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CROP PRODUCTION

Inoculation with arbuscular mycorrhizal fungus *Rhizophagus clarus* on tomato promotes increasing yield under organic farming inputs

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ABSTRACT: Organic agriculture comprises farming practices that discard synthetic pesticides and fertilizers. Tomato production demands huge amounts of fertilizers and pesticides. Improving efficiency of the inputs allowed for organic tomato production is a challenge to upgrade yields. Thereby, we studied the effects of the inoculation of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus clarus,* supplying rock thermophosphate and bioactivator, alone or associated, on tomato development and yield. The experiment was achieved in a greenhouse using undetermined tomato cv. BRS-Nagai sown in polystyrene trays and afterwards transplanted to pots. Treatments included *R. clarus*; thermophosphate (TH) (130 g/pot); bioactivator (PenergeticK® + Penergetic®) (BI); *R. clarus* + TH; *R. clarus* + BI; *R. clarus* + TH+ BI and TH + BI and control (CO). From the flowering onset, plant height, height of insertion of first truss, trusses space, length, and also the diameter and fresh weight of ripe fruits of the three first trusses were assessed. AMF colonization in the roots and macronutrients in leaves and petioles were also measured. Trusses spacing variable was affected by mycorrhiza and thermophosphate. *R. clarus* inoculation incremented 10 and 31.85% of fresh mass of ripe fruits and mass of ripe fruits per plant, respectively. Soluble solids contents in fruits and N, P and K in the leaves and petioles were similar among treatments. AMF colonization decreased on thermophosphate fertilized plants and increased in bioactivator treatment. Results showed that root inoculation with *R. clarus* promoted better plant development and yield and may be used as biological inoculant mostly on organic tomato production.
Key words: agroecology, *Solanum lycopersicu*, organic inputs, nutritional management.

Inoculação com fungo micorrízico arbuscular *Rhizophagus clarus* em tomateiro proporciona aumento de produtividade em cultivo orgânico

RESUMO: A agricultura orgânica preconiza práticas culturais que dispensam o uso de pesticidas e fertilizantes sintéticos. A tomaticultura convencional, por sua vez, demanda grandes quantidades de agroquímicos. Neste contexto, aumentar a eficiência de insumos permitidos em agricultura orgânica consiste em um desafio para a manutenção de altas produtividades. Este estudo objetivou investigar os efeitos, isoladamente e de interação, da inoculação de fungo micorrízico arbuscular (FMA) *Rhizophagus clarus* e da aplicação de termofosfato e de bioativador no desenvolvimento e na produtividade de tomateiros. O experimento foi conduzido em casa de vegetação com a cultivar BRS-Nagai, genótipo de hábito indeterminado do grupo saladete, semeado em bandejas de poliestireno e posteriormente transplantado para vasos. Os tratamentos contemplaram *R. clarus*; termofosfato (TH) (130g/vaso); bioativador (Penergetic K® + Penergetic®) (BI); *R. clarus* + TH; *R. clarus* + BI; *R. clarus* + TH+ BI; TH + BI e controle (CO). A partir do início do florescimento, foram mensuradas altura de plantas, altura do primeiro cacho, distância entre cachos e largura, comprimento e massa fresca de frutos maduros dos três primeiros cachos. Foram determinadas também a colonização micorrízica e os teores de macronutrientes em folhas e pecíolos. A distância entre cachos foi influenciada pela inoculação micorrízica e pela aplicação de termofosfato. *R. clarus* aumentou em 10% e 31.85% a massa fresca de frutos maduros e a massa fresca de frutos por planta respectivamente. Os teores de sólidos solúveis em frutos e de N, P e K em folhas e pecíolos foram similares para os tratamentos. A colonização micorrizica foi menor em plantas que receberam termofosfato e maior na presença do bioativador. Os resultados demonstraram melhor desenvolvimento e maior produtividade em plantas inoculadas com FMA, sugerindo que *R. clarus* apresenta-se como um potencial inoculante biológico para tomateiros, principalmente em cultivos orgânicos.
Palavras-chave: agroecologia, *Solanum lycopersicum*, insumos orgânicos, manejo nutricional.

INTRODUCTION

Organic farming prioritizes renewable inputs with leads to minor environmental impacts and, in a long term, food systems may converge to growing productivity and self-sufficiency (GLIESSMAN, 2016). Regulatory agencies and certifiers prohibit some inputs, mostly synthetic fertilizers and pesticides, and allow others such as manure, compost, rock powders, lime, microbial inoculants and biostimulants.

Root inoculation with arbuscular mycorrizal fungi (AMF) may enhances phosphorus and other low mobile nutrients acquisition by plants in the soil (ORTAS et al., 2013; WATTS-WILLIAMS & CAVAGNARO, 2014), increases plant growth

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and yield (SILVA et al., 2017) what is even more important in lower phosphorus availability soils such as highly weathered tropical soils, due to high adsorption and even lack in original rock (NOVAIS & SMITH, 1999).

Mycorrhiza fungi may establish symbiotic interaction with more than 90% of plant species (DU JARDIN, 2015). AMF *Rhizophagus clarus* is a candidate to be fairly used as inoculant in crops due to high infectivity and low specificity (ADEMAR et al., 2015; SATO et al., 2015; URCOVICHE et al., 2015; CELY et al., 2016; SALGADO et al., 2016; KOYAMA et al., 2017).

Tomato production in organic and even conventional agricultural systems demands large amount of fertilizers. AMF may improve efficiency in the use fertilizers and even decreases the burden. In tomato crops, AMF have been reported to control root diseases (POZO et al., 2002); improving phosphorus content in plant (FINZI et al., 2017); root and shoot dry weight (LEY-RIVAS et al., 2015); plant height and yield (PÉREZ & MARTÍNEZ, 2012) and stem diameter (KILE et al., 2013).

Rock phosphate and bioactivators have been used in organic farming in Brazil, but we did not found reports about studies on the efficiency of these inputs, lonely or even associated with other inputs, on tomato yield and/or plant growth. Thereby we evaluated the inoculation of *R clarus,* rock thermophosphate and bioactivator, alone or associated, on tomato growth and yield.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse in Londrina, PR, Brazil (23^0 23'S e 51^0 11'W); subtropical (Cfa) weather (Köppen).

Undetermined tomato cv. BRS-Nagai was sown in polystyrene trays filled with commercial substrate (MecPlant®). The same substrate was also mixed with *R. clarus* inocula (spores, mycelia and colonized roots) that was acquired by Laboratory of Microbial Ecology (Universidade Estadual de Londrina, Londrina, PR, Brazil), providing a concentration of 50 spores per pit tray. After 32 days of sowing, seedlings were transplanted to pots [10 dm^3 filled with red eutroferric latossol mixed with sand (2:1)] (Sistema Brasileiro de Classificação de Solos, [s.d.])(Santos et al 2014). Chemical analysis of the mixture characterized pH ($CaCl_2$) = 5.4; Ca = 2.36 $cmol_c$ dm^{-3}; Mg = 0.75 $cmol_c$ dm^{-3}; Al = 0; H + Al = 3.42 $cmol_c$ dm^{-3}; K = 0.18 $cmol_c$ dm^{-3}; C = 5.29 g kg^{-1}; MO = 9.1 g kg^{-1} and P = 6.7 mg dm^{-3}. Lime was applied to improve soil base saturation until 80%. Before transplanting, 105 g of Ekosil® (K_2O = 8,0%; Si = 25,0%) fertilizer plus 1 kg of organic manure were also added to each pot. Organic manure composition was pH ($CaCl_2$) = 7.3; Ca = 13.28 $cmol_c$ dm^{-3}; Mg = 7.96 $cmol_c$ dm^{-3}; Al = 0; H + Al = 2.19 $cmol_c$ dm^{-3}; K = 8.33 $cmol_c$ dm^{-3}; C = 60.46 g kg^{-1}; MO = 104 g kg^{-1} e P = 2.868 mg dm^{-3}. Ca and Mg were determined by titration with EDTA and Al by titration with NaOH. Potential acidity was estimated by SMP pH. P and K were extracted using a Melich-1 extracting solution, P was determined by spectrophotometry and K by flame photometry. Organic carbon was quantified using the Walkley-Black method.

Treatments were *R. clarus*; Yoorin® thermophosphate (TH) (130 g/pot); bioativador (PenergeticK® + PenergeticP®) (BI) (1 g L^{-1}); *R. clarus* + TH; *R. clarus* + BI; *R. clarus* + TH+ BI and TH + BI and non-treated plant was considered as control (CO). PenergeticK® and PenergeticP® are composed of bentonite clays which are subjected to application of electric and magnetic fields (BRITO et al., 2012).

From the flowering onset, organic Bokashi (N = 37.67 g kg^{-1}; P = 14.36 g kg^{-1}; K = 21.01 g kg^{-1}; Ca = 12.00 g kg^{-1}; Mg = 8.80 g kg^{-1}) and mineral Potamag® (K_2O = 22,0%; Mg = 11,0%; S = 22,0%) fertilizers were weekly applied in the soil and boric acid by fertigation, according to the cultivar demand. Phytosanitary management was achieved by spraying copper and sodium bicarbonate as fungicides, neem and *Bacillus thuringiensis* insecticides according to Brazilian legislation of organic agriculture (BRASIL, 2014).

Plants were grown on a single stake (single stem) supported by bamboo stakes and pruned (removal of apical meristem) after emission of the 4^{th} truss. Flowers of the 4^{th} truss were also removed (STRECK et al., 1998; GUIMARÃES et al., 2007) .

From the flowering onset, evaluations comprised plant height, height of insertion of first truss, trusses space, length, diameter and fresh weight of ripe fruits (picking point: 60-90% of surface red) of the three first trusses. Fruits out of commercial standards (diameter inferior to 4 cm) (BRASIL, 1995) and damaged fruit were discarded (not included in the yield).

To evaluate the AM colonization, samples of secondary roots was stained with Trypan blue (KOSKE & GEMMA, 1989) and the determination of AM colonization (%) was carried using grid-line method (GIOVANNETTI & MOSSE, 1980). Shoot and root dry weight were also determined after incubated in forced air drying oven until constant weight.

Macronutrients (N, P and K) were determined in dry leaves and petioles after ground in a mill. Nitrogen was determined by sulfuric digestion and distillation in a Kjeldal system and phosphorus and potassium were determined by nitropercloric digestion (SILVA, 2009).

The experimental design was randomized block with three-factor arranged in mycorrhiza (with and without) X thermophosphate (with and without) X bioactivator (with and without), with six replicates and 48 pots. Data were submitted to analysis of variance after testing the normality and variance homogeneity assumptions (Shapiro-Wilk and Bartlett, respectively). Means were compared using Fisher F test ($P < 0.05$). Analyses were achieved using software R (R CORE TEAM, 2018).

RESULTS

Vegetative and productive traits

Similar values for shoot and root dry weight, plant height and height of first truss were observed among treatments. However, trusses spacing variable was affected by mycorrhiza and thermophosphate (Table 1). Reduction of the distance between trusses was observed for plants inoculated with mycorrhiza (9.59%) and those fertilized with termophosphate (8.36%). *R. clarus* inoculation enhanced fresh mass of ripe fruits and mass of ripe fruits per plant (10% and 31.85%, respectively) (Table 2).

Mycorrhizal colonization, macronutrients in leaves and soluble solids in fruits contents

Soluble solids in fruits and N, P and K in the leaves and petioles were similar among treatments. The AMF colonization (Figure 1) was affected by thermophosphate and bioactivator (Table 3).

We observed reduction of AMF colonization in treatments using thermophosphate, compared to those who did not receive the phosphate fertilizer (60.75 *vs*. 71.77%, respectively). In contrast, higher AMF colonization in bioactivator treated than untreated plants were found (71.89 *vs*. 60.62%, respectively).

DISCUSSION

Distance between trusses was reduced in treatments that received mycorrhizal inoculation and thermophosphate (Table 1). In tomato crops, the balance between vegetative and reproductive development is crucial for tomato yield, which may decreased nutrient drain through vegetable tissues (PUIATTI et al., 2010). Fruit are the main drains of photoassimilates produced by leaves (GUIMARÃES et al., 2007) and the relation source/drain is vital of vegetative development and reproductive tissues (OSORIO et al., 2014).

Internodal spacing is directly related with light absorption and energetic efficiency

Table 1 - Shoot (SDW) and root dry weight (RDW); plant height (PHE); height of first truss (H1T) and mean distance between trusses (DBT) in tomato cultivated under different nutritional managements. Londrina, 2018.

R. Clarus	TH	BI	SDW (g)	RDW (g)	PHE (cm)	H1T (cm)	DBT (cm)
Without	Without	Without	75.70	10.92	172.50	43.33	39.22
		With	75.22	11.49	166.50	44.00	37.83
	With	Without	70.47	13.39	159.17	44.00	32.39
		With	84.44	13.01	169.50	45.17	36.94
With	Without	Without	76.68	12.41	167.00	47.33	35.28
		With	76.57	12.66	163.33	46.33	33.11
	With	Without	73.00	13.01	162.50	48.00	32.44
		With	74.45	13.81	158.50	45.33	31.50
Mycorrhiza (A)			0.6042[1]	0.4027	0.2226	0.0829	0.0015
Thermophosphate (B)			0.8544	0.1333	0.1439	0.8000	0.0052
Bioactivator (C)			0.1390	0.5835	0.8014	0.7571	0.9892
A x B			0.3246	0.3958	0.9398	0.7148	0.4278
A x C			0.2231	0.8343	0.3679	0.3562	0.1334
B x C			0.1111	0.9278	0.2320	0.8439	0.0881
A x B x C			0.1973	0.9056	0.2135	0.7148	0.2556

R. clarus (Mycorrhiza); TH (Thermophosphate); BI (Bioactivator).
[1]P-value of Fisher's F test.

Table 2 - Number of ripe fruits per plant (NFPP), fresh mass of ripe fruits (FMRF) and fresh mass of ripe fruits per plant (FMPP) of tomato cultivated under different fertilization systems. Londrina, 2018.

R. clarus	TH	BI	NFPP	FMRF (g)	FMPP (g)
Without	Without	Without	6.83	91.44	640.38
		With	7.00	92.46	704.45
	With	Without	8.83	96.16	827.94
		With	6.33	84.15	574.62
With	Without	Without	8.67	96.23	850.53
		With	8.67	96.14	854.35
	With	Without	8.83	107.45	966.46
		With	9.17	101.11	951.20
Mycorrhiza (A)			0.0782[(1)]	0.0289	0.0228
Thermophosphate (B)			0.5704	0.3271	0.3454
Bioactivator (C)			0.5704	0.3155	0.4508
A x B			0.8496	0.2491	0.7981
A x C			0.4500	0.9105	0.5520
B x C			0.5082	0.2565	0.4578
A x B x C			0.3960	0.8106	0.4391

R. clarus (Mycorrhiza); TH (Thermophosphate); BI (Bioactivator).
[(1)]P-value of Fisher's F test.

(SARLIKIOTI et al., 2011). Variation on this variable may indicate differences in nutritional balance according to treatments. Higher internodal spacing may favor light penetration in crop dossel. In opposition, excessive high internodal spacing may bring about plant stapling and yield reduction (PAPADOPOULOS & ORMROD, 1990) due to allocation of photoassimilates to stem elongation rather than fruit growth (FINZI et al., 2017). Reported prescription of nitrogen amounts for BRS-Nagai cultivar are, in general, lesser than other cultivars due to its highly efficiency in nitrogen usage (VILLAS-BÔAS & JACON, 2016). Besides unbalanced development, N above real needs enhances proportion of green fruits (WARNER et al., 2004; ELIA & CONVERSA, 2012;), delay flowering and fructification (RASHID et al., 2016) and decreases fruit quality (BÉNARD et al., 2009). Besides the extensive reports on the positive effects on P usage efficiency, AMF may also affect N metabolism of plant. Previously, tomato inoculated with *Glomus mossae* increased nitrate reductase and the glutamine synthetase activity and N content (DI MARTINO et al., 2019). The inoculation with *R. clarus*, may be affecting N content and led balance between vegetative/reproductive development as indicated by lesser trusses spacing.

R. clarus inoculation also increased the fresh mass of ripe fruits both individually and per plant (Table 2). These results corroborated previous studies in which increases of 25% tomato yields were found in plants inoculated with AM fungi in organic farming system (BOWLES et al., 2016) and 50% in a low nutrient soil (DI MARTINO et al., 2019). Increasing tomato yields under low nutrient soil was also reported when AMF was associated with plant growth promoting bacteria (*Pseudomonas* spp.) (BONA et al., 2018). The inoculation of AMF fungi also allowed to decrease the amount of fertilizers without reducing productivity (ZIANE et al., 2017). The AMF *R. clarus* was also successfully used to improve crop performance and the effectiveness of fertilizer applied on soybean crop and to reduce amounts of fertilizers in cotton (CELY et al., 2016; BARAZETTI et al., 2019).

Macronutrients in leaves and petioles did not vary significantly between treatments probably due to nutrients drained by tomato fruits (55%, 54% and 56% for N, P and K, respectively) from the vegetative portion (FAYAD et al., 2002).

Significant reductions on mycorrhizal colonization were observed for treatments fertilized with thermophosphate (Table 3). Despite the low water solubility, previous studies showed that thermophosphates can have high agronomic efficiency when compared to soluble sources of phosphorus, enhancing nutrient available to plants in short periods of time (MACHADO et al., 1983). Otherwise, supplying plants with soluble phosphorus

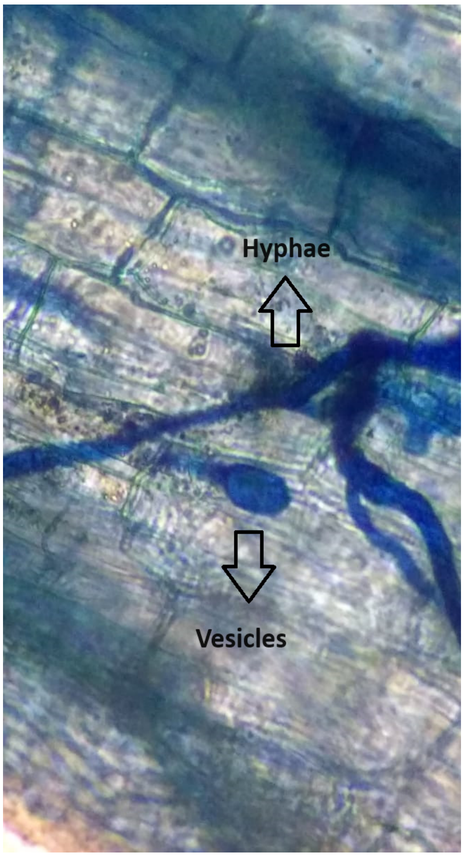

Figure 1 - Structures of *R. clarus* in tomato root.

sources decreases AMF root colonization (WATTS-WILLIAMS & CAVAGNARO, 2012; YANG et al., 2014; KONVALINKOVÁ et al., 2017) while low availability in the soil may increment AMF colonization (BREUILLIN et al., 2010) and enhance root exudation (CARVALHAIS et al., 2010).

Penergetic bioactivator improved microbial activity and AMF colonization (Table 3) as observed previously which incremented yields on sugar beet root, common bean, soybean crops and coffee yields (JAKIENE et al., 2009; COBUCCI et al., 2015; SOUZA et al., 2017; MANTOVANI & FLORENTINO, 2018). However, other studies reported lack of increment in productivity in mayze and soybean yields (ALOVISI et al., 2017), and *Urochloa brizantha* pastures (SILVA et al., 2015). AMF colonization varied among treatments between 60.7% e 71.9%. These values were similar to those obtained in previous studies using *R. clarus* inoculation (LEY-RIVAS et al., 2015) and lesser than other ones in which tomato was inoculated with *G. cubense* (PÉREZ & MARTÍNEZ, 2012), *G. mosseae* e *G. intraradices* (POZO et al., 2002).

CONCLUSION

Tomato inoculated with AMF *R. clarus* improved tomato yield and decreased trusses spacing suggesting a balance development under cultivation

Table 3 - Mycorrhizal colonization (%); N, P, K contents (g kg^{-1}) in leaves and petioles and contents of soluble solids (ºBrix) in tomatoes cultivated under different nutritional managements. Londrina, 2018.

R. clarus	TH	BI	Colonization (%)	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	°Brix
Without	Without	Without	69.25	17.50	5.65	29.19	5.27
		With	75.20	18.08	5.58	26.17	5.32
	With	Without	57.50	17.15	5.80	28.85	5.32
		With	66.50	18.20	5.81	26.84	5.36
With	Without	Without	63.75	16.80	5.78	27.01	5.12
		With	78.88	18.20	5.66	28.52	4.71
	With	Without	52.00	17.97	6.09	24.66	5.31
		With	67.00	16.97	6.35	26.51	5.26
Mycorrhiza (A)			0.7049[1]	0.6785	0.389	0.4482	0.1457
Thermophosphate (B)			0.0217	0.8898	0.257	0.4832	0.1631
Bioactivator (C)			0.0192	0.4373	0.944	0.7685	0.5403
A x B			0.9350	0.9631	0.604	0.4149	0.2772
A x C			0.4546	0.6130	0.868	0.1557	0.3539
B x C			0.9393	0.4373	0.710	0.8138	0.5446
A x B x C			0.7921	0.2592	0.802	0.9062	0.5345

R. clarus (Mycorrhiza); TH (Thermophosphate); BI (Bioactivator).
[1]P-value of Fisher's F test.

with fertilizers allowed in organic agriculture. Thermophosphate inhibited and bioactivator improved AMF root colonization.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

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