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Application of two strains of *Trichoderma asperellum* S. as a growth stimulant in rice cultivation

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ABSTRACT

The research was conducted in the low rainfall periods of 2016 and 2017 at the Scientific-Technological Base Unit "Los Palacios", with the aim of evaluating the effect of two *T. asperellum* strains application on the physiology and agricultural yield in the rice cultivar INCA LP-5. Inoculation was performed prior to sowing by imbibing the seeds in a biopreparation of *T. asperellum* (strains *Ta.13* and *Ta.78*), at a dose of 5 x 10^6 conidia x g seed^{-1} and drying in the shade for 24 h. A Randomized Block Experimental Design was followed, with four replicates and a control with untreated seed was used. At 120 days after emergence, plant height, the percentage of *Trichoderma* spp. colonized in the soil, aerial dry mass, root length and root dry mass, as well as agricultural yield and its components were evaluated. It was obtained that the application of *T. asperellum* (*Ta.13* and *Ta.78*) strains, provoked a tendency to the increase in the *Trichoderma* spp. presence in the Gleysol Nodular ferruginous ferruginous petroferric soil in rice cultivation conditions with irrigation. The growth and rice plant developments were stimulated, as well as the agricultural yield between 20 and 30 %, with respect to the treatment without *Trichoderma*. Strain *Ta.78* was the one that promoted best results.

Key words: agricultural yield, agro-ecology, alternative

INTRODUCTION

Crop yield is usually the attribute that is the main focus of breeding programs and varietal selection decisions. It is the end result of growth and development processes, which are regulated by genetic factors, environmental conditions, and genotype-environment interactions over a plant's growth period [(1)].

Rice (*Oryza sativa* L.) is the most consumed cereal, after wheat, worldwide, with a per capita of 53.9 kg [(2)] and rice and wheat consumption is expected to increase by 1.7 % and 1.0 %, respectively [(3)]. In Cuba, current commercial cultivars have shown a yield potential that exceeds 7 t ha^{-1}; however, despite the existence in the country of favorable climate and soil conditions for the growth and development of this cereal, in the last five years the yield has not exceeded 3.43 t ha^{-1} on average [(4)]. Achieving high yields to meet food demand in the case of rice is a national priority. Since the 1990s, there has been a decline in Cuban rice production, forcing the country to import more than US$100 million annually [(5)].

Non-compliance with the technological regulations for this crop, as well as the low availability of inputs (fertilizers and pesticides) are among the causes that have had a negative impact on the decrease in yields of this crop. Faced with this problem, an alternative could be the use of *Trichoderma* spp. as it presents direct and indirect mechanisms of action as a biological control agent and stimulator of plant growth. These microorganisms have the capacity to solubilize nutritive elements, that in their original form, are not accessible to plants and create a favorable environment to the radical development, which increases the plant tolerance to biotic and abiotic stresses [(6,7)], so it can be considered a biostimulant [(8)].

In a general sense, *Trichoderma* is used as a biological control against soil diseases. National Center for Animal and Plant Health (CENSA) has strains of *Trichoderma asperellum* Samuels, Lieckfeldt and Nirenberg, identified and characterized, physiologically and molecularly, that have manifested a good control on agents of the soil causing diseases in rice, beans and others [(9,10)].

In the scientific literature there is a lack of information on studies that provide a clear picture of how *Trichoderma* enhances growth and development in rice plants, cultivated under flood irrigation system. Therefore, the aim of the present study was to evaluate the effect of two *T. asperellum* strains application on physiology and agricultural yield in the rice cultivar INCA LP-5.

MATERIALS AND METHODS

The research was carried out at the Basic Scientific and Technological Unit "Los Palacios" (UCTB "Los Palacios"), Cuba, at 22°34'32.73" N and 83°14'11.95.95" W, belonging to the Instituto Nacional de Ciencias Agrícolas (INCA), with rice plants cv. INCA LP-5, which were inoculated before sowing with T. asperellum (strains *Ta.13* and *Ta.78*). The experiment was conducted under field conditions, always in the same area in the low rainfall periods of 2016 and 2017, in a soil that was classified as petroferric Ferruginous Nodular Gleysol [(11]) and it was characterized by a slightly acid pH (6.46); low organic matter (OM) content (2.86); exchangeable cations with typical contents for this type of soil and considered low; low assimilable phosphorus (P) (46.80 mg kg^{-1}) [12]. The behavior of the meteorological variables during the experimental period was registered in the Meteorological Station # 317 of Paso Real de San Diego in the municipality "Los Palacios" from Pinar del Río province, Cuba, which is at an average distance of 4 km.

The experimental area (0.6 ha) was prepared starting with the soil rototilling up to 0.20 m depth by dry soil preparation technology, with dry sowing at a dose of 120 kg ha^{-1} of seed [5]. Prior to sowing, rice seeds were washed with running water three times in succession and all those that floated together with foreign matter at the time of washing were discarded. Seeds were then dried in the shade for 24 hours; then the biopreparation was applied at a dose of 5 x 10^6 conidia x g $seed^{-1}$ based on *Trichoderma* strains (*Ta.13* and *Ta.78*) in 1 L of water separately, with 5 mL of 810 SL adherent, to achieve greater adhesion of the product to the seed; which were covered with the biopreparation for a period of 24 hours.

At the same time the standard treatment was prepared without application of *Trichoderma* (control), to conform experimental treatments. After 24 hours of the application, the sowing was carried out in plots of 9 m^2, following a Randomized Block Experimental Design, with four replications and a control with untreated seed was used.

Treatments

Seeds without *Trichoderma* (WT)

Seeds with *Trichoderma* (*Ta.13*)

Seeds with *Trichoderma* (*Ta.78*)

Irrigation, herbicide application and fertilization were carried out according to the Technical Standards for Rice Cultivation [5].

Sampling and evaluations

In both experimental years, sampling and evaluation were carried out following the same methodology. At 120 days after emergence (DAE) in the maturation phase, plant height (PH) was evaluated, which was measured in 10 plants per replicate, for a total of 40 per treatment, from the soil surface to the upper end of the leaf projected in the same direction of the stem [13] and expressed in cm.

The presence of *Trichoderma* was determined by taking five soil samples (0-10 cm) per replicate, for a total of 20 per treatment, close to the root zone of plants in each replicate. The samples were shaken in a beaker and five 100 µL aliquots of the soil suspensions (10^{-3} dilution) were extracted per sample. They were seeded on plates with PDA medium supplemented with amoxicillin (500 mg L^{-1}) in order to avoid contamination of the medium [14].

Subsequently, three plants per replicate were carefully extracted, for a total of 12 per treatment, with the help of a shovel, which made it possible to go deep into the soil and thus extract as much root mass as possible. The aerial part was separated from the root. Roots were washed with abundant water to remove adhering soil and dried with absorbent paper, then the length of the root was measured and expressed in cm. Both sections of the plant were kept in an oven with forced air at 70 ºC until constant mass was reached, which was measured on a technical balance (Denver Instrument PK-601), the result obtained was expressed in g plant^{-1}.

The experiment harvest was carried out at 142 DAE, at which time the number of panicles per square meter (P_m^2) was evaluated. For this purpose, 1 m^2 of rice was cut per replicate, for a total of 4 m^2 per treatment, and all stems with and without spike (P_inf_m^2) or the number of infertile stems per m^2 were counted [(13)]. In addition, 20 panicles were harvested per replicate, for a total of 80 panicles per treatment.

To determine the agricultural yield (t ha^{-1}), the methodology proposed by IRRI [(13)] was followed. A sample of 4 m^2 per replicate was taken, for a total of 16 m^2 per treatment. The samples were dried in the sun until the moisture content reached 14 %, then they were winnowed to eliminate impurities and weighed on a three-digit electronic balance (Ferton Electronic Balance) with an accuracy of 0.046 g.

Statistical analysis

After checking the assumptions of normality and homogeneity of variance (Bartlett's and Kolmogórov-Smirnov test, respectively) for each variable, the data were processed by means of a Simple Rank Analysis of Variance and when significant differences existed, the means were doubled, according to Duncan's Multiple Range Test ($p<0.05$). STATGRAPHICS CENTURION version 16.1 on Windows was used to the statistical analysis.

RESULTS AND DISCUSSION

In general, climatic variables that influenced during the research favored the growth and development of the crop. Temperatures were not extreme over time, the minimum temperatures, in the first stage of crop growth; that is, in the vegetative phase up to 70 DAE were 18.4 ºC in 2016 and in 2017 19.2 ºC, on average. However, temperatures were compensated with maximums of 27.5 ºC in 2016 and in 2017 of 28.6 ºC, on average. These thermal variations conditioned that the crop cycle behaved for this cultivar (INCA LP-5) as a medium cycle. In this regard, it was assured that maximum and minimum temperature ranges above 18 ºC and below 30 ºC are favorable for an efficient development of the rice plant [(15)]. On the other hand, research has shown that the cycle lengthening for the cultivar INCA LP-5 shows a tendency to be below 20 ºC, when it is cultivated in the period with little rainfall and minimum temperatures [(16)].

Regarding rainfall, in 2016, the maximum rainfall occurred in the first decade of June, with values of 254.8 mm and in 2017 it was only 129.7 mm. During the research period, the Relative Humidity in 2016 fluctuated between 66 and 99 % and in 2017, between 66 and 90 %, values that did not constitute a limiting factor for the crop, because the variation ranges are within the permissible parameters for rice [(17)].

When analyzing the result of the soil sampling in function of the presence of *Trichoderma* spp. in the soil (Table 1), it was found a superior tendency of the colonies in treatments where *T. asperellum* strains were applied (*Ta.13* and *Ta.78*). In both years of investigation, the highest presence was observed in the *Ta.78* treatment, an increase that represented 10.11 % in 2016, with respect to the control. In 2017, the increase was 14.67 % for the *Ta.78* treatment and 8.45 % for *Ta.13* with respect to the control.

Table 1. Results of soil sampling and morphological evaluations in uninoculated (WT) and inoculated rice plants with two *T. asperellum* strains (*Ta.13* and *Ta.78*), in a Petroferric Ferruginous Nodular Gleysol soil

Treatments	PTS (%)		PH (cm)		ADM (g plant^{-1})		R_L (cm)		RDM (g plant^{-1})	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
WT	69,61 b	78,05 c	97,23 b	119,47 c	2,96 b	3,24 b	15,72 b	15,67 c	1,18 b	1,22 b
Ta.13	71,80 b	84,65 b	101,30 a	128,30 a	3,31 ab	3,73 ab	20,08 a	17,92 b	1,28 a	1,34 a
Ta.78	76,65 a	89,50 a	103,43 a	124,43 b	3,80 a	4,14 a	17,92 ab	21,33 a	1,33 a	1,38 a
SEx	0,788	0,518	0,865	0,780	0,193	0,176	1,091	1,012	0,023	0,015

Unequal letters differ significantly according to Duncan's Multiple Range Test for p<0,05

(PTS): presence of *Trichoderma spp*. in the soil; (PH): plant height; (ADM): aerial dry mass; (R_L): root length; (RDM): root dry mass; (WT): in uninoculated rice plants

The presence of *Trichoderma spp*. in the Gleysol soil suggests the life of these microorganisms in these soils and evidenced the high ecological plasticity of these soils, because this soil has been cultivated in rice monoculture with two annual productions each year in the UCTB "Los Palacios" and presents all the typical characteristics for this crop [(18)], in flooded conditions [(5)]). Even when these conditions are of anaerobiosis, resident (native) *Trichoderma* species were able to colonize the edaphic medium. However, it should be noted that inoculation by imbibing the seeds with the strains of this fungus (*Ta.13* and *Ta.78*) increased the presence of this fungus and, in turn, promoted growth and development in the plant.

The increase in the presence and colonization of the soil by *Trichoderma spp*. in the treatments with *T. asperellum* indicated that the management under field conditions did not limit the multiplication and development of the fungus, despite the fact that the water depth fluctuated between 10 and 15 cm. The water lamina remained in the crop from the time plants had five true leaves until 50 % of grain maturity, as recommended in the Rice Technical Instructions [(5)]. Even when the period with lamina was superior to 90 days did not affect the presence and the soil colonization by *Trichoderma spp*. The survival of these fungi under these conditions, is possible because *Trichoderma* spores have a thick cellular wall [(9)] that isolates it from the environment and allows it to survive to adverse conditions, keeping it in dominance until the conditions are propitious for the germination. In this respect, it was assured that the *Trichoderma* dormancy spore is the main mechanism that allows this species to adapt to adverse conditions [(9)].

On the other hand, the application of different *T. asperellum* (*Ta.13* and *Ta.78*) strains, caused differences in plant growth, with respect to the control without application (WT), even when a tendency in the presence of these strains in the soil was evidenced. In relation to the behavior of plant height (Table 2), it was found that, in both years (2016 and 2017) always the treatments with *T. asperellum*, showed the highest values in height with respect to the control.

The accumulation of aerial dry mass, root length and root dry mass in both years of research, showed the highest values of these variables (ADM, R_L and RDM) where *T. asperellum* was applied (*Ta.13* and *Ta.78*), although for ADM the treatment with *Ta.13* strain showed no difference with the control (WT), the same happened for the case of root length in the second year of research.

The increase that was appreciated in the physiological variables evaluated (PH, ADM and RDM) indicated the potential of this microorganism on the growth and development of rice plants, even though it showed a variable behavior, depending on *Trichoderma* strain used. Regarding plant height [(10)], a significant increase in height was demonstrated in young rice plants treated with *T. asperellum*. Similar results were reported, but with other *Trichoderma* strains and different heights of water lamina [(14)].

Other causes that could lead to the increase in the growth of plants, was a greater absorption of nutrients, from the symbiosis that is created between this microorganism and the rice plant and the stimulation that was found in the radical growth. In this respect, in other investigations it was evidenced that some species of *Trichoderma* promote the growth and improve the absorption of nutrients [(19)]. The stimulation of growth can be due to the inhibition of minor pathogens in plant root, to the production of vitamins and to the conversion of nutrients (zinc, magnesium and potassium) in the soil, which are in a form not assimilated by plants [(6)]. It was also demonstrated that the application of *Trichoderma* spp. in corn crop (*Zea mays* L.) colonized its roots and required less nitrogen fertilizer, than the untreated corn; which implied a saving of 35 to 40 % of fertilizer [(9)].

Growth stimulation evidenced the possible positive interaction between the plant and *Trichoderma*, which is regulated to ensure the benefits of both symbionts. The plant receives protection and more available nutrients and the fungus obtains organic compounds and a niche for growth. In this regard, it assured that it is possible to stimulate growth from the identification process of the symbionts via microorganism-plant signaling and after the symbiosis, the production of hormones related to plant growth [(7)]. In relation to the production of hormones it was assured that *Trichoderma* species regulate metabolic pathways, as the production of auxins that promote the root growth [(19)]. Another author reported that *Trichoderma* besides competing for the nutrients and the rhizosphere dominance, has the capacity to multiply in the soil and colonize roots of plants and in the multiplication process they produce hormones of growth such as; auxin, gibberilin and cytokinins that stimulate the germination of the seed and plant development [(20)].

Recent research has shown that, in the initial stages of interaction, metabolites such as auxins and protein compounds released by *Trichoderma* are perceived by roots, altering many hormonal mechanisms that control plant growth and development under normal or stress conditions [(19)]. As a consequence, when the root system is colonized, the association is strengthened, providing protection to the root zone against pathogenic microorganisms and also developing the root system, which could improve the absorption of water and nutrients. In addition, plant growth is stimulated and resistance against pathogens is induced [7,21]. This is why this fungus is considered a growth promoter, based on the production of secondary metabolites in the interaction [19].

Regarding the components of rice yield, it was found that the number of panicles per m^2 (P_m^2) was higher in both experimental years in treatments with *T. asperellum* (*Ta.13* and *Ta.78*) compared to the control (Table 2). The highest P_m^2 values corresponded to the *Ta.78* treatment. However, the number of infertile panicles ($P\_inf\_m^2$) showed an inverse behavior in both experimental years, i.e. the control treatment (WT) showed the highest number of infertile stems and there were no differences between the treatments where *T. asperellum* was applied (*Ta.13* and *Ta.78*).

Table 2. Agricultural yield components in rice plants uninoculated (WT) and inoculated with two strains of *T. asperellum* (*Ta.13* and *Ta.78*). Evaluated in the 2016 and 2017 low rainfall seasons

Treatments	P_m^2		$P\_inf\_m^2$		F_G		E_G	
	2016	2017	2016	2017	2016	2017	2016	2017
WT	240,50 c	245,25 c	22,25 a	29,00 a	95,67 b	96,40 b	34,00 a	26,93 a
Ta.13	252,50 b	257,50 b	16,75 b	21,50 b	105,33 a	114,47 a	30,67 b	19,20 b
Ta.78	264,25 a	267,32 a	13,75 b	18,25 b	101,53 a	118,73 a	21,93 c	19,93 b
SEx	3,006	1,338	2,383	1,559	1,326	2,956	0,697	0,841

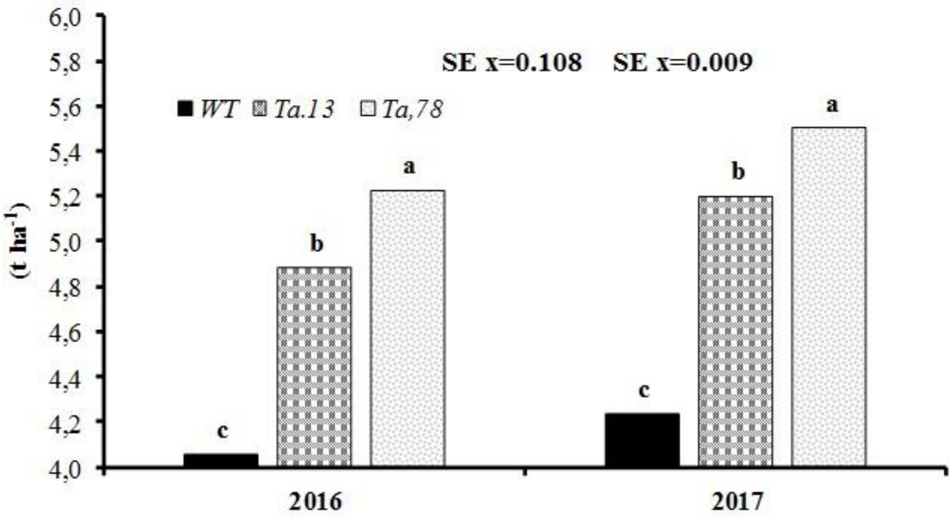
Unequal letters differ significantly according to Duncan's Multiple Range Test for $p<0.05$

(P_m^2): panicles per m^2; ($P\_inf\_m^2$): infertile panicles per m^2; (P_L): panicle length; (F_G: filled grains per panicle and (E_G): empty grains per panicle; (WT): in uninoculated rice plants

Regarding the filled grains per panicle (F_G) the highest values were found in the treatments where both *Trichoderma* (*Ta.13* and *Ta.78*) strains were applied without differences between them. Concerning the empty grains per panicle (E_G) they showed an inverse behavior to the F_G, although in the first experimental year the lowest values of E_G corresponded to the treatment where the *Ta.78* strain was applied.

In relation to agricultural yield components, the variables that contributed to the increase of the same in the treatments inoculated with *T. asperellum* were P_m^2 and FG; and inversely $P\_inf\_m^2$ and E_G, which are determined by the behavior of the variables of growth height of plants, aerial dry mass of the plant, root length and dry mass of the root, which showed increases due to the *Trichoderma* application effect. Specifically the increase of panicle number per plant in the treatments *Ta.13* and *Ta.78*, can be related to the radical system development, expressed in this case as RDM, as other researchers have pointed out in rice crop [22,23], that assured a high correlation between the root growth and agricultural yield components.

Regarding agricultural yield in both years (2016 and 2017), it was found that always the treatments where the seed was treated with *Trichoderma* were superior to the control treatment (Figure 1) and the highest values corresponded to the *Ta.78* treatment in both experimental years. In 2016 the increase in agricultural yield, with respect to the control, in the *Ta.13* treatment was 20.45 %, in *Ta.78* it was 28.99 % and in 2017 it was 22.70 % for the *Ta.13* treatment and 29.80 % for *Ta.78*.



Bars with unequal letters differ significantly according to Duncan's Multiple Range Test for p<0.05

Figura 1. Agricultural performance of rice plants uninoculated (WT) and inoculated with two strains of *T. asperellum* (*Ta.13* and *Ta.78*), grown under field conditions in a Petroferric Ferruginous Nodular Gleysol soil

The increase in agricultural yield is not defined at the end of the cycle [1], it is defined from the moment the soil conditions are prepared, the seed is treated, irrigation water is managed, and weed control, fertilization and phytosanitary control are carried out. Efficiently carrying out all these agro-technical activities on the crop guarantees greater growth and development of the rice plant. The results achieved in this research indicated an agronomic management that allowed obtaining a yield higher than the national average [4] for the control treatment, which was significantly lower than the treatments where *Trichoderma* biopreparation (strains *Ta.13* and *Ta.78*) was applied to the seed as an agroecological alternative.

The treatment to the seed with *Trichoderma*, allowed increases in the physiological variables in the height of the plants, aerial dry mass of the plant, root length and root dry mass. At the same time, it contributed to the increase of yield components (P_m^2 and FG_P), decreased the number of $P\_inf\_m^2$ and the number of E_G per panicle. All these variables are directly related to the agricultural yield achieved. In other researches in rice it was evidenced that the application of different strains of *Trichoderma* in field conditions, additionally showed stimulating effect, which was translated in a greater growth in the plant, which had repercussion in the agricultural yield of the crop [7,14]. Other authors demonstrated similar results regarding the potential that *Trichoderma* application has in promoting the yield in crops such as, garlic "*Allium sativum* L." [19], tomato "*Solanum lycopersicum* L." [23] and in the specific case of rice "*Oryza sativa* L." [14,24], but in other conditions of soil, climate and application methods with different *Trichoderma* strains.

CONCLUSIONS

- The application of *T. asperellum* (*Ta.13* and *Ta.78*) strains, show a tendency to increase the presence of *Trichoderma* spp. in the Gleysol Nodular ferruginous petroferric soil in conditions of rice crop with irrigation.
- The application of bioproducts based on *T. asperellum* strains (*Ta.13* and *Ta.78*), through seed imbibition for a period of 24 hours increase the growth and development of the rice plant, in addition to the agricultural yield between 20 and 30 %.

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