

Mycorrhizal dependency of passion fruit (*Passiflora edulis* f. *flavicarpa*)

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Abstract — Introduction. Brazil is one of the most important producers of passion fruits. Inoculation with arbuscular mycorrhizal fungi (AMF) may reduce or eliminate the need for phosphate fertilization on this crop. We investigated the response of passion fruit seedlings to inoculation with AMF and phosphorus (P) supply. **Materials and methods.** A greenhouse experiment was carried out in a factorial design. The seedlings were inoculated with 100 spores per plant of single or mixed AMF inoculum on sterilized or non-sterilized soil which was amended with (11 or 30) mg P × dm⁻³ soil applied as superphosphate or used unfertilized (4 mg P × dm⁻³ soil). Relative mycorrhizal dependency (RMD) of passion fruit was estimated for each treatment of inoculation, soil sterilization, and P level, by expressing the difference between the dry mass of mycorrhizal plants and non-mycorrhizal plants as a percentage of the dry mass of mycorrhizal plants. **Results.** Inoculation was effective only on plants grown in sterilized soil with (4 or 11) mg P × dm⁻³ soil. Plants were “facultatively mycotrophic” when associated with AMF and when receiving a supply of 30 mg P × dm⁻³ soil. Seedlings in unfertilized soil with 4 mg P × dm⁻³ soil were excessively dependent on the mycorrhizal association. In soil with 11 mg P × dm⁻³ soil, seedlings were marginally to moderately dependent, depending upon the AMF species used. All inoculated seedlings, without considering soil sterilization, were marginally dependent in soil with 30 mg P × dm⁻³ soil. In sterilized soil, independently of P, they were moderately dependent. However, in the same soil, with 30 mg P × dm⁻³ soil, the seedlings were marginally dependent. **Discussion – conclusion.** The relative mycorrhizal dependency (RMD) of the passion fruit cultivar used was influenced by the species of the inoculated AMF, soil sterilization and soil P level. Passion fruit obtained significant benefit from inoculation with AMF; thus, these fungi can be used to improve plant growth even without P fertilization.

Brazil / *Passiflora edulis* / fertilizer application / phosphate fertilizers / phosphorus / vesicular arbuscular mycorrhizae / biomass

Dépendance mycorrhizienne de la grenadille (*Passiflora edulis* f. *flavicarpa*).

Résumé — Introduction. Le Brésil est l'un des plus grands producteurs de grenadilles. L'utilisation de champignons mycorrhiziens à arbuscules (CMA) peut permettre de limiter l'emploi d'engrais phosphatés. Une étude a été entreprise pour évaluer la dépendance mycorrhizienne de *P. edulis*, par rapport à l'application d'engrais phosphatés. **Matériel et méthodes.** L'expérience a été réalisée en serre selon un dispositif factoriel. Les plantules ont été inoculées avec 100 spores par plant de CMA simples ou en mélange, introduits dans des sols stérilisés ou non contenant (11 ou 30) mg P × dm⁻³ de sol, appliqués sous forme de superphosphate ou utilisés sans fertilisation (4 mg P × dm⁻³ de sol). La dépendance mycorrhizienne relative (DMR) de *P. edulis* a été estimée pour chaque traitement d'inoculation, de stérilisation de sol et de doses de P, en exprimant la différence entre la matière sèche des plants mycorrhizés et non mycorrhizés en pourcentage par rapport à la masse sèche des plants mycorrhizés. **Résultats.** L'inoculation n'a été efficace que sur les plantes cultivées dans le sol stérilisé à (4 ou 11) mg P × dm⁻³. Associées à des CMA et en présence d'un sol à 30 mg P × dm⁻³, les plantes ont été ‘mycotrophiques facultatives’. Dans un sol non fertilisé contenant 4 mg P × dm⁻³, les plantules ont été excessivement dépendantes de l'association mycorrhizienne. Dans un sol avec 11 mg P × dm⁻³, les jeunes plantes ont été marginalement à modérément dépendantes, selon les espèces de CMA utilisées. Sans stérilisation du sol, toutes les plantules inoculées ont été marginalement dépendantes dans le sol avec 30 mg P × dm⁻³. Dans le sol stérilisé et indépendamment de P, elles ont été modérément dépendantes. Cependant, dans le même sol, avec 30 mg P × dm⁻³, les jeunes plantes ont été marginalement dépendantes. **Discussion – conclusion.** La dépendance mycorrhizienne relative (DMR) de la grenadille est fonction du CMA inoculé, de la stérilisation du sol, et du taux de P du sol. Étant donné que la production de cette plante augmente avec l'inoculation du CMA, des inoculations de mycorrhizes peuvent permettre de remplacer l'apport d'engrais phosphatés.

Brésil / *Passiflora edulis* / fertilisation / engrais phosphaté / phosphore / mycorrhize à vésicule et arbuscule / biomasse

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1. Introduction

Passion fruit (*Passiflora edulis* Sims. f. *flavicarpa* Deg.) produces fruits that are appreciated for their flavor, high nutritious value, and pharmacological applications. Brazil has been the most important producer, with small and medium farmers being responsible for implementation of this fruit crop throughout the country [1, 2]. The production of seedlings is done mainly by seeds, with the addition of a commercial sterilized substrate, mixed with cow manure and superphosphate, as recommended [2]. A possible alternative is to produce the seedlings without the use of fertilizers but with the inoculation of arbuscular mycorrhizal fungi (AMF). These obligate symbiotic microorganisms may improve the growth rate of the passion fruit plant by increasing nutrient absorption [1] by developing a mycelial net that explores and transfers mineral nutrients from soil to the host plant [3].

The inoculation of fruit trees with AMF has improved nutrition and establishment of seedlings after the field transplant and has increased the host tolerance to soil pathogens [4]. This has been well documented in tropical crops such as cassava [5], pineapple [6], banana [7] and citrus [8].

Plants vary in their mycorrhizal dependency (MD). This parameter was defined as the “degree at which the plant species depend on the mycorrhizal condition for achieving maximum growth or production at a given soil fertility level” [9]. Plant dependency on AMF is related to the genetic interaction between plant and fungus, also being affected by edaphic factors such as phosphorus (P) availability [8, 10], and other environmental conditions [11]. In general, MD decrease with increasing P concentration in the soil solution, and the final response depends on the plant species [12]. Menge *et al.* [8] defined MD numerically as the rate of dry biomass between mycorrhizal and non-mycorrhizal plants, expressed as a percentage. However, with this method, one may obtain extremely high percentage values such as 13000% for *Lepidospermum scoparium* [13], 4000% for citrus and cassava, 800% for papaya, 200% for

mango and avocado [14]. Therefore Plenchette *et al.* [11] proposed the term “relative mycorrhizal dependency” (RMD). With this parameter the highest level of RMD never exceeds 100% and the lowest 0%, where 100% is for plants unable to grow without AMF and 0% when the dry matter of mycorrhizal plants does not differ from that of non-mycorrhizal plants. Applying that concept, the authors found a RMD of 99.2% for carrots, 94.0% for some legumes, 41.9% for potato and 59.2% for tomato. Based on Plenchette *et al.* [11], Habte and Manjunath [12] proposed categories of RMD varying from very highly dependent ($RMD \geq 75\%$ at a soil solution P concentration of $0.02 \text{ mg} \times \text{L}^{-1}$ and responding significantly to inoculation at $0.2 \text{ mg} \times \text{L}^{-1}$) to independent (species that are not colonized or do not respond positively to mycorrhizal inoculation). The determination of the RMD of a species indicates how much the plant is responsive to inoculation with AMF [12]. In non-fumigated soil, RMD will show how much and in which way the introduced AMF competes with the native endophytes [15].

We investigated the RMD of the passion fruit plant, identified promising AMF isolates and determined the most suitable level of soil P for establishment and success of the symbiosis between AMF and seedlings of passion fruit on sterilized and non-sterilized soil.

2. Materials and methods

2.1. Study location

The experiment was conducted in a greenhouse. Temperature and relative humidity, not controlled, were recorded by a digital thermohygrometer, with minimum and maximum values varying from 19.6 °C to 38.6 °C, and 33% to 90% relative humidity, respectively.

2.2. Soil

For production of AMF inoculum, and for the experiments, a Red Yellow Podzol was

used after sterilization with methyl bromide. Soil characteristics were: 4 mg of available P per dm³ of soil (Mehlich method); 0.1 cmol Al, 1.9 cmol Ca, 0.7 cmol Mg, and 0.12 cmol K × dm⁻³ soil; pH 4.0; 1.14 g N, 13.2 g C, and 22.8 g organic matter × dm⁻³ soil. Soil humidity was maintained at 60% of the total pore volume. Two more P levels, other than the natural soil P, were considered (11 mg and 30 mg P × dm⁻³ soil), added as superphosphate before planting. The levels of P were based on determinations of Cavalcanti *et al.* [16] for cultivation of passion fruit.

2.3. Inoculum production

The inocula consisted of spore suspensions from isolates of *Gigaspora albida* Schenck & Smith (INVAM 927 and UFPE 01), *Gigaspora margarita* Becker & Hall (UFPE 02), *Acaulospora longula* Spain & Schenck (UFPE 06) and *Scutellospora heterogama* (Nicolson & Gerdemann) Walker & Sanders (UFPE 12), grown in association with Bahia grass (*Paspalum notatum* Flüggé). Spores were extracted from soil by wet sieving [17] and sucrose centrifugation [18], washed, transferred to Petri dishes and counted under a dissecting microscope.

2.4. Inoculation

At 18 d after planting and 8 d after germination, seedlings with a single true leaf were transferred to plastic cups with 40 g of soil. Inoculated treatments received 100 spores of AMF; those with a mixture of AMF received 25 spores of each isolate, completing 100 spores per treatment. Non-inoculated treatments received 2 mL of the soil solution filtrate (1:100; soil: water; v/v), to recover the original soil microbiota with the exception of the AMF. At 10 d after inoculation, the seedlings were transferred to plastic bags (11 × 26 cm) with 2,000 g of soil.

2.5. Evaluation

Seventy days after inoculation, shoots were separated from roots and allowed to dry. Relative mycorrhizal dependency (RMD) of

Table I.

Degrees of relative mycorrhizal dependency (RMD) of plants after inoculation of seedlings with spores of arbuscular mycorrhizal fungi.

Species dependency	RMD value (%)
Excessive	> 75
High	50–75
Moderate	25–50
Marginal	< 25
Independent	No response to mycorrhizal inoculation

passion fruit was estimated for each treatment of inoculation, soil sterilization, and P level, by expressing the difference between the dry mass of mycorrhizal plants and non-mycorrhizal plants as a percentage of dry mass of mycorrhizal plants [11]. Degrees of RMD were established using the categories proposed by Habte and Manjunath [12], modified by considering values obtained in soil with (4, 11 and 30) mg P × dm⁻³ (*table I*). The experimental design was entirely at random with three replicates in a factorial arrangement of two soil conditions (natural or sterilized), three P levels [(4, 11 and 30) mg P × dm⁻³ soil], and four inoculation treatments: (a) *G. albida*; (b) *S. heterogama*, (c) a mixture of AMF (*G. albida*, *G. margarita*, *A. longula*, *S. heterogama*); (d) control – non-inoculated. The seedlings were maintained in a greenhouse and pots were rearranged every 10 d to minimize possible effects of position on the bench.

Data were subjected to variance analysis using the SANEST Program [19], and means compared by Tukey's at 5% level of significance. Only polynomial regressions were considered in the interactions.

3. Results

In non-sterilized soil, the treatment of non-inoculated seedlings presented 29% of root colonization, produced by the AMF native population while, in those inoculated, colonization varied from 49% to 63%. However, in the treatments performed with

sterilized soil, the rates of colonization were higher than those found in non-sterilized soil (control and inoculated treatments). These results indicate that the native population was not able to colonize the plants as much as the introduced AMF isolates [20].

At the end of the experiments, the spore density of the AMF native population in non-sterilized and non-inoculated soil was $0.57 \text{ spores} \times \text{g}^{-1} \text{ soil}$, which did not significantly differ from the inoculated treatments. However, in sterilized soil, spore density varied from (2.9 to 4.7) $\text{spores} \times \text{g}^{-1} \text{ soil}$ in the mycorrhizal treatments, which was significantly different from the control that presented only $0.05 \text{ spores} \times \text{g}^{-1} \text{ soil}$.

Significant interactions between soil and inoculation treatments on the shoot biomass of passion fruit were registered. Shoot biomass of both control and inoculated plants, was higher in fumigated soils than in non-fumigated soils. All inoculated plants presented higher biomass than the controls when in sterilized soils; however, there were no differences between treatments in non-sterile soils (*table II*).

The analysis of variance showed interaction between AMF inoculum and P level on

shoot biomass of passion fruit. There was a quadratic regression for treatments with inoculation. A maximum peak appeared at $24 \text{ mg P} \times \text{dm}^{-3} \text{ soil}$, after which the biomass did not increase in plants associated with *G. albida* and *S. heterogama* (*figure 1*).

Independent of the AMF species inoculated, passion fruit was considered extremely dependent of the mycorrhizal association when it was maintained in sterilized soil with a natural P concentration of $4 \text{ mg P} \times \text{dm}^{-3} \text{ soil}$. It was moderately dependent when it was associated with any of the AMF and cultivated in sterilized soil with $11 \text{ mg P} \times \text{dm}^{-3} \text{ soil}$ or in non-sterilized soil with $4 \text{ mg P} \times \text{dm}^{-3} \text{ soil}$. However, in the other treatments, it was only marginally dependent on the AMF (*table III*).

4. Discussion

This study demonstrated that passion fruit plants grown in an unfertilized ($4 \text{ mg P} \times \text{dm}^{-3} \text{ soil}$) and non-sterilized soil were highly dependent on the mycorrhizal association with *G. albida*, *S. heterogama*, or mixed inoculum. Plants fertilized with $30 \text{ mg P} \times \text{dm}^{-3} \text{ soil}$ did not benefit from the inoculation but gained from the phosphate fertilization. The results suggest that the association with AMF, on soils with low or medium P values, favored the growth of passion fruit, despite the lack of specificity between the host and the symbiotic species [15]. The efficiency of this symbiotic association is under a genetic control and is affected by fungus and plant species, besides being dependent on environmental conditions [7]. Menge *et al.* [8] cited, among others, root hairs, root geometry, plant growth rates and phosphate transport and utilization, all of which influence P absorption and, consequently, mycorrhizal dependency. Many researchers have shown benefits of inoculations with AMF on fruit crops such as banana [7, 21, 22], plum and apple [23], citrus [24–26] and papaya [27]. In general, these effects are higher when the soil used for cropping is P deficient.

In this study, there was no growth response to AMF inoculations when the

Table II.

Effect of soil fumigation and inoculation with arbuscular mycorrhizal fungi (AMF) on shoot biomass of passion fruit, 70 d after inoculation. For all the inoculation treatments, significant different results ($P < 0.05$) were obtained when sterilized or non-sterilized soils were used.

Inoculation treatment	Shoot biomass (g)	
	in sterilized soil	in non-sterilized soil
Uninoculated	$3.88 \pm 1.07 \text{ c}$	2.60 ± 0.59
<i>Gigaspora albida</i>	$6.54 \pm 0.48 \text{ a}$	2.69 ± 0.58
<i>Scutellospora heterogama</i>	$5.58 \pm 0.44 \text{ b}$	2.85 ± 0.61
AMF mixture ¹	$6.43 \pm 0.53 \text{ ab}$	3.14 ± 0.67
Significance	**	ns

** Values statistically different ($P < 0.05$); means with a different letter (a, b or c) are significantly different.

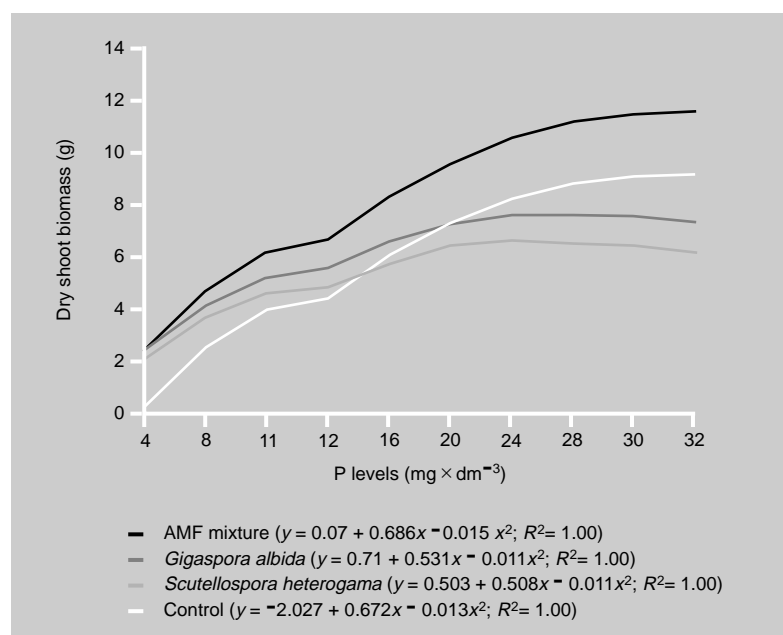
ns: not significant differences.

¹ AMF mixture = mixture of *G. albida*, *G. margarita*, *Acaulospora longula*, *S. heterogama*.

plants were grown in natural, non-sterilized soil. It is possible that competition with the indigenous soil microorganisms has adversely affected the survival, growth and/or effectiveness of the introduced AMF. Soil sterilization was necessary to get a positive growth response from AMF inoculations with *G. albida* or *S. heterogama*, as single or mixed inoculum. However, working with *Passiflora ligularis* L., Rodrigues *et al.* [28] found that soil disinfection had a negative effect on plant growth and efficiency of uptake of P fertilizer. In that case, it seems that pasteurization has eliminated the native but effective mycorrhizal fungi in the soil. On the other hand, the effects of fumigants upon mycorrhizal fungi, which are consistent for many plant species, could be attributed to the toxic effects of the chemicals. Methyl bromide was shown to reduce the root colonization and/or the spore development [29].

Pitcher *et al.* [30] found that mycorrhizal infections are more vigorous and abundant in fumigated or sterilized soil than in non-sterilized soil. Most soil fumigants stimulate crop growth primarily because of the elimination of soilborne pathogens [29] which may also benefit the AMF. Fumigations reduce hyperparasites, predators and competing plant parasites, thereby encouraging rapid dissemination of mycorrhizal fungi.

Kleinschmidt and Gerdemann [31] observed that methyl bromide induced



citrus stunting; however, the application of phosphate or inoculation with AMF eliminated this problem. These authors have also proved that stunted nonmycorrhizal seedlings were P, Zn, and Cu deficient, while mycorrhizal inoculation enabled seedlings to absorb adequate amounts of these nutrients and showed that methyl bromide induced stunting of citrus by killing the mycorrhizal fungi. Conversely, a decrease in the availability of soil elements after fumigation is not the cause of the stunting problem, since there are reports

Figure 1. Effect of arbuscular mycorrhizal fungi (AMF) inoculum and soil P levels on shoot biomass of passion fruit, independently of soil sterilization, 70 d after inoculation. The curves were modeled according to the regression analysis and necessary points were inserted. (AMF mixture = mixture of *G. albida*, *G. margarita*, *Acaulospora longula*, *S. heterogama*).

Table III.

Mycorrhizal dependency (%) of passion fruit evaluated 70 d after inoculation with arbuscular mycorrhizal fungi (AMF), as affected by soil sterilization and phosphorus (P) addition.

AMF treatment	Doses of P (mg × dm ⁻³ of soil)					
	in sterilized soil			in non-sterilized soil		
	4	11	30	4	11	30
<i>Gigaspora albida</i>	88.46	46.26	7.48	38.46	7.26	7.99
<i>Scutellospora heterogama</i>	86.61	40.32	14.50	45.76	11.81	19.83
AMF mixture ¹	87.69	48.67	3.25	38.46	10.00	20.66

¹ AMF mixture = mixture of *G. albida*, *G. margarita*, *Acaulospora longula*, *S. heterogama*.

showing that availability of most soil nutrients is not changed by soil fumigations [29].

Mycorrhizal dependency varies with plant species [14] as well as with the soil nutritional regimes [8]. Kleinschmidt and Gerdemann [31] suggested that citrus are highly AMF dependent. The RMD of passion fruit was influenced by the AMF species, soil conditions (sterilized or non-sterilized) and P supply (*table III*).

Without mycorrhiza, passion fruit plants showed better growth when supplied with medium to high phosphate level (11 and 30 mg P \times dm⁻³ soil). The ability to grow under diverse levels of mineral availability without AMF differs between plants [32] and those that survive with or without the symbioses are considered to be mycotrophic facultative. Passion fruit can be included in this category since it grew well in both situations, whether in association with AMF or fertilized with P. Francis and Read [33] considered that classification of mycorrhizal dependency of plants based on vegetative responses during a short period of time is inappropriate and suggested a review of the terms "facultative" or "obligate mycorrhization", especially if this is to be extended from greenhouse experiments to field situations. However, these terms are useful for characterizing the need for AMF inoculation for each crop.

Considering the classification of RMD [12], passion fruit is highly dependent on mycorrhiza when grown in sterilized soil with low P levels. The degree of RMD of this fruit crop decreased with increasing P levels, as was also observed by Menge *et al.* [8] and Rheinheimer *et al.* [10].

In the present study, passion fruit did not respond to inoculation in non-sterilized soils and, thus, it did not show any mycorrhizal dependency. The native soil microbiota may have affected symbiotic efficiency, acting directly on the external mycelium and competing with the AMF for nutrients, including phosphates [34]. There is also the possibility of formation of complex interactions, such as those with an antagonistic effect [35].

5. Conclusion

Inoculation with 100 spores of *G. albida* and *S. heterogama*, as single species or as mixed inoculum, significantly improved the growth of passion fruit in unfertilized soils with 4 mg P \times dm⁻³ soil. Thus, phosphate fertilization in seedling production may be replaced by AMF inoculation. Furthermore, it was observed that soil sterilization enhanced the plants' response to inoculation with AMF. There was no response to AMF inoculation when non-sterilized soil was used. This study has demonstrated that passion fruit is facultatively mycotrophic, showing significant growth improvement when associated with AMF. The degree of RMD, however, depends on soil P level and on soil sterilization, as well as on the species of mycorrhizal fungi.

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Dependencia micorrízica del maracuyá amarillo (*Passiflora edulis* Sims. f. *flavicarpa* Deg.).

Resumen — Introducción. El maracuyá es apreciado por su valor nutritivo y sus aplicaciones farmacéuticas, siendo el Brasil el productor más importante. El uso de hongos micorrízicos arbusculares (hMA) es una alternativa para la producción de mudas sin aplicación de fertilizante fosfatado. Investigamos la dependencia micorrízica (DM) del maracuyá en función de la fertilización con fósforo (P) y de la esterilización del suelo. **Material y métodos.** El experimento fue realizado en invernadero, con mudas inoculadas con 100 esporas de hMA en suelo esterilizado o no, y con (11 o 30) mg P × dm⁻³ de suelo, bajo la forma de superfosfato simple. El delineamiento experimental fue de tipo completamente casualizado en factorial. **Resultados.** A los 70 días, la inoculación fue eficaz únicamente sobre las plantas cultivadas en suelo esterilizado y con (4 o 11) mg P × dm⁻³. El maracuyá fue “micotrófico optativo” pues respondió a la inoculación con hMA y, en la ausencia de éstos, a la fertilización fosfatada (30 mg de P × dm⁻³ de suelo). Las mudas inoculadas en suelos con (4 y 11) mg P × dm⁻³ fueron “excesivamente” y “ligeramente dependientes”. Las mudas inoculadas, sin considerar la esterilización, fueron “marginamente dependientes” en suelo con 30 mg P × dm⁻³. En suelo esterilizado e independiente de P, las mudas fueron “ligeramente dependientes”; sin embargo, en suelo con 30 mg P × dm⁻³ fueron “marginamente dependientes”. **Discusión – Conclusión.** La DM del maracuyá fue relacionada al hMA, a la esterilización del suelo y al nivel de P en el suelo. Considerando que la inoculación con hMA favorece el crecimiento del maracuyá amarillo, la utilización de esos hongos puede permitir un aumento de la producción, sin necesidad de fertilización fosfatada.

Brasil / *Passiflora edulis* / aplicación de abonos / abonos fosfatados / fósforo / micorrizas arbusculares vesiculares / biomasa

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