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Short communication
EFFECT OF KLAMIC® ON GROWTH STIMULATION OF PLANTAINS AND BANANAS VITROPLANTS

Comunicación corta
Efecto de KlamiC® en la estimulación del crecimiento de vitroplantas de plátanos y bananos

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ABSTRACT. Endophytic fungi protect and benefit the plants in a natural way. The objective of this paper was to determine the entophytic activity of the fungus *Pochonia chlamydosporia* var. *catenulata* (Goddard) Zare and Gams var. *catenulata* strain IMI SD 187 (KlamiC®) and its effect on plant growth promotion of banana and plantain vitroplants. The 'CEMSA ¾' (AAB), 'Pisang Ceilan' (AAB), 'FHIA-01' (AAAB) and 'FHIA-18' (AAAB) cultivars were used. The vitroplants were transplanted to polypropylene trays and black polyethylene bags with bovine compost substrate, and they were then randomly distributed in the acclimatization area. Two applications were made with KlamiC® (5,6 x 10^5 clamidospores.vitroplanta^{-1}) The variables evaluated were the vegetative growth and the substrate and root colonization by the fungus, using a completely randomized experimental design with 70 repetitions per treatment with KlamiC® and the absolute control without KlamiC® for each cultivar. The data were analyzed by a simple analysis of variance followed by Fisher's LSD test. A significant increase of growth was produced in the plants treated with KlamiC® compared with the controls. Substrate and plant rhizosphere was colonized by the fungus, with a lower percentage in the cultivar 'FHIA-18' (AAAB) con 4,15 %.

Key words: *Pochonia chlamydosporia,* endophytic fungus*, Musa*, colonization

RESUMEN. Los hongos endófitos protegen y benefician las plantas de forma natural. El estudio tuvo como objetivo, determinar la actividad endofítica de *Pochonia chlamydosporia* var. *catenulata* (Goddard) Zare y Gams cepa IMI SD 187 (KlamiC®) y su efecto en la promoción del crecimiento de vitroplantas de plátanos y bananos. Se utilizaron los cultivares 'CEMSA ¾' (AAB), 'Pisang Ceilan' (AAB), 'FHIA-01' (AAAB) y 'FHIA-18' (AAAB). Se trasplantaron a bandejas de polipropileno y bolsas de polietileno negro, con sustrato de estiércol vacuno y posteriormente se distribuyeron aleatoriamente en el área de aclimatización. Se efectuaron dos aplicaciones de KlamiC® (5,6 x 10^5 clamidosporas.vitroplanta^{-1}). Como variables se evaluaron el crecimiento vegetativo y la colonización del hongo en el sustrato y raíces, utilizando un diseño experimental completamente al azar, con 70 repeticiones por tratamiento con KlamiC® y el control absoluto sin KlamiC®, por cada cultivar. Los datos se analizaron mediante análisis de varianza simple, seguido de la prueba de comparación múltiple LSD de Fisher. Se produjo un incremento significativo del crecimiento de las vitroplantas tratadas con KlamiC®, en comparación con los controles. El hongo colonizó el sustrato y la rizosfera de las vitroplantas, con menor porcentaje en el cultivar 'FHIA-18' (AAAB) con 4,15 %.

Palabras clave: *Pochonia chlamydosporia*, hongo endófito, *Musa*, colonización

INTRODUCTION

Bananas and plantains (*Musa* spp.) are widely distributed in tropical and subtropical regions, are an important component of food in the human diet (1, 2).

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In Cuba, in recent years, yields are affected, among other factors, by pests, within these are as important, the plant parasitic nematodes, which cause damage to the corm and roots (1, 3). It is therefore necessary to develop new pest management strategies that help reduce the effects on the crop using the medium ambienteA safe products.

In this sense, the use of endophytic fungi technology opens the way for biological plant breeding, which is to introduce beneficial microorganisms to the system of the plant; which can provide early protection seed material and a way to introduce biological control agents fitonematodos[A] (4, 5).

At the National Center for Agricultural Health (CENSA), a bionematicide called KlamiC® occurs, based on the selected strain IMI SD 187 Pochonia chlamydosporia var. catenulata (Goddard) Zare and Gams, which proved to be a biological control agent of forming galls nematode Meloidogyne with satisfactory results in vegetable crops (6). Recently, the endophyte fungus behavior in some species of grasses and Solanaceae were reported; which can result in favor of the defense of the host plant and the promotion of growth (7-9).

This research aimed to determine the endophytic fungus activity

P. chlamydosporia var. catenulata (KlamiC®) and its effect on promoting growth of banana and plantain plantlets.

MATERIALS AND METHODS

The study was conducted in the period from 20 March to 25 April 2015 in the areas of facilities belonging to Biofactory Seed Business Unit. The vegetative growth measurements and microbiological determinations were carried out at the Research and Development Unit Fungal Biological Control Agents belonging to the Agricultural Pest Management Group Plant Health CENSA. Both centers located in San Jose de las Lajas municipality, Mayabeque province, Cuba.

As plant material, vitroplants Phase III were used, at the end of the rooting phase in the culture medium (10) supplemented with 1.3 MIDE indole acetic acid (IAA), 40 g of sucrose and 2 mg L^{-1} thiamin, from the laboratory tissue culture Biofactory at the time that passed the area of adaptation to reach Phase IV of acclimation or ex vitro hardening. All plants that passed this last phase had two or more developed leaves, dark green foliage color, plant height ≥4,5 cm with vigorous appearance and more than three roots, according to the quality parameters of the production process. Commercial cultivars of plantain 'CEMSA ¾' (AAB), 'Pisang Ceylan' (AAB) and banana 'FHIA-01' (AAAB) and 'FHIA-18' (AAAB) were evaluated.

The experimental units were polypropylene trays hardened 47 x 69 cm with 70 small alveoli of 5 x 5 x 5 cm with a volume of 125 cm^3 per $cell^{-1}$, containing 65 g of substrate, and bags new black polyethylene, containing 250 g substrate. The trays were disinfected by submerging for five minutes in a solution of sodium hypochlorite (0,5 %) and washed with tap water. It was used as substrate, compost cow manure (100 %), from the municipal composter.

After planted in trays and bags, vitroplants stayed for 30 days in the shade house (semi-protected, on the deck and walls) with black shade nets color reduce 70 % of the light intensity. In maintenance, air irrigations were applied twice a day for five minutes through a micro-sprinkler system to keep the 80 % field capacity in the substrate and between 85-95 % of ambient relative humidity. The cultural care and observation of the phytosanitary status seedlings during the duration of the experiment was conducted.

Two applications of KlamiC® were made using a sprayer (backpack 16 L). The first spraying was performed at three days of plantlets planted and the second application 20 days after the previous application. In each application received vitroplants 6 mL of the suspension with a concentration of 5,6 x 105 chlamydospores.$vitroplanta^{-1}$. Upon completion of this period of acclimation or hardening *ex vitro* (Phase IV, 30 days), measurements of the variables of vegetative growth were made the whole plant: plant height (cm), number of active leaves, pseudostem diameter (mm), fresh dough (g), leaf fresh mass (g), root length (cm) and fresh root mass plus the corm (g).

For microbiological determinations colonization of *P. chlamydosporia* in substrate techniques were used and roots by dilution and seeding method in semi-selective medium and counting the number of colony forming units (CFU) per gram of sample (11) were used. In addition, the percentage of endophytic colonization of the fungus (7) was determined.

[A] Ramos, M. L. M. *Efecto de Hongos Endofíticos sobre Promoción de Crecimiento en Vitro plantas de Banano y Piña* [en línea]. Tesis de Grado, Escuela Agrícola Panamericana, 2006, Zamorano, 29 p., [Consultado: 2 de agosto de 2016], Disponible en: <http://bdigital.zamorano.edu/handle/11036/932>

A completely randomized experimental design with 70 replications by treatment with the application of the suspension of KlamiC® and absolute control without KlamiC®, for each cultivar. The data of each cultivar were analyzed by a simple analysis of variance. Colonization of the fungus in the substrate and the root and endophytic growth was compared by simple analysis of variance followed by multiple comparison test with Fisher's LSD confidence level of 90 % (12). In both analyzes the statistical package was used InfoStat v. 2.0 (13).

RESULTS DISCUSSION

All plantlets showed an adequate phytosanitary status in treatments with 100% survival during the acclimatization phase. There was a significant increase in the growth variables treated KlamiC®, regarding vitroplants control treatment in all cultivars of banana and plantain plantlets evaluated. The variable number of leaves cultivar 'CEMSA ¾' (AAB) showed no significant difference between both treatments (Table I).

Moreover, colonization of the substrate and rhizosphere of the plant by the fungus treatments KlamiC® and endophytic colonization of the roots of all cultivars tested was confirmed. The substrate allowed colonization of *P. chlamydosporia* in the range of 2.00 x 103 to 2.06 x 104 CFU g-1, whereas the roots reached 8.00 x 102 to 2.35 x 103, most colonization cultivars FHIA -01 '(AAAB) and' FHIA-18 '(AAAB) (Table II). The lowest percentage of endophytic colonization caught in the roots of plants cultivar FHIA-18 '(AAAB) with 4.15 %, the rest was between 16-21 %.

The application of this fungus increased vegetative growth parameters vitroplants as a result of increased biomass production and leaf system radical. These results are consistent with other studies which demonstrated that isolates of endophytic fungi promote growth in banana plants, where the mass of plants and roots and the total root length increased significantly (4, 5). Furthermore, plantlets inoculated with *Fusarium* isolated and *Trichoderma*, had an increase in mass of the roots of 35 % and 19 % leaf system, compared to plants which were not inoculated (14). This effect on growth enabled the planting field vitroplants be held two weeks before the scheduled date.

Table I. Effect of KlamiC® on the variables of growth of four cultivars of bananas and plantains (*Musa* spp.) At 30 days for obtaining plantlets

Cultivar	Tratamiento	PH (cm)	CH	PSD (mm)	LFM (g)	RL (cm)	FRM (g)	FMWPS (g)
'CEMSA ¾'	con KlamiC®	5,14 a	4,56	0,72 a	3,40 a	12,02 a	1,50 a	5,03 a
	sin KlamiC®	4,30 b	4,28	0,48 b	2,06 b	6,06 b	0,86 b	2,79 b
SE		0,19**	0,16 ns	0,03**	0,18**	0,68**	0,09**	0,27**
CV		28,70	25,07	40,13	47,24	53,48	54,06	49,01
PC	con KlamiC®	8,19 a	5,63 a	0,80 a	6,05 a	10,48 a	2,14 a	8,23 a
	sin KlamiC®	5,85 b	4,42 b	0,63 b	3,19 b	6,46 b	0,95 b	4,23 b
SE		0,24**	0,14**	0,02**	0,30**	0,42**	0,12**	0,40**
CV		24,12	19,48	23,09	44,67	34,44	55,65	44,87
'FHIA-01'	con KlamiC®	5,36 a	6,35 a	0,64 a	3,56 a	9,97 a	1,48 a	4,74 a
	sin KlamiC®	4,69 b	5,53 b	0,52 b	2,82 b	6,43 b	0,97 b	3,78 b
SE		0,10**	0,10**	0,01**	0,13**	0,27**	0,09**	0,17**
CV		18,53	14,76	15,75	37,53	29,47	66,1	35,07
'FHIA-18'	con KlamiC®	6,58 a	5,63 a	0,72 a	3,80 a	8,35 a	1,02 a	4,88 a
	sin KlamiC®	5,30 b	4,45 b	0,55 b	2,81 b	5,43 b	0,58 b	3,46 b
SE		0,15**	0,12**	0,02**	0,14**	0,37**	0,05**	0,18**
CV		16,07	15,17	18,79	27,84	35,35	39,56	28,38

AP: plant height; CH: number of active leaves; PSD: pseudostem diameter; LFM: leaf fresh mass; RL: root length; FRM: Fresh root mass; FMWPs: Fresh mass of the whole plant; SE: standard error; CV: coefficient of variation. PC: 'Pisang Ceilan'. Different letters in columns show significant differences (p≤0,05). ns: Non-significant differences (p≤0,05)

Tabla II. Colonization of the substrate and the plantain and banana roots (*Musa* spp.) by *P. chlamydosporia* (KlamiC®) during the production of plantlets 30 days

Cultivar	Colonization substrate x 10^4 (UFC g^{-1}) mean ± SE	Colonization roots x 10^3 (UFC g^{-1}) mean ± SE	Endophytic growth in root (%) mean ± SE
'CEMSA ¾'	1,20±0,38 a	0,80±0,33 b	20,9±4,15 a
PC	2,06±0,62 a	1,68±0,71 ab	16,7±8,35 a
'FHIA-01'	1,72±0,54 a	2,35±0,67 a	20,9±4,15 a
'FHIA-18'	0,74±0,59 b	1,98±0,48 a	4,15±2,15 b

PC:'Pisang Ceilan'. SE: standard error. Different letters in columns show significant differences (p≤0,10), according to Fisher's LSD test

Other studies *in vitro* plants banana cultivar 'Gran Enano (AAA) and pineapple (*Ananas comosus* L.) the hybrid MD-2, inoculated with endophytic fungi (*Trichoderma*, *Fusarium*) and mycorrhizae showed higher growth, expressed in height , diameter, leaf emission, mass and root system development, compared to the control. For the variable number of sheets were no significant differences found; however, the largest number ever presented in endophytics[A] inoculated with fungi treatments.

The application of IMI SD 187 strain in the early stages of development of tomato seedlings (*Solanum lycopersicum* L.) allowed its establishment in the soil and roots, without causing significant damages in physiological variables seedling development (15). This culture is characterized by being a good host fungus where endophytic colonization percentages were noted between 20 and 40 % of several strains of *P. chlamydosporia*; while Pcat (IMI SD = 187) showed a 60 % strain of endophytic colonization in the roots of seedlings on a sterile sand substrate (16).

The endophytic behavior in some species *P. chlamydosporia* grasses and nightshade, indicate that this growth habit leads to benefits for the host plant, including protection against root pathogens (7, 14, 15, 17), promoting growth the aerial and root part, by modulating biochemical and structural responses of the plant or the regulation of expression of genes involved in the biosynthesis of phytohormones (18, 19) as well as antagonist activity in the defense against migratory nematodes as *Radopholus similis* (4, 5, 20).

CONCLUSIONS

The results of this study demonstrate the ability endophytic fungus *P. nematophagous* chlamydosporia var. *catenulata* IMI SD 187 (KlamiC®) in different cultivars of bananas and plantains, with an effect in promoting growth of plantlets; which guarantees the continuation of studies in different ways and times of the bioproduct application in the production of plantlets and field monitoring.

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