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EFFECT OF THE APPLICATION OF THE BIOSTIMULANT *TRICHODERMA ASPERELLUM* MT044384 IN WHITE CREOLO CORN (*ZEA MAYS*)

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Abstract: The present research evaluated the native fungus *Trichoderma asperellum* with registration MT044384 in National Center Biotechnology (NCBI), USA, to know its mechanism of action as a biostimulant for growth and yield of white native corn. A concentration of 1x108 spores/ml of *T. asperellum* was evaluated, which was sprayed on the foliage of the plant every 21 days until spike formation, with a combination of the biostimulant fungus and compost, and control treatments without the fungus and without compost. The experiment was distributed in a completely randomized design with three repetitions, in field and greenhouse conditions. An analysis of variance and comparison of treatment means was performed with the Tukey test with significance of $p<0.0001$. *T. asperellum* stimulated native corn plant height and yield variables in greenhouse and field conditions compared to control treatments. The best corn grain yield was obtained in *T. asperellum* in the greenhouse with 9.40 ton/ha, followed by *T. asperellum* plus compost in the field with 8.92 ton/ha, both treatments were significantly equal, this represents an average of up to 60 percent. percent in relation to control treatments. The highest height was 372.5 cm in *T. asperellum* in the greenhouse. The native strain *T. asperellum* MT044384 is potentially a good alternative to increase the yields of native corn.
Keywords: *Trichoderma asperellum* MT044384, creole.

INTRODUCTION

One of the priorities in the world and particularly in Europe, is the reduction of pesticides and fungicides in agriculture to develop food security for the consumer and environmental biodiversity. Biological control has gained strength in recent years as an alternative to the disuse of synthetic agrochemicals (Woo et al., 2014). *Trichoderma* biostimulants are safe for humans, livestock, bees and plants, including those for horticultural use (López, Pelagio, and Herrera, 2015). *Trichoderma harzianum* is one of the most experienced species in crops and in the world. The T. harzianum T-22 strain demonstrated mechanisms of action in increasing the growth and development of plants and a robust root system. In ornamental plants and grasses, resistance to drought increased. In corn, a higher yield and a reduction of up to 40% in the amount of nitrogen was achieved compared to plants that did not have the T-22 fungus (Harman, 2000).

The effects of biostimulants with microorganisms produce high yield in agricultural crops, which intensify the use of nutrients and increase photosynthetic activity and resistance to biotic and abiotic stress, these contribute to a more sustainable and resilient agriculture (Van Oosten et al, 2017). *Trichoderma* strains have the quality of improving the ability to induce nitrogen use efficiency, in such a way that the application of low doses of nitrogen to plants prevents water pollution and eutrophication, which includes the development of " "dead zones" in bays and river mouths (Harman, 2011). *Trichoderma asperellum* is a biological control agent of phytopathogens of importance in agriculture and a biostimulant for the growth of agricultural plants.

The strain *T. asperellum* Ta. 85 was selected for its capacity to promote the growth of the root system in bean plants cultivar BAT-304, three strains of this same species also had positive effects of promoting foliar growth, these strains are considered as candidates for creating biofertilizers in beans (González, et. al. 2019). *T. asperellum* Ta13-17 at a concentration of 1x108 conidia mL-1 reduced the incidence of Corynespora cassiicola leaf spot in tomato, and also presented greater

yield and photosynthetic activity (Celis et al 2023). *Trichoderma asperellum* MT044384 has the ability to reduce the incidence of soft rot disease in jackfruit caused by the fungus of *Rhizopus stolonifer* (Rodríguez, 2014)

The objective of this work was to evaluate the biostimulatory capacity of the native isolate *Trichoderma asperellum* MT044384 on white native corn, to increase its production yield.

MATERIALS AND METHODS

The environmental conditions of this work were developed during the period from August to December 2018, in a greenhouse and in the field, adjacent to the Department of Biochemical Engineering and Chemical Engineering of the Tepic Institute of Technology, which is located at 21°28' 45´´ north latitude, and 104°51´56´´ west longitude. The average crop temperature in field conditions was 21°C and in the greenhouse it was 32°C.

A mixture of white grain Creole corn was used, from the Academic Unit of Agriculture of `` Universidad Autónoma de Nayarit``, it was sown with a coa, two seeds were deposited per hole. The experiment consisted of eight treatments, of which four were applied Trichoderma and four without the fungus that grew in field and greenhouse conditions. Of these treatments with and without *Trichoderma*, two of these treatments, a dose of 4 tons/ha was applied. of Terra Sana brand compost made from sugarcane bagasse. When the corn plants had four true leaves, a concentration of 1x108 spores/ml of *T. asperellum* MT044384 was sprayed on the foliage. Sprays with the fungus were carried out every 21 days until flowering.

The treatments were distributed in a completely randomized design with three repetitions. The study variables were plant height, stem diameter, and physical qualities of the cob. The grain yield of the treatments was carried out with the weight of 100 g of corn seed that were stored in yellow paper envelopes; these were dried in an oven (Memmer BRAND) at a temperature of 75 °C for 48 hours. At the end of this time, the envelopes with corn were weighed and compared with the initial weight of the seeds. Again, the envelopes with corn were dried for a period of 24 hours at the indicated temperature, until a constant weight was achieved for each of the treatments (Yield Determination Manual, (2012). The treatments were compared in an analysis of variance, and a comparison of means with the Tukey test (α=0.05) in the SAS System for Windows 9.1 statistical package.

The *Trichoderma* fungus was isolated from the jackfruit crop in 2014, in the town of Llano, municipality of San Blas Nayarit. The fungus demonstrated good competition and mycoparasitism characteristics against the phytopathogenic fungus Rhizopus sp. The morphological description of the fungus was carried out with the keys of (Samuels et al., 2002; Samuels et al., 2010). The molecular analysis of *Trichoderma* was carried out with the extraction of genomic DNA with the QUIAGEN kit (DNesay plant mini ki 250, cat. No. 69106) and the amplicons were sequenced by the company Macrogen (Rockville, MD. 20850 USA).

RESULTS AND DISCUSSION

Some native or native corn inoculated with *Trichoderma* species have a negative response, that is, there is no plant and fruit growth, because these species do not colonize the epidermis of the plant due to the little or slight release of biomolecules that modify the mRNA and protein transcriptomes in plants (Harman, 2005). In the present study, the *T. asperellum* MT044384 strain was competent in producing favorable changes to white native corn. The average temperatures at which corn

developed from sowing to harvest with *T. asperellum* applications were 21 °C in the field and 31.4 °C in the greenhouse, which are in the optimal range of *T. asperellum* of 30 ° C, a minimum of 4 °C and a maximum of 35 °C (Lieckfel E. et al, 1999).

The *T. asperellum* treatments with and without compost surpassed or equaled the treatments without the biostimulant fungus, with and without compost applications in the field and greenhouse. *T. asperellum* in the greenhouse obtained the highest yield with 9.40 ton/ha., followed by *T. asperellum* plus compost in the field with 8.92 ton/ha., the compost treatment in the greenhouse without T. asperellum obtained the lowest yield with 4.37 ton/ha. (Figure 1). This means that *T. asperellum* with and without compost increases corn production and outperforms the control. This is similar to the increase in corn production by T. harzianum, also with a nitrogen reduction effect, which prevents soil water contamination and loss of soil fertility (Tavera, 2017).

The yield of *T. asperellum* plus compost in the greenhouse (6.50 ton/ha) would be expected to have a similar yield to that of T. asperellum without compost grown in the greenhouse. It is likely that temperatures higher than 35°C have occurred, exceeding the maximum limit for good growth of the biostimulant fungus and therefore low yield in corn. It is supported by the above that *T. asperellum* that the highest biomass production of *T. asperellum* was recorded at temperatures of 20°C, 25°C and 30°C and in lesser amount of biomass at 35°C (Singh A. et al. al,2014; Lieckfel E. et al, 1999).

The previous results coincide with the investigations of: *Trichoderma asperellum* (Ta13 and Ta.78) increased the height of the rice plant, and its roots, stem, leaves, and yield up to 30% compared to treatments without the fungus. biostimulant (Ruiz S.M. et al, 2022). Similarly, *Trichoderma* viride is a biofertilizer promoter in wheat cultivation by increasing plant height, root weight, leaf length, panicle weight, and number of grains (Mahato et al, 2018). The behavior of the best treatments of this research in their respective variables was: ear length of 30.80 cm in the *T. asperellum* plus compost treatment (I); ear diameter 4.70 cm in T. asperellum (c); rows per ear, 17 rows in T. asperellum (C) and grains per row 42.25 in T. asperellum plus compost (C).

The highest plant height was in *T. asperellum* (I) with 372.5 cm, which exceeded the heights of the treatments without *Trichoderma*, and the largest stem diameter was 3,427 cm in the soil-only treatment (C), which surpassed the treatments with *Trichoderma* in the field and greenhouse. The treatment: *T. asperellum* plus compost in the field, its corn grain yield exceeded the treatments without *Trichoderma* and with the combination of with and without compost in the greenhouse and field (Table 1). This means that the biostimulant mushroom plus compost combination is positive for plants. As indicated that the compost stimulated the population of *Trichoderma harzianum* and the vigor of Pinus radiata in the nursery (Donoso, Lobos, and Rojas 2008).

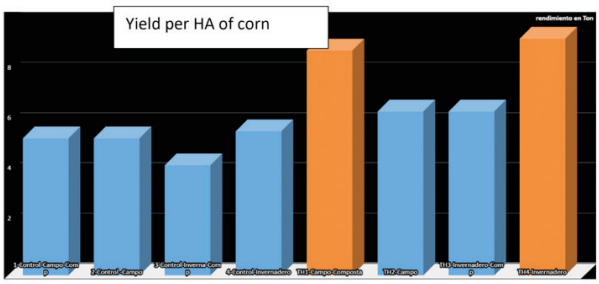

Figure 1. Comparison of grain yield in Creole white corn.

The morphological description of the *Trichoderma* isolate that was used in the present study showed chlamydospores in globose to subglobose, terminal and intercalated shapes (Figures 2 and 3), this coincides with the

Number	Treatments	AP cm	DT cm	LMZ cm	DMZ cm	HMZ	GRH	RD Ton
1	Soil more compost (C)	275.5 cd	3.127 ab	23.500 c	4.52 ab	14.50 ab	29.00 c	5.42 b
2	Just soil (C)	267.5 d	3.427 a	16.00 d	4.46 abc	14.00 b	33.00 c	5.43 b
3	Soil more compost (I)	317.0 abcd	2.600 ab	24.575 bc	4.00 c	14.50 ab	31.00 c	4.37 b
4	Just soil (I)	341.7 abc	2.450 ab	21.375 c	4.10 bc	14.00 b	28.50 c	5.72 b
5	*T. asperellum* more compost (C)	314.5 abcd	3.110 ab	28.750 ab	4.60 a	14.00 b	43.25 a	8.92 a
6	*T. asperellum* (C)	302.7 bcd	3.232 ab	23.625 c	4.70 a	17.00 a	40.25 ab	6.50 b
7	*T. asperellum* more compost (I)	362.0 ab	2.425 ab	30.850 a	4.39 abc	14.50 ab	35.bc	6.50 b
8	*T. asperellum* (I)	372.5 a	2.165 b	23.625 c	4.00 c	12.00 b	42.250 a	9.40 a

Table 1. Results of the morphological characteristics of native corn with and without *T. asperellum* combined with and without compost under field and greenhouse conditions.

Note: The treatments are: (C) = field, (I) = Greenhouse, AP = Plant height, DT = Stem diameter, LMZ = Ear length, DMZ = Ear diameter, HMZ = Rows per ear, GRH = Grains per row and RD = Yield per ha. With a p<0.0005

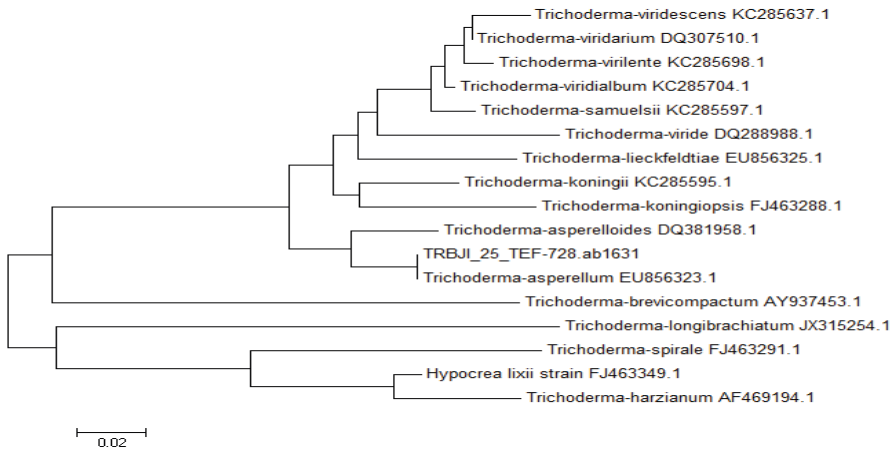

Figure 4. Phylogenetic Tree of *Trichoderma asperellum*

characteristics of *Trichoderma asperellum* cited by Samuels et al 2010. It is one of the most common species worldwide against a wide range of phytopathogens (Tondje et al, 2007).

Figure 2. Subglobose conidia

Figure 3. Phialides

The quality and quantity of DNA of *Trichoderma* isolate Trbji-25 from the jackfruit crop produced clear and sharp bands, properties necessary to guarantee DNA amplification in the PCR technique. The

size of the PCR product of isolate Trbji-25 ranged from 550 to 689 bp for TEF1. The BLAST result presented a 99% homology that corresponds to *Trichoderma asperellum*, with code MT044384) according to the company Macrogen, (Samuels et al., 2002; Samuels et al., 2010; Sánchez et al., 2012). The phylogenetic tree made with the tef gene sequence grouped the Trbji-25 strains with *Trichoderma asperellum* as shown in Figure 4.

CONCLUSIONS

The *Trichoderma Trbji-25* isolate, according to the morphological and molecular characteristics, corresponds to the species: *Trichoderma asperellum* (MT044384). That its evidence from this experiment is considered as a potential biostimulant agent in Creole white corn, by increasing plant height, and cob variables and the feasible increase in yield per hectare.

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