

# MICORRHIZAL BIOTECHNOLOGY AS AN ALTERNATIVE TO POTENTIALIZE THE STRAWBERRY QUALITY

## BIOTECNOLOGIA MICORRÍZICA COMO ALTERNATIVA PARA POTENCIALIZAR A QUALIDADE DO MORANGUEIRO

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**ABSTRACT:** The need to develop and use biotechnology tools to improve management and nutritional techniques in strawberry cultivation are increasing. Based on this, the objective of this study was to test if the mycorrhizal inoculation changes the agronomic and qualitative performance of strawberry fruits. The strawberry daughters plants used were of the cultivar Camarosa. The study was carried out in an agricultural greenhouse, Horticulture Sector of the Faculty of Agronomy and Veterinary Medicine (FAMV) of UPF, in the city of Passo Fundo, Rio Grande do Sul (RS), Brazil. The experiment was developed and maintained from June to December 2015. The treatments consisted of five inoculum: T1 = no inoculum (control); T2 = *Acaulospora morrowiae*; T3 = *Rhizophagus clarus*; T4 = mycorrhizal community; T5 = *Claroideoglossum etunicatum*. The experimental design was a randomized block with four replicates. Each plot consisted of a bag containing 6 plants spaced 0.15 mx 0.15 m. In the pits of the plants, it was applied, with the aid of syringe and water, about 70 infective propagules of the tested inocula. During the conduction of the experiment the temperature and photosynthetically active radiation (PAR) were monitored in the culture environment. It was evaluated the agronomic and qualitative performance of the fruits and the percentage of mycorrhizal colonization of the plants roots. The data were submitted to analysis of variance and the differences between means were compared by the Tukey test at 5% of probability error. The cultivar Camarosa produced more fruits in October. In September, the month before the one with the best agronomic performance, the mean temperature was 17.7 °C. The mean PAR recorded in the growing environment was low in relation to crop requirements, throughout the growing period. Fruits produced by plants inoculated with *A. morrowiae* presented higher values of TSS/TTA ratio in September and October. The percentage of mycorrhizal colonization varied from 26.2% for *A. morrowiae* to 46.2% for *R. clarus*. The inoculation of arbuscular mycorrhizal fungi does not alter the production of strawberry fruits. However, when the plants are inoculated with *A. morrowiae* there is an improvement in the fruit flavor.

**KEYWORDS:** *Fragaria X ananassa* Duch. Arbuscular mycorrhizal fungi. Total soluble solids. Titratable total acidity.

### INTRODUCTION

The strawberry (*Fragaria X ananassa*) is a fruit of great economic and nutritional importance, being cultivated in Europe, Asia, North and South America (TORUN et al., 2014). It is appreciated by its juicy texture and nutritive value. In Brazil, among the small fruits, the strawberry is the main cultivated species, with an approximate production of 150 thousand tons, in a planted area corresponding to 4.2 thousand ha (FAGHERAZZI et al., 2017).

Although the strawberry conventional cropping system (soil planting) is still the most used (COSTA et al., 2014) among producing countries,

there is a growing migration to substrate cultivation. However, this system presents drawbacks, such as the high use of pesticides and fertilizers, mainly in where criteria and technical recommendations are not considered (PASZT et al., 2015).

In this way, there is a need to develop and use biotechnology tools to improve water use efficiency (DEPARDIEU et al., 2016) and to reduce the use of chemical fertilizers in fertigation (HARTZ; HOCHMUTH, 1996), which, in addition to having a high cost, have negative consequences for the environment, such as soil contamination, water (eutrophication by excess nutrients) and extraction of nonrenewable resources, such as P

sources, which are limited (ELSER, 2012). One of the alternatives to make the strawberry production system more sustainable, maintaining and/or improving the yield and the qualitative potential of the fruits, is the use of arbuscular mycorrhizal fungi (AMF).

The AMF, representative of the phylum Glomeromycota, are obligate biotrophic microorganisms, which perform mutual symbiotic associations with 80% of the vascular plants, altering the morphology and physiology of the roots (AHANGER et al., 2014). They perform various ecological functions in exchange for host photosynthetic carbon (WILLIS; RODRIGUES; HARRIS, 2013). The benefit to the plant stems is the increase of the area of mycorrhizal roots exploration by means of hyphae network (GUTJAHR, 2014). The process of nutrient exchange between plant and fungus occurs in the arbuscules and depends on environmental and biological variables (MORCILLO et al., 2016).

The AMF have the potential to greatly improve the rhizospheric soil characteristics, improving plant growth and development (AHANGER et al., 2014; NAVARRO; PEREZ-TORNERO; MORTE, 2014) by means of increased water and nutrient uptake (GUTJAHR, 2014; GÓMEZ-BELLOT et al., 2015). The symbiosis between AMF and strawberry plants benefits yield (MARTINEZ; WEILAND; PALENCIA, 2013) and fruit quality (CECATTO et al., 2016; CHIOMENTO et al., 2019). The use of AMF can reduce the use of phosphate fertilizers in vegetables (ELBON; WHALEN, 2015), as is the case with strawberries. However, the studies that evaluate the maintenance and/or improvement in fruit production and quality of this species are still incipient. Thus, the hypothesis of the study is that, in addition to the AMF potentiate the absorption mainly of phosphorus present in the materials used as substrate, already described in the literature (SHARMA; ADHOLEYA, 2004), production gains and fruit quality can be visualized.

This study has relevance since this fruit is marketed worldwide. Therefore the objective of the study was to test if the mycorrhizal inoculation changes the agronomic and qualitative performance of strawberry fruits.

## MATERIAL AND METHODS

### Plant material and experiment site

The strawberry daughters plants used was from the Camarosa cultivar, obtained from *in vitro*

cultivation (micropropagation) carried out at the Plant Biotechnology Laboratory at the University of Passo Fundo (UPF). After micropropagation, they were planted in polyethylene trays of 72 cells, filled with sterilized sand and acclimatized in an agricultural greenhouse for one month.

The study was carried out in an agricultural greenhouse of 430 m<sup>2</sup>, with a semicircular roof, installed in the northwest-southeast direction, located in the Horticulture Sector of the Faculty of Agronomy and Veterinary Medicine (FAMV) of the UPF, in the municipality of Passo Fundo (28°15'46" S; 52°24'24" W), Rio Grande do Sul (RS), Brazil. The structure was made of galvanized steel and covered with low density polyethylene (LDPE) film, with anti-glare additive and with a thickness of 150 microns. The experiment was developed and maintained from June to December 2015.

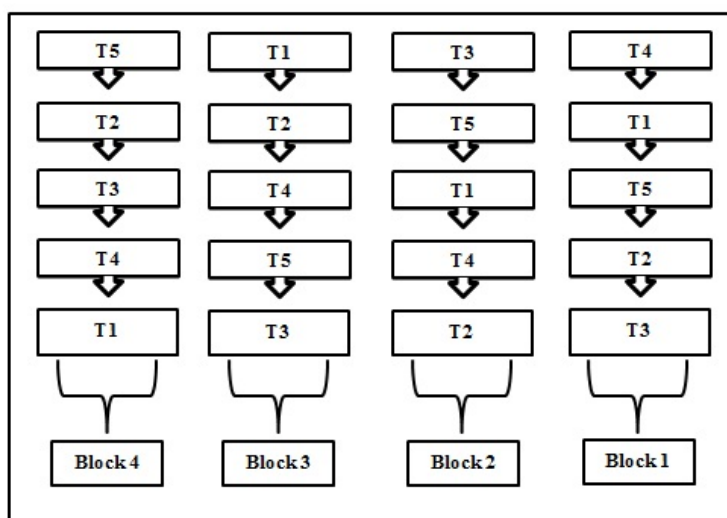
### Treatments and experimental design

The treatments consisted of five inoculum: T1 = no inoculum (control); T2 = *Acaulospora morrowiae* Spain & Schenck; T3 = *Rhizophagus clarus* (T.H. Nicolson & N.C. Schenck) C. Walker & A. Schüßler; T4 = mycorrhizal community; T5 = *Claroideoglossum etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler. The experimental design was a randomized block with four replicates. Each plot consisted of a bag containing 6 plants spaced 0.15 m x 0.15 m.

### Procedures

After acclimatization, the strawberry daughters plants were transplanted in bags 0.5 m long by 0.3 m wide, filled with Mec Plant Horta 2<sup>®</sup> substrate, arranged horizontally on the ground according to the sketch of the experiment (Figure 1).

The isolates of AMF (*A. morrowiae*, *R. clarus* and *C. etunicatum*) were obtained from the International Collection of Glomeromycota Cultures (CICG), located at the Regional University of Blumenau (FURB), Santa Catarina (SC), Brazil. The mycorrhizal community was obtained from soil collected at the strawberry cultivation site in the UPF, Horticulture Sector. In the first year of cultivation (2014), the plants served as matrices for the production of new strawberry daughters plants. After this period, they were subjected to drastic cleaning (removal of all leaves) to start the present study.



**Figure 1.** Configuration of the experimental study: T1 = no inoculum (control); T2 = *A. morrowiae*; T3 = *R. clarus*; T4 = mycorrhizal community; T5 = *C. etunicatum*.

Samples were collected of the substrate for chemical analysis (Table 1). Throughout the crop evaluation period, irrigation was distributed through a drip system at the spacing of 0.3 x 0.3 m.

According to the substrate chemical analysis, it was not necessary to administer fertigation during the growing period.

**Table 1.** Substrate chemical analysis.

Clay (%)	pH <sup>1</sup> H <sub>2</sub> O	SMP index	P K		OM (%)	Al Ca Mg H+Al CEC					Saturation				
			mg dm <sup>-3</sup>			cmol <sub>c</sub> dm <sup>-3</sup>					Bases Al K				
													%		
32.4	6.0	6.2	>51	493	>6.7	0	10.4	4.9	3.5	20.0	83	0	6.3		

<sup>1</sup>pH H<sub>2</sub>O: hydrogen potential in water; SMP index: Shoemaker-Mac'Lean-Pratt index; P: phosphorus; K: potassium; OM: organic matter; Al: aluminum; Ca: calcium; Mg: magnesium; H+Al: potential acidity; CEC: cation exchange capacity.

### Mycorrhizal inoculation

The inoculation of the AMF was carried out shortly after the cleaning of the plants. Using a syringe, they were applied in the pit, approximately 70 infective propagules of each isolate and mycorrhizal community that were diluted in 10 ml of water. The application of the inocula occurred according to the distribution of the treatments.

### Microclimate

During the conduction of the experiment the temperature and photosynthetically active radiation (PAR) were monitored in the culture environment. For this, a meteorological mini-station was used, located inside the agricultural greenhouse, from which the data were extracted.

### Agronomic performance

In the fruit yield, the number (total and commercial) and weight (total and commercial) of fruits per plant were evaluated weekly. Commercial fruits were those weighing more than 6 g and devoid of injuries.

### Qualitative performance

The chemical quality of the fruits was based on the evaluation of the total soluble solids (TSS) content, expressed in °brix, and the titratable total acidity (TTA), expressed in % of citric acid. The TSS content was determined using an analog refractometer, while the TTA was performed according to the standards of the Adolfo Lutz Institute (IAL, 2008). To evaluate the taste of the fruits, the TTS/TTA ratio was determined.

### Mycorrhizal colonization

To determine the percentage of mycorrhizal colonization of the plants' roots, at the end of the experiment, 1 g of root per plant was collected. They were washed in running water, then clarified in 10% KOH, acidified with 1% HCl, colored with 0.05% tripan blue and preserved in lactic acid (PHILLIPS; HAYMAN, 1970). The percentage of segments colonized by mycorrhizae was determined according to Trouvelot, Kouch and Gianinazzi-Pearson (1986) by the formula: % MC = ((total number of fragments with mycorrhized roots)/(total number of fragments)) x 100. The segments that

presented hyphae (external and internal), vesicles or arbuscules were considered colonized.

**Data analysis**

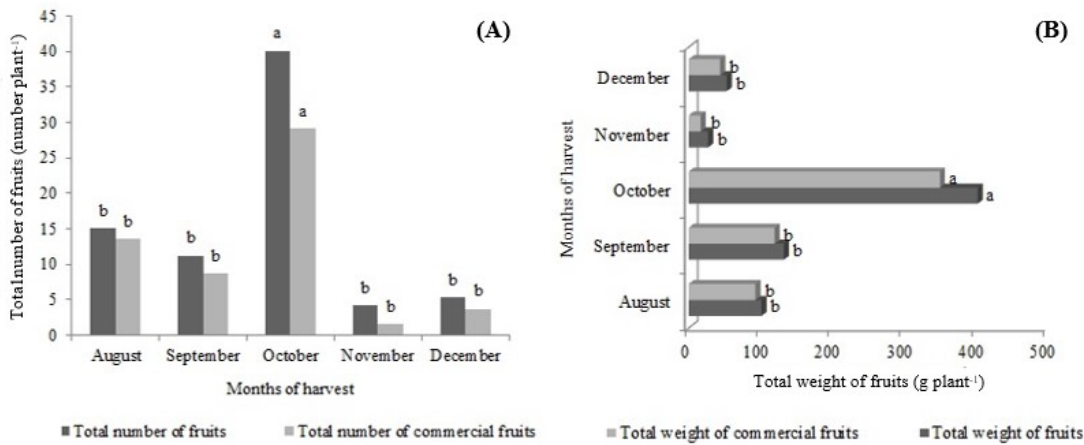
For fruit yield, the harvests were grouped according to five harvesting periods (months) constituting a bifactorial (5 inocula x 5 months of harvest). For fruit quality, the quantification of the TSS/TTA ratio also corresponded to a bifactorial (5 inocula x 4 months of harvest).

All data, including the percentage of mycorrhizal colonization of the plants' roots, were submitted to analysis of variance (Anova) and the

differences between means were compared by the Tukey test at 5% of probability error.

**RESULTS**

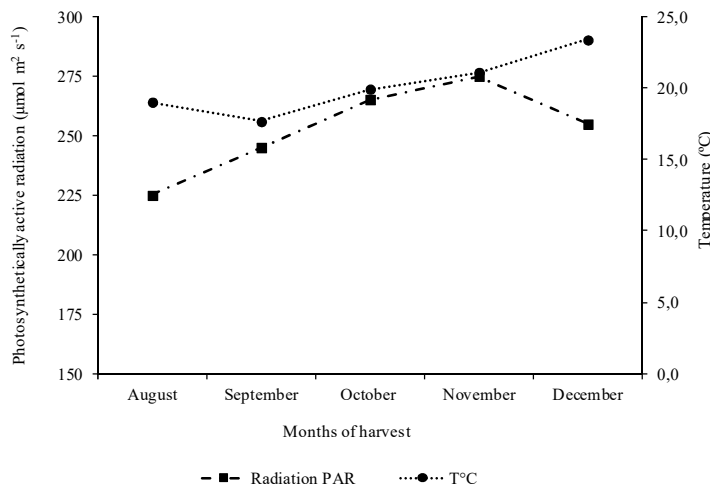
The cultivar Camarosa produced the largest number of fruits in October, with a total and commercial number of fruits of 40.1 g plant<sup>-1</sup> and 29.1 g plant<sup>-1</sup>, respectively (Figure 2A). Considering strawberry production, it was also in October that the highest yield was obtained, with a total and commercial weight of fruits of 402.1 g plant<sup>-1</sup> and 349.4 g plant<sup>-1</sup>, respectively (Figure 2B).



**Figure 2.** Total and commercial number of fruits, in number plant<sup>-1</sup> (A); total and commercial weight of fruits, in g plant<sup>-1</sup> (B). Means followed by the same lowercase letter do not differ by the Tukey test at 5% of error probability (p≤0.05, n = 4).

In September, the month preceding the one with the best agronomic performance, the average temperature was 17.7°C (Figure 3). From this period, the average temperatures inside the environment were higher. The average PAR

recorded in the growing environment (Figure 3) was considered low in relation to strawberry cultivation requirement. In October, the month of the greatest fruit production, the average PAR was higher (268.2 μmol m<sup>2</sup> s<sup>-1</sup>) in comparison to the previous months.



**Figure 3.** Averages of PAR and temperature recorded in the environment during the harvest period.

Considering the quality of the fruits harvested, there was interaction between inocula and harvest months for TSS/TTA ratio (Table 2). In the interaction, considering the months within each inoculum, October was highlighted with higher value for this relation (10.6) for plants colonized by *A. morrowiae*. Considering the inocula within each

month, fruits produced by plants inoculated with *A. morrowiae* showed higher values of the TSS/TTA ratio in September and October (11.6 and 10.6, respectively) and September for mycorrhizal community and *C. etunicatum* (9.2 and 10.6, respectively).

**Table 2.** TSS/TTA ratio of the Camarosa cultivar submitted to different AMF treatments.

Treatments	Harvest months			
	September	October	November	December
Control	10.3 aA	8.4 bA	7.9 aA	6.5 aA
<i>A. morrowiae</i>	11.6 aA	10.6 aA	6.8 aB	6.5 aB
<i>R. clarus</i>	11.4 aA	8.5 bA	5.9 aA	5.5 aA
Mycorrhizal community	9.2 aA	6.0 cB	6.0 aB	5.2 aB
<i>C. etunicatum</i>	10.6 aA	8.7 dAB	5.2 aB	5.3 aB
Mean	10.6	8.5	6.3	5.8
Coefficient of variation (%)	20.6	4.4	12.3	17.0

Means followed by the same lowercase letter in the column and upper case in the line do not differ by the Tukey test at 5% error probability ( $p \leq 0.05$ ,  $n = 4$ ).

Mycorrhizal colonization (MC) of the strawberry roots did not differ between treatments (Table 3). At the end of cultivation the plants' roots

inoculated with *A. morrowiae* presented only 26.2% of colonization, while *R. clarus*, 46.2%.

**Table 3.** Anova referring to mycorrhizal colonization (MC) of strawberry roots, inoculated with AMF.

Causes of variation	Degrees of freedom	Mean square
		MC (%)
Blocks	3	393.64 <sup>ns</sup>
Inocula	4	212.50 <sup>ns</sup>
Error	12	
Total	19	

<sup>ns</sup>: not significant at 5% error probability ( $p \leq 0.05$ ,  $n = 4$ ).

## DISCUSSION

The inoculation of AMF did not alter the agronomic performance of strawberry. However, it provided an improvement in the quality of the fruits of the Camarosa cultivar (Table 2).

In the month (September) that preceded the highest productive expression (October), the average temperature (17.7 °C) recorded in the environment (Figure 3) was considered optimal for floral induction (18°C) and fruit production (24°C) (OPSTAD et al., 2011; SØNSTEBY; HEIDE, 2008; WANG; CAMP, 2000), which was confirmed by the best results obtained in October. From this period, the average temperatures inside the environment were higher, mainly affecting the induction and differentiation of vegetative buds in floriferous, compromising the agronomic performance of the plants. It is worth mentioning that the sensitivity of plants to high temperatures varies with their size and age. Allied to temperature,

the PAR is recorded in the growing environment (Figure 3). Temperature and solar radiation are primary factors in the control of growth and development of strawberry plants (PALENCIA et al., 2013).

In October, the PAR was higher (268.2  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) compared to the months preceding that period, but still below the range considered ideal for floral induction and, consequently, fruit production (400 to 450  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). The intensity and quality of light are important for strawberry production. The production of flowers and fruits can be improved with light intensity between 400 and 450  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of PAR (KIRSCHBAUM, 1998). The quality and intensity of light is of particular importance for the different strawberry cultivars, being the necessary stimulus for the floral evocation, induction and differentiation of the meristematic regions, determining the activation of specific genes (SOC1 and LFY) (HEIDE; STAVANG; SØNSTEBY, 2013; JUNG et al., 2012) responsible by the

formation of floral whorls (CORBESIER; COUPLAND, 2005; TAIZ et al., 2017), also helping, in fruit maturation, which is the main index for classification and harvesting (XU; ZHANG, 2007). It is inferred that the quality of the light, together with the elevation of the temperature in the environment from October may have negatively affected the flowering process, thus compromising the formation and development of the fruits.

Considering the qualitative performance of the strawberry fruits of the cultivar studied, the inoculation with AMF showed interference in the attribute used to determine the fruit flavor (TSS/TTA ratio). Ideal values of this relationship which determines the taste are found when the titratable acidity quantification is between 0.5 to 1.87% and the total soluble solids content (°brix) between 5 and 12 (PERKINS-VEAZIE; COLLIN, 1995). With these values, the fruits present good palatability and acceptance.

In this study, it was understood that the inoculation in the strawberry plants with AMF configured plant stress by the low percentage of mycorrhizal colonization found in the roots, regardless of the inoculum, diverting the carbon that would be destined to the formation of biomass for the production of compounds of the secondary metabolism (such as anthocyanins and polyphenols) in an attempt, at first, to defend against the fungus. Depending on the environmental conditions such as moderate temperatures around 20°C, sugar is released by the degradation of these compounds, determining an increase in sugar content in fruits (FRANCIS, 1989).

The accumulation of sugar in the fruits is higher when the fruit develops at lower temperatures (KIMURA, 2008) confirming higher TSS/TTA ratios in the month of October, since the month before that time was the fruit development period. The higher the TSS/TTA ratio, the more acceptable the consumer is and the tastier the fruit.

The low mycorrhizal colonization (26.2% for *A. morrowiae* and 46.2% for *R. clarus*) presented by the plants' roots may also be related to the temperatures recorded in the environment (Figure 3). The ideal temperature range is variable with the adaptation of the fungal species. In general, at temperatures around 30°C there is an increase in the mycorrhizal colonization and sporulation by AMF (SILVEIRA, 1998), which would potentiate root colonization. In the present study mean temperatures throughout the growing season were below this range.

The pH of the substrate used to fix the plants' roots is also one of the factors that influence

the plant/fungus association. Low pH values favor species of the genus *Acaulospora* whereas species of the genus *Claroideoglomerum* prefer pH close to neutrality (TRUFEN, 1990). This fact was confirmed in our study because plants inoculated with the *Acaulospora* isolate had a lower colonization rate (26.2%) while the isolate *Claroideoglomerum* had a higher value (46.2%) because the pH of the substrate used was 6.0 (Table 1).

The phosphorus content is another factor that may have negatively affected the mycorrhizal colonization of the roots of the inoculated plants. For the substrate used presented >51 mg dm<sup>-3</sup> of phosphorus (Table 1). It is known that the higher the phosphorus content lower the possibility of colonization, consequently the mycorrhizal colonization tends to be low (SMITH; READ, 2008). In conditions of low levels of phosphorus, the occurrence of mycorrhizal association provides better use of phosphate fertilizers and benefits in obtaining nutrients (JOBIM, 2015). Thus, temperature, pH and phosphorus levels directly influenced the infection capacity of the AMF used, and the plants inoculated with *A. morrowiae* were the most impaired, where colonization of the plants was stressful, increasing the TSS/TTA ratio, resulting in flavored fruits more acceptable by the consumer market.

Studies that investigate the conservation strategy so that biological resources can be approved in some way to increase the production or quality of natural products, from a perspective of sustainable development (JOBIM; OLIVEIRA; GOTO, 2016) should be incentives, such as the applicability of AMF.

## CONCLUSIONS

The inoculation of AMF does not alter the production of strawberry fruits.

In addition, we verified that when the plants are inoculated with *A. morrowiae* there is improvement in the taste of the fruits of the Camarosa cultivar. Studies that seek to develop biotechnological tools to improve the production and quality of fruits, besides potentiating the use of a biological resource, as is the case of arbuscular mycorrhizal fungi deserve to be highlighted and further investigations.

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**RESUMO:** A necessidade de desenvolver e usar ferramentas biotecnológicas para melhorar o manejo e as técnicas nutricionais no cultivo do morangueiro está aumentando. Com base nisso, o objetivo desse estudo foi testar se a inoculação micorrízica altera o desempenho agrônômico e qualitativo de frutos de morangueiro. As mudas de morangueiro usadas foram da cultivar Camarosa. O estudo foi realizado em estufa agrícola, no Setor de Horticultura da Faculdade de Agronomia e Medicina Veterinária (FAMV) da UPF, na cidade de Passo Fundo, Rio Grande do Sul (RS), Brasil. O experimento foi desenvolvido e mantido de junho a dezembro de 2015. Os tratamentos consistiram em cinco inóculos: T1 = sem inóculo (controle); T2 = *Acaulospora morrowiae*; T3 = *Rhizophagus clarus*; T4 = comunidade micorrízica; T5 = *Claroideoglossum etunicatum*. O delineamento experimental foi em blocos casualizados, com quatro repetições. Cada parcela consistiu de um saco contendo 6 plantas espaçadas de 0.15 mx 0.15 m. Na cova de plantio das plantas, foi aplicado, com auxílio de seringa e água, cerca de 70 propágulos infectivos dos inóculos testados. Durante a condução do experimento, a temperatura e a radiação fotossinteticamente ativa (PAR) foram monitoradas no ambiente de cultivo. Foi avaliado o desempenho agrônômico e qualitativo dos frutos e a porcentagem de colonização micorrízica das raízes das plantas. Os dados foram submetidos à análise de variância e as diferenças entre médias foram comparadas pelo teste de Tukey, a 5% de erro de probabilidade de erro. A cultivar Camarosa produziu mais frutos em outubro. Em setembro, no mês anterior ao de melhor desempenho agrônômico, a temperatura média foi de 17.7°C. A PAR média registrada no ambiente de cultivo foi baixa em relação às necessidades da cultura durante todo o período de cultivo. Frutos produzidos por plantas inoculadas com *A. morrowiae* apresentaram maiores valores da relação SST/ATT em setembro e outubro. A porcentagem de colonização micorrízica variou de 26.2% para *A. morrowiae* a 46.2% para *R. clarus*. A inoculação de fungos micorrízicos arbusculares não altera a produção de frutos de morangueiro. No entanto, quando as plantas são inoculadas com *A. morrowiae*, há uma melhora no sabor dos frutos.

**PALAVRAS-CHAVE:** *Fragaria X ananassa* Duch. Fungos micorrízicos arbusculares. Sólidos solúveis totais. Acidez total titulável.

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