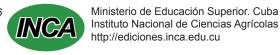
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ALTERNATIVES IN NUTRITION OF TRANSGENIC MAIZE FR-BT1 (Zea mays L.): RESPONSE IN GROWTH, DEVELOPMENT AND PRODUCTION

Alternativas en la nutrición del maíz transgénico FR-Bt1 de (*Zea mays* L.): respuesta en crecimiento, desarrollo y producción

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ABSTRACT. The maize is one of the most important cultures in most of the countries, whose yield been has limited by the attack of pathogens and weed affectation. The variety modified genetically FR-Bt1 has the characteristics to protect against the attack of Fall armyworm (Spodoptera frugiperda Smith) and tolerance to the ammonium glufosinate herbicide. In order to study the behavior in growth and yield of the variety in response to alternatives of nutrition with the combine use of a NPK fertilizer, the biofertilizer inoculant (EcoMic®) and the phytostimulant (FitoMas®), two field experiments and an extension test in a commercial field were carried out. Different variables of growth and yield were observed. The crop had a good answer to the application of the different products and the combination of FitoMas®-E, EcoMic® and the 50 % of mineral fertilization level proposed for the FR-Bt1 variety. had a similar response to 100 % of NPK dose and to each of the other different treatments. Finally, in low fertility soils, similar to the used in the experiments, the FR-Bt1 corn variety don't fulfill its potential yield when NPK dose are lower than the established in the technic instructive; it is possible with the combination of mineral fertilizer, EcoMic[®] y FitoMas®-E, including 25 to 50 % reduction in the mineral fertilizer, without affecting crop behavior.

Key words: inorganic fertilizer, biostimulating, maize, Mycorrhizae

Palabras clave: bioestimulante, fertilizante inorgánico, maíz, Mycorrhizae

RESUMEN. El maíz es uno de los cultivos más importantes en la mayoría de los países, cuyo rendimiento está limitado por ataques de patógenos y la afectación por arvenses. La variedad sintética cubana de maíz FR-Bt1 transgénica posee la capacidad de protección contra el ataque de la palomilla del maíz (Spodoptera frugiperda Smith) y la tolerancia al herbicida glufosinato de amonio. Para conocer el comportamiento en crecimiento y rendimiento de variedad FR-Bt1 en respuesta a alternativas en la nutrición, combinando un fertilizante NPK, el inoculante micorrícico EcoMic® y el estimulador de crecimiento vegetal FitoMas®-E, se realizaron experimentos en condiciones de campo y una prueba de extensión, observándose variables de crecimiento, desarrollo, rendimiento y sus componentes. El cultivo respondió positivamente a la aplicación de los productos; en los indicadores estudiados, con la combinación del FitoMas®-E, EcoMic® y el 50 o 75 % de la dosis de fertilización mineral recomendada para la variedad, la respuesta fue similar a la aplicación del 100 % de la dosis, siendo ambos tratamientos superiores a cualquiera de los productos por separado y sus combinaciones. En suelos pocos fértiles, similares a los de los experimentos realizados, la variedad de maíz FR-Bt1 no alcanza su rendimiento potencial cuando se utilizan dosis de NPK inferiores a las establecidas en el instructivo técnico, lo que si se logra con la combinación de la fertilización mineral con EcoMic® y FitoMas®-E permitiendo incluso la reducción de la dosis de fertilizante en un 25 o 50 %, sin afectar el comportamiento del cultivo.

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INTRODUCTION

Corn is grown in most countries of the world, which plays a fundamental role in animal and human food, being one of the most important cereals due to its use and volume of planting (1). However, its production is affected by the impossibility of effectively controlling the devastating attack of the corn moth, Spodoptera frugiperda (Lepidoptera: Noctuidae) and the affectations by the weeds, with the consequent use of herbicides (2). The control of these affectations increases the production and damages the environment due to the use of pesticides (3). One solution is the genetic transformation of crops, with the introduction of genes that transfer to the modified species the characteristics of control to these insects-pests and tolerance to herbicides (4). Precisely the Cuban synthetic variety of transgenic FR-Bt1 corn has the capacity to produce the insecticidal protein Cry1 Fa of Bacillus thurigensis and the detoxifying protein of glufosinate ammonium Pat of Streptomyces viridochromogenes, which provide protection against the attack of the corn moth (Spodoptera frugiperda Smith) and tolerance to the herbicide glufosinate ammonium in its different commercial variants (5). These characteristics make them more productive than the unmodified varieties from which they originate. In the case of FR-Bt1, it is FR-28, which is used in agricultural practice in Cuba (6). It should be expected that their nutritional requirements are equal or superior to the unmodified variety, however, there is no related information. For this reason the same fertilization doses are applied based on their productive potential, which could be different.

Chemical fertilization is a common agronomic practice in the cultivation of corn; a nutritional deficiency can reduce performance between 10 and 30 %, before clear symptoms of the deficiency appear. Investment in maize fertilization represents approximately 30 % of the production costs of the irrigated areas and up to 60 % in rainfed areas, which reduces the profits of the farmers and influences the soil fertility degradation (7). To resolve both problems, the resizing of the use of technologies is being imposed, suggesting the use of biofertilizers (8-10) and growth stimulators (11).

The plant stimulant FitoMas®-E is a derivative of the sugar industry developed by the Cuban Institute of Investigations in Derivatives of Cuba Sugarcane (ICIDCA) that produces a positive effect on the growth and yield of crops given its influence in the physiological activity of plants (11). Its effect in the cultivation of corn is related, among others, because it enhances the action of other bioproducts used in agriculture and allows the reduction of

the doses of mineral fertilizers, guaranteeing the nutritional needs of crops such as corn (12).

The arbuscular mycorrhizal symbiosis is an important component in agroecosystems, in which there is an exchange of nutrients, essential metabolites and hormonal substances between the symbionts and new structures are created (13), which have an impact on the development of the system contributing to transport of photosynthates and the exchange of nutrients, as well as protection against biotic and abiotic stresses (14). Therefore, the use of biofertilizers based on arbuscular mycorrhizal fungi (AMF), such as the commercial inoculant EcoMic®, developed by the National Institute of Agricultural Sciences, promotes the maintenance and regeneration of soils, helps control pathogens and they can reduce approximately 50 % of the recommended chemical fertilization doses, without loss in potential yields (15,16). In nongenetically modified corn plants inoculated with AMF, increases in yields between 21 and 77 %have been reported, depending on the type of soil. In the same way, it has been observed that the application of EcoMic® and FitoMas®-E combined with different doses of NPK have a positive effect on the yields of different crops (16,17).

In the literature, information on the nutrition of transgenic maize varieties and their relationship with mineral fertilizers or biofertilizers and growth stimulants is not abundant, being directed especially to the effect of AMF on arbuscular mycorrhizal colonization and crop response (18-20) and more directly related to the FR-Bt1 corn variety (16,21).

With the introduction of the FR-Bt1 genetically modified variety, it is necessary to evaluate if the nutrition of the same allows the integration of mycorrhiza inoculation, the use of growth stimulators and mineral fertilizer as in the non-modified varieties. The objective of the present work was to determine if it is possible to optimize the nutrition of the variety FR-Bt1 with the combined use of mineral fertilizers, AMF and FitoMas-E, without affecting the indicators of growth, development and yield.

MATERIALS AND METHODS

Two experiments were carried out in the field. The first experiment consisted of a preliminary evaluation of the FR-Bt1 maize variety behavior with increasing doses of NPK chemical fertilizer and the combination of the mycorrhizal inoculant EcoMic® and the plant growth stimulator FitoMas®-E. The objective of the second experiment was to determine the effect of the treatments using a greater number

of combinations and observations of maize growth and yield. Finally, a validation test was carried out under production conditions with the treatment that best behaved in the experiments.

GENERAL EXPERIMENTAL CONDITIONS IN THE TWO EXPERIMENTS

Original seeds of the variety FR-Bt1 obtained in the Center of Genetic Engineering and Biotechnology were used through the event TC1507 (5). As refuge (sowing of four rows as edge area, two for each side of the plot) the original variety FR 28 was used, supplied by the Artemisa Seed Company. The preparation of the soil and the rest of the cultivation work were carried out according to the technical norm for the cultivation of the modified variety (22).

As a mycorrhizal inoculant, the mycorrhizal species *Glomus cubense* (23), (commercial product EcoMic®) was used, from the stock of the National Institute of Agricultural Sciences, with a superior fungal richness to 20 spores per gram of inoculum. The inoculant was applied by the method of coating the seeds, mixing the inoculum with water and the seed (24).

In both experiments, plots of 8 m long by 5,4 m wide with six rows per plot and a plantation frame of $0,45 \times 0,90$ m were used.

In experiment 1, irrigation was performed by watering; while, in experiment 2, a furrow irrigation system was used. In both cases, seven irrigations were carried out during the entire crop cycle, with a delivery equivalent to 250 m³ ha⁻¹.

To control weed plants in the areas planted with the FR-Bt1 transgenic variety, the herbicide "Fínale" (active ingredient glufosinate ammonium) was applied 15 days after emergence (dae), at a dose of 1,5 L ha ⁻¹. In the refuge, two manual controls were carried out between days 10 and 35 dae.

The FitoMas®-E came from the ICIDCA, with a composition of 150 g L⁻¹ of organic extract; 55 g L⁻¹ of total N; 60 g L⁻¹ of K_2O and 31 g L⁻¹ of P_2O_5 (13). A first foliar application was applied by sprinkling at a rate of 2,5 L ha⁻¹ at 35 days after the emergence (dae) and a second application at the same dose at the beginning of the flowering phase in the crop. The applications were made with a 15 L manual sprayer.

Experiment 1. It was the preliminary evaluation of the behavior of the FR-Bt1 corn variety with the use of the AMF inoculant EcoMic® and the plant growth stimulator FitoMas®-E.

The experiment was carried out in the "Niña Bonita" Cattle Company, located in the Artemisa province, on a Ferricitic Red typical eutric soil (25), according to the Classification of Soils of Cuba (Nitisol Ferrálico Líxico, Eutrico,

Ródico), according to the World Reference Base for Soil Resources WRB (26). Its main chemical characteristics are presented in Table I.

Table I. Chemical characteristics of the soil of the "Niña Bonita" Cattle Company area, used in Experiment 1

$pH_{_{H_2O}}$	MO	P ₋₁	Ca ²⁺	Mg^{2+}	Na ⁺	K ⁺	ССВ
2	(%)	(mg P kg)		(cmol kg)			
6,50	3,25	14,00	9,70	2,20	0,15	0,21	12,26

Analysis Method: pH $_{(H_2O)}$, organic matter (Wakley-Black), P (Oniani, H $_2SO_4$ IN), K and other changeable cations, Maslova (NH $_4$ Ac pH 7), la CCB by sum of the bases (27)

According to the analyzes, this soil is acidic, with medium organic matter contents, low levels of K, Mg and base change capacity, very low in Ca and Na (28) and low in phosphorus (29).

The following treatments were tested, distributed in a randomized block design with three replicas:

- ♦ 50 % NPK
- ◆ 50 % NPK + EcoMic® + FitoMas®-E
- ♦ 75 % NPK + EcoMic® + FitoMas®-E
- ♦ 100 % NPK

Table II shows the doses of each of the primary macroelements used in the treatment of 100 % of mineral fertilization, according to the instructions of the variety (22).

Table II. Dose of nutrients applied in the cultivation of corn (Kg ha⁻¹), corresponding to 100 % of the complete formula of NPK 9-13-17

Fertil	Fertilización de base		Fertilización nitrogenada adiciona		
N	P,O,	K,O	N		
45	65	85	138		

The chemical fertilizer was applied prior to sowing, in a narrow band at the bottom of the furrows, separated from the seed with a layer of soil. 30 days after the emergency (dae) the second application of nitrogen with urea was carried out, next to the row of plants, covered by a hilling.

The experiment was harvested when the beans had approximately 18 % moisture. The yield was expressed in tons per hectare (t ha⁻¹), for which the dry mass of the grains was weighed in 76 plants of each plot, adjusting to 14 % humidity.

To evaluate the components of the yield, 20 cobs of corn were collected per plot and the fresh mass of the cob (g) and the dry mass of the beans per ear (g per cob) were determined.

Experiment 2. It was study of the mineral effect of the fertilizer combination, EcoMic® mycorrhizal inoculant and FitoMas®-E growth stimulator on the vegetative development, yield and its components.

The experiment was carried out in the experimental plot of the Center for Genetic Engineering and Biotechnology, on a typical leached Ferralitic Red eutric soil according to Classification of the Soils of Cuba (25), which corresponds to the soils Nitisol Ferrálico Líxico, Eutrico, Ródico, according to the WRB (26), whose chemical characterization is presented in Table III.

Table III. Chemical characteristics of the soil of the experimental plot of the Center for Genetic Engineering and Biotechnology, where the study was carried out

$pH_{H_2^O}$	MO	P ₋₁	Ca ²⁺	Mg^{2+}	Na ⁺	K ⁺	ССВ
2	(%)	(mg P kg)		(c	mol kg)	
6,65	2,72	4,70	18,10	7,40	0,17	0,95	26,62

Analysis Method: pH $_{(H_2O)}$, organic matter (Wakley-Black), P (Oniani, H2SO4 IN), K and other changeable cations, Maslova (NH4Ac pH 7), la CCB by sum of the bases (27)

According to the analyzes the soil is slightly acidic, low in organic matter and Ca, it has high K content, medium Mg, very low Na and medium CCB (28) and low phosphorus (29).

Three doses of NPK were studied: 0, 50 and 75 % similar to the doses used in experiment 1, which were combined with EcoMic® and FitoMas®-E. Fertilization and urea were applied in a similar way as was done in the experiment 1. A randomized block design was used with 13 treatments and three replications.

Treatments

- ♦ 0 % NPK 50 % NPK + EcoMic + FitoMas-E
- ♦ 0 % NPK + EcoMic 75 % NPK
- ♦ 0 % NPK + FitoMas-E 75 % NPK + EcoMic
- ◆ 0 % NPK + EcoMic + FitoMas-E 75 % NPK + FitoMas-E
- ◆ 50 % NPK 75 % NPK + EcoMic + FitoMas-E
- ◆ 50 % NPK + EcoMic 100 % NPK
- ♦ 50 % NPK + FitoMas-E

VEGETATIVE DEVELOPMENT EVALUATION

Twenty plants were selected by random treatments, in which they evaluated the height (cm) measured from the neck of the root to the axilla of the youngest leaf at the time of change from vegetative primordium to floral primordium.

The dry mass of the foliage was determined at the time of grain filling, for which the foliage of 20 plants was randomly harvested and placed in Kraft paper, in a stove at a temperature of 70 °C until constant weight was reached.

DETERMINATION OF PERFORMANCE AND ITS COMPONENTS

The experiment was harvested when the beans had approximately 18% moisture. To evaluate the components of the yield, 20 ears of corn were collected per plot at the time of harvest, in which the number of ears per plant, the number of rows per ears and the fresh mass of the ear were determined and dry mass of the grains per cob in a similar way as was described in experiment 1.

In addition, the mass of 1000 grains (g) at 14 % humidity was evaluated. The yield was determined in a manner similar to that described in experiment 1, and the IRRN Performance Response Index (30) was calculated by means of the expression:

IRRN = kg yield with the nutrient per kg yield without the nutrient

Extension of results in production conditions

In order to know how the best treatment of experiments in experimental plots behaves under production conditions, extension tests were carried out during two years in the spring stage, in areas of the municipality of Venezuela in the province of Ciego de Ávila, on a Red compacted Ferralitic soil (25), which corresponds to Nitisol Ródico soils, according to the WRB (26). The chemical characteristics of the soil are shown in Table IV. To ensure the development of the crop potentialities, the technical standard for corn cultivation was applied (29), modifying the fertilization doses according to the results obtained in the experiments already described. In the first year we worked on 3 590 ha and the second on 786 ha.

Tabla IV. Características químicas del suelo del área de producción del Municipio Venezuela de la provincia Ciego de Ávila, donde se realizaron los trabajos de extensión

pH _H O	MO	P	Ca ²⁺	Mg^{2+}	Na ⁺	K ⁺	ССВ
2	(%)	(mg P kg-1)		(c	mol kg	·-1)	
6,50	1,50	16,00	8,52	2,17	0,20	0,44	11,12

Analysis Method: pH $_{(H_2O)}$, organic matter (Wakley-Black), P (Oniani, H $_2$ SO $_4$ IN), K and other changeable cations, Maslova (NH $_4$ Ac pH 7), la CCB by sum of the bases (27)

According to the results of the chemical characterization the soil is acidic, low in organic matter, CCB and Mg, medium in K; very low in Ca and Na (28) and low phosphorus content (29).

In this extension the nutrition scheme was used with the combined use of the mycorrhizal inoculant, the growth stimulator and 50 % of the NPK dose and the economic savings obtained solely by using less mineral fertilizer were calculated.

STATISTICAL ANALYSIS

For the statistical analysis in both experiments, the compliance of the ANOVA premises was verified, such as the normality and homogeneity of the variance and subsequently the data were statistically processed by comparing the means by the Duncan Multiple Range Test with a level of significance of 5 % error probability (31). For the processing of all information, the statistical package Statgraphics Centurión XVII (32) was used.

RESULTS AND DISCUSSION

EXPERIMENT 1. IT WAS THE PRELIMINARY EVALUATION OF THE BEHAVIOR OF THE FR-BT1 CORN VARIETY WITH THE USE OF AMF INOCULANT ECOMIC® AND THE FITOMAS®-E GROWTH STIMULATOR

The results in fresh mass of the cob, dry mass of the grains and yield are shown in Table V. In the treatment where only 100 % of NPK was applied and in which the mineral fertilizer was combined with the AMF and the stimulator of growth, higher values were obtained when only 50 % of NPK was applied without the bioproducts.

The fresh mass of the cob showed higher values than those referred in the descriptor of the distinctive agronomic characteristics of the variety FR-Bt1 (33), for this indicator, corresponding to 194.21 (33).

In relation to the dry mass of the grains per cob (14% humidity), for the first ear of this variety an average value of 164,01 g is reported. However, those obtained in the present work were inferior to those referred in the descriptor, which may be due to the fact that they correspond to averages of 20 cobs of different positions in the plant, where the first cob is the one with the largest size and mass, the others being lower.

Regarding the yield, the variety can reach levels higher than 4 t ha⁻¹, (5) which were reached in all the treatments except for applying only 50% of NPK.

With the application of bioproducts it was possible to achieve levels similar to those obtained with 100 % NPK, which shows the positive effect of the AMF and the plant growth stimulator, by allowing the reduction of chemical fertilizer by 50 % without affecting the productivity of the crop, in spite of the fact that soil had low phosphorus and potassium content.

These results coincide with those reported for the variety FR 28 with the application of AMF (16), which was the parent used to obtain the FR-Bt1 variety. Similar results are also reported in other crops, when using EcoMic® and FitoMas®-E, in an isolated or combined form (15,21, 34-39).

EXPERIMENT 2. STUDY OF THE COMBINATION
EFFECT OF THE MINERAL FERTILIZER, MYCORRHIZAL
INOCULANT ECOMIC® AND GROWTH STIMULATOR
FITOMAS®-E ON VEGETATIVE DEVELOPMENT AND
VIELD

Vegetative development

Table VI shows the height values of the plant at the time of the change from vegetative primordium to floral primordium and the total dry mass of the plants at the time of harvest.

Table V. Evaluation of fresh dough cobs and dry mass of grains per cob (18% humidity) and yield (14% humidity) in the FR-Bt1 corn variety, with different doses of NPK and the EcoMic[®] and FitoMas[®]-E bioproducts

Tratamientos	Compone	Rendimiento		
	Masa fresca de las mazorcas (g)	Masa seca de los granos por mazorca	(g)	(t ha ⁻¹)
50 % NPK	182,00 b	104,20 b		3,77 b
50 % NPK + EcoMic® + FitoMas®-E	210,00 a	130,60 a		5,16 a
75 % NPK + EcoMic® + FitoMas®-E	208,50 a	117,50 a		4,71 a
100 % NPK	206,60 a	121,40 a		4,79 a
EE +/-	17,80	0,22		0,48

Means with common letter are not significantly different according to Duncan (p \leq 0,05)

Table VI. Height of the plant and total dry mass obtained by the corn plants variety FR-Bt1, with different doses of NPK and the products EcoMic® and FitoMas®-E

Tratamientos	Altura de la planta (cm)	Masa seca total (g)
0 % NPK	59,9 d	156,0 d
0 % NPK + EcoMic®	67,8 d	167,0 d
0 % NPK + FitoMas®-E	72,4 c	205,0 bc
0 % NPK+ EcoMic® + FitoMas®-E	79,1 c	214,0 bc
50 % NPK	92,9 c	219,0 b
50 % NPK + EcoMic®	100,1bc	224,0 a
50 % NPK+ FitoMas®-E	87,9 c	221,0 b
50 % NPK + EcoMic® + FitoMas®-E	186,9 a	224,0 a
75 % NPK	92,2 c	219,0 b
75 % NPK + EcoMic®	121,9 b	224,0 a
75 % NPK + FitoMas®-E	102,0 bc	221,0 b
75 % NPK + EcoMic®+ FitoMas®-E	202,2 a	224,0 a
100 % NPK	200,2 a	223,0 a
EE +/-	0,41	0,21

Means with common letter do not differ significantly as multiple range test Duncan ($p \le 0.05$)

In general, when analyzing the indicators height of the plant and total dry mass, with the application of 50 or 75 % of the dose of NPK, without bioproducts, similar results were achieved, which were lower than the 100 % treatment which responds to the conditions of low soil fertility and the particularities of the crop.

The treatments where 50 or 75 % of the NPK dose combined with EcoMic® and FitoMas®-E and the one corresponding to 100 % of NPK were applied were the best response but only when applied 75 % of the NPK dose , they approached plant height values according to the descriptor corresponding to the variety, (205-225 cm) (33), although they showed statistical similarity with the application of 50 % NPK with EcoMic® and FitoMas®-E. Considering that the descriptor refers to the height in the finished flowering stage and the data shown corresponds to the phase of change from vegetative primordium to floral primordium, the need for adequate nutrition is revealed, since in the rest of the variants are reached values well below the one referred by the descriptor.

When analyzing the total dry mass, the response was similar to that obtained in the evaluation of the height of the plants. The highest values were reached in the treatments in which 50 or 75 % of NPK and both bioproducts were jointly applied, which showed statistical similarity with 100 % NPK.

This result shows that mineral fertilization plays a very important role in the production of dry matter throughout the vegetative and reproductive process of crops.

The unique application of the EcoMic® independently did not achieve a positive effect on both indicators, but the FitoMas®-E was superior to the control. However, when applied together with chemical fertilizer, the treatments that included the EcoMic® reached equal yields when the three products were applied. This result is explained because in order to achieve an effective arbuscular mycorrhizal symbiosis, the inoculation must be combined with low or medium doses of fertilizers (40), which significantly increases the growth and development of the plants, the phosphorus content and the accumulation of dry mass (41,42). However, good results have been found with the strain used in low P-available soils (43), but in experiments in pots where its root system explores practically all the soil.

Similar results have been found with the combination of chemical fertilizer with mycorrhiza EcoMic® and the plant growth stimulator FitoMas®-E in non-genetically modified corn varieties (34). The positive effect of FitoMas®-E has also been obtained in indicators of vegetative development in other crops such as beans and sugar cane (37,38).

YIELD AND ITS COMPONENTS

Table VII shows the evaluation results of the performance components and in Table VIII the performance results.

All treatments had a positive and similar effect on the number of cobs, being less marked with the independent application of the mycorrhizal inoculant, which does not differ from the treatment that did not receive any of the products studied. In the other indicators, the response to the chemical fertilizer was ascending with the dose, responding to the low content of nutrients in the soil. The individual application of the mycorrhizal inoculant did not have a positive effect on the yield components except the number of rows per ear. The applied plant growth stimulator was only superior to the control and the same as when applied together with the AMF, without the application of mineral fertilizer. When the AMF and the growth stimulator, individually, were combined with 50 or 75 % of the NPK dose, the results were superior to the control that did not receive any product, however only when both products were combined with 50 or 75 % of NPK reached values similar to those higher than those obtained in the present study. The highest values, equal to adding 100 % of NPK were found when the bioproducts were combined with 50 or 75 % of the dose of the chemical fertilizer.

These results corroborate those obtained in the first experiment, where response was observed to the increase of fertilizer levels, which responds to the fertility of the soil where it was worked. The limitation on nutrition also determined that the response to the individual or combined application of bioproducts did not allow the variety to reach its potential of 4 t ha⁻¹, which was achieved when 50 % of the proposed dose was applied for variety. since the application of nitrogen, phosphorus and potassium, allows the crop to respond optimally and achieve an increase in yields; However, a deficit can lead to a decrease in yields and its components (40). Similar results have been reported for non-transgenic maize, where, the lack of nutrients in the corn crop, produced a reduction of 10 to 30 % yield (15,39,42). The 50 % reduction in the dose of chemical fertilization by 50 % also means significant savings in fertilizer, similar to that reported by other authors (15,39).

The IRRN Performance Response Index (30), shown in Table VIII, highlights that the best treatments exceed about six times, being important for work under production conditions, that each bioproduct cambering only with 50 % of the NPK dose, the yield of maize can practically be tripled.

Only with the addition of doses higher than 50 % of NPK combined with the two products studied and with only 100 % of the fertilizer were the potential yield values of 4 t ha⁻¹ of the variety reached. This result shows that only under these conditions in the soils of the characteristics similar to those studied, would be possible to express the crop's yield potential, from the point of view of crop nutrition.

The lack of response to the addition of the AMF and the growth stimulator, individually, with or without application of NPK, which did not allow the expression of crop yield potential, can respond to the low P content of the soil where the experiment was developed, together with a very low percentage of organic matter. When the soil has low nutrient contents, being limiting elements potassium, nitrogen and even more important phosphorus, the arbuscular mycorrhizal symbiosis, can be reduced or inhibited, not achieving the plant express a positive response (9).

Table VII. Evaluation of yield and yield components in the FR-Bt1 corn variety, with different doses of NPK and the EcoMic® and FitoMas®-E bioproducts

	Componentes del rendimiento							
Tratamientos	Número de mazorcas por planta	Número de hileras por mazorca	Masa fresca de la mazorca (g)	Masa seca de granos por mazorca (g) 18 % humedad	Masa de 1000 granos (g) 14 % humedad			
0 % NPK	1,0b	10,9f	46,2 d	26,2 e	140 e			
0 % NPK + EcoMic®	1,1ab	11,9e	54,3 d	28,6 e	145 e			
0 % NPK + FitoMas®-E	1,2a	12,8cd	74,7 c	41,4 de	213 d			
0 % NPK+ EcoMic®+ FitoMas®-E	1,2 a	12,9bc	76,5 c	45,6 de	231 d			
50 % NPK	1,2 a	12,3cd	79,5 bc	57,2 cd	246 d			
50 % NPK + EcoMic®	1,2 a	13,8bc	87,1 bc	61,2 cd	305 cd			
50 % NPK+ FitoMas®-E	1,2 a	13,6bc	90,6 bc	72,8 bc	326 bc			
50 % NPK + EcoMic®+ FitoMas®-E	1,2 a	14,4a	174,1 a	154,1 a	366 a			
75 % NPK	1,2 a	13,15bc	102,5 bc	76,1 bc	335 b			
75 % NPK + EcoMic®	1,2 a	12,95bc	136,7 b	81,7 b	337 b			
75 % NPK + FitoMas®-E	1,2 a	13,5b	137,9 b	89,9 b	345 b			
75 % NPK + EcoMic®+ FitoMas®-E	1,2 a	14,4a	181,9 a	153,9 a	381 a			
100 % NPK	1,2 a	14,4a	176,1 a	155,1 a	373 a			
EE +/-	0,22	0,46	11,2	17,8	21,6			

Means with common letter do not differ significantly as multiple range test Duncan (p \leq 0,05)

Table VIII. Evaluation of performance components in the FR-Bt1 corn variety, with different doses of NPK and the EcoMic® and FitoMas® bioproducts - E

Tratamientos	Rendimiento (t ha ⁻¹)	IRRN	Tratamientos	Rendimiento (t ha ⁻¹)	IRRN
0 % NPK	0,82 e		50 % NPK + EcoMic®+ FitoMas® - E	4,88 a	6,0
0 % NPK + EcoMic®	0,90 e	1,1	75 % NPK	2,41 bc	2,9
0 % NPK + FitoMas®-E	1,31 de	1,6	75 % NPK + EcoMic®	2,56 b	3,1
0 % NPK+ EcoMic®+ FitoMas®-E	1,44 de	1,76	75 % NPK + FitoMas® - E	2,84 b	6,0
50 % NPK	1,81 cd	2,2	75 % NPK + EcoMic®+ FitoMas® - E	4,87 a	6,0
50 % NPK + EcoMic®	1,94 cd	2,4	100 % NPK	4,94 a	6,0
50 % NPK+ FitoMas®-E	2,31 bc	2,8	EE +/-	25,00	
EE +/-	25,00				

Means with common letter do not differ significantly as multiple range test Duncan (p \leq 0,05)

The effect of FitoMas®-E responds to its stimulating action, since with the application of $5\,L\,ha^{-1}$, which is used in the experiment, only the plant in leaf form is supplied with 275 g of N, 150 g of P_2O_5 and 300 g of K_2O per hectare, which do not compensate the nutrient contents, which are no longer applied with 50 % of the recommended dose for the variety (11).

EXTENSION OF RESULTS IN PRODUCTION CONDITIONS

The results obtained with the application of 50 % of the recommended dose of NPK, combined with EcoMic® and FitoMas®-E, in productive areas of Ciego de Ávila are shown in Table IX.

Table IX. Productive results of the 2011 and 2012 campaign in Ciego de Ávila

Campaña	Área sembrada (ha)	Rendimientos (t ha ⁻¹)
2011	3590	4,45
2012	786	4,32

Analyzed the yields, it was observed that, in both years, the levels were similar to those obtained in the experiments and even higher than the one referred in the descriptor of the variety, which shows a positive response to the fertilization variant used and it is verified that with Using the EcoMic® and FitoMas®-E it is possible to reduce the dose of NPK chemical fertilizer by up to 50 % (15-17,34,36). Similar responses were found in validations carried out in areas of Matanzas and Sancti Spíritus provinces (16). These results mean bringing fewer chemicals to the soil and saving in the use of fertilizer carriers.

CONCLUSION

In less fertile soils, similar to those of the experiments carried out, the growth, development and yield of the FR-Bt1 corn variety (genetically modified) is affected when lower NPK doses are used than those established to reach the potential yield of variety. The combination of mineral fertilization with EcoMic® and FitoMas®-E allows the reduction of the fertilizer dose by 25 or 50 %, without affecting the behavior of the crop, similar to how it has been reported by different authors for corn and other crops that are not genetically modified.

BIBLIOGRAPHY

- FAO. Anuario Estadístico de la FAO [Internet]. FAOSTAT. 2014 [cited 2015 Jun 27]. Available from: http://faostat3. fao.org/home/E
- Binning RR, Coats J, Kong X, Hellmich RL. Susceptibility and Aversion of Spodoptera frugiperda (Lepidoptera: Noctuidae) to Cry1F Bt Maize and Considerations for Insect Resistance Management. Journal of Economic Entomology. 2014;107(1):368–74. doi:10.1603/EC13352
- 3. Blanco Y, Leyva Á. Las arvenses y su entomofauna asociada en el cultivo del maíz (*Zea mays*, L.) posterior al periodo crítico de competencia. Cultivos Tropicales. 2009;30(1):11–7.
- 4. James C. Situación mundial de la comercialización de cultivos GM/transgénicos en 2008 [Internet]. International Service for the Acquisition of Agri-biotech Applications (ISAAA); 2014 [cited 2015 Jul 22] p. 26. Available from: https://www.isaaa.org/resources/publications/briefs/41/executivesummary/pdf/Brief %2041 %20- %20Executive %20Summary %20- %20Spanish.pdf
- Ayra C. Variedad transgénica de maíz FR-Bt1, una alternativa viable para el desarrollo del cultivo en Cuba. Revista de Biotecnología Aplicada. 2009;26(4).
- Rabí O, Permuy N, García E. Nueva variedad de maíz (Zea mays) FR-28 con alto potencial de rendimiento. Agrotecnia de Cuba. 1997;27(1):43–44.

- Lafitte HR. Identificación de problemas en la producción de maíz tropical: guía de campo [Internet]. México, DF: Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT); 1994 [cited 2017 Jul 7]. 122 p. Available from: https://books.google.com.cu/books?id=OUVBwJBXYecC
- Aguado-Santacruz GA. Uso de microorganismos como biofertilizantes. In: Introducción al uso y manejo de los biofertilizantes en la agricultura [Internet]. Celaya, Guanajuato, México: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) - Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA); 2012 [cited 2017 Jul 7]. p. 1–11. Available from: http://www.bioqualitum.com/sites/ default/files/publicaciones/Libro-biofertilizantes.pdf
- Bosques-Macías G. Introducción. In: Aguado-Santacruz GA, editor. Introducción al uso y manejo de los biofertilizantes en la agricultura [Internet]. Celaya, Guanajuato, México: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) - Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA); 2012 [cited 2017 Jul 7]. p. 12–4. Available from: http://www.bioqualitum.com/sites/ default/files/publicaciones/Libro-biofertilizantes.pdf
- Martínez-Viera R, Dibut B, Ríos Y. Efecto de la integración de aplicaciones agrícolas de biofertilizantes y fertilizantes minerales sobre las relaciones suelo-planta. Cultivos Tropicales. 2010;31(3):27–31.
- Montano R, Zuaznabar R, García A, Viñals M, Villar J. Fitomas E: Bionutriente derivado de la industria azucarera. ICIDCA. Sobre los derivados de la caña de azúcar. 2007;41(3):14–21.
- 12. Martínez-Plácido N, Alfredo-González J, Beatríz-Piñeiro D. Efectos del Fitomas-E en el maíz (Zea mays L.) variedad Tuzón, en las condiciones edafoclimáticas del municipio "Amancio Rodríguez", Las Tunas. Innovación Tecnológica. 2013;19(1):1–12.
- Yan S, Du X, Wu F, Li L, Li C, Meng Z. Proteomics insights into the basis of interspecific facilitation for maize (*Zea mays*) in faba bean (*Vicia faba*)/maize intercropping. Journal of Proteomics. 2014;109:111–24. doi:10.1016/j. jprot.2014.06.027
- 14. Fusconi A. Regulation of root morphogenesis in arbuscular mycorrhizae: what role do fungal exudates, phosphate, sugars and hormones play in lateral root formation? Annals of Botany. 2014;113(1):19–33. doi:10.1093/aob/mct258
- 15. Díaz A, Loredo C, García J, Cortinas H, Peña-del Río MA. Inoculantes Microbianos como Promotores de la Producción Sostenible de Maíz en Condiciones Semiáridas. In: Aguado-Santacruz GA, editor. Introducción al uso y manejo de los biofertilizantes en la agricultura [Internet]. Celaya, Guanajuato, México: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA); 2012 [cited 2017 Jul 7]. p. 241–68. Available from: http://www.bioqualitum.com/sites/default/files/publicaciones/Libro-biofertilizantes.pdf
- Rivera R. Avances y retos en el manejo de los inoculantes micorrícicos en Cuba. In La Habana, Cuba: Ediciones INCA; 2010.

- 17. Fundora LR, Cabrera JA, González J, Ruiz LA. Incrementos en los rendimientos del cultivo de boniato por la utilización combinada del fitoestimulante FitoMas-E® y el biofertilizante EcoMic® en condiciones de producción. Cultivos Tropicales. 2009;30(3):14–7.
- 18. Cheeke TE, Rosenstiel TN, Cruzan MB. Evidence of reduced arbuscular mycorrhizal fungal colonization in multiple lines of Bt maize. American Journal of Botany. 2012;99(4):700–7. doi:10.3732/ajb.1100529
- 19. Cheeke TE, Cruzan MB, Rosenstiel TN. Field Evaluation of Arbuscular Mycorrhizal Fungal Colonization in Bacillus thuringiensis Toxin-Expressing (Bt) and Non-Bt Maize. Applied and Environmental Microbiology. 2013;79(13):4078–86. doi:10.1128/AEM.00702-13
- Cheeke TE, Schütte UM, Hemmerich CM, Cruzan MB, Rosenstiel TN, Bever JD. Spatial soil heterogeneity has a greater effect on symbiotic arbuscular mycorrhizal fungal communities and plant growth than genetic modification with *Bacillus thuringiensis* toxin genes. Molecular Ecology. 2015;24(10):2580–93. doi:10.1111/mec.13178
- 21. Calderón AA, Marrero YJ, Martín JV, Mayo I. La fertilidad de los suelos y su importancia en el empleo de bioproductos en la provincia de Sancti Spíritus. Cultivos Tropicales. 2013;34(2):16–23.
- 22. CIGB (Centro de Ingeniería Genética y Biotecnología). Guía técnica para el manejo de la variedad FR-Bt1. La Habana, Cuba: CIGB; 2012. 5 p.
- 23. Rodríguez Y, Dalpé Y, Séguin S, Fernández K, Fernández F, Rivera RA. *Glomus cubense* sp. nov., an arbuscular mycorrhizal fungus from Cuba. Mycotaxon. 2011;118(1):337–47. doi:10.5248/118.337
- 24. Fernández F, Gómez R, Vanegas LF, de la Noval BM, Martínez MA. Producto inoculante micorrizógeno. La Habana, Cuba: Oficina Nacional de Propiedad Industrial; 22641, 2000.
- Hernández JA, Pérez JJM, Bosch ID, Castro SN. Clasificación de los suelos de Cuba 2015. Mayabeque, Cuba: Ediciones INCA; 2015. 93 p.
- 26. IUSS Working Group WRB. World Reference Base for soil resources 2014: international soil classification system for naming soils and creating legends for soil maps. Rome: Food and Agriculture Organization of the United Nations; 2014. 191 p. (World Soil Reports).
- 27. Paneque PVM, Calaña NJM, Calderón VM, Borges BY, Hernández GTC, Caruncho CM. Manual de técnicas analíticas para análisis de suelo, foliar, abonos orgánicos y fertilizantes químicos [Internet]. 1st ed. La Habana, Cuba: Ediciones INCA; 2010 [cited 2016 Jan 27]. 157 p. Available from: http://mst.ama.cu/578/
- Paneque PVM, Calaña NJM. La fertilización de los cultivos aspectos teórico prácticos para su recomendación. La Habana, Cuba: Ediciones INCA; 2001. 27 p.
- Cancio R. El Servicio Agroquímico. La Habana, Cuba: Dirección General de Suelos y Fertilizantes, MINAG; 1982. 17 p.
- 30. Syers JK, Johnston AE, Curtin D. Efficiency of soil and fertilizer phosphorus use: reconciling changing concepts of soil phosphorus behaviour with agronomic information [Internet]. Rome, Italy: Food and Agriculture Organization of the United Nations; 2008 [cited 2017 Jul 7]. 108 p. (Fertilizer and plant nutrition bulletin). Available from: ftp://ftp.fao.org/docrep/fao/010/a1595e/a1595e00.pdf

- 31. Duncan DB. Multiple Range and Multiple F Tests. Biometrics. 1955;11(1):1–42. doi:10.2307/3001478
- 32. StatPoint Technologies. Statgraphics Centurion [Internet]. Version 17. 2015. (Centurion). Available from: http://www.statgraphics.net/descargas-centurion-xvii/
- 33. CIGB (Centro de Ingeniería Genética y Biotecnología). Descriptor de las características agronómicas distintivas de la variedad FRBt 1. La Habana, Cuba: CIGB; 2013. 4 p.
- 34. Ramos L, Reyna Y, Lescaille J, Telo L, Arozarena NJ, Ramírez M, et al. Hongos micorrízicos arbusculares, Azotobacter chroococcum, Bacillus megatherium y FitoMas-E: una alternativa eficaz para la reducción del consumo de fertilizantes minerales en Psidium guajava, L. var. Enana Roja cubana. Cultivos Tropicales. 2013;34(1):5–10.
- 35. Álvarez-Rodríguez A, Campo-Costa A, Batista-Ricardo E, Morales-Miranda A. Evaluación del efecto del bionutriente Fitomas-E como alternativa ecológica en el cultivo del tomate. ICIDCA. Sobre los Derivados de la Caña de Azúcar. 2015;49(1):3–9.
- 36. Cruz-Hernández Y, García-Rubido M, León-González Y, Acosta-Aguiar Y. Influencia de la aplicación de micorrizas arbusculares y la reducción del fertilizante mineral en plántulas de tabaco. Cultivos Tropicales. 2014;35(1):21–4.
- 37. Guevara TE, Méndez GJC, Vega LJ, González POS, Puertas AA, Fonseca del C. Influencia de diferentes dosis de FitoMas-E en el frijol común. Centro Agrícola. 2013;40(1):39–44.
- 38. Zuaznabar-Zuaznabar R, Pantaleón-Paulino G, Milanés-Ramos N, Gómez-Juárez I, Herrera-Solano A. Evaluación del bioestimulante del crecimiento y desarrollo de la caña de azúcar FITOMAS-E en el estado de Veracruz, México. ICIDCA. Sobre los Derivados de la Caña de Azúcar. 2013;47(2):8–12.

- 39. Aguirre-Medina JF, Durán-Prado A, Peña-del Río Á, Grageda-Cabrera O, Irizar-Garza M. Micorriza INIFAP: Biofertilizante para el Campo Mexicano. In: Aguado-Santacruz GA, editor. Introducción al uso y manejo de los biofertilizantes en la agricultura [Internet]. Celaya, Guanajuato, México: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA); 2012 [cited 2017 Jul 7]. p. 219–40. Available from: http://www.bioqualitum.com/sites/default/files/publicaciones/Libro-biofertilizantes.pdf
- 40. Rivera R, Fernández F, Fernández K, Ruiz L, Sánchez C, Riera M. Advances in the Management of Effective Arbuscular Mycorrhizal Symbiosis in Tropical Ecosystems. In: Mycorrhizae in crop production. Binghamton, N.Y.: Haworth Food & Agricultural Products Press; 2007. p. 151–95.
- 41. Irañeta I, Armesto AP, Segura A, Arregui L, Merina M, Baroja E, et al. Herramientas de ayuda a la decisión II: para el manejo correcto de los fertilizantes nitrogenados en maíz. Navarra Agraria. 2003;(138):10–8.
- 42. Amado-Álvarez J, Ávila-Moroni M, Ramírez-Valle O. Micorriza INIFAP y el Incremento de la Productividad de Avena y Maíz en el Estado de Chihuahua. In: Aguado-Santacruz GA, editor. Introducción al uso y manejo de los biofertilizantes en la agricultura [Internet]. Celaya, Guanajuato, México: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA); 2012 [cited 2017 Jul 7]. p. 269–95. Available from: http://www.bioqualitum.com/sites/default/files/publicaciones/Libro-biofertilizantes.pdf
- 43. Mena A, Fernández K, Olalde V, Serrato R. Diferencias en la respuesta del maíz (*Zea mays* L.) a la inoculación con *Glomus cubense* (y. Rodr. & Dalpé) y con un conglomerado de especies de hongos micorrízicos arbusculares (HMA). Cultivos Tropicales. 2013;34(2):12–5.

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