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Development Physiological and Stimulation of Roots Architecture in *Cucumis sativus* L. Plants Inoculated with *Trichoderma longibrachiatum*

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We performed inoculation of *Cucumis sativus* L., seedlings with *T. longibrachiatum* strain *ICA*-4, with the objective to evaluate the physiological changes on the development of the seedlings. The results indicated that inoculated seedlings had higher development with respect to non-inoculated seedlings. The range values of relative growth rate, doubling time, height and number of leaves (0.06 g g^{1}days^{1}, 11.55 days, 29.26 cm and 6.34, respectively) were significantly higher that those observed in uninoculated plants (0.02 g g^{1}days^{1}, 34.65 days, 14.08 cm and 3.2, respectively). With respect to the radical architecture inoculated seedlings showed a higher percentage of roots 2nd (256 %), 3rd (237 %) and 4th (222 %) order with respect to control plants.

Key words: *Cucumis sativus* L., *Trichoderma* sp, Roots architecture, Mexicali valley.

Mexicali valley is located in the District of Rural Development 002, which covers the municipality of Mexicali, Baja California, and the municipality of San Luis Rio Colorado, Sonora. Its arable irrigated area is 210,930 hectares (ha.) of which 184,283 hectares belong to Mexicali and 26,648 hectares belong to San Luis Rio Colorado. A total of 15,177 people are employed in agriculture in the Mexicali valley producing mainly cotton, wheat, alfalfa, and vegetables. In this sense, in the Mexicali valley, the most commonly grown vegetable crops are tomato, watermelon, pepper, eggplant, onion and cucumber. The cucumber (*Cucumis sativus* L.), is a popular vegetable crop of the family Cucurbitaceae, is rich in phosphorus, potassium and oxalic acid and is popularly used in salads. Its seeds are diuretic, tonic and refreshing (Pandey, 2000). In México the total greenhouse area for cucumber production increased from 3 hectares in 2002 up to 73 hectares in 2006 (SIAP, 2011) and production increased from 335 ton in 2002 up to 5365 ton. However, the overuse of fertilizers in the Mexicali valley can cause negative effects unanticipated environmental impacts

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(Adesemoye *et al*., 2008). For example, 70% of the cultivated land in Mexicali is irrigated with water from the Colorado River by which elevated concentrations of fertilizer chemistry and heavy metals in agricultural drain waters might be expected (Daesslé *et al*., 2009). On the other hand, the fungus *Trichoderma* spp., is a biological control organism against a wide range of soil-borne pathogens and showing of plant growth promoter capacity (Kubicek *et al*., 2001). In this sense, this microorganism could help to remedy the problems of pollution of the environment across the reduction of chemical fertilizer application at soils. The induction of plant growth by *Trichoderma* sp. has been reported in some commercial crops such as wheat and tomato (Lo and Lin, 2002; Bal and Altintas, 2006). However, the potential of *Trichoderma* sp., as consistent plant growth stimulators in cucumber plants under green house condition has been scarcely studied.

In this context, the purpose of this study was to examine the effect of *Trichoderma longibrachiatum* isolate *ICA*-4 on plant growth and root system architecture in cucumber seedling.

MATERIALS AND METHODS

Inoculant Preparation

Trichoderma longibrachiatum strain ICA 4 was used in this study. This strain was characterized molecularly and registered in the Genbank (HQ667667). The formulation of inoculant was carried out by a scaling process that included the following steps: a) 100 grams of rice with 20% moisture were put into flasks (350 ml) and were inoculated with 5 ml of mycelia and spores suspension (approximate concentration of 1 x 10^3 spores / ml water); b) the rice inoculated was incubated for seven days at 32 ± 2 ° C in a growth chamber Lumistell (ICP-19) with 40% relative humidity ; c) at the end of the incubation period 50 g of the rice inoculated was mixed with sterile soil (250 g), water (sterile) and calcium carbonate to obtain a soil with 30% humidity and pH of 7.0. Subsequently, the inoculum was incubated in complete darkness for seven days 30 ± 2 ° C before application to cucumber seedlings.

Inoculation of cucumber plants with *T. longibrachiatum*

Seeds of cucumber seedling were surface sterilized with 1% solution of sodium hypochlorite for 30 seconds and rinsed thoroughly with several changes of sterile and distilled water and then dried with sterile blotting paper. The seeds were sown individually into plastic pots (150 ml) at the rate of one plant per pot. Each pot contained two grams of inoculant (treatment 1) and two grams of sterile soils (treatment 2) mixed with a commercial potting soil mix combined with quartz sand and peat moss (50% soil, 20% sand and 30% peat moss, respectively). The seedlings were grown in a growth chamber with a photoperiod of 12/9 h in a photosynthetic photon flux density of 120 ìmol m^{-2} s^{-1}, 30 ± 2 °C, 60 % relative humidity.

Each treatment with and without inoculants was replicated 10 times and were daily irrigated with sterile water and fertilized each week with strength Hoagland's solution.

Evaluation of growth parameters

After thirty days of germination, relative growth rate (RGR); duplication time of plants (DT); height (H) and number of leaves (Ho) of both cucumber seedling treatment were evaluated. RGR was defined as the rate of increase in plant dry weight relative to the total dry weight of that plant at a single given time (Stadt *et al*., 1992) and was calculated as: $RGR= (\ln W_1 – \ln W_0) / t_1$. Where, W_0 = initial dry weight of seed; W_1 = final dry weight after germination of seed; t_1 = number of days after germination of seed. DT was defined as time required for growth increase and it is expressed in days and was calculated as: $DT= \ln 2/ RGR$

The radical architecture was classified according to the number of roots: 1st, 2nd and 3rd orders using an stereomicroscope (Velab, Mexico).

Photochemical Efficiency measurement

The Photochemical efficiency (*Fv/Fm*) is an effective and sensitive parameter which may be used as an efficient indicator of stress in plants leaves. This parameter was measured using a Plant Efficiency Analyser (PEA, Hansatech Instruments Ltd., King's Lynn, Norfolk PE32 1JL, UK) according to Strasser and Strasser (1995). Reading was collected after thirty days of germination using five single leaves per treatments. The randomly selected leaves were subjected to a 3 min period of adaptation to darkness under to induce the complete oxidation of the reaction centers. The Photochemical efficiency (*Fv/Fm*) was calculated according to the method of Rapacz (2007).

Statistical analysis

The experiment was set up in a completely randomized design with 10 replications. The significant differences between the inoculated seed (treatment 1) and control samples (treatment 2) were analyzed using the Student t test with a significance level of contrast: *a* = 0.05 (Statistical Package version 5.5, Statsoft, USA).

RESULTS AND DISCUSION

The results showed that the inoculation of cucumber seedlings with *T. longibrachiatum* strain *ICA-4* had a significant effect on seedling growth (Figure 1). The range of relative growth variables (RGR) in seedling inoculated with *T. longibrachiatum* (0.06 ± 0.32) was significantly increased compared with the untreated control seedling (0.02 ± 0.07) (Table 1). Additionally, height and number of leaves from the seedling inoculated with *T. longibrachiatum* strain *ICA*-4 were significantly higher compared with the control plants (Table 1). In contrast, the DT in seedling inoculated with *T. longibrachiatum* (11.55 ± 2.63) was significantly decreased compared with the untreated control seedling (34.65 ± 3.42) (Table 1). On the other hand, the inoculation of plants with the *T. longibrachiatum* strain *ICA*-4 showed an increase in the number of roots of 2nd, 3rd and 4th-order (256 %, 237 % and 222 % respectively) , with respect to control plants (Table 2). On the other hand, in the present study, the used of *T. longibrachiatum* strain *ICA*-4 on *Cucumis sativus* L resulted in significant changes on Photochemical efficiency (*Fv/Fm*) with respect to control plants (Figure 2). Where, the increased on *Fv/Fm* values observed in our study may be in part due to the positive effects of *T. longibrachiatum* strain *ICA*-4 that helped stabilize the chloroplast thylakoid membrane retarding the chlorophyll degradation and stimulating photosynthetic processes in the plant with respect to control plants. The stimulation of plant growth and the increased on *Fv/Fm* values by *Trichoderma* include the interactions with plant roots similar to mycorhizae, in which *Trichoderma* penetrates and colonizes root tissues without eliciting specific defense responses against the colonizing strain (Yedidia *et al*., 2001). Similar, results were found in maize plants inoculated with arbuscular mycorrhiza where the inoculation could increase their leaf chlorophyll content, photosynthesis, and chlorophyll fluorescence, resulting in the promotion of host plant growth and the increase of host plant biomass (Zhu *et al*., 2010).

Table 1. Growth effect in cucumber plants inoculated with *Trichoderma* strain *ICA* 4, at 30 days after inoculation

Plants	RGR	DT (days)	H (cm)	Ho
Inoculated	0.06 ± 0.32 a	11.55 ± 2.63 a	29.26 ± 1.54 a	6.34 ± 0.11 a
Control	0.02 ± 0.07 b	34.65 ± 3.42 b	14.08 ± 1.32 b	3.2 ± 0.27 b

Values with different letters in the same column are significantly different (α = 0.05) according to the Student's t-test. The results are averages of 10 replicates for each treatment.

Table 2. Root architecture changes in cucumber plants: inoculated, and control at 30 days after inoculation with *Trichoderma* strain *ICA* 4

Plants	Root architecture		
	(2nd order)	(3rd order)	(4th order)
Inoculated	63.50 ± 6.03 a	132.25 ± 6.34 a	39.5 ± 4.20 a
Control	24.75 ± 3.40 b	55.75 ± 4.03 b	17.75 ± 1.70 b

Values with different letters in the same column are significantly different (α = 0.05) according to the Student's t-test. The results are averages of 10 replicates for each treatment

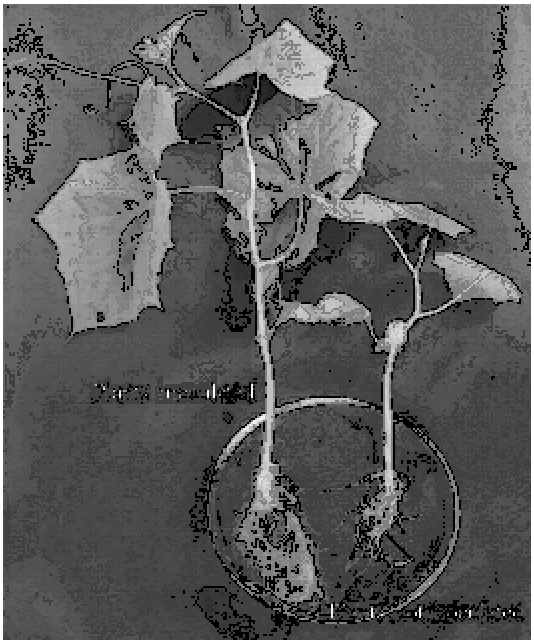

Fig. 1. *Cucumis sativus* plants of one month old inoculated and not inoculated with *T. Longibrachiatum*

On the other hand, the production of plant growth hormones or analogues is another mechanism by which *Trichoderma* can enhance plant growth. According to several authors the plant growth promotion seems to be mediated by the synthesis of auxin by *Trichoderma* spp. and the activity of the enzyme 1-aminocyclopropane-1-carboxylate deaminase, which induced changes in root architecture in plants inoculated with this fungus (Mastouri *et al*., 2010; Hermosa *et al*., 2012). In this sense, the higher development in roots architecture observed in the transgenic plants inoculated with *Trichoderma* could be results of increased of auxin in roots system (Figure 1). Similar results has been observed in other plants where *Trichoderma* also increases root development and crop yield, the proliferation of secondary roots, and seedling fresh weight and foliar area (Harman, 2000; Hajieghrari, 2010). In summary, future studies should be designed to evaluate the expression of genes involved in biosynthesis of plant hormone that could be involved in root development.

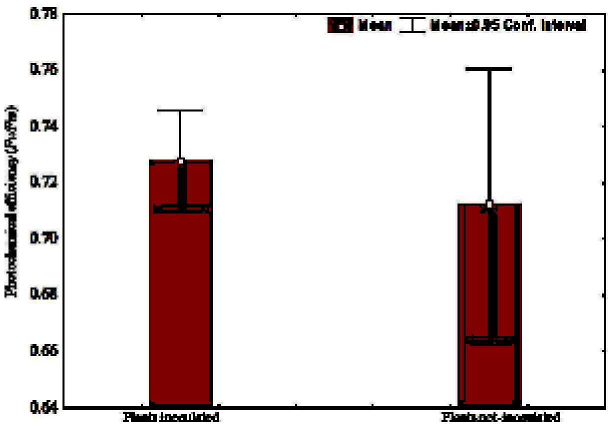

Fig. 2. Changes on Photochemical efficiency (*Fv/Fm*) in inoculated plants and not inoculated plant with *T. longibrachiatum*

CONCLUSION

In this study we found *Trichoderma* strain ICA-4 that could stimulate the development of roots architecture and growth in *Cucumber sativus* plants. Additionally, this study is a first step to using this strain for formulation of novel bioinoculants in agriculture for increased crop yields in the Mexicali valley, Baja California, México.

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REFERENCES

1. Adesemoye, A.O., Torbert, H.A., Kloepper, J.W. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. *Can J Microbiol.,* 2008*;* **54**: 876-886
2. Bal, U., Altintas, S. Effects of Trichoderma harzianum on yield and fruit quality characteristics of tomato (*Lycopersicon esculentum* Mill) grown in an unheated greenhouse. *Aus. J. Exp. Agr*., 2006; **46**: 131-136.
3. Daesslé, L.W., Lugo-Ibarra, K.C., Tobschall, H.J., Melo, M., Gutierrez-Galindo, E.A, García-Hernández J., Alvarez L.G. Accumulation of As, Pb and Cu Associated with the Recent Sedimentary Processes in the Colorado Delta, South of the United States-Mexico Boundary. *Arch Environ Contam Toxicol.,* 2009; **56**: 680-692
4. Hajieghrari, B. Effects of some Iranian *Trichoderma* isolates on maize seed germination and seedling vigor. *Afr. J. Biotechnol*., 2010; **9**: 4342-4347
5. Harman, G.E. Myths and dogmas of biocontrol – changes in perceptions derived from research on Trichoderma harzianum T-22. *Plant Dis.,* 2000; **84**: 377–393
6. Hermosa, R., Viterbo, A., Chet, I., Monte, E. Plant-beneficial effects of Trichoderma and of its genes. *Microbiology*., 2012; **158**: 17–25.
7. Kubicek, C.P., Mach, R.L., Peterbauer, C.K., Lorito, M. *Trichoderma*: From genes to biocontrol. J. Plant Pathol., 2001; **83**: 11–23.
8. Lo, C.T., Lin, C.Y. Screening strains of *Trichoderma spp* for plant growth enhancement in Taiwan. *Plant Pathol. Bull*., 2002; **11**: 215-220.
9. Mastouri, F., Bjorkman, T., Harman, G.E. Seed treatment with Trichoderma harzianum alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. *Phytopathology.,* 2010; **100**: 1213–1221.
10. Pandey, B.P. Economic Botany., 2000. pp 76-77. New Delhi: S. Chand and Co. Ltd
11. SIAP. Servicio de Información Agroalimentaria y Pesquera. Sagarpa, México.2011.http://reportes.siap.gob.mx/Agricola_siap/ResumenProducto.do.
12. Yedidia, I., Srivastva, A.K., Kapulnik, Y., Chet, I. Effects of Trichoderma harzianum on microelement concentrations and increased growth of cucumber plants. *Plant Soil*., 2001; **235**: 235-242.
13. Zhu, X.C., Song, F.B., Xu, H.W.Effects of arbuscular mycorrhizal fungi on photosynthetic characteristics of maize under low temperature stress. *Ying Yong Sheng Tai Xue Ba*., 2010; **21**: 470-475.
14. Stadt, K.J,, Taylor, G,J., Dale, M.R.T. Control of relative growth rate by application of the relative addition rate technique to Plant Soil a traditional solution culture. *Plant Soil*., 1992;**142**:113-122.
15. Strasser, B.J., Strasser, R.J. Measuring fast fluorescence transients to address environmental questions: the JIP-test. In: Mathis P, ed. Photosynthesis: from light to biosphere. Dordrecht: Kluwer Academic Publishers. 1995. p 977-980.

13\. Rapacz .M. Chlorophyll a fluorescence transient during freezing and recovery in winter wheat. *Photosynthetica .,* 2007*;* **45**:409-418.