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Biofertilization of Yacon with *Azospirillum brasilense* and Native Mycorrhical Fungi, Cultivated in the Central Valley of Catamarca, Argentina

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Abstract

In order to evaluate the effect of *Azospirillum brasilense* and mycorrhizal fungi in the soil on the nutrition of the yacon crop (*Smallanthus sonchifolius* [(Poeppig & Endlicher) H. Robinson]), determinations of agronomic parameters and the health status of the plants were carried out, under field conditions. The tests were carried out, at the time of the implantation of the culture: the propagules were inoculated with *A. brasilense* and with native mycorrhizal fungi, generating four treatments including the control and the co-inoculation of the consortium of the microorganisms under study (T0: control without inoculation; T1: inoculation with native *A. brasilense*; T2: inoculation with native mycorrhizal fungi and T3: joint inoculation with *A. brasilense* and native mycorrhizal fungi). The results indicate that co-inoculation with *A. brasilense* and with native mycorrhizal fungi, increased significantly the growth of plants in height, leaf area, biomass, dry matter and yields, in field production. It was determined that the application of the selected microorganisms has a promoting effect of plant growth, increasing growth and productivity of the cultivation of yacon.

Keywords

Inoculation, Interactions, *Smallanthus sonchifolius*, Nutraceutical

1. Introduction

The yacon (*S. sonchifolius* [(Poeppig & Endlicher) H. Robinson]) of the botanical family Asteraceae is a tuber plant that originated in the Andes [1]. It was domesticated centuries ago by pre-Inca cultures whose original habitat is the highlands of the Andes, from southern Colombia to northern Argentina, in the provinces of Jujuy and Salta, between 800 and 2,800 meters above sea level, in temperate mountainous climates [2].

In contrast to other root crops, which store carbohydrates in the form of starch, yacon accumulates carbohydrates such as inulin and fructooligosaccharides (FOS), fructose polymers, which cannot be hydrolyzed by the human body and pass through the digestive tract without being metabolized, they do not raise the blood glucose level, providing lower calories than sucrose, excellent for low-calorie diets and diets for diabetics [1] and their consumption is associated with other properties such as the reduction of cholesterol and triglycerides; improves calcium absorption, strengthens the immune system, prevents and reduces the risk of colon cancer, prevents constipation, and restores intestinal flora [2, 3, 4].

Inulin is considered a biological fiber, the ingestion of which confers several health benefits: it lowers the level of cholesterol in the blood, promotes the activity of bifidobacteria in the intestine, and reduces blood sugar [5, 6, 7]. It is poorly digested by humans and therefore has the potential to be used in low calorie food formulations [8, 9]. Long inulin chains can be used to replace fat in foods, as they simulate its texture. This is used in the production of low calorie dairy products [10]. Inulin acts as a prebiotic, favoring the development of beneficial bacteria in the colon.

Yacon is a healthy alternative to the usual diet due to its high inulin content and the benefits it brings to human health. Therefore, at present the consumption of yacon is associated with the prevention of chronic diseases (dyslipidemia and insulin resistance), colon cancer since it reconstitutes the beneficial microflora of the colon, improves calcium assimilation, corrects constipation, reduces blood cholesterol and includes prebiotic, antidiabetic, antioxidant and antimicrobial effects, among other properties [11, 12]. It is an ideal food for diabetics and for people who want to lose weight since its consumption does not raise the concentration of glucose in the blood and provides very few calories to the diet. These properties are strongly associated with phenolic compounds and fructooligosaccharides (FOS) and it is proven that these compounds have beneficial effects on nutrition and health. The storage root of yacon has a pleasant sweet taste, it is usually consumed after a period of drying in the sun. This procedure increases the sweetness of the roots and they are ready when the skin begins to wrinkle [2, 3].

The importance of yacon lies in the presence of bioactive components present mainly in the tuber and leaves of the plant, which has aroused great interest due to its content of FOS and phenolic compounds with beneficial properties for health. It contains FOS (50%-70% of its dry weight) and is therefore considered a prebiotic, and recent studies have suggested that feeding yacon prevents and controls diabetes by lowering blood glucose [13]. In addition, the consumption of *S. sonchifolius* tubers is increasingly popular in the Japanese diet due to its low caloric value and high fiber content.

In Peru, yacon is a traditional crop, it is part of the food and medicinal biodiversity and both the roots and the leaves are used there for their antioxidant, hypoglycemic and antibacterial properties. Due to these characteristics, its use as a functional and/or nutraceutical food is popular and promising [14]. It is sold fresh and or in the form of juices, syrups, capsules and tea in boxes with filter envelopes.

For all the previously discussed, it is considered valuable to increase the production of yacon by incorporating more productive and profitable cultivation technologies such as the use of biofertilizers, which in turn reduces production costs and reduces the use of agrochemicals.

The use of plant growth promoting microorganisms has been investigated for many years, the genus *Azospirillum* being one of the most prominent, mainly due to its ability to produce a wide range of active metabolites such as indole acetic acid, cytokinins, gibberellins and siderophores [15, 16], which positively influence the healthy growth and development of plants. Meanwhile, mycorrhizae are mutualistic associations between soil fungi and higher plant roots [17, 18]. As in other symbiotic relationships, both participants get benefits. These fungi depend on the plant for the supply of energy, carbohydrates and vitamins, which the fungus itself is unable to synthesize while the plant can do so thanks to photosynthesis and other internal reactions. At the same time, they deliver mineral nutrients to the plant, especially those that are not very mobile such as phosphorus and water [19, 20, 21, 22]. Biofertilizers can contain one or more selected microorganisms, which can be applied to the seed or the soil in order to increase its density and its association with the root system of the plant to promote its nutrition. This improves the vegetative and productive development of the plant. Among the microorganisms most used for their potential contribution to plant development are the rhizobacteria *A. brasilense* and the arbuscular mycorrhizal fungus *Glomus intraradices* [23].

Therefore, the objective of this work was to evaluate the effect of *A. brasilense* and native soil mycorrhizal fungi on the nutrition of the yacon crop (*S. sonchifolius* [(Poeppig & Endlicher) H. Robinson]), by determining parameters agronomic and plant health status.

2. Materials and Methods

Two bioassays were carried out in the field. The treatments carried out on yacon were: T0: Control (without inoculation); T1: Inoculation with *A. brasilense*; T2: Inoculation with native mycorrhizal fungi and T3: Joint inoculation with *A. brasilense* and native mycorrhizal fungi.

The inoculated treatments consisted of applying the selected microorganisms to the yacon propagules, by immersing them in the inoculant just before implantation. While the propagules of the control treatments were placed in sterile tap water. For the plantations, propagules of yacon (*S. sonchifolius*) of around 30 grams were used.

The native strain Pi 8 of *A. brasilense* was used, isolated from the endorhizosphere of paprika (*Capsicum annum* var. Elephant trunk) cultivated in the Province of Catamarca, whose identification was carried out biochemically and molecularly [24, 25, 15]. The concentration of *A. brasilense* used for the inoculations was $5x10^7$ azospirilos. mL^{-1} quantified in a Neubauer chamber [26].

The inoculum of mycorrhizal fungi native to the province was constituted by roots of *Melilotus officinalis* L., *Avena sativa* L., *Hordeum vulgare* L., *Secale cereale* L., *Panicum maximum* Jacq. and *Cenchrus ciliaris* L. colonized by these. The percentage of mycorrhizal colonization of the roots used as inoculum was 81.38%, estimated by the method of line intersections and microscopic observation of roots of Sieverding (1983) [27] and Mc Gonigle *et al.* (1990) [28].

The following data were recorded on each evaluation date: height of plants; number of stems; leaf area index (LAI) [29], fresh weight (FW), dry (DW) and% DM of stems, leaves, roots, propagules, tubers and whole plant; number of tubers per plant; average weight and tuber yield.

With the data of dry weight (DW) and fresh weight (FW) the % of dry matter (% DM = DW x 100/FW) was calculated, both for stems, tubers, roots and other parts of the plant.

The results were statistically analyzed by analysis of variance (ANAVA) and the means were compared by Fisher's LSD (Least Significant Difference) test at a significance level of 0.05 using the Infostat statistical program [30].

Two types of bioassays were carried out, in one of them periodic evaluations of plant growth were carried out in different phenological stages of the yacon crop, while, in the other type of bioassay, the plants were evaluated at harvest time, in the phase phenological delivery and commercial maturity of the crop with mature tubers.

The experimental design used was randomized blocks with three repetitions per treatment. Each repetition corresponded to a 3m x 3m plot with 25 plants (experimental units), in 5 cultivation lines 70 cm apart.

Field bioassay with evaluation at the end of the crop cycle: At the end of the crop cycle, 15 plants located in the central part of the plot of each treatment were evaluated.

Field bioassays with periodic evaluation of the crop cycle: In each collection, 3 plants were taken from each treatment and repetition. Collections were made at 35, 64, 114, 160 and 215 days after implantation.

3. Results

The tests were carried out in the Valle Viejo Department (28° 28'19.52"S; 65° 43'54.40" W) in lots with sandy loam textural class soil.

3.1 Field bioassay with evaluation at the end of the crop cycle:

The main indicators of the harvest of yacon tubers are: yellowing and falling of leaves, cessation of flowering and dry inflorescences, dry, brownish and stretched senescent stems, regrowth of new stems [31] and this is when the crop is considered "delivered" when the aerial material is completely dry, for which the harvest was carried out 10 months after its implantation.

The evaluation was carried out at the time of harvest and the consistent variables are related to the production of tubers, such as: Fresh Weight (FW), Dry Weight (DW), quantity, yield and percentage of dry matter. Although other variables of growth of the crop were evaluated, due to the characteristics of the phenological phase, it will not be expanded on the most unstable variables (leaf area, mainly fresh and dry weight of leaves).

Plant height: The highest plant height was observed in the treatments inoculated with the microbial consortium made up of *A. brasilense* and mycorrhizal fungi (T3) and with mycorrhizal fungi (T2), registering differences with statistical significance between the treatments (T2 and T3) compared to controls (T0).

Table 1. Height comparison of yacon plants produced in the field according to treatments. Evaluation at the end of the crop-cycle

Treatment	Height (cm)
Control	75.07±23.58**a**
Azospirillum	79.47±22.26**a**
Mycorrhizae	96.67±10.24**b**
Azosp.+Myco.	120.47±6.58**c**

Uncommon letters in the same variable denote significant differences according to the LSD test (Minimum significant difference) for P <0.05.

On the other hand, research on the growth of yacon has shown that plants can reach 3 meters in height [32, 33]. Meanwhile, in this study yacon plants with average heights between 75 cm (T0) and 120 cm (T3) were observed at 300 days of cultivation (Table 1). These values coincide with those reported by Cabrera (2005) [31] and Cano Romero (2016) [34].

Percentage of dry matter of aerial stems: Differences with statistical significance were observed in favor of the co-inoculation treatments with the microorganisms selected in study (T3) (Table 2). The high percentage of dry matter in aerial stems is due to the phenological state of the crop at the time of evaluation, presenting dry and brittle stems.

Table 2. Comparison of agronomic parameters of yacon (number, fresh weight, dry weight, dry matter of aerial stems), Evaluation at the end of the cycle

Variable	Treatments			
	Control	*Azospirillum*	Mycorrhizae	*Azosp.*+Myco.
No.	1.29±0.49**a**	1.50±0.97**a**	1.57±0.79**a**	1.43±0.51**a**
FW (g)	26.03±16.15**a**	29.12±20.13**a**	38.06±22.31**ab**	54.61±6.58**b**
DW (g)	14.71±9.12**a**	16.45±11.37**a**	21.50±12.61**ab**	30.86±9.57**b**
DM (%)	30.13±29.18**a**	41.43±25.86**ab**	41.43±25.86**ab**	56.50±0.00**b**

Uncommon letters in the same variable denote significant differences according to the LSD test (Minimum significant difference) for P <0.05.

Number of tubers per plant: The highest number of tubers were observed in the inoculated treatments (T1 and T3), registering differences with statistical significance compared to the control (T0) (Table 3).

Weight of tubers per plant: With microbial co-inoculation (T3), plants with a greater mass of tubers were obtained than with the rest of the treatments, achieving significant statistical differences between the inoculation treatments with the consortium of *A. brasilense* and mycorrhizal fungi (T3) and the control treatments (T0) (Table 3).

Yield (t of tubers per ha^{-1}): The highest yields were achieved with the co-inoculation of the selected microorganisms (T3), where significant statistical differences were recorded with respect to the control treatment (T0). The highest yield was observed with the co-inoculation of the selected microorganisms (T3), but in all inoculated treatments, increases with statistical significance were achieved with respect to the control (Table 3). Compared with the control treatment (T0), average increases of 662% were obtained with coinoculation (T3), an average 572% with the inoculation of *A. brasilense* (T1) and an average 286% with the inoculation of mycorrhizal fungi (T2). However, magnitudes much lower than those recorded in the yacon-producing regions were estimated. In Sao Paulo (Brazil) yields of up to 100t.ha^{-1} have been reported, the average yield in high Andean conditions varies between 20 to 40 t.ha^{-1} [35, 36, 31, 4]. While in Germany yields of 6 to 46 t.ha^{-1} were reported, according to different genotypes [37].

Percentage of dry matter of the tubers: the dry matter content in yacon tubers, differences with statistical significance were detected between the inoculated treatments and the controls, registering the best results in T1 and T3 (Table 3).

Table 3. Comparison of agronomic parameters of yacon (number, fresh weight, dry weight, dry matter and tuber yield), Evaluation at the end of the crop cycle

Variable	Treatments			
	Control	*Azospirillum*	Mycorrhizae	*Azosp.*+Myco.
No.	0.6±0.91**a**	2.67±1.4**b**	1.67±0.9**ab**	2.53±0.99**b**
FW (g)	27.79±37.75**a**	186.78±94.72**b**	134.36±91.3**b**	218.42±97.55**b**
DW (g)	12.14±16.49**a**	104.34±52.91**b**	76.34±51.87**b**	126.25±56.39**b**
DM (%)	17.47±22.14 **a**	55.87±0.00**c**	41.67±26.01 **b**	53.95±14.92**bc**
Yield (t.ha^{-1})	0.567±0.770**a**	3.811±1.933**c**	2.190±2.271 **b**	4.322±2.223**c**

Uncommon letters in the same variable denote significant differences according to the LSD test (Minimum significant difference) for P <0.05.

Average weight of tubers: For this variable, significant differences were manifested between the treatments. With the inoculations of the microbial consortium (T3) there were heavier tubers than in the rest of the treatments (Table 4).

The highest yields of yacon tubers were achieved with the inoculation of the microbial consortium made up of *A. brasilense* and native mycorrhizal fungi (T3), generating the highest tuber production and the highest average size thereof.

Table 4. Comparison of average, minimum and maximum weight of yacon tubers according to treatments produced in the field, Evaluation at the end of the crop cycle

Treatments	Average tuber weight(g)	Minimum tuber weight(g)	Maximum tuber weight(g)
Control	20.64±28.46**a**	0	49.1
Azospirillum	75.57±29.51**bc**	46.06	105.08
Mycorrhizae	54.07±46.73**b**	7.34	100.80
Azosp.+Myco.	91.30±61.43**c**	29.87	152.73

Uncommon letters in the same variable denote significant differences according to the LSD test (Minimum significant difference) for P <0.05.

Root dry matter percentage: Root DM percentages were similar between the treatments without registering differences with statistical significance. The highest FW and DW of yacon roots were observed in T1, registering statistically significant differences between the microbial inoculation treatments (T1, T2 and T3) compared to the control (T0) (Table 5).

Table 5. Comparison of agronomic parameters in yacon (fresh weight, dry weight, dry matter of roots) produced in the field, Evaluation at the end of the cropcycle

Variable	Treatments			
	Control	***Azospirillum***	**Mycorrhizae**	***Azosp.*+Myco.**
FW (g)	158.77±79.30**a**	270.08±143.68**b**	247.05±92.06**b**	235.01±94.1**ab**
DW (g)	32.6±16.28**a**	56.27±29.93**b**	51.79±19.3**b**	49.68±19.9**b**
DM (%)	20.53±0.28**a**	20.83±0.51 **a**	20.96±0.34 **a**	21.14±0.61 **a**

Uncommon letters in the same variable denote significant differences according to the LSD test (Minimum significant difference) for P <0.05.

Number of propagules: The highest quantity of yacon propagules was observed in inoculated plants, registering statistically significant differences with respect to the control plants (Table 6). The maximum quantity obtained was 12 propagules per plant, which came from the inoculation treatment with *A. brasilense* (T1) and the average quantity of 6.53 propagules per plant. Similar amounts to those reported by Muñoz Jáuregui (2010) [4], who maintains that between 6 to 14 propagules can be obtained, rooting being favored using growth regulators (auxins). This explains the results, since the bacterium *A. brasilense* is widely known for its ability to synthesize phytohormones, especially auxins [38].

Table 6. Comparison of agronomic parameters in yacon (fresh weight, dry weight and number of propagules) produced in the field, Evaluation at the end of the cropcycle

Parte de Yacon	Variable	Tratamientos			
		Control	***Azospirillum***	**Mycorrhizae**	***Azosp.*+Mico.**
Propagules	*FW (g)*	33.78±55.56**a**	322.81±286.85**b**	251.27±156.6**b**	387.49±82.59**b**
	DW(g)	8.22±13.52**a**	80.52±71.53**b**	63.13±39.35**b**	97.85±20.86**b**
	No.	1.13±1.86 **a**	6.53±5.28 **b**	5.47±4.1 **b**	6±2.14 **b**

Uncommon letters in the same variable denote significant differences according to the LSD test (Minimum significant difference) for P <0.05.

The highest fresh weight values of the underground yacon organs, which include tubers, propagules and roots, were observed in the plants from the inoculated treatments (T1, T2 and T3), registering highly significant statistical differences (p <0.0001) compared with the control plants (T0) (Tables 3, 4, 5 and 6). Productivity values much lower than those registered in Sao Paulo (Brazil) were estimated, in which average yields of 29 $t.ha^{-1}$ [36] and from 2000 to 3000 grams of roots per plant [33] (Table 5) are obtained.

Yacon plants inoculated with the combination of *A. brasilense* plus mycorrhizal fungi (T3) were those that showed a higher production of fresh and dry biomass (Table 7). DM production was higher in yacon plants inoculated with the consortium made up of *A. brasilense* and mycorrhizal fungi (T3), but when comparing with the values obtained with the treatment inoculated with *A. brasilense* (T1) the differences achieve statistical significance, but if statistically significant differences were registered with the reference yacon plants (T0) in which they were not inoculated with the selected microorganisms (Table 7).

Table 7. Comparison of agronomic parameters of yacon (fresh weight, dry weight and dry matter of whole plants) produced in the field, Evaluation at the end of the crop cycle

Variable	Treatments			
	Control	***Azospirillum***	**Mycorrhizae**	***Azosp.*+Myco.**
FW (g)	454.78±277.63**a**	1,573.35±813.30**bc**	1,235.42±606.58**b**	1,723.20±468.01**c**
DW (g)	60.92±42.40**a**	251.90±121.34**bc**	192.06±114.94**b**	300.82±91.51**c**
DM (%)	12.90±2.12**a**	16.59±2.27**bc**	5.13±2.72**b**	17.30±1.25**c**

Uncommon letters in the same variable denote significant differences according to the LSD test (Minimum significant difference) for P <0.05.

3.2 Bioassays in the field with periodic evaluation during the crop cycle:

Collections were made at 35, 64, 114, 160 and 215 days after implantation.

Plant height: The inoculated yacon plants (T1, T2 and T3), presented higher height throughout the crop cycle, registering statistically significant differences with respect to the control treatments (T0) (Table 8).

Table 8. Comparison of the agronomic parameters of yacon (height, quantity, fresh weight, dry weight and dry matter of stems)

Treat.	Variable	Days after yacon implantation				
		35	64	114	160	215
Control	*Height (cm)*	2.83±1.04**a**	11.5±1,5**a**	24.33±5.9**a**	47.7±1.9**a**	73.0±7.2**a**
	No.	2.33±0.58**a**	2.33±0.58**a**	3.33±0.58**a**	4.0±1.0**a**	4.0±2.65**a**
	FW (g)	0.53±0.23**a**	1.10±0.44**a**	3.37±0.51**a**	27.6±1.9**a**	51.27±8.08**a**
	DW (g)	0.10±0.05 **a**	0.22±0.11**a**	0.6±0.08**a**	4.98±0.4**a**	9.32±1.5 **a**
	DM (%)	18.08±0.63 **a**	19.47±2.1**a**	17.75±0.6**a**	18.1±0.21**a**	18.2±0.01**a**
Azosp.	*Height (cm)*	7.17±1.61**b**	17.2±2.6**b**	33.5±3.12**b**	61.8±6.75**b**	94.7±8.5**b**
	No.	2.33±1.15**a**	2.33±1.15**a**	2.67±0.58**a**	5.0±1.0**a**	4.0±1.0**a**
	FW (g)	1.0±0.17**b**	1.73±0.4**ab**	17.03±5.9**ab**	37.9±4.1**b**	67.7±4.9**b**
	DW (g)	0.20±0.03 **b**	0.36±0.1 **ab**	3.62±1.26**b**	8.03±0.9**b**	14.4±1.1**b**
	DM (%)	20.37±0.64 **b**	20.42±0.4 **ab**	21.23±0.04**b**	21.2±0.01**b**	21.2±0.03**b**
Myco.	*Height (cm)*	6.33±2.52**b**	15.7±2.75**b**	39.0±3.6**bc**	60.0±6.2**b**	101.7±7.4**b**
	No.	2.33±0.58**a**	1.67±0.58**a**	3.0±1.0**a**	3.0±1.0**a**	4.33±0.58**a**
	FW(g)	1.03±0.23 **b**	1.83±0.31**b**	12.5±1.2**b**	37.5±3.14**b**	74.8±7.7**bc**
	DW (g)	0.20±0.05 **b**	0.38±0.06**b**	2.65±0.25**b**	7.96±0.7**b**	15.9±1.6**b**
	DM (%)	19.26±1.3 **ab**	20.56±0.1**ab**	21.23±0.01**b**	21.22±0.02**b**	21.26±0.07**b**
Azosp. +Myco.	*Height (cm)*	6.17±1.04**b**	17.7±1.53**b**	42.2±3**c**	68.2±4.3**b**	104.7±12.6**b**
	No.	1.67±0.58**a**	2.33±0.58**a**	3.0±0**a**	3.67±1.5**a**	2.67±0.58**a**
	FW (g)	0.97±0.06**b**	2.0±0.1**b**	13.2±0.31**b**	42,1±2.1**b**	82.9±9.35**c**
	DW(g)	0.22±0.02 **b**	0.44±0.03 **b**	3.06±0.07**b**	9.8±0.5**c**	19.3±2.14**c**
	DM (%)	22.41±0.53 **c**	22.2±0.2**b**	23.22±0.04**c**	23.2±0.01**c**	23.2±0.03**c**

Uncommon letters in the same variable denote significant differences according to the LSD test (Minimum significant difference) for P <0.05.

Number of stems: During the development of the yacon crop, plants with similar amounts of aerial stems were observed (Table 8).

Percentage of dry matter of aerial stems: In almost all the collections there were no differences with statistical significance between the treatments (Table 8). The highest values of fresh weight of stems were observed in the inoculated plants (T1, T2 and T3), registering statistically significant differences, mainly in the phase of full vegetative growth (first collections), when the stems are more turgid and palatable (Table 8).

Number of tubers per plant: Statistically significant differences were determined between the treatments, where the inoculated plants generated a greater number of tubers (Table 9).

Weight of tubers per plant: The highest weights of tubers per yacon plant were recorded in the treatments with the inoculation of the microbial consortium of *A. brasilense* and mycorrhizal fungi (T3) and with the bacterial inoculation (T1), where differences were observed statistically significant between the microbial inoculation treatments and the controls, during the development of the cultures (Table 9).

Percentage of dry matter of the tubers: A higher percentage of dry matter of tubers from the inoculated treatments was determined, mainly with the microbial consortium (T3), observing in most of the collections significant statistical differences with respect to the controls (T0) (Table 9).

Average weight of the tubers: The yacon tubers, with the highest average weight, were observed in the inoculated treatments and fundamentally in the last collections, registering significant statistical differences in most of the collections, with respect to the control treatment (Table 9).

Yield (kg or t of tubers per ha^{-1}): In the harvest of mature yacon tubers, the highest yields were achieved with the inoculation of the microbial consortium of *A. brasilense* and mycorrhizal fungi (T3), at which time differences were recorded significant statistics compared to the control treatments (T0) (Table 9).

In the yacon culture, the highest average yield was estimated with the inoculation of the microbial consortium (T3) throughout the entire crop cycle, achieving increases with respect to the control treatment, of 118.1% in the 3rd. collection, in the 4th. 61.8% collection and in the 5th. 74% collection (Table 9).

Table 9. Comparison of the agronomic parameters of yacon (fresh and dry weight, dry matter, quantity and yield of tubers)

Treat.	Variable	Days after yacon implantation				
		35	64	114	160	215
Control	*FW (g)*	0.0±0.0	0.0±0.0	15.8±6.1**a**	49.2±13.2**a**	88.8±7.1**a**
	DW(g)	0.0±0.0	0.0±0.0	2.17±0.53**a**	5.24±1.13**a**	9.97±0.9**a**
	DM (%)	0.0±0.0	0.0±0.0	14.3±3.57**a**	10.8±0.7**a**	11.2±0.4**a**
	No.	0.0±0.0	0.0±0.0	1.67±0.58**ab**	2.33±0.58**a**	2.67±1.53**a**
	A.W. (g)	0.0±0.0	0.0±0.0	11.7±9.5**a**	21.34±5.4**a**	46.4±34.7**a**
	Yield (kg.ha^{-1})	0.0±0.0	0.0±0.0	323.1±124.2**a**	1,004±124.2**a**	1,812.9±144**a**
Azosp.	*FW (g)*	0.0±0.0	0.0±0.0	29.5±4.2**ab**	71.4±10.3**b**	123.6±19.6**b**
	DW(g)	0.0±0.0	0.0±0.0	3.41±0.53**ab**	8.51±1.33**b**	15.17±2.3**b**
	DM (%)	0.0±0.0	0.0±0.0	11.5±0.15**a**	11.9±0.3**bc**	12.3±0.4**b**
	No.	0.0±0.0	0.0±0.0	1.0±0**a**	2.33±1.15**a**	2.33±0.58**a**
	A.W. (g)	0.0±0.0	0.0±0.0	29.5±4.2**b**	41.94±34.4**a**	55.3±17.1**a**
	Yield (kg.ha^{-1})	0.0±0.0	0.0±0.0	602.7±85.5**ab**	1,457.8±211**b**	2,521.8±400**b**
Myco.	*FW (g)*	0.0±0.0	0.0±0.0	24.2±11.1**ab**	78.8±9.1**b**	134.7±21.9**bc**
	DW(g)	0.0±0.0	0.0±0.0	3.16±0.79**ab**	8.9±0.9**b**	15.56±2.5**bc**
	DM (%)	0.0±0.0	0.0±0.0	14.6±5.9**a**	11.4±0.31**ab**	11.56±0.16**a**
	No.	0.0±0.0	0.0±0.0	2.0±0**b**	2.0±1.0**a**	2.67±1.15**a**
	A.W. (g)	0.0±0.0	0.0±0.0	12.1±5.55**a**	49.97±32.8**a**	56.8±24.1**a**
	Yield (kg.ha^{-1})	0.0±0.0	0.0±0.0	493.9±226**ab**	1,607.5±186**b**	2,748±446**bc**
Azosp+ Myco.	*FW (g)*	0.0±0.0	0.0±0.0	34.5±7.02**b**	79.6±2.8**b**	154.7±9.3**c**
	DW(g)	0.0±0.0	0.0±0.0	4.05±0.95**b**	9.91±0.16**b**	18.9±1.0**c**
	DM (%)	0.0±0.0	0.0±0.0	11.7±0.58**a**	12.5±0.3**c**	12.22±0.09**b**
	No.	0.0±0.0	0.0±0.0	1.33±0,58**ab**	2.67±0.58**a**	2.67±0.58**a**
	A.W. (g)	0.0±0.0	0.0±0.0	27.95±9.01**b**	30.8±6.85**a**	60.1±14.9**a**
	Yield (kg.ha^{-1})	0.0±0.0	0.0±0.0	704.8±143.3**b**	1,624.5±56.4**b**	3,157.1±189**c**

Uncommon letters in the same variable denote significant differences according to the LSD test (Minimum significant difference) for P <0.05.

Percentage of dry matter of propagules: After four months plantation, propagules were obtained and the largest ones, with the highest biomass and dry matter values, were obtained with microbial co-inoculation (T3), where significant statistical differences were recorded. The smallest propagules were those belonging to the control treatments (T0).

Table 10. Comparison of the agronomic parameters of yacon (fresh weight, dry weight and dry matter of propagules)

Tratamiento	Variable	Days after yacon implantation				
		35	64	114	160	215
Control	*FW (g)*	0.0±0.0	0.0±0.0	8.63±1.66**a**	32.0±14.9**a**	117.1±8.6**a**
	DW (g)	0.0±0.0	0.0±0.0	0.9±0.15**a**	3.75±1.8**a**	12.5±0.66**a**
	DM (%)	0.0±0.0	0.0±0.0	10.5±0.31**a**	11.6±0.2**a**	10.7±0.26**a**
Azospirillum	*FW (g)*	0.0±0.0	0.0±0.0	18.2±4.01**b**	73.3±12.4**b**	182.9±16.3**b**
	DW (g)	0.0±0.0	0.0±0.0	1.99±0.45**b**	7.99±1.05**b**	20.3±1.42**b**
	DM (%)	0.0±0.0	0.0±0.0	10.9±0.38**ab**	10.9±0.45**a**	11.1±0.28**ab**
Mycorrhizae	*FW (g)*	0.0±0.0	0.0±0.0	21.6±7.3**b**	71.4±15.9**b**	176.3±20.9**b**
	DW (g)	0.0±0.0	0.0±0.0	2.46±0.82**b**	8.09±1.65**bc**	20.1±1.58**b**
	DM (%)	0.0±0.0	0.0±0.0	11.4±0.52**b**	11.4±0.74**a**	11.5±0.51**b**
Azosp. +Myco.	*FW (g)*	0.0±0.0	0.0±0.0	21.3±4.5**b**	88.8±17.6**b**	197.5±4.3**b**
	DW (g)	0.0±0.0	0.0±0.0	2.43±0.4**b**	11.2±2.97**c**	24.13±0.71**c**
	DM (%)	0.0±0.0	0.0±0.0	11.5±0.6**b**	12.7±0.2**b**	12.2±0.09**c**

Uncommon letters in the same variable denote significant differences according to the LSD test (Minimum significant difference) for P <0.05.

Fresh weight of leaves per plant: The highest production of foliar mass was recorded in the inoculated yacon plants, establishing differences with the control with statistical significance in the phenological stage of vegetative growth (Table 11), characterized by the production of leaves and stems, an important quality for the development and yield of crops.

Percentage of dry matter of leaves: In most of the collections there were no statistically significant differences between the treatments for this variable (Table 11).

Table 11. Comparison of yacon agronomic parameters (fresh weight, dry weight, dry matter of leaves and leaf area index)

Treat.	Var.	Days after yacon implantation 35	64	114	160	215
Control	*FW (g)*	0.30±0.1**a**	0.7±0.35**a**	10.5±2.3**a**	25.1±1.85**a**	48.3±7.7**a**
	DW (g)	0.03±0.01**a**	0.09±0.05**a**	0.32±0.04**a**	2.69±0.21**a**	0.5±0.07**a**
	DM (%)	9.8±1.04**a**	16.63±13.1**a**	3.15±0.4**a**	10.7±0.16**a**	1.04±0.03**a**
	LAI(cm²)	15.0±5.0**a**	35.0±17.3**a**	523.3±117.2**a**	1,255±92.6**a**	2,415±384.5**a**
Azosp.	*FW (g)*	0.5±0.1**b**	1.4±0.6**b**	960±39.7**b**	36.4±3.08**bc**	65.4±5,5**b**
	DW (g)	0.05±0.01**b**	0.21±0.05**b**	4.77±0.81**b**	3.85±0.28**b**	7.14±0.26**b**
	DM (%)	10.7±0.3**ab**	15.6±3.14**a**	12.42±3.2**c**	10.6±0.2**a**	10.94±0.5**b**
	LAI(cm²)	25.0±5.0**b**	70.0±27.8**b**	738.3±40.7**b**	1,818.3±154.1**bc**	3,271.7±276**b**
Myco.	*FW (g)*	0.57±0.12**b**	1.63±0.3**b**	17.8+1.5**c**	35.9±3.23**b**	73.7±7.6**bc**
	DW (g)	0.07±0.01**b**	0.22±0.05**b**	1.37±0.08**b**	3.91±0.16**b**	8.04±0.82**b**
	DM (%)	11.9±1.5**b**	13.37±0.7**a**	7.73±0.51**b**	10.9±0.6**a**	10.9±0.1**b**
	LAI(cm²)	28.3±5.8**b**	81.7±15.3**b**	891.7+75.2**c**	1,797±161.6**b**	3,686.7±380**bc**
Azosp. +Myco.	*FW (g)*	0.57±0.06**b**	1.8±0.1**b**	19.2±0.8**c**	40.9±1.7**c**	84.7±7.9**c**
	DW (g)	0.06±0.01**b**	0.25±0.02**b**	1.5±0.06**b**	4.42±0.2**c**	9.38±0.8**c**
	DM (%)	11.2±0.3**ab**	13.9±0.6**a**	7.79±0.23**b**	10.8±0.06**a**	11.09±0.89**b**
	LAI(cm²)	28.3±2.9**b**	90.0±5.0**b**	960±39.7**c**	2,045±87.2**c**	4,236.7±395.7**c**

Uncommon letters in the same variable denote significant differences according to the LSD test (Minimum significant difference) for P <0.05.

Leaf Area Index (LAI): The highest LAI was obtained in the inoculated treatments, mainly with T3, during the vegetative growth period of the crop, registering statistically significant differences between the inoculated treatments compared to the control treatments (Table 11). As the crop evolves, the temperature drops and winter approaches, the leaves of yacon plants turn yellowish and droopy, which is why the number of leaves and consequently the LAI decreases. The higher production of leaves of the inoculated treatments, evidenced by the variables evaluated, explain the higher production of photosynthates and its direct effect on the growth of the crops.

Root dry matter percentage: The highest root dry matter production was obtained in the inoculated plants, with significant statistical differences between the inoculated treatments in comparison with the controls (Table 12). Because the highest fresh and dry root biomass was observed in the inoculated treatments, in magnitudes much higher than the controls, results that show the growth-promoting activity of the roots of the microorganisms under study.

Table 12. Comparison of agronomic parameters of yacon (fresh weight, dry weight and dry matter of roots)

Treatment	Variable	Days after yacon implantation 35	64	114	160	215
Control	*FW (g)*	0.17±0.12**a**	1.33±0.12**a**	10.9±2.66**a**	75.33±7.6**a**	108.5±48.8**a**
	DW (g)	0.02±0.01**a**	0.14±0.01**a**	1.18±0.3**a**	7.81±0.78**a**	11.23±4.98**a**
	DM (%)	9.78±1.95**a**	10.83±0.3**ab**	10.79±1.03**a**	10.37±0.09**a**	10.37±0.07**a**
Azospirillum	*FW (g)*	0.4±0.17**ab**	4.8±1.71**b**	31.5±9.07**b**	99.67±27**a**	160.4±40**ab**
	DW (g)	0.04±0.02**ab**	0.51±0.18**b**	3.44±0.81**b**	11.09±3.4**a**	18.09±4**bc**
	DM (%)	10.61±1.19**ab**	10.55±0.07**a**	11.09±0.98**a**	11.07±0.3**b**	11.34±0.41**b**
Mycorrhizae	*FW (g)*	0.47±0.21**ab**	4.73±1.59**b**	36.3±11.9**b**	93.27±27**a**	160±3.44**ab**
	DW (g)	0.05±0.02**bc**	0.53±0.17**bc**	4.18±1.31**b**	11.22±3.03**a**	16.35±0.61**ab**
	DM (%)	11.41±0.82**ab**	11.21±0.21**b**	11.57±0.44**a**	12.07±0.58**c**	10.22±0.26**a**
Azosp. +Myco.	*FW (g)*	0.63±0.15**b**	7.63±2.24**b**	35.23±9.3**b**	106.9±24.8**a**	194.2±11**b**
	DW (g)	0.08±0.01**c**	0.83±0.21**c**	4.03±1.0**b**	12.48±2,76**a**	22.86±1.01**c**
	DM (%)	12.33±0.76**b**	10.93±0.57**ab**	11.51±0.6**a**	11.7±0.2**b c**	11.78±0.24**b**

Uncommon letters in the same variable denote significant differences according to the LSD test (Minimum significant difference) for P <0.05.

The evaluations of whole plants during the development of the crop showed a greater growth of the inoculated plants throughout their cycle, registering significant statistical differences with respect to the control (Table 13).

Table 13. Comparison of the agronomic parameters of yacon (fresh weight, dry weight and dry matter of the whole plant)

Treatment	Variable	Days after yacon implantation				
		35	64	114	160	215
Control	*PF (g)*	1.0±0.44**a**	3.13±0.67**a**	57.83±14.5**a**	241.3±26.9**a**	531.1±24.8**a**
	PS (g)	0.09±0.03**a**	1.38±0.39**a**	4.93±0.91**a**	22.09±1,69**a**	39.06±3.87**a**
	MS (%)	9.53±0.81**a**	43.5±3.4**b**	8.64±0.83**a**	9.18±0.31**a**	7.34±0.43**a**
Azospirillum	*PF (g)*	1.9±0.44**b**	7.93±2.62**b**	129.3±9.2**b**	392±35.2**b**	782.9±21.2**b**
	PS (g)	0.21±0.07**b**	2.52±0.62**b**	12.9±0.92**b**	35.52±2.88**b**	69.05±3.26**b**
	MS (%)	10.84±0,94**ab**	32.27±2,64**a**	9.97±0.17**ab**	9.07±0.28**a**	8.82±0.2**bc**
Mycorrhizae	*PF (g)*	2.03±0.59**b**	8.2±1.2**bc**	133.9±19.5**b**	388.3±51.5**b**	795.7±78,2**b**
	PS (g)	0.23±0.07**b**	2.63±0.25**b**	13.4±1.48**b**	36.26±4.6**bc**	68.76±4.2**b**
	MS (%)	11.54±0,13**b**	32.63±6.17**a**	10.06±1.01**ab**	9.34±0.29**a**	8.66±0.34**b**
Azosp. +Myco.	*PF (g)*	2.17±0.25**b**	11.43±2,04**c**	144.8±15.9**b**	447.2±54.8**b**	911.6±15.8**c**
	PS (g)	0.3±0.05**b**	3.12±0.09**b**	15.25±2,09**b**	42.69±4.3**c**	84.48±1.73**c**
	MS (%)	13.93±0.75**c**	27.84±4.46**a**	10.52±0.79**b**	9.56±0.23**a**	9.27±0.18**c**

Uncommon letters in the same variable denote significant differences according to the LSD test (Minimum significant difference) for P <0.05.

4. Discussion

Although the results obtained are in many cases inferior to those achieved in other parts of the world, there are also many reasons that can explain the differences. Yacon cultivation has a good water demand (greater than 800 mm), but can survive long periods of drought, its productivity being significantly affected under these conditions [4]. These circumstances are frequent in the central valley of the Province of Catamarca, immersed in the semi-arid region with problems of access to irrigation water. Furthermore, there is a direct relationship between the size of the "seed" propagule and the productivity of the plant. The plantation of 50-gram yacon propagules gave an average tuberous root yield of 2 kg/plant or 26.6 t/ha, 200 grams gave 5 kg/plant or 66.5 t/ha and 500 grams produced 6 kg/plant or 79.8 t/ha, showing that the weight of the propagules has a great effect on the tuberous root yield [4]. In this work, the yacon plantations were started with propagules of 30 g average, this is one of the main reasons for the low productivity values obtained. It is recommended that a 180-200 g piece of propagules be used to establish yacon cultures for tuberous root production. Using this seeding weight increased the crowns in weight 10 times [4].

However, the results obtained showed an increase in the different variables of vegetable production evaluated (plant height, number of tubers and propagules, fresh weight and dry weight of stems, roots, propagules and tubers) in yacon plants due to the effect of the inoculation with native microorganisms of *A. brasilense* and mycorrhizal fungi, registering significant statistical differences with respect to the control plants without inoculation. Therefore, the highest productivity of these crops is obtained through the microbial inoculation of the "seeds" (propagules) at the time of the implantation of the cultures.

The microbial consortium used, made up of native, bacterial and fungal strains, generated the best results due to the greater intake of water and nutrients, and mainly nitrogen that can be incorporated into the soil by biological nitrogen fixation, since the bacterium *A. brasilense* has this capacity, in addition to synthesizing auxins and other phytohormones [38]. The greater uptake of water and nutrients, especially those that are not very mobile such as phosphorus, facilitate their availability and assimilation by plants [19, 20, 21, 22]. In addition, these microorganisms locate and colonize sites in the rootlets, which could potentially be occupied by phytopathogens [39].

The selection of effective microorganisms in promoting the growth of cultures is a great challenge. The adaptation to the environment to which they are introduced and the compatibility between the microorganisms that make up the microbial consortia and these with the plants, may be the factors that prevent their use in agricultural production.

This work contributes to making evident the potential of the selected microorganisms as an alternative to improve the nutrition and productivity of the yacon culture. These results could support the possible use of microbial inoculants in the production of this crop, which would avoid or reduce the use of chemical fertilizers. They also indicate that there was a direct effect of microbial inoculation on the growth and yields of the yacon culture. In addition, it is important to note that these microbial interactions with yacon roots were achieved with native microorganisms and that the cultures were carried out in batches with no previous production history of this crop, in addition to the fact that it is a non-traditional crop in the province and that it is produced almost exclusively by a single farmer in the Central Valley area of Catamarca.

5. Conclusions

- The inoculations of the "seed" propagules at the time of the implantation of the yacon (*S. sonchifolius*) culture with the selected microorganisms generated a positive effect in all the cultivation conditions and variables evaluated, improving their development and productivity due to the better and increased nutrition.
- Significant differences were detected in the variables evaluated due to the effect of the treatments applied to the yacon crop. The harvest of the crop in the phenological stage of "delivery" and tuber maturity, together with the variables associated with the production of tubers (quantity, fresh weight, dry weight, yield, etc.) are the most consistent.
- The application of the microbial consortium increased the potential of the culture obtaining the best results, due to the co-inoculation with the consortium of *A. brasilense* and mycorrhizal fungi (T3), achieving yield increases of 77% average, widely exceeding the controls.
- The application of microorganisms in studies, in the implantation of yacon in field trials, allowed the establishment of beneficial relationships, ensuring survival, promoting the growth of plants in their first phases of growth fundamentally and increasing the yield of the crop.
- The microbial inocula used in these experiments are native species, and due to their origin they have generated more adequate adaptation mechanisms to the environmental conditions, for which it is estimated that this is one of the reasons for which promising results have been presented for the growth of yacon.
- This study is a pioneer in the studied area, so it is considered very promising to obtain a higher production and innovate with the cultivation of yacon, for its application with multiple purposes.

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