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Yeast-mycorrhizae interaction as a strategy to improve tomato production

La interacción levadura-micorriza como estrategia para la producción de tomate

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Abstract

Inoculation with plant growth-promoting microorganisms such as bacteria, mycorrhizal fungi, and soil yeasts may play a promising role in sustainable plant production. This study evaluated the potential of Patagonian yeasts and mycorrhizal fungi to enhance the growth and productivity of tomato plants (*Lycopersicum esculentum* var. *platense*) during the production season in Patagonia. A greenhouse experiment was conducted, where plants were inoculated with the mycorrhizal fungus *Funneliformis mosseae* and the yeasts *Candida saitoana*, *Saccharomyces eubayanus*, or *Tausonia pullulans*. None of the 45-day-old seedlings exhibited mycorrhizal colonization, although *F. mosseae* inoculation significantly influenced seedling growth. By the end of the production season (135-day-old plants), all plants showed mycorrhizal colonization, and those inoculated with *F. mosseae* demonstrated increased plant growth and yield. Inoculation with *S. eubayanus* enhanced both plant yield and mycorrhizal colonization. Conversely, co-inoculation with *T. pullulans* and *F. mosseae* was detrimental to mycorrhizal colonization.

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Nevertheless, *Tausonia pullulans* independently improved plant growth and yield, suggesting that this yeast may benefit tomato production without relying on mycorrhizal associations. These findings highlight the complex interactions between mycorrhizal fungi and soil yeasts in agronomic systems.

Keywords: *Funneliformis mosseae*; *Candida saitoana*; *Saccharomyces eubayanus*; *Tausonia pullulans*; sustainable agriculture.

Resumen

La inoculación con microorganismos promotores del crecimiento vegetal, como bacterias, hongos micorrícicos y levaduras del suelo, puede tener un rol prometedor en la producción agrícola sostenible. Este estudio evaluó el potencial de hongos micorrícicos y levaduras patagónicas para mejorar el crecimiento y la productividad de plantas de tomate (*Lycopersicum esculentum* var*. platense*) durante la temporada de producción en Patagonia. Se llevó a cabo un experimento en invernadero, en el cual las plantas fueron inoculadas con el hongo micorrícico *Funneliformis mosseae* y las levaduras *Candida saitoana, Saccharomyces eubayanus* o *Tausonia pullulans*. Ninguna de las plántulas de 45 días presentaban colonización micorrícica, aunque la inoculación con *F. mosseae* influyó significativamente en el crecimiento de las plántulas. Al final de la temporada de producción (plantas de 135 días de edad), todas las plantas presentaron colonización micorrícica, y aquellas inoculadas con *F. mosseae* mostraron un aumento en el crecimiento y el rendimiento. La inoculación con *S. eubayanus* mejoró tanto el rendimiento de las plantas como la colonización micorrícica. En contraste, la co-inoculación con *T. pullulans* y *F. mosseae* resultó perjudicial para la colonización micorrícica. Sin embargo, *T. pullulans* mejoró de forma independiente el crecimiento y el rendimiento de las plantas, lo que sugiere que esta levadura puede beneficiar la producción de tomate sin depender de las asociaciones micorrícicas. Estos resultados destacan las complejas interacciones entre hongos micorrícicos y levaduras del suelo en sistemas agronómicos.

Palabras clave: *Funneliformis mosseae; Candida saitoana; Saccharomyces eubayanus; Tausonia pullulans*; agricultura sustentable.

INTRODUCTION

Addressing the global challenge of food security is imperative, and the increase in food production must go hand in hand with the implementation of safe practices for people and the environment (Wezel, 2014). Therefore, when considering sustainable practices in agricultural production, plant growth promoting microorganisms (PGPM) arise as a biotechnological tool to reduce the use of chemical fertilizers and enhance food production.

Several reports support a promising role of yeasts as PGPM due to the production of plant's growth regulators and, indirectly, as result of the interaction with symbiotic microorganisms (Carvajal *et al.*,2024; Mohamed, 2015; Nimsi *et al.*, 2023; Radić *et al.*, 2022; Vargas *et al.*, 2024). The symbiotic association between arbuscular mycorrhizal fungi (AMF) and plants is the most widespread symbiosis in the plant kingdom (Smith and Read, 2008) and it is relevant for plant development and production (Van Der Heijden and Horton, 2009). Inoculation with AMF is been explored as an environmentally friendly practice in agriculture (Aguilera *et al.*, 2021; Dejana *et al.*, 2022), while the co-inoculation of AMF and other PGPM is not so widespread. The effect of yeasts on mycorrhizal colonization is not well understood yet; several publications reported enhanced (Fracchia *et al.*, 2003; Sampedro *et al.*, 2004; Sarabia *et al.*, 2018), neutral (Sarabia *et al.*, 2018) or decreased (Gollner *et al.*, 2006) mycorrhizal colonization of roots. It is of particular interest to further investigate the yeast-AMF-plant interaction, as yeasts could provide new strategies for sustainable agriculture.

A large number of yeasts were isolated from cold-temperate *Nothofagus* native forests in Patagonia (Mestre *et al.*, 2011, 2014; Fernández *et al.*, 2012). These indigenous soil yeasts are considered psychrotolerant, as they are periodically exposed to low temperatures: mean annual temperature of 10 °C, winter temperatures are often below 0 °C with precipitation in the form of snow (Mestre *et al.*, 2016). Several of the isolated yeasts presented PGPM features such as phytohormone's production, phosphate solubilization and plant-pathogen inhibition (Mestre *et al.*, 2016). In Western-Patagonia, agriculture practices are closely related to native forest due to the closeness of production sites to the forest and the use of the surrounded forest soil and litter in several steps of production. Here, the agriculture is lead by seasonal dynamics due to cold and short light-period during winter. Some times in economically important crops, such as tomato and pepper, tunnel greenhouse without temperature control are used to complete fruit maturity (Venegas Jaque *et al.*, 2021). The use of native cold adapted yeasts with PGPM features could be an attractive strategy to promote sustainable agriculture in Patagonia and other cold-temperate regions.

Tomato is an economically important horticultural crop worldwide and in Patagonia; and it also has been used as model plant in several PGPM-plant studies. In the present study, we aim to evaluate the potential use of Patagonian yeasts and mycorrhizal fungus to enhance growth and productivity of tomato (*Lycopersicum esculentum* var. platense) plant during the production season in Patagonia. The guiding hypothesis of the study was that yeast inoculation would improve tomato growth and yield, as well as enhance mycorrhizal colonization, with a synergistic effect expected from the combined yeast and mycorrhizal inoculation.

MATERIAL AND METHODS

Mycorrhizal and yeast inoculum preparation

The arbuscular mycorrhizal fungus used as inoculum was *Funneliformis mosseae* (ex *Glomus mosseae*) gently supplied by Dr. J. A. Ocampo. Mycorrhizal fungus was contained in a soil inoculum, produced under greenhouse conditions from trap plants at Estación Experimental del Zaidín, CSIC (Spain), and included colonized root fragments, fungal mycelia and spores (with approximately 110 sporocarps per 10 g inoculum). The mycorrhizal inoculum was added to one-half of seedling production substrate in a 2 % W/V ratio, before planting the germinated seed.

The yeast used as inoculum were *Tausonia pullulans* CRUB1772, *Candida saitoana* CRUB1770 and *Saccharomyces eubayanus* CRUB2014; they have already been proposed as plant growth promoters (Mestre *et al.*, 2016; Mestre *et al.*, 2021). The three yeast strains are deposited in the Yeast Collection of the Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, Argentina.Each yeast strains was cultivated on solid MYP medium (Malt extract 7 g L^{-1}, Yeast extract 0.5 g L^{-1}, Soy peptone 2.3 g L^{-1}, Agar 15 g L^{-1}) at 20 °C for 72 h. Biomass from cultures was harvested and then suspended in peptone water (Soy peptone 1% W/V) up to a turbidity of 0.3 at 600 nm (equivalent to 10^6 cells mL^{-1}). Nine mL of cell suspension from each yeast or peptone water without yeast were used to inoculate early stages of tomato seedlings production.

Plant production experiment

Lycopersicum esculentum var. platense plants, a commercially important variety of tomato in Argentina, were produced following an experimental design with two factors: mycorrhizal inoculation (with or without mycorrhizal inoculum) and yeast inoculation (with one of the three yeast species or without yeasts). We combined control conditions for seedling production -sterile substrate-, with conditions that mimics common production technique -use of natural non-sterile soil and water. By completing tomato production season we were able to evaluate plant growth as well as plant yield.

Seedling production

Tomato seeds were surface disinfected by immersion in 20% sodium hypochlorite for 2 min, followed by triple washing with sterile water. Disinfected seeds were germinated in a sterilized moistened filter paper under controlled conditions (16 hours light at 25ºC, 8 hours darkness at 22ºC) for 4 days. Germinated seeds were individually planted in alveolar trays with tyndallized commercial organic amendment (Lombriquen®), with or without mycorrhizal inoculum. We produced 192 tomato seedling, by using 12 replicates in each of the 8 treatments in the experiment. All seedlings were incubated under controlled conditions (10 hours darkness at 20°C, 14 hours light at 28°C) and periodically watered with sterile tap water.

When the first true leaves appeared in tomato seedlings (20 days after germination), the yeasts were inoculated at the base of the shoot of seedling growing in substrate with *F. mosseae* (co-inoculation treatments) and without the mycorrhizal fungus inoculum (single inoculation treatments). Seedling were transplanted 45 days after germination.

Seedling recorded variables: Half of the seedling (96) were harvested to evaluated seedling quality using destructive determinations. We recorded shoot length, root and aerial biomass (material was harvested separately and dried at 90 °C to constant weight in an oven) and stem diameter. Slenderness index (SI) was calculated as the rate between shoot length and stem diameter, $cm.mm^{-1}$ (Gallegos-Cedillo *et al.*, 2021). A fraction of the seedling roots were collected and preserved in 70% alcohol for fungal colonization determination.

Tomato plant growth and yield evaluation

The 45 day-old seedlings (96) were placed manually into 7-litre pots (one seedling per pot) containing a non-sterile mixture of perlite, peat and soil in a 1:1:2 ratio. The soil was sourced from the vicinity of the greenhouse, and it was used as a source of nutrients and natural microorganisms (including native mycorrhizae). The pots were transferred to the greenhouse and randomly distributed on greenhouse benches. The greenhouse was equipped with air conditioning to prevent room-temperature from dropping below 10°C during early stages of cultivation, as nighttime temperatures during late spring and early summer, could approach 0°C. In summer, during flowering and fruiting, temperature was regulated using a combination of fogging and ventilation systems to keep the maximum temperature under 40°C. Plant were watering using a drip irrigation system, and water regime was adjusted based on plant requirement throughout the production season. Weed control was carried out every 15 days and axillary buds were eliminated to simulate productive conditions. The plants grew in the greenhouse until harvest (3 month after transplant) to complete the production season in Patagonia.

Adult plant recorded variables: We harvested complete adult plants (135 day-old) to record aerial and root biomass (as describe above); we also recorded the number and weight of fruits per plant. We calculated the probability of fruit production per treatment by comparing plant with fruit (1) vs. plant without fruit (0) within each treatment. We measured chlorophyll concentration in plant leaves by optical density difference at 660 nm vs. 940 nm, usually known as SPAD (Soil Plant Analysis Developmental), using Clorofilio® (Cavadevices). For each plant, an average chlorophyll value was calculated from three leaves (one apical, one intermediate, and one basal). SPAD readings are positively correlated with chlorophyll concentration in tomato leaves (Jiang *et al.*, 2017). A portion of roots was collected and preserved in 70% alcohol for fungal colonization determination.

Fungal colonization determination

We determined fungal colonization in seedling and adult plant roots using the non-vital Trypan Blue histochemical staining procedure (Phillips and Hayman, 1970). Stained roots were observed under optical microscope (Olympus BX40) and the presence of arbuscules was used as diagnostic for arbuscular mycorrhizal colonization (AM). For quantification of fungal colonization, stained root fragments of 1 cm length were placed on a slide and observed with 200x magnification, resulting in the observation of at least 300 fields per plant. Percentage of mycorrhizal colonization (AM%) was estimated using the intersect method described by McGonigle *et al.* (1990).

Statistical Analysis

Data from seedlings (45 day-old) and adult plants (135 day-old) were separately analysed. We used two-way ANOVA for seedling's slenderness index, seedlings dry biomass, and for adult plants dry biomass and chlorophyll concentration. The variable AM% was transformed with arcsin's square-root prior to statistical analysis using two-way ANOVA. Tukey´s post-hoc tests were used to form homogenous groups when necessary. We used a generalized linear model with binomial distribution to analyse the probability of fruit production per treatment. All statistical analyses were performed with *agricolae* (de Mendiburu, 2020), *car* (Fox and Weisberg, 2019) and *DHARMa* 0.4.6 (Hartig, 2020) packages on R software version 4.4.0 (2024-04-24 ucrt); differences at $p < 0.05$ were considered significant.

RESULTS

Quality of the 45 day-old seedlings was assessed using total dry biomass and slenderness index (SI). None of these variables showed significant interactive effects between the inoculation with yeast and the inoculation with *Funneliformis mosseae* ($F_{Biomass}=2.28$, $p_{Biomass}=0.08$; $F_{SI}=2.25$, $p_{SI}=0.08$). Total plant biomass showed significant differences for mycorrhizal inoculation as main effect ($F=37.9$, $p=2\cdot10^{-8}$; Fig. 1): plant with *F. mosseae* inoculum were smaller (about 40% less biomass) than plant without the mycorrhizal inoculum. Total plant biomass showed no significant differences for yeast inoculation ($F=0.62$, $p=0.6$; Fig.1). Slenderness index showed significant differences for each factor as main effect (Fig. 1). Seedlings without *F. mosseae* inoculum showed lower SI than those with the mycorrhizal inoculum ($F=5.57$, $p=0.02$; Fig 1). Seedlings inoculated with *T. pullulans* or *C. saitoana* showed lower SI than those inoculated with *S. eubayanus* ($F=3.9$, $p=0.01$; Fig 1). The 45 days-old seedlings did not show any fungal colonization in their roots.

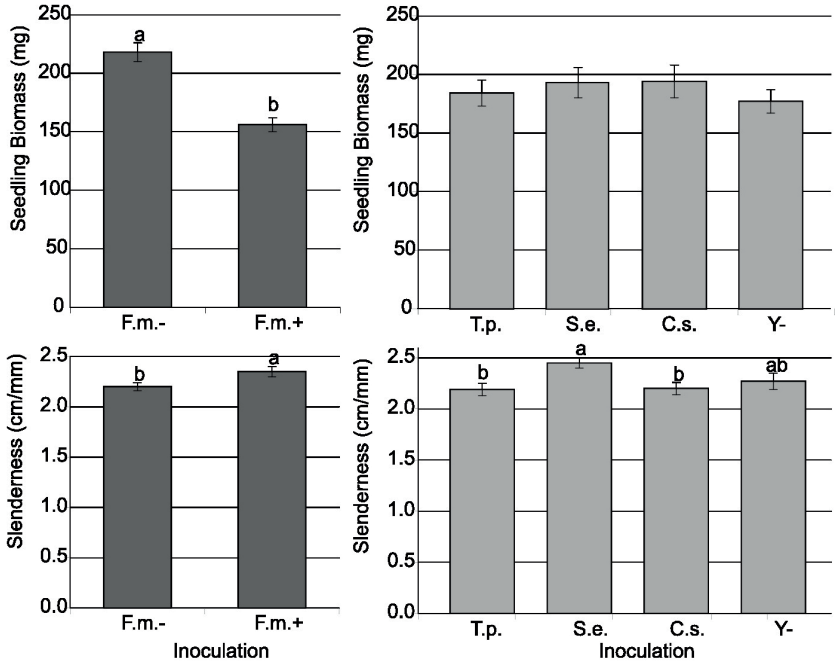

Fig. 1. Quality indicators for tomato seedling (45-days-old). Statistical significance was determined with $\alpha=0.05$ using two-way ANOVA. Treatments with the same letter are not significantly different. F.m.+: with *Funneliformis mosseae* inoculum; F.m.-: without *Funneliformis mosseae* inoculum; C.s.: *Candida saitoana* CRUB1770; S.e.: *Saccharomyces eubayanus* CRUB2014; T.p.: *Tausonia pullulans* CRUB1772; Y-: without yeast inoculum.

Fig. 1. Indicadores de calidad de las plántulas de tomate (45 días). La significancia estadistica se determinó con un $\alpha=0,05$ usando un ANOVA de dos factores. Los tratameintos con la misma letra no son significativamente diferentes. F.m.+: inoculado con *Funneliformis mosseae*; F.m.-: sin inoculo de *Funneliformis mosseae*; C.s.: *Candida saitoana* CRUB1770; S.e.: *Saccharomyces eubayanus* CRUB2014; T.p.: *Tausonia pullulans* CRUB1772; Y-: sin inóculo de levaduras.

At final harvest (135 day-old plants), plant dry biomass did not show significant interactive effects between the inoculation with yeasts and the inoculation with *F. mosseae* (F=0.25, p=0.85), nor significant effects of yeast inoculation (F=1.59, p=0.19). The plants which received *F. mosseae* inoculum (with and without yeast) have significantly (F=24.08, $p=4\cdot10^{-6}$) higher biomass (42%) than those without the inoculum (with and without yeast; Fig. 2). The plants singly inoculated with *F. mosseae* (F.m.) showed 64% increase in biomass respect the plants without any inoculation (Fig. 2). We observed significant interactive effects (F=5.07, p=0.002) of yeasts and mycorrhizal inoculation for chlorophyll concentration in leaves (Fig. 2).

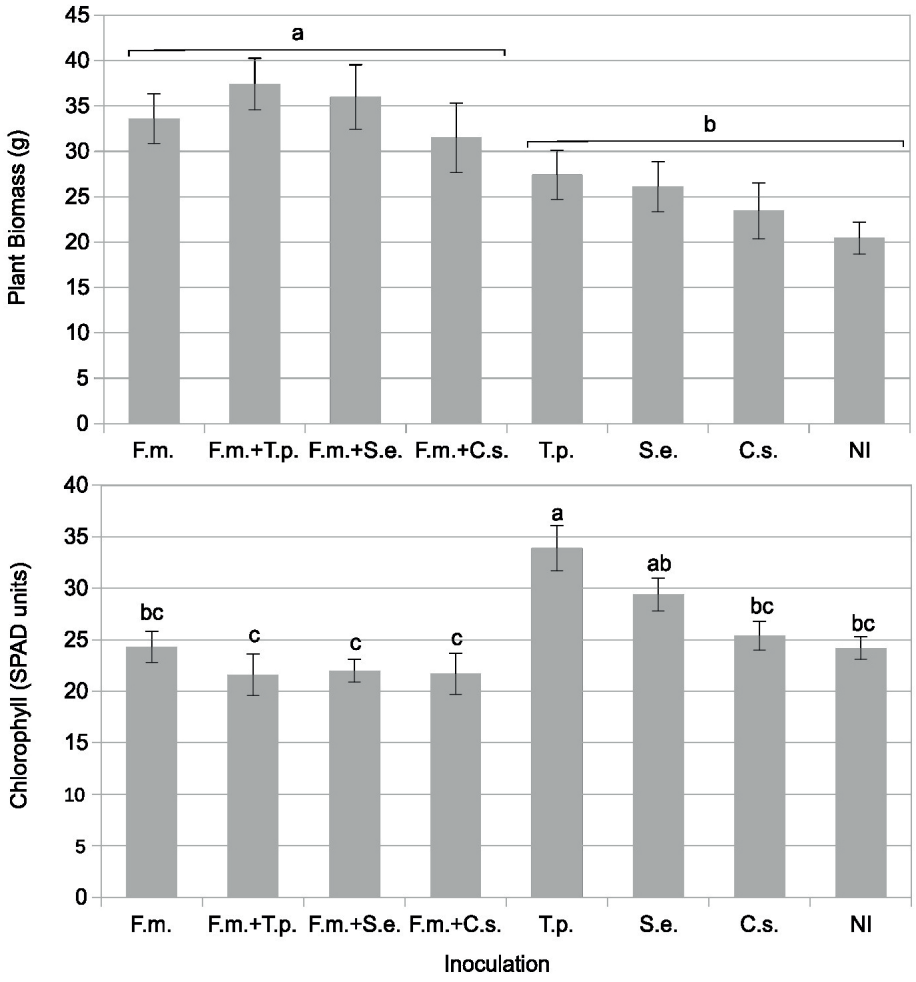

Fig. 2. Biomass and chlorophyll concentration in tomato plants at final harvest (135 days). Statistical significance was determined with $\alpha=0.05$ using two-way ANOVA. Treatments with the same letter are not significantly different. F.m.: *Funneliformis mosseae* inoculation; C.s.: *Candida saitoana* CRUB1770; S.e.: *Saccharomyces eubayanus* CRUB2014; T.p.: *Tausonia pullulans* CRUB1772; NI: without inoculation.

Fig. 2. Biomasa y concentración de clorofila en las plantas de tomate en la cosecha final (135 días). La significancia estadistica se determinó con un $\alpha=0{,}05$ usando un ANOVA de dos factores. Los tratameintos con la misma letra no son significativamente diferentes. F.m.: *Funneliformis mosseae*; C.s.: *Candida saitoana* CRUB1770; S.e.: *Saccharomyces eubayanus* CRUB2014; T.p.: *Tausonia pullulans* CRUB1772; NI*:* sin inoculación.

Plants singly inoculated with *T. pullulans* (T.p.) showed the highest chlorophyll concentration, 40% higher than plants without any inoculation (Fig. 2). The plants co-inoculated with yeast and *F. mosseae* showed similar chlorophyll concentration (independently of yeast inoculum), and it was lower than for plant singly inoculated with *T. pullulans* or *S. eubayanus*. Plants inoculated with *F. mosseae* showed similar chlorophyll concentration than plants without any inoculation (Fig. 2b).

The adult plant yield was evaluated using the total weight of fruits per plant, the percentage of plant with fruits within each treatment and the probability of fruits occurrence in each treatment (Fig. 3). Co-inoculation of *F. mosseae* and *S. eubayanus* resulted in a higher yield (with at least 2 fruit per plants when fruiting) than most treatments. Plants with *F. mosseae* inoculum showed significantly higher probability of fruit occurrence than plants without *F. mosseae* inoculum. Co-inoculation of *F. mosseae* and *T. pullulans* treatment (Fig. 3) presented the highest percentage of plants with fruits (83%), and the lowest percentage (25%) was observed for the treatment without any inoculation (Fig. 3). Plants singly inoculated with *S. eubayanus* (S.e.; Fig. 3) showed the highest percentage (67%) of plants with fruit among plants which didn't received *F. mosseae* inoculum.

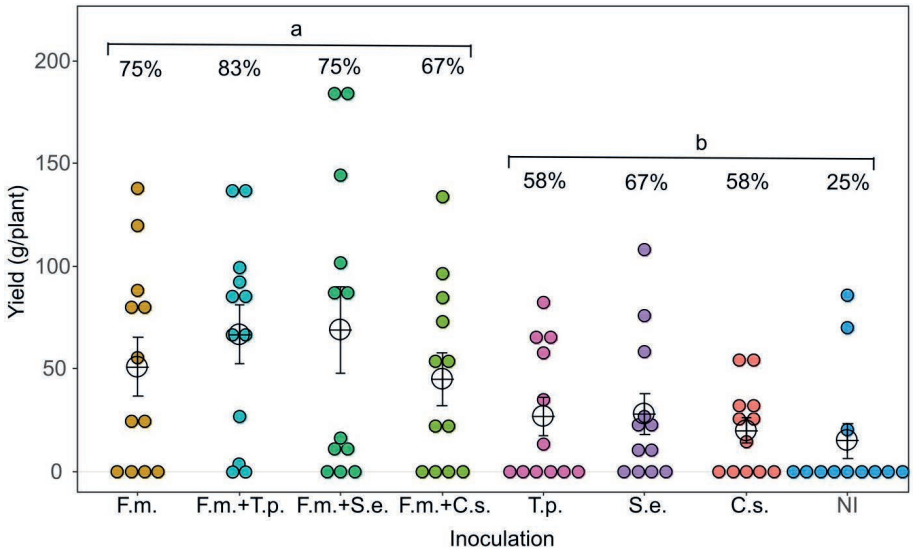

Fig. 3. Tomato yield per adult plant (dots), percentage of plants with fruit (upper numbers) and the probability of fruit production, at final harvest (135 days). Statistical significance was determined with $\alpha=0.05$ using a generalized linear model with binomial distribution. Treatments with the same letter are not significantly different F.m.: *Funneliformis mosseae;* C.s.: *Candida saitoana* CRUB1770; S.e.: *Saccharomyces eubayanus* CRUB2014; T.p.: *Tausonia pullulans* CRUB1772; NI: without inoculation.

Fig. 3. Rendimiento por planta adulta (puntos), porcentaje de plantas con frutos (número superior) y probabilidad de producción de frutos, en la cosecha final (135 días). La significancia estadistica de la probabilidad de producción de frutos se determinó con un $\alpha=0,05$ usando un modelo lineal generalizado con una distribución binomial. Los tratamientos con la misma letra no son significativamente diferentes. F.m.: *Funneliformis mosseae;* C.s.: *Candida saitoana* CRUB1770; S.e.: *Saccharomyces eubayanus* CRUB2014; T.p.: *Tausonia pullulans* CRUB1772; NI: sin inoculación.

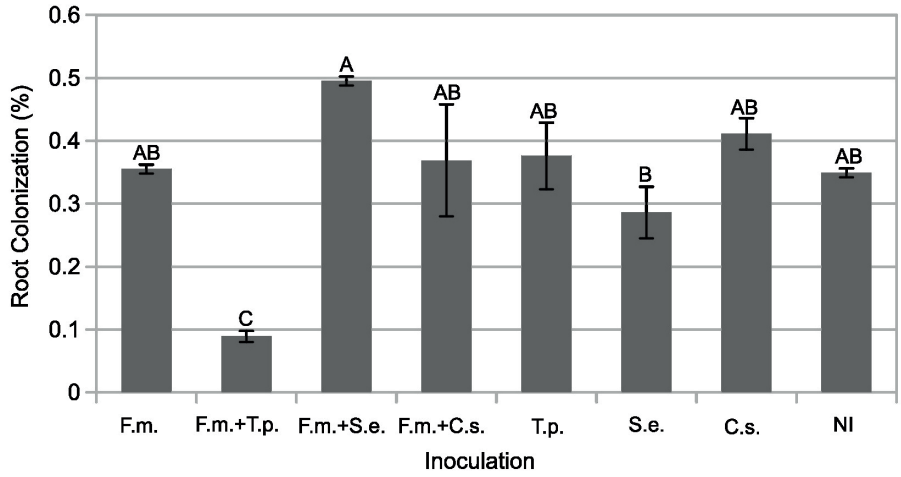

Fig. 4. Mycorrhizal (AM) colonization of adult tomato plants at final harvest (135 days). Statistical significance was determined with $\alpha=0.05$, using a two-way ANOVA. Treatments with the same letter are not significantly different. F.m.: *Funneliformis mosseae*; C.s.: *Candida saitoana* CRUB1770; S.e.: *Saccharomyces eubayanus* CRUB2014; T.p.: *Tausonia pullulans* CRUB1772; NI: without inoculation.

Fig. 4. Colonización por micorrizas (AM) de las plantas adultas de tomate en la cosecha final (135 días). La significancia estadística se determinó con un $\alpha=0,05$ usando un ANOVA de dos factores. Los tratamientos con la misma letra no son significativamente diferentes. F.m.: *Funneliformis mosseae*; C.s.: *Candida saitoana* CRUB1770; S.e.: *Saccharomyces eubayanus* CRUB2014; T.p.: *Tausonia pullulans* CRUB1772; NI: sin inoculación.

All adult plants showed typical mycorrhizal colonization, within their roots. The interaction between the inoculation with yeasts and the inoculation with *F. mosseae* showed significant effects ($F=15.204$, $p=6.05\cdot10^{-5}$; Fig. 4). Plants without *F. mosseae* inoculation showed colonization percentage ranging from 28 to 40 %. The highest mycorrhizal colonization (50%) was observed in plants co-inoculated with *F. mosseae* and *S. eubayanus*; the lowest percentage (under 10%) was observed in plants co-inoculated with *F. mosseae* and *T. pullulans* (Fig.4).

DISCUSSION

We studied the interaction between yeasts, arbuscular mycorrhizal fungi and tomato plants, in a greenhouse trial. Most studies published on the potential of yeast and AMF to enhanced plant production involve in-vitro tests or short-term in-vivo evaluations conducted under axenic conditions (Carvajal *et al.*, 2024; Radić *et al.*, 2022; Vargas *et al.*, 2024), while studies dealing with conditions close to field production are scarce (Biel *et al.*, 2021). One of the main cause for this bias, is the complexity of the production systems, where inoculated microorganisms compete with and are challenged by native microbiota in agronomical soils, alongside the long duration required for experiments.

Our experiment combined control conditions –surface-disinfected seeds and production in pots– with typical agronomic practices, which included the production of plants during the natural growing season and the use of natural soil and water. Therefore the yeast and mycorrhizal inocula were tested against the native microbiota in a soil from native forest which is typically used for horticultural and forest production in the region. We included the mycorrhizal inoculum during seedling substrate preparation, as it would be an easy adoptable step for seedling producers. Yeast inoculum as a cell suspension could be included in irrigation systems and its addition to the plant, when first true leaves appeared, coincide with the first fertigation for seedling, which means that yeast inoculation would not be a disrupting or time adding step for agricultures. Although conducted under experimental conditions, our experimental design mimics common production technique in Patagonia which enables us to establish connections between the recorded results and real-world agronomic scenarios.

The seedlings produced were primarily exposed to the inoculated microorganisms, either yeasts or mycorrhizae (up to 45 days of growth), as we used sterile substrate and water while growing. Gallegos-Cedillo *et al.* (2021) reported that slenderness index (SI) could be used to estimate the potential success and survival after seedling transplanting and good quality seedling has SI under 6 cm mm^{-1} (low value for SI correspond to shorter and thicker plants). All treatments in our trial produced seedlings of good quality (SI under 6). The seedlings inoculated with *T. pullulans* CRUB1772 or *C. saitona* CRUB1770 would have better potential survival (lower SI) than seedlings inoculated with *S. eubayanus* CRUB2014. Although mycorrhizal colonization was not detected in any of the seedlings, those without *F. mosseae* inoculation appear to be of better quality, exhibiting higher biomass and a lower slenderness index, compared to seedlings that received the mycorrhizal inoculum. Lack of mycorrhizal colonization in plant with *F. mosseae* inoculum was an unexpected result. One possible explanation could be the lack of infectivity of mycorrhizal inoculum. Another explanation could be a delay in colonization due to the fulfilled nutritional requirement of the seedling by the seedling substrate (Dejana *et al.*, 2022). Mycorrhizal colonization is a fine-tuned process which involve changes in fungal and plant chemistry, and depends on plant age (Lidoy *et al.*, 2023). Seedlings that received the mycorrhizal inoculum exhibited significant differences compared to those that did not. While this complicates the interpretation of the results, it also opens the door to explore previously unexamined phenomena. The synthesis of plant chemical products by plant to attract mycorrhizal forming fungi (Bonfante and Requena, 2011) before fungal colonization suppose an energy cost. Therefore, the decreased biomass observed in seedling inoculated with *F. mosseae* but lacking mycorrhizal colonization, could be explained by the resource invested in setting future mycorrhizal colonization.

Adult plants were produced in pots containing no-sterilised substrate, using forest soil and water from natural mountain spring, therefore the plants were exposed to natural microbiota as well as to the previous inoculated microorganisms. At final harvest (135 days) plant biomass and yield was higher for plants inoculated with *F. mosseae*, which is opposite to the result obtained for seedling biomass. The growth variables measured in seedling failed to predict the level of growth or production in adult plants. The reduced chlorophyll concentration in plants inoculated with *F. mosseae*, compared to those without the inoculum, may be linked to higher fruit production, as a decrease in chlorophyll during flowering and fruit setting has been previously reported in tomato (Soval-Vila, 2002).

In our study, adult plants were expose to two sources of mycorrhizae: *F. mosseae* inoculated during seedling production and the native mycorrhizal fungi from soil (and possibly water) used in the pot's substrate. We observed mycorrhizal colonization in plants without *F. mosseae* inoculation, which showed that native mycorrhizae were able to colonized tomato roots. Therefore, colonization values observed should be interpreted as a mix effect of the *F. mosseae* inoculum and the AMF from native soil. Harnessing native AMF for agricultural production could be and economically and friendly practice (Aguilera *et al.*, 2021). Microorganisms inoculated in plant production systems, interact with the target plant and also with the indigenous soil microbiota; such interaction must be evaluated and could reveal novel strategies to enhance plant growth. Plant singly inoculated with *T. pullulans* (exposed to native mycorrhizae) showed similar colonization percentage than plants singly inoculated with *F. mosseae* (exposed to the two source of mycorrhizae) or plants without yeast nor *F. mosseae* inoculum (exposed to native mycorrhizae); but the co-inoculation of *T. pullulans* and *F. mosseae* (exposed to the two source of mycorrhizae) resulted in the lowest percentage of mycorrhizal colonization. In another study, we observed a negative effect of *S. eubayanus* on *Rizhophagus irregularis* colonization of tomato roots (Mestre *et al.*, 2022), which was linked to the activation of the plant Induced Systemic Resistance. In the present study, a similar phenomenon may result in response to pressure by the inoculated microorganisms added to the indigenous microorganism from the soil used in the pot's substrate. Although the low mycorrhizal colonization, the *T. pullulans-F. mosseae* co-inoculation treatment rendered high average plant biomass, high percentage of plants with fruits and high yield per plant. For most variables, this treatment was not different than co-inoculation of *S. eubayanus* and *F. mosseae* which showed the highest colonization percentage. Therefore, the inhibitory effect on colonization is not affecting the general growth and production of the plants which received *F. mosseae* and *T. pullulans* inocula.

CONCLUSION

Some of the unexpected and challenging-to-interpret results obtained in our study underscore the complexity of agronomic research, where fungal and plant species, plant age, microbial interactions, and environmental conditions play critical roles. The experimental design employed allowed us to partially simulate conditions closer to those encountered by plant producers. The growth variables measured in seedlings failed to predict growth or production levels in mature plants, highlighting the importance of conducting *in-vivo* trials encompassing the plant's productive age. The application of *Funneliformis mosseae* inoculum demonstrated a significant increase in plant yield, despite the possibility that the inoculum was not fully infective. Inoculation with *S. eubayanus CRUB2014* showed good result for plant yield and mycorrhizal colonization. *Tausonia pullulans* CRUB1772 exhibited the ability to enhance plant growth and yield independently of its effects on mycorrhizal colonization, suggesting that this yeast may independently benefit tomato production. Although, we failed to observed synergist effect of yeast and mycorrhizal inoculation, we identified the yeast strains *S. eubayanus CRUB2014 and Tausonia pullulans* CRUB1772 as promising candidates to explore sustainable crop production practices.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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