USE OF PLANT GROWTH REGULATORS TO OBTAIN MINI TUBERS OF *Dioscorea remotiflora* Kunth IN MICROPROPAGATED PLANTS †

[USO DE REGULADORES DEL CRECIMIENTO VEGETAL PARA OBTENCIÓN DE MINI TUBÉRCULOS DE *Dioscorea remotiflora* Kunth EN PLANTAS MICROPROPAGADAS]

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SUMMARY

Background: Camote de cerro (*Dioscorea remotiflora* Kunth) stands out as a crop option in protected agriculture in Mexico due to its agronomic benefits and its versatility in the food and pharmaceutical industries. Despite its potential, it is not commercially cultivated in the country motivating the need to develop an efficient propagation method from vegetative planting material. Previous research suggests that the use of growth retardants stimulates root growth and carbohydrates accumulation in tubers, while applying gibberellic acid (GA_3) is strongly related with greater tuber production. **Objective:** To evaluate the effect of different growth regulators to obtain *D. remotiflora* mini tubers. **Methodology:** Two experiments to obtain mini tubers were carried out. The first one evaluated mediums of culture *in vitro*: MS and MS + 0.5 mg L^{-1} paclobutrazol (PBZ). In the second, effects of foliar application of GA_3, PBZ and trinexapac ethyl (TNE) were evaluated. Both experiments were carried out in two accesions of *D. remotiflora* plants propagated *in vitro* and adapted to greenhouse conditions. **Results:** An increase in mini tubers yield and in their weight was observed when seedlings were propagated in MS + 0.5 mg L^{-1} PBZ and GA_3 was applied to foliage. **Implications:** The use of growth retardants showed efficiency in developing mini tubers; nevertheless, more research focusing on concentrations and combinations of these substances is needed to improve the protocol to obtain planting material. **Conclusions:** The observed effect of growth retardants and gibberellic acid application in *Dioscorea remotiflora* plants suggests a favorable influence in the development of mini tubers. In the long term, this phenomenon could contribute significantly to promoting viability of its commercial cultivation and rehabilitation of previously exploited areas.
Key words: Growth retardants; gibberellic acid; paclobutrazol; trinexapac ethyl; camote de cerro.

RESUMEN

Antecedentes: El camote de cerro (*Dioscorea remotiflora* Kunth) destaca como una opción de cultivo en la agricultura protegida en México por sus bondades agronómicas y su versatilidad en la industria alimentaria y farmacéutica. A pesar de su potencial, no se cultiva comercialmente en el país motivando la necesidad de desarrollar

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un método eficiente de propagación a partir de material de siembra vegetativo. Investigaciones previas sugieren que el uso de retardantes de crecimiento estimula el crecimiento de las raíces y la acumulación de carbohidratos en los tubérculos, mientras que la aplicación de ácido giberélico (GA_3) está fuertemente relacionada con una mayor producción de tubérculos. **Objetivo:** Evaluar el efecto de diferentes reguladores de crecimiento para la obtención de mini tubérculos de *D. remotiflora*. **Metodología:** Se realizaron dos experimentos para la obtención de mini tubérculos. En el primero se evaluaron medios de cultivo *in vitro*: MS y MS + 0.5 mg L^{-1} paclobutrazol (PBZ). En el segundo, se evaluaron los efectos de la aplicación foliar de GA_3, PBZ y trinexapac etil (TNE). Ambos experimentos se realizaron en dos accesiones de plantas de *D. remotiflora* propagadas *in vitro* y adaptadas a condiciones de invernadero. **Resultados:** Se observó un incremento en el rendimiento de mini tubérculos y en su peso cuando las plántulas se propagaron en MS + 0.5 mg L^{-1} de PBZ y se aplicó GA_3 al follaje. **Implicaciones:** El uso de retardantes de crecimiento mostró eficiencia en el desarrollo de mini tubérculos; sin embargo, se necesita más investigación enfocada en las concentraciones y combinaciones de estas sustancias para mejorar el protocolo de obtención de material de siembra. **Conclusiones:** El efecto observado de la aplicación de retardantes de crecimiento y ácido giberélico en plantas de *Dioscorea remotiflora* sugiere una influencia favorable en el desarrollo de mini tubérculos. A largo plazo, este fenómeno podría contribuir significativamente a promover la viabilidad de su cultivo comercial y la rehabilitación de áreas previamente explotadas.
Palabras clave: Retardantes del crecimiento; ácido giberélico; paclobutrazol; trinexapac etil; camote de cerro.

INTRODUCTION

The genus *Dioscorea* (family Dioscoreaceae) comprises dioecious, herbaceous, perennial plants propagated vegetatively from tubers (Eggli and Rowley, 2020; Bömer *et al*., 2019), widely distributed in tropical and temperate regions worldwide (Jing *et al*., 2017). They are primarily cultivated for food as they serve as a source of carbohydrates, essential nutrients, minerals, and vitamin C (Wu *et al*., 2016; Raymundo, 2014; Morris and Taylor, 2010). *Dioscorea* species are recognized for their high levels of diosgenin, a sapogenin used in the pharmaceutical industry to synthesize steroid hormones and cortisone pills (Sharlina *et al*., 2017). In addition to pharmaceutical applications, the presence of diosgenin has been reported to be responsible for a wide range of medicinal properties in the genus, including antimicrobial, anti-inflammatory, and anticancer effects; consequently, they are widely used in alternative medicine for treating menopause, joint pain, arthritis, and also find application in the food industry for making flours and thickeners (Lima *et al*., 2013; Sharlina *et al*., 2017; Obidiegwu *et al*., 2020; Waris *et al*., 2021).

In Mexico, there are more than 60 *Dioscorea* species (Castañeda-Nava *et al*., 2017), one of which is *Dioscorea remotiflora*, an endemic species with a native distribution area in central and southwestern Mexico, mainly in mountainous regions (POWO, 2024). The tubers of this species are not cultivated in Mexico but are extracted from their natural habitat, collected by individuals known as "camoteros," who depend economically on their sale for human consumption (Jones *et al*., 2011). The extraction of tubers leads to various agroecological problems, such as the decrease in wild plant populations and, primarily, soil erosion, as harvesting requires digging more than 1 meter deep, and camoteros often do not refill the holes (Bernabe-Antonio *et al*., 2012). Agroforestry practices, such as agro-silviculture, involving the combination of crops, are widely employed in tropical regions for soil erosion control (Atangana *et al*., 2014). The enhancement of soil under trees and agroforestry systems is largely associated with increased organic matter, either in the form of surface litter or soil carbon (Gupta, 2020). It has been reported that various *Dioscorea* species can be cultivated within these agrosystems, thriving under the low light intensity resulting from tree shade (Hapsari *et al*., 2023; Hamadina and Asiedu, 2015; Oka and Floquet, 2018).

In main producing countries, tubers of several *Dioscorea* species are propagated from tuber sections which represent up to 30% from total harvest (Asiedu and Sartie, 2010; Zulu *et al*., 2020). This method tends to have a high rate of losses as there is an increased probability of rotting, as well as transmission of pests and diseases to new plants resulting in low quality harvested tubers. To prevent this problem insecticides and fungicides are used causing high economic expenses (Aighewi *et al*., 2015; Morse and McNamara, 2018).

Producing mini tubers from cuttings is one alternative, among others, for obtaining vegetative planting material (Maroya *et al*., 2014). In the case of potato crops, tubers are developed from seedlings propagated *in vitro* and adapted to greenhouse conditions (Struik, 2007). Propagating plants *in vitro* before adapting them to greenhouse conditions increases production rate and generates disease-free planting material due to being propagated under aseptic conditions (Sharma *et al*., 2018; Phillips and Garda, 2019). Growth retardants are compounded by triazoles that play an important role in abiotic stress tolerance. These compounds also help to distribute root assimilates avoiding excessive foliage formation and increasing tuber carbohydrates concentration (Soumya *et al*., 2017; Ellis *et al*., 2020).

The tubers of *D. remotiflora* contribute to the diet with 8.75% proteins, 2.15% fats, 5.59% crude fiber, and 79% carbohydrates, they also serve as an excellent source of minerals, including potassium, iron, and phosphorus, however, their fatty acid content is very low, and their caloric contribution is similar to that of potatoes (Soto *et al*., 2014). This makes them highly agronomically promising for food security, and considering the presence of diosgenin, they hold potential in medicine and the pharmaceutical industry (Udensi *et al*., 2008; Bernardo *et al*., 2018; Waris *et al*., 2021). Given that *Dioscorea remotiflora* is not yet cultivated in Mexico, it is pertinent to devise an efficient method for propagating vegetative planting material to integrate it as a commercial crop under an agroecological system such as agro-silviculture.

The objective of this work was to evaluate the effects of gibberellic acid application and growth retardants paclobutrazol and trinexapac ethyl in *D. remotiflora* plants micropropagated and adapted to greenhouse conditions for mini tuber induction.

MATERIALS AND METHODS

Place

The research was carried out at Laboratorio de Cultivo de Tejidos, Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA), Universidad de Guadalajara, in Nextipac, Zapopan, Jalisco, México at 20º 44' 47" N and 103º 30' 43" O, and 1661 masl altitude.

Vegetable material

For starting material, two seedlings accessions of *Dioscorea remotiflora*, one designated as number 112 and the second as "aerial potato" (PA), provided by Laboratorio de Cultivo de Tejidos Vegetales, were propagated *in vitro* in MS medium (Murashige and Skoog, 1962) adding 2 mg L^{-1} kinetin (2KIN medium), incubated at 27 ºC, with 16 h light/8 h darkness photoperiod and 2,000 Luxes of luminous intensity, to increase vegetable material quantity and maintain the collection of those accessions.

In vitro culture medium evaluation of two accessions of *D. remotiflora* for mini tubers induction

Nodal segments with one or two axillary buds from accessions 112 and PA seedlings that were in 2KIN medium were taken and placed in two different types of medium culture chosen for comparison: MS and MS + 0.5 mg L^{-1} paclobutrazol (PBZ). They were placed in an incubator at 27 ºC ± 2 °C, with 16 h/ 8 h light/darkness photoperiod and luminous intensity of 2,000 Luxes for seedlings to develop roots.

After two months of incubation, seedlings had developed roots, so the adaptation process began, which consisted of removed from the jars and washed under running water to completely remove the root gelling agent and avoid fungus proliferation. Seedlings roots were permeated with indol-3-butiric acid at 10,000 ppm concentration (RADIX 10000®) and then placed in 60 cavity unicell trays with 70% peat moss, 30% agrolite substrate and placed in greenhouse conditions at 30 ± 5 ºC temperature approximately and humidity fluctuations during the day between 60% (at high temperature hours) and 90% (at low temperature hours).

Five mL of 20-10-20 (NPK) at 3 g L^{-1} concentration were applied once a week until the end of the plants cycle as fertilizer. In this case, 30 replications were used for accession 112 and 40 for accession PA.

Response variables were number of mini tubers developed in the plant at 90 d, and average weight of mini tubers obtained per experimental unity (seedling).

Determining paclobutrazol, trinexapac ethyl and gibberellic acid application effect in *D. remotiflora* mini tuber induction

Accessions 112 and PA seedlings in 2KIN medium were separated in nodal segments with one or two axillary buds and placed in MS + 0.5 mg L^{-1} PBZ culture medium at 27 ºC ± 2 °C incubator with 16 h light/ 8 h darkness photoperiod and luminous intensity of 2,000 Luxes for seedlings to develop roots.

After two months of incubation, the seedlings had developed roots, so the adaptation process described above was carried out.

Thirty days after the seedlings had acclimatized to *ex vitro* conditions and had at least 8 leaves (Fig. 1), seven foliar treatments were applied at a rate of 2 mL each: (T1): 300 mg L^{-1} PBZ, (T2): 600 mg L^{-1} PBZ, (T3): 300 mg L^{-1} trinexapac ethyl (TNE), (T4): 600 mg L^{-1} TNE, (T5): 50 mg L^{-1} gibberellic acid (GA_3), (T6): 100 mg L^{-1} GA_3 and (T7): water as control treatment.

Figure 1. Seedlings of *D. remotiflora* 30 days post-*ex vitro* acclimatization.

A week later 5 mL of 20N, 10P, 20K, at 3 g L^{-1} concentration were applied once a week until the end of the plants cycle as fertilizer.

For this experiment, 10 repetitions per treatment for accession 112 and 15 repetitions per treatment for accession PA were carried out and response variables were number of obtained mini tubers per plant and obtained mini tubers weight.

Statistical analysis

As data did not correspond to a normal distribution it was analyzed with Kruskal-Wallis test with 95% confidence level. Comparisons were carried out based on Tukey test ($\alpha = 0.05$).

RESULTS AND DISCUSION

In vitro* culture medium evaluation for inducing mini tubers in two accessions of *D. remotiflora

Kruskal-Wallis test showed that *in vitro* medium had a significant effect for obtaining mini tubers at 120 d in accession 112 ($P = 0.004$) as well as in accession PA ($P = 0.000$). Propagation in culture medium MS + 0.5 mg L^{-1} PBZ resulted more favorable in both accessions, 112 (Fig. 2) and PA (Fig. 3), due to the favorable effect of PBZ in developing roots and increasing nutrients assimilation into the reserve organs (Tekalign and Hammes, 2004; Ellis *et al*., 2020).

Regarding obtained mini tubers weight, culture medium was significant in accession 112 ($P = 0.011$), as well as in accession PA ($P = 0.018$), and an increase in mini tubers weight from seedling accession 112 (Fig. 4) and accession PA (Fig. 5) previously propagated in MS + 0.5 mg L^{-1} PBZ medium was observed. The obtained tubers can be observed in Fig. 6.

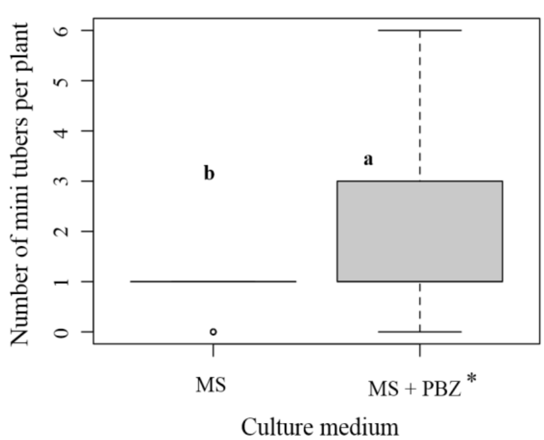

Figure 2. *In vitro* culture medium effect in mini tubers number per plant of *Dioscorea remotiflora* accession 112 at 120 d, Kruskal-Wallis test ($P = 0.004$). Data with different letters are statistically different ($P<0.05$), based on Tukey test. *MS + PBZ indicates a culture medium MS with 0.5 mg L^{-1} paclobutrazol added, n = 30.

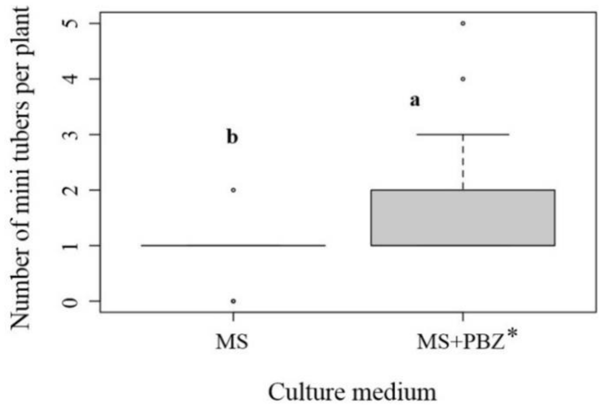

Figure 3. *In vitro* culture medium effect in mini tubers number per plant of *Dioscorea remotiflora* accession PA at 120 d, Kruskal-Wallis (P = 0.000). Data with different letters are statistically different (P<0.05), based on Tukey test. *MS + PBZ indicates culture medium MS with 0.5 mg L^{-1} paclobutrazol added, n = 40.

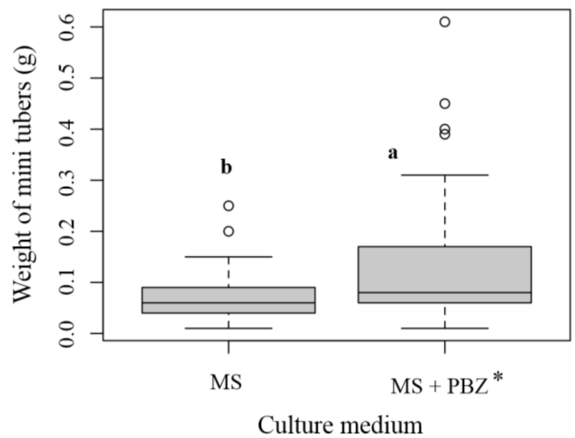

Figure 4. *In vitro* culture medium effect in obtained mini tubers weight (g) from *Dioscorea remotiflora* accession 112 plants, Kruskal-Wallis test (P = 0.011). Data with different letters are statistically different (P<0.05) based on Tukey test. *MS + PBZ indicates culture medium MS with 0.5 mg L^{-1} paclobutrazol added.

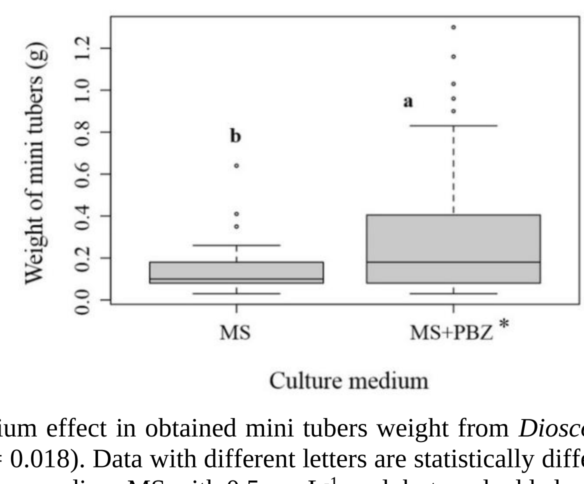

Figure 5. *In vitro* culture medium effect in obtained mini tubers weight from *Dioscorea remotiflora* accession PA plants, Kruskal-Wallis test (P = 0.018). Data with different letters are statistically different (P<0.05) based on Tukey test. *MS + PBZ indicates culture medium MS with 0.5 mg L^{-1} paclobutrazol added.

Figure 6. Mini tubers derived from plantlets originating from *in vitro* culture and adapted to greenhouse conditions. a) Mini tubers from plantlets propagated in MS medium; b) Mini tubers obtained from plantlets propagated in MS medium + 0.5 mg L^{-1} paclobutrazol.

Determining paclobutrazol, trinexapac ethyl and gibberellic acid application effect in inducing mini tubers in two accessions of *D. remotiflora*

Data showed that foliar application had a significant effect in accession 112 (P = 0.002) and in accession PA (P = 0.000) on mini tubers per plant number at 120 d, where development of up to four mini tubers per plant were obtained when applying 100 mg L^{-1} GA_3, and between one and two mini tubers when applying 300 mg L^{-1} and 600 mg L^{-1} TNE for accession 112 (Fig. 7). For accession PA, application of 100 mg L^{-1} GA_3 was the dosage which increased the number of mini tubers per seedling, obtaining up to three mini tubers more compared to control treatment (Fig. 8).

Kim *et al* (2003) report a high rate in mini tuber production obtained from *D. opposita* cuttings, size ≥ 4 mm when GA_3 at 100 mg L^{-1} concentration and TNE at 600 mg L^{-1} concentration were applied to foliage. They also mention that applying GA_3 has an effect in the mini tuber development stage increasing the number of mini tubers per plant. Finally, they state that applying GA_3 to foliage in low concentrations (50 and 100 mg L^{-1}) and TNE in a 600 mg L^{-1} concentration increases tuber production in plants obtained from *D. opposite* cuts. The relation between demand and supply assimilation has an important influence on plant growth and development in various crops. Several reports point out the use of growth retardants as generating balance between source and sink which helps to have higher yields (Bera *et al.,* 2022).

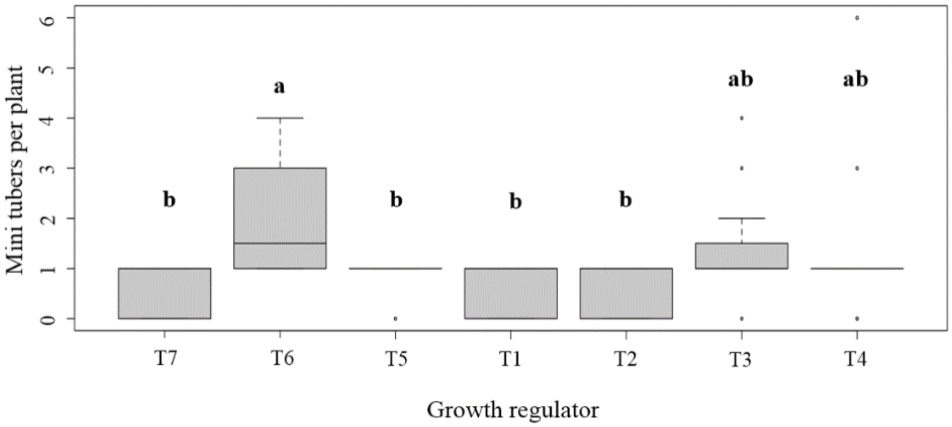

Figure 7. Effect in number of obtained mini tubers per plant of *Dioscorea remotiflora* accession 112 at 120 d when a growth regulator was applied to foliage. T1 = 300 mg L^{-1} paclobutrazol, T2 = 600 mg L^{-1} paclobutrazol, T3 = 300 mg L^{-1} trinexapac ethyl, T4 = 600 mg L^{-1} trinexapac ethyl, T5 = 50 mg L^{-1} gibberellic acid, T6 = 100 mg L^{-1} gibberellic acid and T7 = water, Kruskal-Wallis test (P = 0.002). Data with different letters are statistically different (P<0.05) based on Tukey test, n = 10.

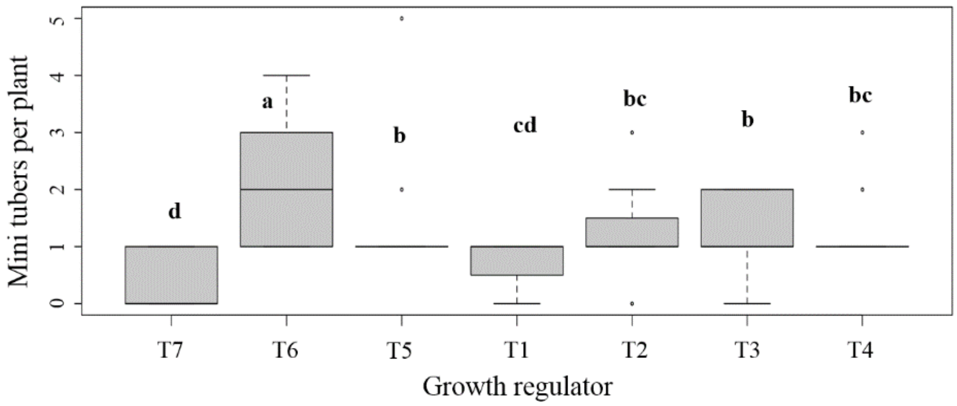

Figure 8. Effect in number of obtained mini tubers per plant of *Dioscorea remotiflora* accession PA at 120 d when a growth regulator was applied to foliage. T1 = 300 mg L^{-1} paclobutrazol, T2 = 600 mg L^{-1} paclobutrazol, T3 = 300 mg L^{-1} trinexapac ethyl, T4 = 600 mg L^{-1} trinexapac ethyl, T5 = 50 mg L^{-1} gibberellic acid, T6 = 100 mg L^{-1} gibberellic acid and T7 = water, Kruskal-Wallis test (P = 0.000). Data with different letters are statistically different (P<0.05) based on Tukey test, n = 15.

Regarding mini tuber weight, applying foliar growth regulators was significant in mini tubers obtained from accession 112 (P = 0.000) and PA (P = 0.000) weight, where an increase, compared to control treatment of up to 0.3 g was observed when 100 mg L^{-1} GA_3 and up to 0.4 g when 50 mg L^{-1} GA_3 were applied to accession 112 seedlings (Fig. 9). Also, applying GA_3 to obtained mini tubers increased their weight up to three times compared to control treatment (Fig. 10). Degebasa (2020) reported an increase in potato tubers weight when applying GA_3 and mentions that this is related to the assimilates production, especially carbohydrates. As reported by Abeytilakarathna (2022) foliar application of GA_3 to potato plants 40 d after planting increases the yield of harvested tubers. The obtained tubers can be observed in Fig. 11.

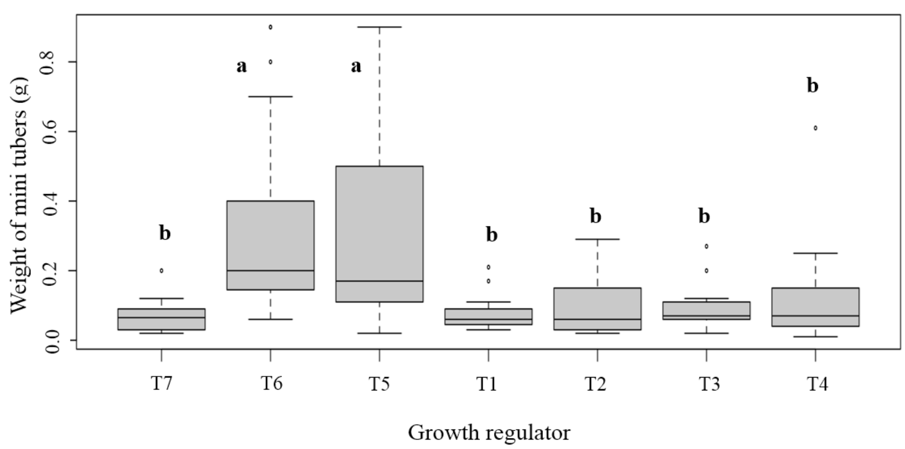

Figure 9. Growth regulator effect in obtained *Dioscorea remotiflora* accession 112 mini tubers weight when applied to foliage. T1 = 300 mg L^{-1} paclobutrazol, T2 = 600 mg L^{-1} paclobutrazol, T3 = 300 mg L^{-1} trinexapac ethyl, T4 = 600 mg L^{-1} trinexapac ethyl, T5 = 50 mg L^{-1} gibberellic acid, T6 = 100 mg L^{-1} gibberellic acid, and T7 = water. Kruskal-Wallis test (P = 0.000). Data with different letters are statistically different (P<0.05), based on Tukey test.

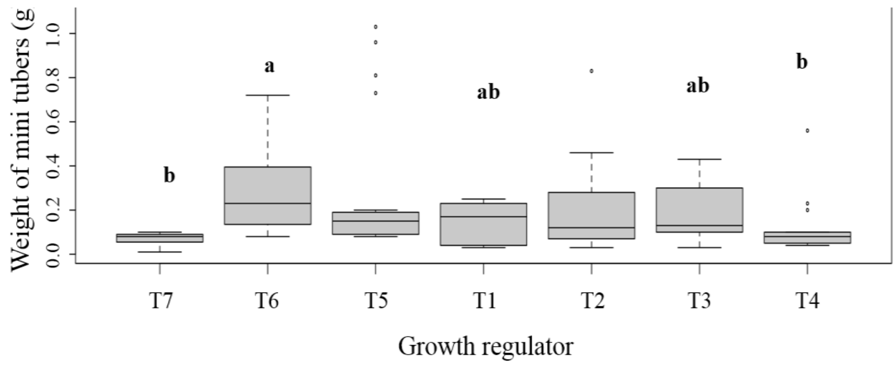

Figure 10. Growth regulator effect in obtained *Dioscorea remotiflora* accession PA mini tubers weight when applied to foliage. T1 = 300 mg L^{-1} paclobutrazol, T2 = 600 mg L^{-1} paclobutrazol, T3 = 300 mg L^{-1} trinexapac ethyl, T4 = 600 mg L^{-1} trinexapac ethyl, T5 = 50 mg L^{-1} gibberellic acid, T6 = 100 mg L^{-1} gibberellic acid, and T7 = water, Kruskal-Wallis test (P = 0.000). Data with different letters are statistically different (P<0.05), based on Tukey test.

Figure 11. Mini tubers derived from plantlets originating from *in vitro* culture in MS medium + 0.5 mg L^{-1} paclobutrazol, acclimatized to greenhouse conditions, and sprayed with 3 mL of gibberellic acid at a concentration of 100 mg L^{-1}.

CONCLUSIONS

Propagating seedlings of *Dioscorea remotiflora* in MS medium with paclobutrazol added and applying gibberellic acid to foliage of seedlings adapted to *ex vitro* environment favors mini tuber induction in greenhouse conditions. Micropropagation ensures clean vegetable material that is obtained in a short time and with little space. Using this technique can be a feasible alternative for obtaining mini tubers due to the high productivity obtained with *in vitro* propagation.

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Conflict of interest. The authors declare that they have no conflict of interest.

Compliance with ethical standards. Does not apply.

Data availability. All data was presented in this manuscript. Experimental data are available upon request to the corresponding author.

Author contribution statement (CRediT). M. M. Espíritu-Rodríguez: Conceptualization, research, methodology, writing – original draft, data curation, formal analysis. **F. Santacruz-Ruvalcaba:** Conceptualization, methodology, project resources and supervision. **J. J. Castañeda-Nava:** Methodology, supervision, and validation. **R. M. Hernández-Herrera:** Data analysis, manuscript reviewing and editing. **L. De la Cruz Larios:** Results interpretation, writing- review and editing. **E. Salcedo-Pérez:** Data analysis, writng - review and editing.

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