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# Biobras-16 application effect on the growth and quality of pineapple fruits 'MD-2'

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## ABSTRACT

Biostimulants can increase yields in the 'MD-2' pineapple crop. The following work evaluated the effect of foliar application of a brassinosteroid analogue (Biobras-16) on the vegetative growth index of 'MD-2' pineapples plants and fruits. A biweekly foliar treatment of Biobras-16 at 2.0 mg $L^{-1}$ was applied, at a rate of one liter every 160 plants of plants five months old and subsequently to fruits 90 days after the floral induction (DAFI). At five months of age, 20 random plants were selected and evaluated in fresh weight of the plant (g), leaves number, roots number, fresh and dry mass (g) and length and width of "D" leaves (cm). Three months after start of the experiment, the same variables were evaluated in each treatment. At 150 DAFI bromatological analyses were performed on 20 random fruits per treatment. The Biobras-16 increased the fresh weight of the plants (402 g) and the number of roots emitted (20.4) and don't had effects on the new foliage emission. In "D" leaves the fresh weight and length were significantly increased, and the width decreased. The fresh weight of crowns on the treatment with Biobras-16 reached the highest and significant values with respect to the control. No differences were observed in the fruit/crown relationships. The chemical analyses showed no significant differences. Biobras-16 positively affected the growth of plants and crowns or pineapple fruits ‘MD-2’, without affecting the chemical-physical characteristics evaluated.

**Key words:** Brassinosteroids, development, *Ananas comosus*, plant growth regulators

# INTRODUCTION

Pineapple (*Ananas comosus* var. Comosus) is one of the most widely produced tropical fruit trees. It ranks third in world production, after bananas and mangoes. It is cultivated in order to satisfy the nutritional needs of the population and constitutes an important line for preserve production and sale of fresh fruit. Since the last decade of the last century, new cultivar introduction has been promoted, including the hybrid ‘MD-2’, which, despite its excellent organoleptic qualities and good yield, it is more demanding in terms of its agrotechnics, susceptible to fungal diseases and abiotic stress.

Since the discovery of brassinolide, more than forty compounds of brassinosteroid family of natural origin have been identified. However, the main difficulty for the practical use of compounds such as brassinolide is the low stability of their effects under field conditions; for this reason, its large-scale applications were not continued (1). Based on these results, the production and use of analogous compounds capable of being transformed by the plant into active brassinosteroids is increased, with higher indices of biological activity, its duration over time and the persistence of its effect at the field level (2,3).

In Cuba, since the end of the 1980s, the biosynthesis and study of brassinosteroid analogues began at the Center for the Study of Natural Products of Chemistry Faculty of the Havana University. In 1990, the obtaining of formulations based on brassinosteroid spirostanic analogues was reported for the first time: BIOBRAS-6 (BB-6), widely used in plant biotechnology. Subsequently, other analogues such as BIOBRAS-16 (BB-16) and MH-5 are synthesized, which have stood out for their biological activity in germination, morphogenesis, growth, yield and crop quality processes. However, MH-5 studies have been fundamentally at the *in vitro* level in biotechnological processes, while BB-16 has been used more at a productive level. The BB-16 biosynthetic process is the least cumbersome and costly, so in recent years efforts have been directed to continue improving the use and handling of this formulation (4).

Several studies of BB-16 biological applications have been carried out and beneficial effects have been found on growth (5), rooting and increase in fruit and vegetable yield (6,7). Furthermore, it has been shown to reduce heat stress in bananas (8) and saline stress in young rice plants, which improved their morpho-physiological and biochemical indicators when sprayed with BB-16 (9).

However, in pineapple plants grown under field conditions there is very little evidence of brassinosteroid analogues use, results have been reported in pineapple vitroplants under acclimatization conditions and nursery conditions (10-12). Therefore, this work aimed to evaluate the foiar application effect of a brassinosteroid analog (Biobras-16) on the vegetative growth of plants and on pineapple 'MD-2' fruit quality under field conditions.

## MATERIALS AND METHODS

This study was carried out in the Base Business Unit "Pineapple Production" (UEB) belonging to the Ceballos Agroindustrial Company (21º47´N 78º48´O). The experiments were carried out on plants obtained from carnation sprouts of pineapple (*Ananas comosus* var. Comosus) 'MD-2' of approximately 300 g of fresh mass. After the disinfection treatment with fungicides and insecticides, established in the seed disinfection methodology by the UEB, they were planted and the agrotechnical package established in the UEB for this crop was applied throughout the experiment. In the 15$^{th}$ month of planting (November, 2018) the Biobras-16 foliar application began with a fortnightly frequency at a concentration of 2.0 mg $L^{-1}$ (studied in pineapple (10,12) and in other crops such as rice, beans and bananas (6,8,9)). Five applications were made until now before the artificial induction of flowering (January, 2019). In addition, applications were made on the fruits every 15 days from the 90 days after flower induction and up to 135 days after flower induction the treatments applied, during the vegetative growth of the plants and during the growth of the fruit, were constituted as follows:

1. Control (Water)
2. Biobras-16 (2,0 mg $L^{-1}$)

A randomized block experimental design was used in which each treatment was replicated in three plots 13 m long by 6.70 m wide (six double row rows with approximately 850 plants per plot) which were planted in the June 2018 at a rate of 68,000 plants per hectare. The foliar applications were carried out in the early hours of the morning using a MATABY backpack with a capacity of 16 liters, at a rate of 1 L per 160 plants. Table 1 shows the morphological plant characteristics at the time the experiment started.

**Table 1.** Morphological characteristics of the plants at the initial moment of the experiment (five months after planting)

| Fresh mass of plant (g) | Number of roots per plant | Number of leaves per plant | Fresh mass of leaf "D" (g) | Length of leaf "D" (cm) | Width of leaf "D" (cm) |
|---|---|---|---|---|---|
| 1 190 ± 45 | 33.2 ± 10.2 | 20.9 ± 1.5 | 44.9 ± 1.6 | 71.7 ± 2.5 | 6.08 ± 0.7 |

The evaluations were carried out on 20 randomly selected plants. The mean ± standard deviation is presented

## Determination of the morpho-physiological variables of the plants and leaves "D"

Morpho-physiological variables were evaluated: fresh mass of the plant (kg), length of the plant (cm), number of roots, number of emitted leaves, fresh mass of the leaf "D" (g), length of the leaf "D" (cm), leaf width "D" (cm), dry mass of leaf "D" (g), content of chlorophyll a (µg $g^{-1}$MF), chlorophyll b (µg $g^{-1}$ MF) , total chlorophylls (µg $g^{-1}$ MF) and chlorophyll a/b ratio.

For these determinations, 20 plants were taken at random for each treatment and the final evaluation was carried out before the induction of flowering (3 months after starting the experiment). In the case of the evaluation of the dry masses (g), the samples were placed in an oven at 105±2 ºC until obtaining a constant mass.

## Chlorophyll contents

The determinations were made on 6 leaves per treatment, which were collected in the field before 9:00 am and immediately placed in liquid nitrogen until their evaluation, the results were expressed in micrograms of chlorophyll per gram of fresh mass (µg $g^{-1}$ MF). The determination of the chlorophyll content was carried out [(13)], for this approximately 2 g of leaves were macerated with liquid nitrogen and when a fine powder was obtained, 0.20 g of macerated plant material was weighed at which was added 1 mL of the extraction solvent composed of acetone at 80 % (v/v) and Tris HCl 300 mM pH 7.2. Measurements were made directly on a visible spectrophotometer (Rayleigh vis 723 G) at the wavelengths indicated by the method.

### Determination of bromatological variables of fruit quality

For these determinations, 20 fruits were taken at random for each treatment at 150 DAFI. The following physical and chemical variables were evaluated.

*Physical variables*: mass of fruit with crown (g), mass of crown (g), fruit/crown length ratio.

*Chemical variables*: these determinations were made with the respective methodologies of the AOAC (1998 and 1990). To carry out the evaluations, pulp juice of each fruit was obtained separately and the following determinations were made.

*Soluble solids content*: In the case of soluble solids, a hand refractometer was used and the results were expressed in ºBrix.

*Titratable acidity content* (*% citric acid*): exactly 2 milliliters were taken from each juice sample, 20 mL of distilled $H_2O$ was added and titrated with NaOH (0.1N). The result obtained was expressed in percent acidity as anhydrous citric acid (g of citric acid in 100 mL of juice).

*Maturity index*: it was calculated by dividing the values of the total soluble solids (ºBrix) by the percentage of titratable acidity.

The statistical treatment of the results was developed using the “STATISTIC 8.0” utility from StatSoft (2007). Parametric analyzes (T-students, $p<0.05$) were performed after checking the normal distribution (Kolmogorov-Smirnov, $p<0.05$) and variance homogeneity (Levene, $p<0.05$).

## RESULTS AND DISCUSSION

Table 2 shows Biobras-16 application effect on growth variables in pineapple plants ‘MD-2’ under field conditions after three months of starting the applications.

The results show that in the variables plants fresh mass and root number, the Biobras-16 treatment achieves significant differences with control treatment plants. However, there was no difference in the variable: number of leaves issued.

**Table 2.** Biobras-16 effect application on growth variables in pineapple plants 'MD-2' under field conditions

| Treatments | Fresh mass of the plant (g) | Increase (g) | Number of leaves issued | Increase (g) | Number of roots | Increase (g) |
|---|---|---|---|---|---|---|
| Control | 1 800 | 610 | 24.7 | 3.8 | 45.1 | 11.9 |
| BB-16 | 2 202 | 1012 | 23.9 | 3.0 | 65.5 | 32.3 |
| SE | 75.8 * | 75.8 * | 2.6 NS | 2.6 NS | 8.6 * | 8.6 * |

The increase was calculated as the difference between the initial values (Table 1) and the evaluated values. NS: not significant, * significant for t-Students. $p<0.05$. n = 20. SE, Standard error

The difference of 402 g found in the plants that applied BB-16 with respect to the control plants, shows that, in just three months, the plants of this treatment approach the mass recommended by the UEB (2.5 kg) to induce flowering in plantations and achieve higher commercial quality fruits. It is recognized that there is a close relationship between the fresh mass of the plant and the leaf "D" with respect to the final mass of the fruit at the time of harvest (14).

The increase in plant fresh mass to which BB-16 was applied is closely related to the greater number of roots observed in this treatment, which managed to emit 20.4 more roots than control treatment plants. It has been recognized that the foliar application of Brassinosteroids stimulated root emission in vegetables (4) at low concentrations, but there was no information that BB-16 could cause a similar effect in pineapple plants under field conditions with more than five months of planted. Perhaps BB-16 was able to increase the activity of auxins or perform synergism with them, to increase the number of roots emitted in the pineapple 'MD-2' plants. Brassinosteroids are recognized to promote root growth by affecting auxin synthesis, as has been observed in model plants (15,16).

The use of a brassinosteroid analog in foliar applications at low doses also promoted an increase in all the morphological variables evaluated in smooth cayenne pineapple plants (10). Also the application of MH5 (brassinosteroid analog), stimulated the formation of roots and leaf number of *Vriesea* plants, which presupposes a synergistic effect with auxins in this process (17).

On the other hand, interactions between BRs and other plant hormones have been observed in plant growth responses and under stress conditions (18). Auxins have been suggested to exert a direct control in the biosynthesis of BRs in plants; BRs positively influence ethylene biosynthesis through the regulation of the activities of the enzymes 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACC oxidase) and that there are interactions and cross paths between BRs and cytokinins in various biological processes [19]. Effects that apparently are also present in pineapple plants 'MD-2' under field conditions, by observing increases in fresh masses and root emission.

If the increase in the fresh mass is compared with the values obtained with the evaluation carried out at the initial moment of the experiment (Table 1), it can be seen that the masses in the plants treated with BB-16 increased 1 012 g, while the Control treatment only increased 610 g in the three months after establishing the experiment. This shows the positive effect of BB-16 applications on this variable. Brassinosteroids have been shown to regulate the expression of genes linked to cell expansion, enhancing the extension of cell walls [20].

On the other hand, the increase in root number was also favored in the BB-16 treatment with respect to the control. The plants to which BB-16 were applied increased 32.3 roots with respect to the initial moment of the experiment. While the control plants barely increased 11.9 roots in the same period. Similar results were observed in Imperial variety pineapple vitroplants in the acclimatization phase [21].

In the case of pineapple, the characterization of "D" leaf as an indicator of growth during its vegetative development is of great importance. This indicator is related to the nutritional level of the plant and serves to evaluate the environment effect on the hydric and development status of plant. For this reason, in this study leaf "D" characterization was carried out at the end of the experiment (moments before flower induction) (Table 3).

**Table 3.** Effect of the application of Biobras-16 on growth variables in "D" leaves of pineapple 'MD-2' under field conditions

| Treatments | "D" leaf fresh mass (g) | "D" leaf dry mass (g) | "D" leaf length (cm) | "D" leaf width (cm) |
|---|---|---|---|---|
| Control | 51.5 | 4.3 | 64.6 | 5.9 |
| BB-16 | 52.7 | 4.5 | 73.8 | 5.2 |
| SE | 4.8 NS | 0.07 * | 3.7 * | 0.04 * |

The increase was calculated as the difference between the initial values (Table 1) and the evaluated values. NS: not significant, * significant for t-Students. $p<0.05$. $n = 20$. SE, Standard error

The fresh mass of “D” leaves did not show significant differences between both treatments, while in the dry mass an increase was observed in the BB-16 treatment, which differs significantly from the control treatment. This increase is 0.2 g, which indicates that these leaves formed a greater amount of photosynthetic and reserve structures than the control plants.

On the other hand, regarding the “D” leaf length, significant differences were observed between both treatments, with BB-16 being the best again, this difference was 9.2 cm in length, which indicates a greater cell multiplication and tissue growth, as a greater photosynthesis function, which may be related to the greater dry mass found in this treatment. While in the “D” leaf width, the control treatment statistically exceeded BB-16 by 0.7 cm, which shows that BB-16 promotes leaf elongation.

It is well documented that brassinosteroids stimulate specific physiological processes including cell elongation, division, and differentiation. Spirostanic analogs and in particular, the formulation known as BIOBRAS-16, is capable of stimulating pineapple plant growth. However, it is necessary to evaluate lower application concentrations, since it is recognized that these effects occur when very low concentrations are applied, similar to those used when natural brassinosteroids are applied [(4)].

Brassinosteroids are involved in cell elongation processes through their effects on gene expression and enzyme activity [(22)]. These plant growth promoters act synergistically with auxins and additively with gibberellins [(23)]. The existence of synergism or additive effects between exogenous brassinosteroids and other plant hormones such as auxins, gibberellins, cytokinins, ABA and ethylene has also been proposed, mainly in *in vitro* plant elongation experiments [(23)].

The changes that are induced in the growth and plant development by brassinosteroid application are the result of a cascade of biochemical events, which can be initiated directly on the genome or through routes that do not involve the direct action of the genes. Both pathways assume the participation of a secondary messenger system: an important characteristic is the ability of these compounds to act at extremely low concentrations [(24)].

The greater growth found in the plants to which BB-16 was applied is perhaps also related to a greater photosynthetic activity of the plants of this treatment and therefore knowing chlorophyll concentration could give an idea of this process. Since it is recognized that, during leaf development, photosynthetic cells progressively acquire the ability to differentiate plastids within chloroplasts.

Table 4 shows the effect of the application of Biobras-16 on the chlorophyll concentration and carotenes in pineapple leaves under field conditions.

**Table 4.** Biobras-16 application effect on chlorophyll and carotene concentration in pineapple leaves under field conditions

| Treatments | Chlorophyll a (μg g$^{-1}$ MF) | Chlorophyll b (μg g$^{-1}$ MF) | Ratio a/b | Total chlorophylls (μg g$^{-1}$ MF) | Carotenes (μg g$^{-1}$ MF) |
|---|---|---|---|---|---|
| Control | 47.79 | 53.69 | 0.89 | 101.48 | 5.32 |
| BB-16 | 47.91 | 55.13 | 0.85 | 103.04 | 6.06 |
| SE | 2.12 NS | 4.18 NS | 0.09 NS | 3.85 NS | 1.17 NS |

NS: not significant, * significant for t-Students. $p<0.05$. n = 20. SE, Standard error. MF, Fresh mass

As can be seen, no significant differences were found in the chlorophyll contents, nor in the carotenes between the controls did plants and those that were apply BB-16. Apparently BB-16 had no direct effect on the chloroplasts of pineapple leaves under field conditions or on the plant's ability to produce photosynthetic pigments. However, it has been reported that brassinosteroids do affect photosynthetic efficiency by acting on photosystems, both in the production of protective pigments and in the protection of active centers [(25)].

A high damage percentage to chlorophylls related to stress due to water deficit has been observed in pineapple ‘MD-2’ seedlings at the end of the acclimatization phase, since photosynthesis is more sensitive to dehydration than any other metabolic process [(25,26)]. Under field cultivation conditions, there are no stress levels that could affect the chlorophyll pigment contents, as cultural practices are applied to avoid them, therefore photosynthetic pigments are at the levels necessary for the plant and do not increase or decrease under the action of BB-16. There are various works where the influence of BB-16 on photosynthesis and the content of photosynthetic pigments of different crops is evaluated. The use of DI-31 concentrations (active principle of Biobras-16) of 8 mg L$^{-1}$ increased net photosynthesis in pepper plants (*Capsicum annuum* L) [(27)].

On the other hand, its use in beans (*Phaseolus vulgaris*), despite increasing plant growth indicator values, did not significantly affect photosynthetic pigment values, which coincides with the results obtained in this work [(28)]. It should be said that in other plants, such as rice (*Oryza sativa* L.), BB-16 did increase plant chlorophyll content, but when they were subjected to saline stress [(29)].

Table 5 shows Biobras-16 application effects on bromatological variables of pineapple fruits ‘MD-2’ at 150 days after flower induction.

**Table 5.** Biobras-16 application effect on bromatological variables of pineapple fruits‘MD-2’ at 150 days after flower induction

| Treatments | Fresh fruit mass without crown (g) | Fresh mass crown (g) | Ratio fruit-crown |
|---|---|---|---|
| Control | 1 223.9 | 366.4 | 3.3 |
| BB-16 | 1 238.7 | 408.3 | 3.0 |
| SE | 9.8 NS | 10.7 * | 0.08 NS |

NS: not significant, * significant for t-Students. $p<0.05$. n = 20. SE, Standard error

The results show that once again the BB-16 treatment reached the highest values regarding the fresh mass of the crowns, which show significant differences with respect to the control. However, in the fruit/crown ratio and fresh mass of fruits without crown, no differences were observed between both treatments. The increase in crown masses of 41.9 grams, observed in the BB-16 treatment, shows its positive effect on cell elongation and dry mass accumulation that was observed during vegetative growth. The crown mass increase is important because they represent an important weight of fruits when exported fresh and it is an indicator of their quality. The crowns should remain green with no visible damage and no kinks of the leaves. An increase in crown mass is also important for its use as propagation material when the fruit is destined for industrial processing.

On the other hand, in the chemical analyzes carried out on the fruits, no differences were observed in the content of soluble solids (°Brix), the content of total titratable acidity (%) and maturity index, as seen in the results shown in the Table 6.

**Table 6.** Biobras-16 application effect on chemical-physical variables of pineapple fruits ‘MD-2’ at 150 days after flower induction

| Treatments | Soluble solids content (ºBrix) | Titratable acidity content (%) | Maturity index |
|---|---|---|---|
| Control | 9,48 | 0,80 | 11,85 |
| BB-16 | 10,2 | 0,78 | 13.08 |
| SE | 0,33 NS | 0,17 NS | 1.62 NS |

NS: not significant, * significant for t-Students. $p<0.05$. n = 20. SE, Standard error

## CONCLUSION

The BB-16 use increased the growth variables of pineapple plants under production conditions. In addition, the crown fresh mass also increased, which improves the harvest yields of this crop and allows this organ to be used as propagation material.

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