, we tested whether wild-type, old and modern tomato cultivars differ in the parameters of the AM interaction. Moreover, the bioprotective effect of AM against the soilborne tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) was tested in the different cultivars. Ten tomato cultivars were inoculated with the arbuscular mycorrhizal fungus (AMF) *Glomus mosseae* alone or in combination with *Fol*. At the end of the experiment, AM root colonization, *Fusarium* infection, and the plant fresh weight was determined. The tomato cultivars differed in their susceptibility to AMF and *Fol*, but these differences were not cultivar age dependent. In all the cultivars affected by *Fol*, mycorrhization showed a bioprotective effect. Independent of the cultivar age, tomato cultivars differ in their susceptibility to AMF and *Fol* and the bioprotective effect of mycorrhization, indicating that the cultivar age does not affect the AM parameters tested in this study.

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Introduction

Arbuscular mycorrhizal fungi (AMF) colonize roots of more than 80% of vascular plants and form a symbiotic association, the arbuscular mycorrhizal symbiosis (AM), improving the nutritional status of the host plant and enhancing its tolerance to biotic and abiotic stress (Smith and Read 2008). The effect of the AM varies in the interaction with their hosts and this variation can be observed even at the plant cultivar level (recently reviewed by Estaún et al. 2010).

Most data on mycorrhizal dependence of plant cultivars are available with cereals (Azcón and Ocampo 1981; Lackie et al. 1987; Kapulnik and Kushnir 1991; Vierheilig and Ocampo 1991a, b; Hetrick et al. 1992, 1993; Kirk et al. 2008; Castellanos-Morales et al. 2011). In wheat, it was suggested that depending on the age of the cultivar, the degree of AM colonization and the responsiveness varies (Hetrick et al. 1993). Compared to modern cultivars, old wheat cultivars showed higher degrees of root colonization and were more responsive to AM colonization; however, in a more recent study, this could not be confirmed (Kirk et al. 2008).

Apart from improving the nutritional status of the host plant, the mycorrhizal symbiosis provides bioprotection against a number of soilborne fungal pathogens (St-Arnaud and Vujanovic 2007). *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) is the cause of a severe wilt disease and is an important

pathogen in tomato cultures (Jones 1991); however, data on the interaction of AMF with *Fol* are scarce. Recently, the germination of microconidia, which are efficient propagules of *Fol* for the infection of tomato plants, has been shown to be stimulated by tomato root exudates (Steinkellner et al. 2005, 2008a, b). Interestingly root exudates of plants colonized by AMF have been shown to exhibit a different effect on microconidia germination of *Fol* than root exudates of non-mycorrhizal plants (Scheffknecht et al. 2006, 2007). Moreover, co-inoculation of tomato with AMF and *Fol* resulted in a certain bioprotection (Dehne and Schönbeck 1979; Akköprü and Demir 2005).

To our knowledge, no comparison of tomato cultivars has been performed yet for the AM interaction and the bioprotective effect of AM against *Fol*. In the present work, we tested whether in wild-type, old, and modern tomato cultivars, these parameters differ.

Materials and methods

Plant and fungal materials

Ten tomato cultivars (Table 1) representing heirloom (old) tomatoes, modern cultivars, and wild types were selected. Tomato seeds were surface sterilized by soaking in 50% household bleach (Dan Clorix, 3.8% NaOCl) for 10 min. After three rinses with tap water, the seeds were transferred to pots containing autoclaved perlite (Granuperl S 3-6, Knauf Perlite GmbH, Vienna, Austria) and incubated in a growth chamber (York International) with a 16-h light (light intensity 296 mol m^{-1} s^{-1}) and 8-h dark photoperiod at 24°C for 2 weeks. The plants were irrigated with tap water during this germination period.

Fol isolate Fol007 (race 2) was subcultured for 2 weeks at 24°C in darkness on Czapek Dox Agar before using it for plant inoculation. A suspension containing microconidia was obtained by flooding the colonies with sterile, distilled water; separating the microconidia from the mycelium using a trigalski spatula; and filtering the resulting suspension through three layers of filter paper. The concentration of the microconidia suspension was adjusted to 10^5 microconidia ml^{-1}.

For the inoculation of the plants with the AMF *Glomus mosseae* (BEG 12), a commercially available inoculum was purchased from BIORIZE/Agrauxine (Quimper, France).

Plant bioassay

After 2 weeks, the plantlets (plant growth stage 11, BBCH scale) were transferred into pots containing an autoclaved (20 min at 121°C) mixture of sand, soil, and expanded clay (1:1:1, *v*/*v*/*v*). The plants were grown in a random design in the greenhouse under natural conditions. The transplanted, inoculated plants were cultivated further for 11 weeks. According to their moisture requirements, they were watered every second day with a low concentration nutrient solution (Steinkellner et al. 2005). If necessary, during the days in between, plants were watered with tap water.

The experimental set-up included (1) a control treatment without AMF and without *Fol*; (2) an AMF treatment; (3) a *Fol* treatment; and (4) a combined AMF–*Fol* treatment. Each treatment comprises eight plants.

For the AMF treatment, 4 ml of the AMF inoculum were added directly into the planting hole, to the roots of each plantlet, when the plantlets were transferred to the sterile substrate mixture. To inoculate the plantlets with *Fol*, the roots were dipped in a microconidial suspension for 5 min. Water was used for mock inoculation. For the combined AMF–*Fol* treatment, the root dip method as described above was used; afterwards, 4 ml of the AMF inoculum was added.

Eleven weeks after transplanting, the plants were removed from the substrate, gently washed under running tap water to preserve the root system, and dried between folded paper towels. The *Fusarium* infection was determined visually according to Wellman (1939). Afterwards, roots and shoots were separated and the fresh weights were

Table 1 Tomato cultivars selected to represent various cultivar ages

Cultivar	Type	Cultivar age	Reference source
Lycopersicon hirsutum	Wild type		Arche Noah
Lycopersicon peruvianum	Wild type		Arche Noah
Yellow Pearshaped	Heirloom (old)	Older than 1900	Austrosaat
Kremser Perle	Heirloom (old)	Older than 1900	Austrosaat
Marmande	Heirloom (old)	Older than 1900	Austrosaat
Rheinlands Ruhm	Heirloom (old)	Older than 1960	Austrosaat
Vitamina	Heirloom (old)	Older than 1960	Arche Noah
Apero F1	Modern cultivar	Younger than 1970	Austrosaat
Myriade F1; F2	Modern cultivar	Younger than 1970	Austrosaat
Supersweet F1	Modern cultivar	Younger than 1970	Austrosaat

F1 resistance to *Fol*, race 1; *F2* resistance to *Fol*, race 2 (used in the present study); *Arche Noah* The Austrian Seed Savers Association, Schiltern, Austria; *Austrosaat* Österreichische Samenzucht- u. Handels-Aktiengesellschaft, Vienna, Austria

Table 2 AM colonization percentage (means±standard error) of tomato cultivars inoculated with AMF (*G. mosseae*) and co-inoculated with the AMF and *Fol* (*F. oxysporum* f. sp. *lycopersici*)

Cultivar	AMF	AMF and *Fol*	Mean
L. hirsutum	19.75 (±3.88)	9.38 (±1.46)	14.56 (±2.05) b, c
L. peruvianum	14.00 (±2.75)	17.86 (±2.64)	15.78 (±2.12) b, c
Yellow Pearshaped	14.25 (±2.69)	10.00 (±10.00)	13.22 (±2.63) b, c
Kremser Perle	27.00 (±1.39)	17.25 (±2.68)	22.13 (±2.05) d, e
Marmande	2.63 (±1.69)	4.63 (±1.34)	3.63 (±2.05) a
Rheinlands Ruhm	11.25 (±2.93)	22.20 (±3.81)	15.39 (±2.28) b, c
Vitamina	9.75 (±2.99)	11.38 (±2.68)	10.56 (±2.05) b
Apero	22.33 (±1.90)	26.88 (±4.71)	24.97 (±2.20) e
Myriade	17.38 (±3.05)	19.25 (±1.75)	18.31 (±2.05) c, d
Supersweet	13.13 (±3.06)	8.00 (±3.14)	10.71 (±2.12) b
Mean	15.22 (±0.93)A	14.63 (±1.01)A	

Cultivar means with different letters indicate a statistically significant difference following the LSD test (p=0.0000). There were no significant differences between the *Fol* and the AMF and *Fol* treatment (p=0.7321). The interactions between cultivar and treatment were significant (p=0.0071)

determined. To confirm the infection with *Fol*, segments of 2-cm length starting upwards the shoot basis were dipped in 70% ethanol, flamed, and put into Petri dishes containing potato dextrose agar (containing 10 mg/l Streptomycinsulfat and 10 mg/l Chloramphenicol to prevent bacterial growth). The presence of *Fol* was determined by visual and microscopic analysis of the morphology of the mycelium and the conidia based on Nelson et al. (1983). The degree of mycorrhization was estimated on defined fresh root segments of 1-cm length, starting 2 cm down the shoot. The roots were cleared by boiling for 4 min in 10% KOH, rinsed three times with tap water, and stained by boiling for 4 min in a 5% ink (Sheaffer black) according to the method of Vierheilig et al. (1998). Thereafter, the percentage of root colonization was determined according to the counting procedure of McGonigle et al. (1990).

Statistical analysis

The effects of cultivars and treatments were determined by two-factorial analysis of variance. Analysis of variance was done after a variance check by the Levene's test. Mean values were compared using Fisher's least significant difference (p<0.05). These analyses were performed using appropriate standard statistical methods (Statgraphics Plus 5.0).

Results

The percentage of AM root colonization was determined in roots of plants co-inoculated with the AMF and *Fol* (AMF/*Fol*) and in the roots of plants inoculated only with the AMF (Table 2). The examination of the roots of the control plants and the Fol-inoculated plants for a possible contamination with AMF was negative.

AM root colonization levels differed between the cultivars. One cultivar that is older than 1900 showed the highest (Kremser Perle), whereas another one that is older than 1900 showed the lowest levels of AM root colonization (Marmande) and the modern cultivar Apero (younger than 1970) showed relatively high AM root colonization levels. All other cultivars including the two wild-type tomatoes (*Lycopersicon hirsutum* and *Lycopersicon peruvianum*) showed intermediate levels of AM root colonization.

Looking over all the cultivars, none of the cultivars co-inoculated with *Fol* affected the percentage of AM root

Table 3 *Fusarium* infection (diseases index, means±standard error) of tomato cultivars inoculated with Fol (*F. oxysporum* f. sp. *lycopersici*) and co-inoculated with AMF (*G. mosseae*) and *Fol*

Cultivar	Fol	AMF and *Fol*	Mean
L. hirsutum	0.38 (±0.26)	0.13 (±0.13)	0.25 a
L. peruvianum	2.13 (±1.86)	0.00 (±0.00)	1.06 a
Yellow_Pearshaped	12.00 (±2.04)	11.75 (±2.16)	11.88 e
Kremser Perle	2.63 (±0.50)	1.38 (±0.38)	2.00 a, b, c
Marmande	4.86 (± 0.72)	2.86 (±0.77)	3.86 b, c
Rheinlands_Ruhm	7.00 (±1.76)	7.25 (± 2.36)	7.13 d
Vitamina	1.88 (±0.44)	1.25 (±0.53)	1.56 a, b
Apero	1.86 (±0.35)	2.75 (±0.31)	2.31 a, b, c
Myriade	0.00 (±0.00)	0.00 (±0.00)	0.00 a
Supersweet	5.00 (±2.22)	3.13 (±1.75)	4.06 c

Columns within a cultivar with different lowercase letters indicate a statistically significant difference following the LSD test (p=0.0000). There were no significant differences between the *Fol* and the AMF and *Fol* treatment (p=0.1875)

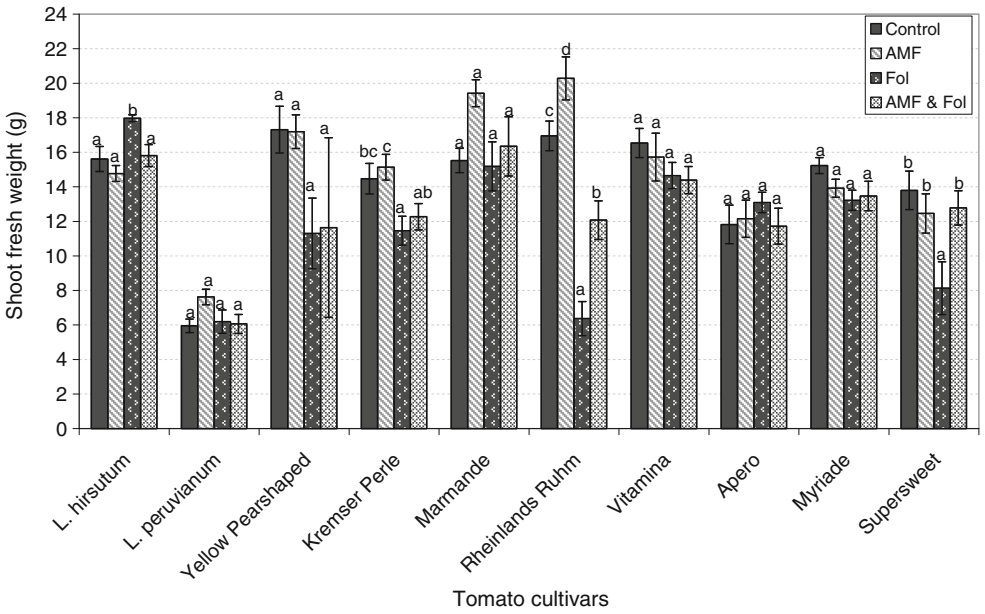

Fig. 1 Shoot fresh weight of uninoculated tomato cultivars and cultivars inoculated with *F. oxysporum* f. sp. *lycopersici* (*Fol*) and/or the AMF *G. mosseae* (Means±SE). Columns within a cultivar with different *lowercase letters* indicate a statistically significant difference following the LSD test ($p<0.05$)

colonization compared to the "AMF only" treatment. Comparing the percentage of AM root colonization within a cultivar in the AMF/*Fol* treatment and the "AMF only" treatment, co-inoculation with *Fol* reduced the percentage of AM root colonization in *L. hirsutum* and Kremser Perle, whereas an increase could be observed in Rheinlands Ruhm.

The level of *Fusarium* infection (disease index) was determined in the roots of plants co-inoculated with *Fol* and the AMF and in the roots of plants inoculated only with *Fol* (Table 3). The control plants and the AMF-inoculated plants did not show any contamination with *Fol*.

Fusarium infection differed between the cultivars. Infection was highest in the cultivar Yellow Pearshaped (older than 1900), followed by the cultivars Rheinlands Ruhm (older than 1960) and Supersweet (younger than 1970). All other cultivars showed a lower *Fusarium* infection. The cultivar Myriade did not show any signs of *Fusarium* infection.

Looking over all the cultivars, none of the cultivars co-inoculated with AM were affected by *Fusarium* infection compared to the "*Fol* only" treatment. Comparing the *Fusarium* infection within a cultivar in the AMF/*Fol* treatment and the "*Fol* only" treatment, co-inoculation with AMF reduced the *Fusarium* infection in *L. peruvianum*, Kremser Perle, and Marmande.

Inoculation with AMF and/or *Fol* affected the shoot and root fresh weight of only a few cultivars. AM inoculation

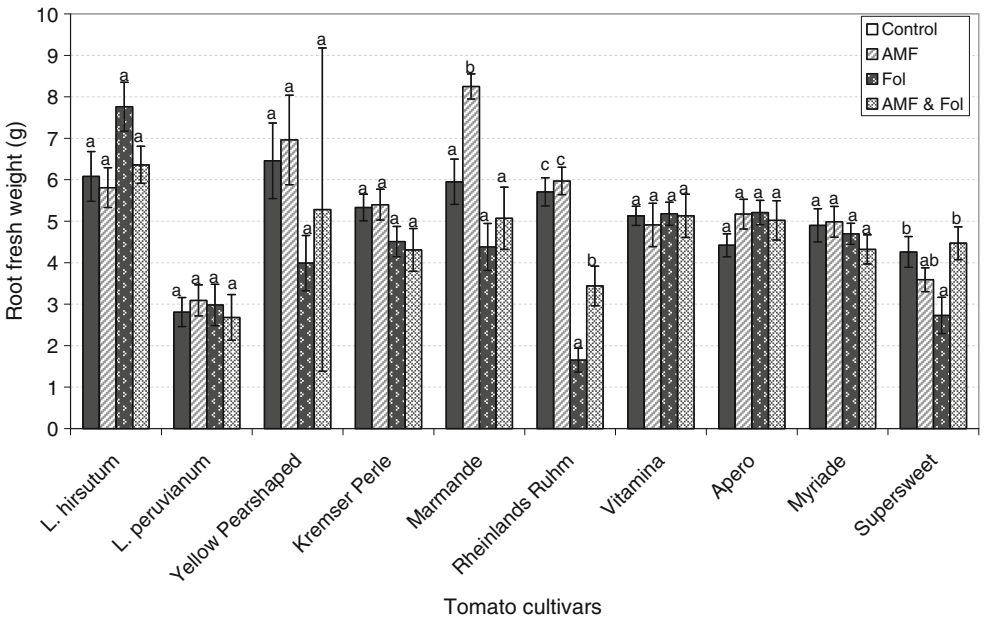

Fig. 2 Root fresh weight of uninoculated tomato cultivars and cultivars inoculated with *F. oxysporum* f. sp. *lycopersici* (*Fol*) and/or the AMF *G. mosseae* (means±SE). Columns within a cultivar with different *lowercase letters* indicate a statistically significant difference following the LSD test ($p<0.05$)

alone showed an effect with Rheinlands Ruhm (older than 1960) by increasing the shoot fresh weight (Fig. 1) and with Marmande (older than 1900) by increasing the root fresh weight (Fig. 2). *Fol* inoculation alone reduced the shoot and root fresh weight of only two cultivars (Rheinlands Ruhm and Supersweet) (Figs. 1 and 2), whereas in these two cultivars co-inoculation of AMF and *Fol* had a positive effect on the shoot and root fresh weight, when compared to the *Fol* only treatment (Figs. 1 and 2).

Discussion

The effect of AMF varies in the interaction with their hosts, and this variation can be observed even at the plant cultivar level (Estaún et al. 2010). In our study, when looking at AM root colonization, we found a large variability between the tested tomato cultivars, confirming data on the variability of AM root colonization between cultivars from other plant families such as legumes, cereals, and trees (Estaún et al. 2010).

In the tested tomato cultivars, these differences in AM root colonization could not be linked with the age of the cultivar as found in wheat (Hetrick et al. 1993). In the old tomato cultivars (older than 1900), we detected the highest and the lowest levels of AM root colonization, and the modern cultivar Apero also showed high levels of AM root colonization. All other tomato cultivars showed similar intermediate levels of AM root colonization, thus confirming the data with wheat and barley (Kirk et al. 2008; Castellanos-Morales et al. 2011) that the age of the cultivar is not linked with the level of AM root colonization.

In several studies, it has been reported that not only the level of AM root colonization varies between the cultivars but also the growth response (see review Estaún et al. 2010). We found a growth response by mycorrhization in two of the ten tested cultivars showing that in the used experimental system, a positive mycorrhizal growth effect can become evident; however, in our system, no growth effect could be observed in most cultivars.

As *Fol* is an important pathogen in tomato cultures (Jones 1991), through plant breeding, tomato cultivars have been obtained showing resistance to different *Fol* races (see Table 1). In our study, cultivars' resistant to certain *Fol* races had similar levels of AM root colonization as cultivars not resistant to *Fol*, showing that resistance to *Fol* does not affect AM root colonization.

Tomato cultivars differ in their susceptibility to *Fol* and this susceptibility also depends on the *Fol* race (Jones and Crill 1974; Abawi and Barker 1984; Gao et al. 1995; Larkin and Fravel 2002). In our experiment with *Fol* race 2, independent of the cultivar age, a high and low susceptibility to *Fol* was observed indicating that the cultivar age is not linked with the susceptibility to *Fol*.

In all the cultivars affected by *Fol*, co-inoculation of cultivars with AMF and *Fol* resulted in a reduced *Fusarium* infection and/or an increased plant growth, corroborating a bioprotective effect of mycorrhization against *Fol*, as reported before (Dehne and Schönbeck 1979; Akköprü and Demir 2005). Looking at our data, it seems that this bioprotective effect of mycorrhization is not linked with the age of the cultivar.

To summarize, independent of the cultivar age, tomato cultivars differ in their susceptibility to AMF and *Fol* and the bioprotective effect of mycorrhization.

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