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Effect of Five Biostimulants on Sugar Cane Seedling Strengthening and Resistance to Transplantation

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Abstract

Context: The stress undergone by sugar cane during the seedling strengthening stage may be caused by various reasons, such aspest infestation, nutritional deficiencies, and mechanical damages due to manipulation that leads to the loss of genetic material.

Objective: To evaluate the effect of five biostimulants on sugar cane seedling strengthening and resistance to transplantation.

Methods: A completely randomized experimental design with six treatments (absolute control and samples with the application of azotobacter, phosphorine, improved natural liquid humus, and mineral-fortified liquid humus (BoCalZn), and four repetitions, was used. The seedlings were placed on a 60-well tray filled with a mix of soil and filter cake. Seven foliar applications were made between days 7 and 56 following transplantation. Plant height and thickness, foliar area, root length, number of active roots, and fresh and dry weightswere evaluated 50 days after. For evaluation of resistance to transplantation, the strengthened seedlings were placed in the field, and 45 days later, their survival percentage was determined.

Results: The positive effects of the biostimulants applied were observed on the morphophysiological indicators.

Conclusions: The application of biostimulants showed the positive effect on the morphophysiological indicators evaluated. The best transplantation resistance of the mineral-fortified liquid humus (BoCalZn) was observed in field conditions.

Key words: humus, sugar cane, azotobacter, phosphorine, genetic breeding.

Introduction

Sugar cane is an important source of nutrition and bioenergy, a significant element in the economy of many tropical and subtropical countries. Its economic value lies in three attributes: high productivity, efficient use of cropping inputs (water, fertilizers, pesticides, management), and possibility of being locally processed to obtain various derivatives, like sugar, molasses, ethanol, and energy, which are all easy to transport and store. These attributes make sugar cane one of the basic items of global economy.

Sugar cane cultivars are likely to suffer irreversible damages that demand renovation and replacement of useless plants with new ones, which can provide a better response to different environmental conditions with higher industrial and phytosanitary features (Fernández et al., 2017). Accordingly, the main producing countries pay significant attention to the genetic breeding programs of sugar cane.

The Sugar Cane Research Institute (INICA) comprises a network of stations across Cuba, whose main goals are generation and recommendation of new commercial cultivars. These are selected from the national scheme that goes through a series of stages with a lifespan of 10-12 years (Jorge et al., 2011).

The seedlings (seed clones) stage is critical, since it marks the beginning of the selection process after hybridization. A large number of seedlings offer more genetic material for evaluation in each of the stages of the scheme; however, they undergo stress during the strengthening period, which may be caused by several reasons, such as, pests, nutritional deficiencies, and mechanical damage due to manipulation. These produce loss of genetic material in this stage, so it is necessary to deploy a set of strategies devised to minimize the effects of these adversities (Fernández et al., 2017). Today, there are numerous biologicals with the capacity to enhance crop growth and productivity (Almenares et al., 2002). These compounds are natural biostimulants that benefit plant growth and improve the conditions of the soil that favor plant germination, development, and production (Garcés, Arteaga & Díaz, 2002).

Hence, the use of biostimulants might become a viable and sustainable alternative to produce healthy and strong sugar cane seedlings that can survive after transplantation, and more genetically variable material for further selection of new commercial cultivars.

Materials and methods

The experiment was conducted at the Territorial Station for Sugar Cane Research (ETICA), Camagüey, in the municipality of Florida, on brown soil with carbonates (Soil Institute, 1975; Hernández et al., 1999), coordinates 21º 31' north latitude, and 78º 04' west longitude, 57.08 m above sea level (Agro-meteorological Station of Florida, Camagüey, 2016). This researchwas designed in two stages to better meet the preset goals. First stage: A randomized block design with six treatments and four repetitions was used (Table 1).

Table 1. Dosage per treatments in the study.

Treatments	Dose
T1 Absolute control.	-
T2 Azotobacter.	2 kg ha^{-1}
T3 Phosphorine.	2 kg ha^{-1}
T4 Improved liquid humus (Natural liquid humus with , phosphorine, azotobacter, and glucose)	2 L ha^{-1}
T5 Fortified liquid humus (phosphorine, azotobacter, macro and micro. nutrients and molasses as adherents).	2Lha^{-1}
T6 Fortified liquid humus with minerals (BoCalZn)	2 Lha^{-1}

The biostimulantswere applied according to the recommendations of the Basic Technological and Scientific Unit (UCTB) of the Soils Institute, Camagüey. To achieve the recommended dose of biostimulants, 0.02 kg of both azotobacter and phosphorinewere weighed, dissolved in 80 mL of running water, and filtered with a mosquito net or colander. The solution was recovered and washed again with 30 mL of water. The substrate was filtered again, and then the two suspensions were mixed (110 mL). The final solution was applied to the four repetitions. The three variants of liquid humus, 20 mL of each biostimulant, were initially measured. Then the volume was increased to 2 L, and it was applied to the four repetitions of each treatment.

The repetitions were constituted by the seedlings derived from C568-75 x Ja60-5 crossing, which were placed in twenty-four 60-well plastic containers (10 x 10 x 15 cm), filled with a substrate of soil and compost in a 3:1 proportion. Each container became a repetition *per se*.

The seedlings selected for the study were planted on October 17, 2017, and were trimmed (roots and leaves) before transplantation. Seven foliar applications weremade with a 7-day interval, between days 7 and 56 following transplantation. Plant height and thickness, foliar area, root length, number of active roots, and fresh and dry weights were evaluated 50 days after. Ten plants per repetition were evaluated in each treatment. Plant height was determined with a measure ruler (cm), from the base to the first visible dewlap. Plant thickness was determined by measuring the stem in the middle with a gauge caliper (mm). The foliar area was determined according to Lerchet et al. (1977). The white roots were considered active, and their length was determined with a ruler (cm). The fresh weight of the plant was determined with a technical balance (Kern). Before determining dry weight, the plants were placed in a forced-air circulation system, at 65 ºC, for 48 hours. Accordingly, the roots from all the plants evaluated had been collected and washed.

The second stage included field plantation of all the treated seedlings in the study. After 45 days of transplantation, the survival percentage was determined using the equation below:

Where survival % $= \frac{PF}{PP} 100$

PS: Physical seedlings

PS: Planted seedlings

Data normality was analyzed for statistical processing of all the variables studied. The standard means and errors were determined in each case. Analyses of variance were performed, and the Turkey's multiple mean comparison test was performed ($p<0.05$). All statistics were analyzed with SPSS, for Windows, version 15.0 (2006).

Results and discussion

Seedling height was influenced by the type of treatments (Table 2). There was evidence that the application of phosphorine produced the highest mean value for this indicator, which did not differ statistically among biostimulant sazotobacter, improved liquid humus, and mineral-fortified liquid humus, but it did differ in relation to the fortified liquid humus and the control. Moreover, the seedlings treated with the fortified liquid humus showed the lowest mean value during the evaluation.

The positive effect of bioorganic alternatives was evidenced, since these products are composed of humic acids; phosphorine, azotobacter, and essential chemical elements that influence the metabolic processes of plants, thus developing efficiently. Likewise, another aspect associated to the positive response of plants to these products is the moment when the bioproductswere applied, early in the morning, when the stomas open for better nutrient intake and plant assimilation. The results observed with phosphorine supported the report made by Khan, Zaidi & Wani (2007) on the importance of phosphate-solubilizing microorganisms as an alternative to chemical fertilizers, since nitrogen, phosphorus, and potassium (NPK) are the main nutrients needed for plant growth. However, the availability of phosphorus is limited, because it transforms almost entirely into its soluble type, leading to inefficient assimilation by the plant (Gyaneshwar et al., 2002).

Table 2. Effect of biostimulant application on plant height.

Treatments	Plant height (cm)
T1 Absolute control.	13.9^{bc}
T2 Azotobacter	15.53^{ab}
T3 Phosphorine	16.28^{a}
T4 Improved liquid humus	15.51^{ab}
T5 Fortified liquid humus	13.14^{b}
T6 Mineral-fortified liquid humus	15.35^{ab}
ESx	0.29

Note: values with different subscript letters indicate significant differences for p: 0.05.

The application of biostimulants increases stem thickness (Table 3). The best results were achieved with the application of improved liquid humus, which did not differ statistically from those based on mineral-fortified liquid humus; however, they differed from the other treatments. The lowest value was observed in the untreated seedlings, using biostimulants (control). This response was caused by the higher nutritional composition of improved liquid humus and mineral-fortified liquid humus, which provided plants with the necessary elements for growth and development, along with more efficient metabolic processes. Presumably, these results were attributed to the fact that the three evaluation studies of different biostimulants did not use seedlings from the same genetic crossings. It corroborated the study of Bernal et al. (1997), cited by Quiñones (2017), who stated that this trait depends largely on the genetic characteristics of every particular genotype. Meanwhile, Díaz et al. (2004) stressed on the positive effect of micropropagated sugar cane acclimatization using worm humus. Likewise, Velasco (2014) remarked the positive effect of biostimulants on sugar cane growth and development.

Table 3. Effect of the application of biostimulants on plant thickness.

Treatments	Plant thickness (mm)
T1 Absolute control.	3.02^{c}
T2 Azotobacter	3.30^{bc}
T3 Phosphorine	3.46^{b}
T4 Improved liquid humus	3.90^{a}
T5 Fortified liquid humus	3.45^{b}
T6 Mineral-fortified liquid humus.	3.58^{ab}
ESx	0.06

Note: values with different subscript letters indicate significant differences for p: 0.05.

The foliar area of the seedlings evaluated was influenced by biostimulants (Table 4). No statistically significant differences were observed among the treatments for this growth indicator. This result was associated to optimum temperatures during that period, along with proper distribution of light in the different treatments on the surface of photosynthetically active leaves. The results of this research in foliar growth were better than the reports made by Fernández et al. (2015 and 2017), which confirmed the adequate physiological state in which the evaluated seedlings developed. Meanwhile, Borges, Barrios & Escalona (2012) pointed out that the application of biostimulants every seven days favors an increase of the fresh weight of leaves greatly (22.9%), compared to the 14-day application also evaluated. The increase of foliar mass could contribute to a faster vegetative growth of young plants, which might survive long-lasting environmental stress, and grow properly after definitive plantation.

Table 4. Effect of the application of biostimulants on foliar area.

Treatments	Foliar area (cm^2)
T1 Absolute control.	87.37^{a}
T2 Azotobacter	88.25^{a}
T3 Phosphorine.	91.43^{a}
T4 Improved liquid humus	82.19^{a}
T5 Fortified liquid humus	84.25^{a}
T6 Mineral-fortified liquid humus	78.49^{a}
ESx	5.47

Note: values with different subscript letters indicate significant differences for p: 0.05.

There are statistically significant differences in root length among the treatments (Table 5). The highest average values were observed in the seedlings treated with the fortified liquid humus, which did not differ statistically from the plants treated with azotobacter and phosphorine, but did differ from the other treatments. The lowest average value was observed in the control treatment. These results evidenced that the best treatments were benefited by their contents of Zn, Ca, Bo, phosphorine, and azotobacter, AIA, because they stimulated root development; even at very low concentrations, they could take part in stem and root growth response.

The root length results achieved in this research in foliar growth were better than the reports made by Fernández et al. (2015 and 2017). The high significance lies in how the indicator behaves when the seedlings adapt to transplantation, when a larger number of roots is equivalent to better possibility to absorb water, nutrients, and minerals deeper in the soil.

Table 5. Effect of the application of biostimulants on root length.

Treatments	Root length (cm)
T1 Absolute control.	11.70^{c}
T2 Azotobacter.	15.55^{ab}
T3 Phosphorine.	13.20^{abc}
T4 Improved liquid humus	12.76^{bc}
T5 Fortified liquid humus	12.35^{c}
T6 Mineral-fortified liquid humus.	15.85^{a}
ESx	0.45

Note: values with different subscript letters indicate significant differences for p: 0.05.

The number of active roots is influenced by the application of biostimulants (Table 6). No statistically significant differences were observed among the treatments. The best average values were reached by the seedlings treated with phosphorine, which only differed statistically from the control treatment (the lowest average value). This may have been caused by the effect of phosphorine as a soil phosphorus solubilizer, which favors better intake, since phosphorus is linked to the increased growth rate of roots. When soluble phosphate compounds are applied to the soil, plant roots extend thoroughly, especially in the treated soil areas.

The average values of active roots achieved in this research were below the reports made by Fernández et al. (2015 and 2017). Despite their lower number, the seedlings were vigorous at the time of washing before weighing. The phosphorine treatment corroborated the reports of Mora (2011) and Padrón et al. (2012) about the important role of phosphorus in the development of the root system of plants, as well as in early shoot growth, increased early productivity, and internode extension growth.

Table 6. Effect of the application of biostimulants on the number of active roots.

Treatments	Number of active roots
T1 Absolute control	2.40^{b}
T2 Azotobacter	3.60^{ab}
T3 Phosphorine	4.10^{a}
T4 Improved liquid humus	2.90^{ab}
T5 Fortified liquid humus	3.30^{ab}
T6 Mineral-fortified liquid humus	2.60^{ab}
ESx	0.07

Note: values with different subscript letters indicate significant differences for p: 0.05.

The effect of biostimulant application can also be seen on the fresh weight of plants (Table 7), with statistically significant differences among the treatments. The highest average values were observed in the seedlings treated with the mineral-fortified liquid humus, which did not differ statistically from the plants treated with fortified liquid humus and improved liquid humus, but they did differ from the other treatments. The lowest average value was observed in the control treatment.

This was a favorable response thanks to the fact that foliar enhancers carry a whole nutritional solution which stimulates plant development more. When these products are applied to the soil, they save fertilizer use, since the micro and macro nutrients are thoroughly assimilated, thus preventing salt concentration. Besides, they contribute with an ideal environment for the proliferation of beneficial organisms, bacteria, fungi, etc., which hinder pathogen development, significantly reducing the risk of diseases. Moreover, they promote soil humification by incorporating and breaking down plant residues.

The results achieved in this research were better than the reports made by Fernández et al. (2015 and 2017). Furthermore, Alfaro (1999) in a study of lower plants evaluated the application of five biostimulant types on sugar cane germination and growth. The average values achieved after the application of liquid humus treatments to each variant were outstanding. These results corroborated the reports of Díaz et al. (2004); Casco and Iglesias (2005); Borges et al. (2014); and Huanio (2017), who claimed that liquid fertilizers containing soil worm humus provide humic and fulvic acids, live organisms for nitrification and solubilization of soil minerals. Plant fresh weight can be considered an indicator of the plant's physical state, because greater weight is linked to optimum intake of nutrients and high efficiency of physiological and metabolic processes in the cells. These are translated into an increase in different plant tissues and organs.

Table 7. Effect of the application of biostimulants on plant fresh weight.

Treatments	Fresh weight of plants (g)
T1 Absolute control	2.6^{d}
T2 Azotobacter	3.8^{c}
T3 Phosphorine	4.2^{bc}
T4 Improved liquid humus	5.0^{ab}
T5 Fortified liquid humus	4.5^{abc}
T6 Mineral-fortified liquid humus.	5.6^{a}
ESx	0.15

Note: values with different subscript letters indicate significant differences for p: 0.05.

Statistically significant differences among the treatments were observed in the effect of biostimulants on plant fresh weight (Table 8). The highest average values were observed in the seedlings treated with the mineral-fortified liquid humus, which did not differ statistically from the plants treated with fortified liquid humus and improved liquid humus, but they did differ from the other treatments. The lowest average value was observed in the control treatment. This response was caused by the higher nutritional composition of the mineral-fortified liquid humus, which provided plants with the necessary elements for growth and development, along with more efficient metabolic processes. The results observed with the use of mineral-fortified liquid humus (BoCalZn) corroborated the importance of microelements (boron, calcium, and zinc) to plant development. They promote adequate cell division, and are involved in the synthesis of tryptophan, the main precursor of auxins, which are the growth hormone regulators (cell elongation) of plants. Additionally, they stimulate various enzymatic activities; intervene in nitrogen metabolism and the formation of favorable pigments and ascorbic acid; participate in the metabolic process of absorption of other nutrients; and help protect the plant from high temperature stress, and fungal and bacterial diseases (Zérega, 2003; Aguado, 2012).

Equally important is dry weight, it is the result of metabolic and physiological processes in the cell, and it reveals plant efficiency in those processes. The moredry weight, the greater the number of nutritional and water needs that will be met throughout plant growth. Hence, this indicator allows for assessment of the most efficient biostimulant for sugar cane seedling strengthening.

Table 8. Effect of the application of biostimulants on plant dry weight.

Treatments	Dry weight of plants (g)
T1 Absolute control	0.9^{d}
T2 Azotobacter	1.11^{c}
T3 Phosphorine	1.19^{b}
T4 Improved liquid humus	1.2^{b}
T5 Fortified liquid humus	1.11^{c}
T6 Mineral-fortified liquid humus	1.3^{a}
ESx	0.01

Note: values with different subscript letters indicate significant differences for p: 0.05.

Statistically significant differences were observed among treatments in relation to seedling survival in field conditions. The best average values were reached by the seedlings treated with biostimulants (Table 9), which only differed statistically from the control treatment. The mineral-fortified liquid humus treatment was the most remarkable due to the high seedling survival percentage in field conditions, 12.67% higher than the control. These results clearly showed the positive effect of this treatment containing boron, calcium, and zinc. These microelements take part in metabolic processes of absorption of other nutrients. They also help protect the plants from stress caused by high temperatures, and prevent fungal and bacterial diseases, facilitating more efficient seedling strengthening.

The survival results observed in this research were higher than the reports made by Molina (2013) and González (2016), who applied FitoMas-E and *Trichoderma harzianum*, and a combination of the two for sugar cane seedling strengthening to evaluate survival after transplantation.

These findings have a great practical significance, considering that the annual seedling production plan is 40 000 individuals, which marks the starting of strengthening. Then, the highest possible quantity of individuals should be planted in the field, in compliance with the existing Norms of the Genetic Breeding Program for Sugar Cane in Cuba, which according to Jorge et al. (2011), demands 35 000 physical seedlings in the field to start the first selection stage of the current scheme. Taking into account the percentages of seedling survival in the field, collected after evaluation of the five biostimulants, the figure suggested by Jorge et al. (2011) was comparable. However, the control seedlings were insufficient in number to meet the annual plan required for this stage.

Table 9. Effect of the application of biostimulants on seedling survival.

Treatments	Seedling survival (%)
T1 Absolute control.	87.32^{b}
T2 Azotobacter	96.14^{a}
T3 Phosphorine	95.10^{a}
T4 Improved liquid humus	97.21^{a}
T5 Fortified liquid humus	97.68^{a}
T6 Mineral-fortified liquid humus.	98.39^{a}
ESx	4.08

Note: values with different subscript letters indicate significant differences for p: 0.05.

Conclusions

The application of biostimulants showed positive effects on the morphophysiological indicators evaluated.

The best transplantation resistance of the mineral-fortified liquid humus (BoCalZn) was observed in field conditions.

Author contribution

Yaima de las Mercedes Daniel Ortega: research planning, program design, analysis of results, paper redaction, final review.

Yoslen Fernández Gálvez: research planning, analysis of results, paper redaction, final review.

Elianis Rodríguez Ramírez: Assembly of experiment, analysis of results, interpretation of results.

Arelys Valido Tomes: analysis of results, paper redaction, final review.

Dania González Gort: analysis of results, paper redaction, final review.

Conflicts of interest

The authors wish to declare the existence of no conflict of interest.

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