Agricultural Sciences, 2017, 8, 751-758
http://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

Dose of Paclobutrazol in the Growth of Sugarcane Seedlings *in Vitro* in the Acclimatization Stage

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How to cite this paper: Valdés, T.D., Ruvalcaba, L.P., Tafoya, F.A., Orona, C.A.L. and de Jesús Velázquez Alcaraz, T. (2017) Dose of Paclobutrazol in the Growth of Sugarcane Seedlings *in Vitro* in the Acclimatization Stage. *Agricultural Sciences*, **8**, 751-758.
https://doi.org/10.4236/as.2017.88056

Received: July 5, 2017
Accepted: August 15, 2017
Published: August 18, 2017

Abstract

Micropropagation has been widely used for the rapid multiplication of many plant species, where the final quality of the plant depends on the acclimatization stage. To obtain compact plants, with desired characteristics and adaptable to field conditions, the use of chemical inhibitors of growth is necessary. In sugarcane of the variety CP 72-2086 the effects caused by the growth regulator paclobutrazol (PBZ) *in vitro* plants applied in the acclimatization phase are unknown. The objective of this research was to know the effect of paclobutrazol in the acclimatization phase in the growth and development of the *vitro* plants of sugarcane of the variety CP 72-2086. The research was carried out in the greenhouse of the Faculty of Agronomy of the Autonomous University of Sinaloa, located at 24˚37'29"N and 107˚26'36"W, in Sinaloa, Mexico. Micro-propagated sugarcane seedlings were used by tissue culture; *vitro* plants were extracted from the flasks 24 hours before being transplanted into the greenhouse. The treatments of PBZ applied were the doses of 0 (control), 100, 150, 200, 250 and 300 mg·L^{-1}. The PBZ was applied when the seedlings had seven true leaves [(31 days after transplant (ddt)], and did not show gutting. At 36 ddt, the *vitro* plants of the trays were extracted and the variables evaluated were height (cm), stem diameter (mm), root length (cm), root volume (mL), root dry weight (g), aerial biomass dry weight (g), leaf area (cm^2) and greenness in spad units. A randomized complete block design with three replicates was used. The results of the study indicated that the dose of 150 g·L^{-1} of PBZ induced the growth of plants more compact and of better quality for the transplant.

Keywords

CP 72-2086, Micropropagation, Spad Units, Root Volume, Stem Diameter

1. Introduction

Micropropagation has been widely used for the rapid multiplication of many plant species, but their more widespread use is limited by a high percentage of plants lost or damaged when transferred to greenhouse or field conditions [1].

The process of acclimatization of plants grown *in vitro* proves to be one of the most important phases in a biofactory, when the sugarcane plantlet is extracted from the laboratory to the greenhouses for the acclimatization, the change to different environmental conditions has the consequence that these are very susceptible to different stress, because they have not developed or have not adapted their organs to the new conditions, so they need to respond with new anatomical, morphological and physiological characteristics [1] [2] [3] [4].

The final quality of the plants produced by micropropagation depends on the acclimatization stage [2]. In this phase, different substances and microorganisms have been tested in order to reduce losses and improve the quality of plants [3] [5]. For the survival of plants must be made that these are compact with appropriate growth of the root system and leaf area [2]. The production of compact plants with characteristics that are desirable and adaptable to the field conditions necessitates the use of chemical growth inhibitors that possess qualities to handle the size and the natural form of plants in a relatively short time [6]. The use of growth regulators, such as paclobutrazol (PBZ), leads to retarding growth, decreasing height and, consequently, making compact plants, due to the inhibition of gibberellic acid synthesis [6] [7].

However, since there is currently no record of the effect of PBZ on the quality of *vitro* plants of sugarcane, variety CP 72-2086, applied in the phase of acclimatization of seedlings for field transplantation, the objective of this research was to know the response in growth and development of said plants after applying the PBZ in the mentioned phase.

2. Materials and Methods

The research was carried out in a greenhouse of the Faculty of Agronomy of the Autonomous University of Sinaloa, located at 24˚37'29"N and 107˚26'36"W, in Sinaloa, Mexico. Sugarcane seedlings of the variety CP 72-2086 were micropropagated by tissue culture. The *vitro* plants were extracted from the flasks 24 hours before being transplanted into the greenhouse, separated by size and rinsed to remove residues from culture media. Rooted seedlings were transplanted on November 22, 2013, in polypropylene trays with 54 holes filled with peat moss (organic peat) and 32 cm^3 capacity. In this phase of acclimatization, the *vitro* plants were kept for 38 days after transplant (ddt), in the greenhouse that had the conditions of humidity, light and temperature required for the growth and development of plants.

With microspring the plants were irrigated until 36 ddt with a duration of 1.5 minutes every 4 hours for seven days, and during the other 29 days the duration of the irrigations was 2.0 minutes every 8 hours. Three Nitrogen (N) applications

were made from lobby urea (44% N), using an aqueous solution of 2.2 $g \cdot L^{-1}$ of N. The doses of PBZ applied were 0 (control), 100, 150, 200, 250 and 300 $mg \cdot L^{-1}$. Each dose was applied only once with a hand-held atomiser. In order to avoid contamination, an atomizer was used for each treatment. The solution was sprayed with the same number of shots (7) of the atomizer in each experimental unit, and each shot was attempted to be carried out with almost the same force, until dew-like droplets were formed on the surface of the leaves of the seedlings, without that these droplets had fallen; distilled water was applied to the control seedlings. The PBZ was applied when the seedlings had seven true leaves (31 ddt) without gutation by bundle. The already sprayed seedlings were isolated by glass barriers in order not to contaminate the rest of the experimental units.

For statistical analysis, a randomized complete block design with three replicates was used; each tray represented an experimental unit with 54 seedlings per tray, evaluating 10 of them randomly. At 36 ddt, the *vitro* plants of the trays were extracted and they were determined the variables of plant height (cm), stem diameter (mm), roots length (cm), roots volume (mL), roots dry weight (g), dry weight of biomass aerial (g), leaf area (cm^2) and greenness in spad units. The height of plants was measured from the base of the stem to the apex of the last expanded leaf; The stem diameter was measured with a vernier, the volume of roots was determined by the displacement of water through a graduated cylinder, the root dry matter and aerial part was obtained in stove at 70˚C for 48 h until constant weight, and its weight was determined with digital scale. With the statistical package SAS Institute [8], analyzes of variance and comparison of means were made with the Tukey test at 0.05.

3. Results and Discussion

3.1. Plant Height

Was no detect significant effect of the PBZ on plant height (**Figure 1**), numerically the control was superior to PBZ-treated plants, the results show a tendency to decrease plant height by increasing PBZ concentration; Similar results were obtained by Chacón *et al*. [9] in the species *Dioscorea trifida* and *D. alata*, where no significant differences were observed between the effects caused by the treatments in the growth of the seedlings during the acclimatization process due to the addition of paclobutrazol to the crop and with a linear relationship inverse between height and dose of PBZ. The observed effect of this triazole is that it slows growth because it inhibits the biosynthesis of gibberellin, which affects cell elongation, resulting in more compact plants (Chacon *et al*. [9]; Chaney [10].

3.2. Stem Diameter

In the stem diameter of the *in vitro* sugarcane seedlings treated with PBZ, it was observed that this was higher than in the control (**Figure 2**), where the 150 $mg \cdot L^{-1}$ dose caused an increase of 15.1% with respect to the control. The effect of PBZ on tomato stem thickness has been reported by Berova and Zlatev [11] after

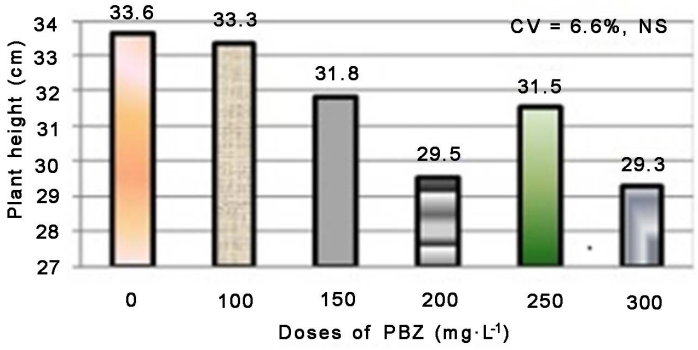

Figure 1. Effect of Paclobutrazol on the height of sugarcane seedlings (*Saccharum* sp.) *in vitro* in the acclimatization phase. (NS: Non-significant difference).

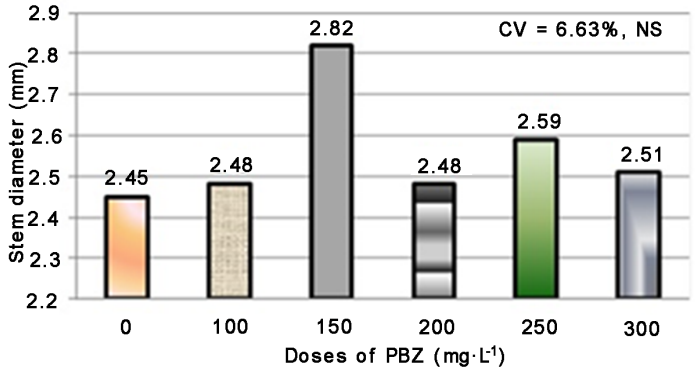

Figure 2. Effect of Paclobutrazol on the stem diameter of sugarcane seedling (*Saccharum* sp.) *in vitro* in the acclimatization phase. (NS: Non-significant difference).

applying it on the foliage in solution of 25 g·L^{-1}, likewise by Giovinazzo *et al.* [12] with an increase of 9.0%.

3.3. Roots Length

The analysis of variance indicated highly significant differences in roots length ($p < 0.01$), so that with Tukey's test ($\alpha = 0.05$) it was detected that the effect caused by the doses of 150 and 250 mg·L^{-1} of PBZ was statistically the same, but higher than those observed in the control plants and in those treated with 100, 200 or 300 mg·L^{-1}. With 150 and 250 mg·L^{-1} the increments respective were 9.9 and 17.7% relative to the control (**Figure 3**). Similar results were obtained by Bello-Bello *et al.* [7] after applying solution with 2 mg·L^{-1} of PBZ.

3.4. Roots Volume

This response variable was expressed without significant statistical differences between the averages obtained with the different doses of PBZ and the control (**Figure 4**); however, with 150 mg·L^{-1} an increase of 36.1% was achieved in comparison to the control. These results are closely related to those of Partida-Ru-

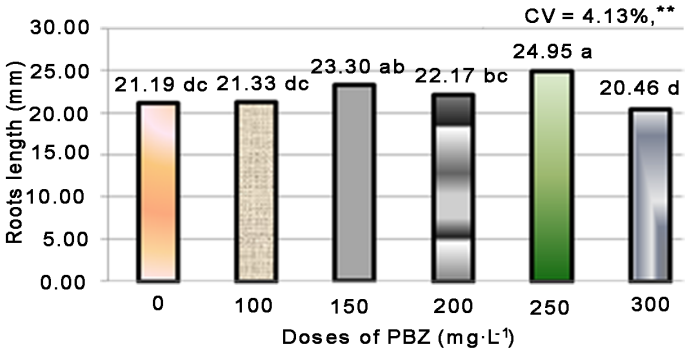

Figure 3. Effect of Paclobutrazol on the root length of the sugarcane seedling (*Saccharum* sp.) *in vitro* in the acclimatization phase. (NS: Non-significant difference).

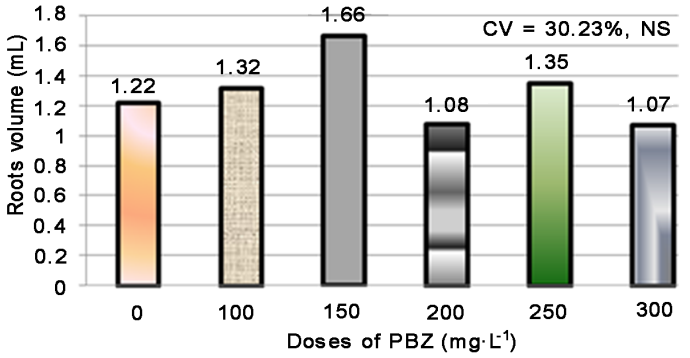

Figure 4. Effect of Paclobutrazol on roots volume of sugarcane seedling (*Saccharum* sp.) *in vitro* in the acclimatization phase. (NS: Non-significant difference).

valcaba *et al.* [13], since they reported that with 150 mg·L^{-1} of PBZ the root system was increased in seedlings of bell pepper and eggplant.

3.5. Weight Dry of Roots

This other study variable was also expressed without statistical differences between the means that induced the different doses of PBZ and the control (**Figure 5**); however, with 150 mg·L^{-1} it was have an increase of 15.0% with respect to the control. These results are also closely related to those of Partida-Ruvalcaba *et al.* [13], mentioned in the previous paragraph.

3.6. Dry Weight of Biomass Aerial

The PBZ did not significantly affect this variable in comparison to the control (**Figure 6**), but in absolute values the highest production was presented in seedlings that received 150 or 200 mg·L^{-1}, whose averages exceeded in 10% that was obtained in the control. Similar trends observed Partida-Ruvalcaba *et al.* [13] in seedlings of bell pepper and eggplant cultivated with 150 mg·L^{-1} of PBZ in the solution applied on the foliage.

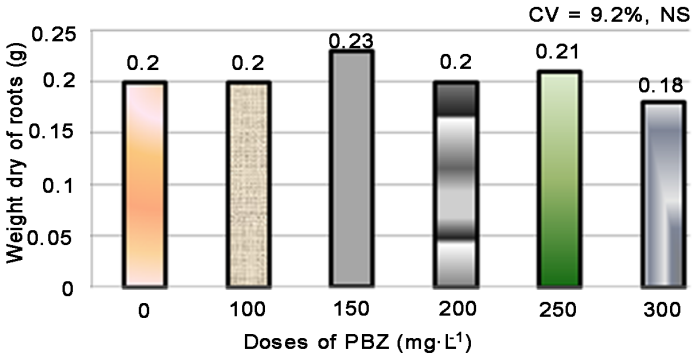

Figure 5. Effect of Paclobutrazol on roots dry weight of sugarcane seedling (*Saccharum* sp.) *in vitro* in the acclimatization phase. (NS: Non-significant difference).

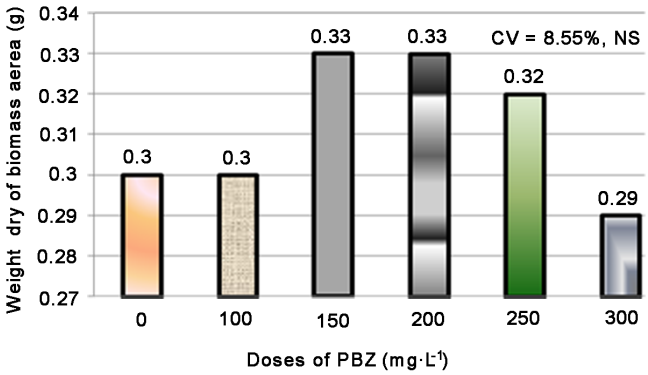

Figure 6. Effect of paclobutrazol on dry weight of biomass aerial of sugarcane seedling (*Saccharum* sp.) *in vitro* in the acclimatization phase. (NS: Non-significant difference).

3.7. Leaf Area

In this other characteristic of the seedlings, no significant statistical differences were observed between the average values that induced PBZ doses in relation to the control (**Figure 7**), but with 150 mg·L^{-1} of PBZ the seedlings had an increase of 7.7% compared with control, although with 100, 250 and 300 mg·L^{-1} the tendency was to decrease. The increase caused by the 150 mg·L^{-1} is related to what was discovered by Silva *et al.* [14] in sunflower plants cultivated with 31 mg·L^{-1} in the solution applied twice in the foliage, resulting in an increase of 5.3% of leaf area with respect to the control.

3.8. Greenness

This character varied in a range of 26.1 to 30.7 and the analysis of variance indicated highly significant differences between the values achieved in the PBZ treated seedlings and the control, so that the Tukey test (α = 0.01) established that the greenness was higher in the order of the solutions with 200, 300, 250, 150 and 100 mg·L^{-1} applied leaf (**Figure 8**) compared to the control seedlings.

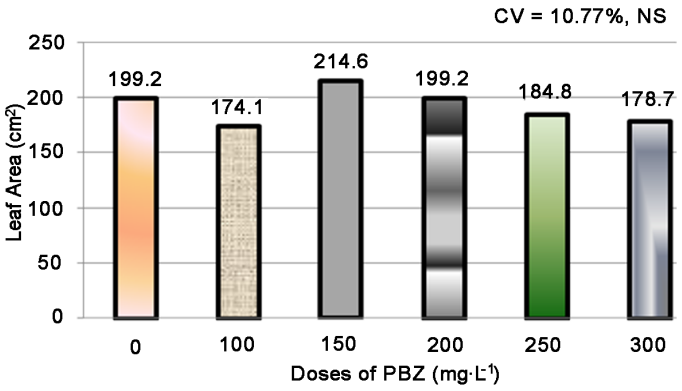

Figure 7. Effect of Paclobutrazol on the leaf area of sugarcane seedlings (*Saccharum* sp.) *in vitro* in the acclimatization phase. (NS: Non-significant difference).

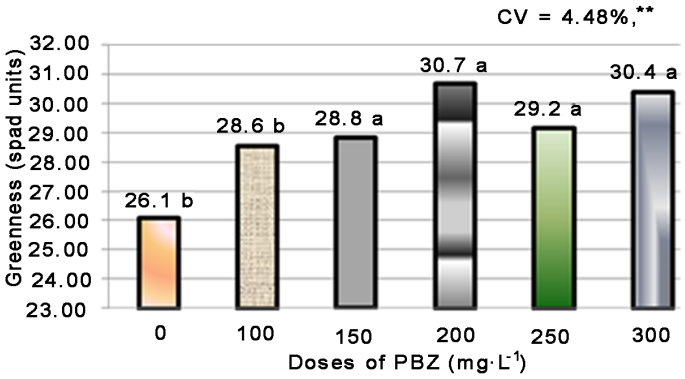

Figure 8. Effect of paclobutrazol in the greenness of sugarcane seedlings (*Saccharum* sp.) *in vitro* during the acclimatization phase. (**Highly significant difference).

4. Conclusion

Paclobutrazol caused a reduction in plant height; the dose of 150 mg·L^{-1} promoted a greater growth of stem diameter, root volume, root dry matter and aerial part and greenness, so that this growth regulator, applied in the acclimatization phase, induces the production of seedlings of sugarcane *in vitro* with quality and greater probability of success since the transplant in field.

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