International Journal on Agriculture Research and Environmental Sciences

Effect of Viusid Agro® on the Growth of Banana (*Musa* Spp.) Seedlings Under Nursery Conditions

Research Article

Volume 4 Issue 2- 2023

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Article History

Received: August 25, 2023 Accepted: September 1, 2023 Published: September 1, 2023

Abstract

The commercial production of plantains and bananas (*Musa* spp.) involves several aspects to overcome, including those ones related to abiotic and biotic factors. Catalysis, S. L. Agroveterinary Division has developed the product VIUSID agro® as a plant growth promoter in agriculture. Research centers from several countries have shown field studies that reveal the product efficacy. Studies conducted on this topic support the importance of the use of this product in crops, such as cassava, papaya, pumpkin, cucumber, bean, chickpea, garlic, among others at the Research Institute of Tropical Roots and Tuber Crops (INIVIT). This research is carried out with the aim of determining the effect of VIUSID agro® on the growth of banana seedlings. The experimental design was conducted in February 2020, using banana *Musa* AAA seedlings, Cavendish subgroup, 'Gran enano' cultivar of six months of age from meristem cultures, transplanted to polyethylene bags with a mixture of red soil and organic matter (70:30%), located at the Center for Accelerated Seed Reproduction (CRAS). VIUSID agro® (0,2 mL L^{-1}) was applied as foliar application during the first, third and fifth weeks after transplanting. The treated seedlings showed significant statistical differences in terms of: basal diameter (mm), height (cm), number of leaves, foliar area (cm2), radial length of emerged roots directly from the corm (cm), biomass of the foliar and belowground area with respect to the control treatment. VIUSID agro® is an excellent biostimulant, which allows the farmer to take to his farms more vigorous and better adapted seedlings to adverse conditions.

Keywords: Acclimatization; Biostimulants; *Musa* spp; Vitroplants

Introduction

Bananas (*Musa* spp) are the world's most important fruit crop in terms of production volume and trade. Although a major staple in Africa, Asia and Latin America, only 13% of bananas produced are internationally traded [1], indicating the fruit's importance for domestic markets and food security. In addition, Latin American and Caribbean region is also a key exporter of plantains, with 72% of plantains traded on international markets. Nevertheless, 62% of the banana and plantain production in LAC (20 million tons) is consumed locally, which indicates its high importance in diets and food security throughout the region [1]. In Cuba, the production of plantains and bananas has great significance within the production of "Viandas", since they represent more than 40% of this indicator annually. The execution of a Local Self-sufficiency Program of Agricultural Productions, pursues the satisfaction of the per capita of 6.8 kg (15 pounds) of "viandas", where 40% correspond to plantains and bananas, crops with a high nutritional value and ingrained eating habits of the Cuban population [2].

Traditional banana propagation methods neither satisfy the crop demand nor guarantee disease-free plants or high yields when propagated by the traditional way, because of the attack by diseases. The *in vitro* micropropagation is the tool that allows obtaining plants with excellent features: health, high vigor and yield of fruits [3]. The acclimatization of *in vitro* plants is a significant aspect in the culmination of such process. Growing them under *in vitro* conditions makes them develop very weak, since they grow under simulated conditions and in a culture flask, whose relative humidity is high. The *ex vitro* environment reaches a relative humidity of 70% [4], so when plants are placed in *ex vitro* conditions, their survival is affected. In addition to, the leaves do not have cuticle and have a low photosynthetic activity. This is the most difficult phase of the culture, when *in vitro* plants are transferred from the aseptic and nutrient-rich environment of the culture flask to start their development in *ex vitro* conditions.

This requires adequate conditions and to be careful to avoid losses, due to water excess and the attack by pathogens. The economic importance of biostimulants has increased in recent years, because of market factors and the increasing predominance of a sustainability culture in the economic development. These are the two main phenomena that encourage the production and consumption of these compounds in the world [5]. In recent years, in order to make production systems more efficient, several agrochemical industries have placed on the market nutrient complexes containing micronutrients, amino acids and plant extracts, which have been called growth "promoters" or biostimulants. They influence on the growth and development of crops; they increase flowering and improve fruiting. Moreover, it is demonstrated that this type of product does not affect either the environment or the population health [6].

In Cuba it is used in the municipality of Taguasco in the province of Sancti Spíritus by Expósito [7], Hernández [8], Peña et al. [9] and Pérez [10], on tobacco (Nicotiana tabacum L), tomato (*Solanum lycopersicum* L.), bean (*Phaseolus vulgaris* L.) and onion (*Allium cepa* L.) crops, respectively for the first time. In all cases there were obtained important results related to the growth of plants and final yields. The use of the biostimulators VIUSID agro® and FitoMas-E® had a positive effect on the growth and development of sugarcane *in vitro* plants at the *ex vitro* acclimatization phase [11].

Nowadays, many farmers are acclimatizing banana plants produced *in vitro* in shadehouses on their own farms, which are used as acclimatization area, where there may be difficulties in survival percentages and on the growth of seedlings, so the use of alternatives, such as the use of bioproducts could reduce this problem. Taking into account the above mentioned, the present research was carried out with the aim of determining the effect of VIUSID agro® on the growth of banana seedlings.

Materials and Methods

The research was carried out in the ex *vitro* acclimatization phase of the Biofactory belonging at the Research Institute of Tropical Roots and Tuber Crops (INIVIT), located in Santo Domingo municipality, Villa Clara province. The experimental design was conducted in February 2020.

General Procedures

Plant material: Banana *Musa* AAA seedlings, Cavendish subgroup, 'Gran enano' cultivar of six months of age were used, from meristem cultures, transplanted to polyethylene bags (12.5 X 20 cm) with a capacity of 480 g of substrate composed a mixture of red soil and organic matter (70:30%), located at the Center for Accelerated Seed Reproduction (CRAS) Figure 1.

VIUSID agro® (Catalysis, Spain) was foliar applied (0,2 mL L^{-1}) during the first, third and fifth weeks after transplanting Figure 2. The foliar applications in the morning hours (9:00 am) were made with a spray backpack (Matabi, Spain) of 16 L capacity, with a Lurmark AN 2.5 flood-jet nozzle with a pressure of 1, 5 to 2.0 bar. The experimental design used was completely randomized. A total of 50 *in vitro* seedlings were used per treatment. The experimental trial was repeated and evaluated twice over time.

Figure 1: Arrangement of banana Musa AAA seedlings, Cavendish subgroup, 'Gran enano' cultivar at the CRAS.

Figure 2: VIUSID agro® (0,2 mL L^{-1}) application during the first, third and fifth weeks after transplanting.

Culture conditions: Polyethylene bags were placed in a shadehouse, it is a metallic structure without a plastic roof and covered with a black shade net (Saran), that allowed a 50% reduction in light intensity, and irrigated with micro-sprinklers for five minutes twice a day. The cultural services during this stage were carried according to the protocol developed for the propagation of the banana in the CRAS, detailed in the Technical Instructions for the Acclimatization Phase of Bananas [4].

The following variables evaluated 20 randomly selected seedlings ten weeks after planting:

- Diameter (mm) Basal diameter with a caliper aid
- Height (cm) Measure from the pseudostem base until the insertion point of the flag leaf
- Number of leaves
- Foliar area (cm^2)
- Radial length of emerged roots directly from the corm (cm) with the use of a graduated ruler

- Biomass of the foliar and belowground area (pseudostem and roots), dry weight (g)
- Survival percentage at the end of the acclimatization phase

Plants from the two treatments were taken for the determination of the fresh and dry flesh. The roots were washed with water to eliminate substrate. The fresh flesh was determined with a technical balance (Sartorius, Germany). Later, for obtaining the dry flesh, the plant material was dried in an oven (Verticell MMM Medcenter Einrichtugen GmbH, Germany) at a temperature of 80°C during 72 hours until constant weight, which was determined on a technical balance (Sartorius, Germany).

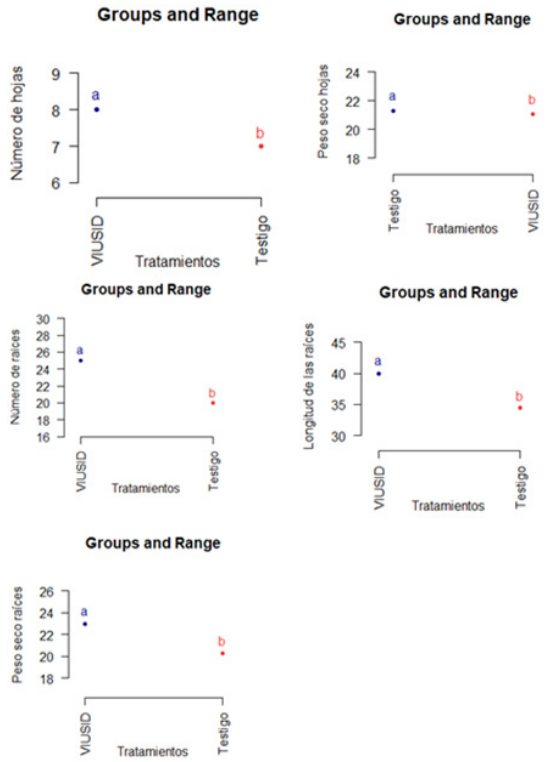

Figure 3: Variables with statistical differences in the treatment with VIUSID respect to the Control.

Statistical analysis: The variance analysis was performed using the aov( ) function described in the *Stats* package. If a Variance Analysis is significant, it suggests that at least two of the compared means are significantly different between them, but it is not indicated which one. To identify them, multiple comparisons are performed to detect differences among treatment means. In this case, Tukey's honestly significant difference method was used, implemented at the *agricolae* package in the *HSD.test*() function.

Results and Discussion

VIUSID Agro® showed a positive effect on the survival of *in vitro* plants under *ex vitro* acclimatization conditions Table 1.

Table 1: The survival of in vitro plants of *Musa* AAA, Cavendish subgroup, cultivar 'Gran enano' at 15 days after transplantation under ex vitro acclimatization conditions.

Treatments	Survival (%)
VIUSID Agro® (0,2mL L^{-1})	97,3 a
Control	93,7 b

Percentages with different letters in the same column differ significantly according to the Proportions test for $p<0.05$ [11] verified the anti-stress effect of the biostimulants FitoMas-E and VIUSID Agro® by increasing the survival of *in vitro* sugarcane plants in shade conditions by more than 20%. The biostimulant VIUSID agro® showed a positive effect on the evaluated variables at ten weeks of cultivation. The treated seedlings showed significant statistical differences with the control treatment in terms of: number of leaves, radial length of emerged roots directly from the corm (cm), biomass of the foliar and belowground area Figure 4. Regarding with the survival percentage at the end of the acclimatization phase, there were no statistical differences between treated and untreated seedlings.

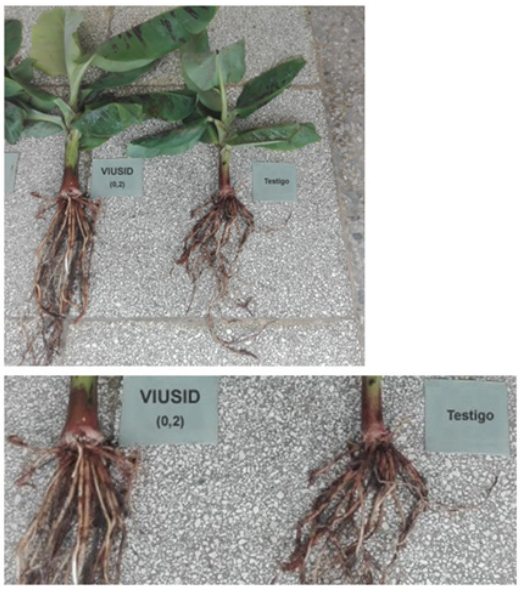

Figure 4: Root system development of banana Musa AAA seedlings, Cavendish subgroup, 'Gran enano' cultivar at ten weeks after transplanting.

Banana Musa AAA seedlings, Cavendish subgroup, 'Gran enano' cultivar with the bioproduct application showed a greater number of leaves, which allowed an increase in chlorophyll content and, therefore a possible greater photosynthetic activity. Seon *et al*. [12]; Sáez *et al*. [13] pointed out that chlorophyll content is not directly related to photosynthetic capacity, but it is a good indicator of the state of the photosynthetic apparatus. The variable number of leaves plays an important role, when obtained plants by *in vitro* culture are transferred to the acclimatization phase. In this regard, Pahlares et al. [14] pointed out that the quality of the *in vitro* plant material has a great influence on the survival percentage. These results can be attributed to the composition of VIUSID agro® formulation, since according to Catalysis [6] it contains potassium phosphate, which is necessary for energy transfer and storage in plants. Besides, it promotes the formation of carbohydrates.

Zinc sulfate influences on the creation and development of new tissues. Glycine is another component and it is a vital amino acid for the growth process, a structural pillar of chlorophyll and cytochromes. It also favors the formation of new shoots and foliar tissue. Another no less important component is folic acid, which acts as a transporter of compounds. It is also a very important coenzyme in the metabolism of amino acids and in the synthesis of nitrogenous bases, required for the formation of new tissues, therefore they positively influence on the growth of vegetative organs. The results obtained in this research could be due to the anti-stress effect of the bioproduct, where the presence of several amino acids stands out [6]. In this regard, Azcón-Bieto and Talón [15] pointed out that the plant´s leaves and roots easily absorb amino acids. Between 5 and 20% of them, penetrate at the first day of foliar application. They have an anti-stress effect, and in the water balance of the plant cells.

According to Szabado and Savoure [16] proline is an essential amino

acid in the primary metabolism of plants. It has a profound effect on the development of plants and in the response to adverse environmental conditions. Its biosynthesis is, therefore an important indicator of the plant adaptation to stress. VIUSID agro® used in the present work has this amino acid in its composition. According to Malabadi *et al*., [17] the transfer of *in vitro* plants to *ex vitro* conditions is one of the critical stages of any *in vitro* propagation protocol. It is essential that they develop a good root system, since their nutrition largely depends on the functionality of the roots, as well as on the accumulated nutrients in the seedlings before reaching *ex vitro* conditions. Beovidez et al. [18] han realizado estudios en fase de aclimatización y destacan que dosis de VIUSID agro® iguales o superiores a 0,7 L ha^{-1} producen un efecto positivo y significativo en el crecimiento general de las plantas de malanga 'INIVIT MC-2012' (producidas in vitro) respecto a las que no son tratadas con el producto.

The variables radial length of emerged roots directly from the corm, number of roots, and dry weight of roots also had higher results with the bioproduct application Figure 5 with significant differences respect to the control. In the evaluation of the main morphological variables of the seedlings, it is significant to highlight that the number of roots increased by five units (25) in the treated seedlings in relation to the Control (20). The variable length of roots emerged directly from the corm showed a superior result in the treated seedlings (40 cm) in relation to the Control, where the mean values obtained were 34.5 cm. In this regard, Rodríguez *et al*. [19], stated that the most important aspect at the *ex vitro* acclimatization phase is that *in vitro* plants form a good root system, due to their nutrition will depend on this system effectiveness to a large extent, and for a long time.

ex vitro acclimatization consists of transplanting obtained seedlings under controlled in vitro conditions to conditions where they are developed for cultivation [20]. It is considered one of the most important stages of micropropagation (Chandra et al., 2010). This is mainly due to the fact that its continuous growth and development requires an adaptation process to biotic and abiotic factors [21]. Beovidez *et al*. [18] refer positive and significant effects on the general growth of malanga plants 'INIVIT MC-2012' in the acclimatization phase with the use of VIUSD agro® (0,7 L ha^{-1}). [22] Improvement of *ex vitro* acclimatization technology in banana, through the use of VIUSID agro® allowed the development of better quality seedlings for planting on farms, and it also offers the possibility of reducing the time spent at this phase. This minimizes costs, as the plant material is for less time at this phase, thus reducing the cultural attentions for its commercialization. [23] VIUSID agro® (0,2 mL L^{-1}) proved to be an excellent biostimulant, which allowed the farmer to take to his farms more vigorous and better adapted seedlings to adverse conditions. [24] Nowadays, banana plantation from treated and untreated seedlings is being monitored through technical assistance on the farms of David Díaz Credit and Service Cooperative (CCS), located in the municipality of Santo Domingo, Villa Clara province.

Conclusions

1) The use of VIUSID agro® had a positive effect on the growth and development of *in vitro* plants of banana *Musa* AAA, Cavendish subgroup, 'Gran enano' cultivar at the *ex vitro* acclimatization phase under nursery conditions.

2) Improvement of *ex vitro* acclimatization technology in banana, through the use of VIUSID agro® offers the possibility of reducing the time of the seedlings at this phase, with the consequent reduction of costs by reducing the time the plant material spends in this space.

Acknowledgment

The authors express their gratitude to the Direction of the Scientific Department of Catalysis, S.L. for the bioproduct availability, technical advice and possibility, through the workshops given of socializing experiences in Cuba. In addition to, our appreciation to David Díaz Credit and Service Cooperative (CCS) in Santo Domingo, Villa Clara province, and especially to the producer Eduardo Chávez for monitoring the results with VIUSID agro® on his banana farm.

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