

Respuesta de *Agave potatorum* Zucc a dos dosis de fertilizante de liberación lenta y reguladores de crecimiento

Response of *Agave potatorum* Zucc to two doses of slow-release fertilizer and growth regulators

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ABSTRACT

In Oaxaca, Mexico, *Agave potatorum* Zucc, a wild species, is extensively harvested for mezcal production. This study evaluated the effects of applying two doses (5 and 10 g plant⁻¹) of the slow-release fertilizer (SRF) Osmocote Plus (OS) and growth regulators (GR) Biozyme TF[®] (BI) and Agromil Plus[®] (AG) on the growth and accumulation of total soluble solids in the stem (TSS) of *A. potatorum* Zucc plants under nursery conditions. A completely randomized design with a 3×3 bifactorial arrangement was employed. After 12 months, measurements were taken for plant height (PH), unfolded leaves number (ULN), stem diameter (SD), root length (RL), root volume (RV), root density (RD), fresh weight of leaves (FWL), stem (FWS), and roots (FWR), as well as TSS. Relative to the control, OS 10 g increased by 40.1 % PH, 17.7 % SD, 58.8 % FWL, 43.2 % FWS, 43.7 % FWR, 33.3 % RD, and 25.3 % TSS. OS 5 g increased 24.4 % PH, 19.3 % SD, 49.0 % FWL, 61.1 % FWS, and 22.6 % TSS. BI increased PH by 9.7 %, SD by 19.6 %, FWS by 77.3 %, FWR by 50.0 %, RV by 72.2 %, and TSS by 18.6 %. AG increased ULN by 23.8 %, SD by 24.5 %, and FWL by 101.7 %. OS 5 g + BI increased PH by 43.0 %, SD by 81.6 %, FWS by 144.2 %, FWS by 332.2 %, RV by 137.1 %, and TSS by 75.8 %. OS 5 g + AG increased ULN by 52.3 % and RV by 136.1 %. OS 10 g + no GR (NGR) increased FWL by 141.1 %. OS 10 g + BI increased PH by 43.7 %, FWR by 129.8 %, and TSS by 75.0 %. OS 10 g + AG increased RD by 46.1 %. The individual or combined application of SRF and GR significantly enhanced growth and TSS accumulation in *A. potatorum* Zucc plants. Further research is recommended to evaluate the application of SRF and GR in more advanced growth stages of *A. potatorum* Zucc under open field conditions.

KEY WORDS: Wild agave, Plant growth, Phytohormones, Plant nutrition, Osmocote.

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RESUMEN

En Oaxaca (México), el *Agave potatorum* Zucc, una especie silvestre, es intensamente aprovechada para la producción de mezcal. Este estudio evaluó el efecto de la aplicación de dos dosis (5 y 10 g planta⁻¹) del fertilizante de liberación lenta (SRF) Osmocote plus (OS) y de reguladores de crecimiento (GR) Biozyme TF[®] (BI) y Agromil Plus[®] (AG) sobre el crecimiento y acumulación de sólidos solubles totales en el tallo (TSS) de plantas de *A. potatorum* Zucc en condiciones de vivero. Se utilizó un diseño completamente al azar con arreglo bifactorial 3×3. Después de 12 meses se determinó altura de planta (PH), número de hojas desplegadas (ULN), diámetro de tallo (SD), longitud radicular (RL), volumen radicular (RV), densidad radicular (RD), peso fresco de hojas (FWL), tallo (FWS) y raíz (FWR) así como TSS. Con relación al control, OS 10 g incrementó 40.1 % PH, 17.7 % SD, 58.8 % FWL, 43.2 % FWS, 43.7 % FWR, 33.3 % RD y 25.3 % TSS. OS 5 g incrementó 24.4 % PH, 19.3 % SD, 49.0 % FWL, 61.1 % FWS y 22.6 % TSS. BI aumentó 9.7 % AP, 19.6 % SD, 77.3 % FWS, 50.0 % FWR, 72.2 % RV y 18.6 % TSS. AG incrementó 23.8 % ULN, 24.5 % SD y 101.7 % FWS. OS 5 g + BI incrementó 43.0 % PH, 81.6 % SD, 144.2 % FWL, 332.2 % FWS, 137.1 % RV y 75.8 % TSS. OS 5 g + AG incrementó 52.3 % ULN y 136.1 % RV. OS 10 g + sin GR (NGR) incrementó 141.1 % FWL. OS 10 g + BI aumentó 43.7 % PH, 129.8 % FWR y 75.0 % TSS. OS 10 g + AG incrementó 46.1 % RD. La aplicación individual o combinada de SRF y GR favoreció el crecimiento y TSS en plantas de *A. potatorum* Zucc. Se sugiere seguir evaluando la aplicación de SRF y GR en etapas más avanzadas del crecimiento de *A. potatorum* Zucc bajo condiciones de campo abierto.

PALABRAS CLAVE: Agave silvestre, Crecimiento vegetal, Fitohormonas, Nutrición vegetal, Osmocote.

Introduction

Agave has been of great importance to indigenous American cultures for approximately 10,000 years due to its diverse uses. Mexico is considered a center of origin and diversification of *Agave* genus species, which hold significant ecological, cultural, and economic relevance (Colunga-GarcíaMarín *et al.*, 2017). Worldwide, this country harbors 75 % of agave species (García-Mendoza, 2007), many of which are economically important in Oaxaca state, where they serve as the raw material for mezcal production, a traditional alcoholic beverage (García-Mendoza, 2010). Intensive mezcal production has led to overexploitation and seriously threatens natural agave populations, also known as maguey (García-Mendoza, 2007). Thus, research focused on their agronomic management is crucial. Overexploitation is particularly pronounced in *Agave potatorum* Zucc, colloquially known as maguey tobalá or papalomé, as it is one of the

most heavily exploited wild species. Unlike most agaves, its ability for asexual propagation is nearly nonexistent, relying heavily on seeds for survival (García-Mendoza, 2010; Aguirre-Dugua & Eguiarte, 2013; Delgado-Lemus *et al.*, 2014). Agaves are robust plants capable of thriving and reproducing in shallow, low-fertility soils, steep slopes, and with limited water for several months each year (Verduzco-Martínez *et al.*, 2009). Despite their ability to adapt to harsh conditions, studies have shown that organic and inorganic fertilization can significantly enhance their growth and the accumulation of sugars in the stem (Sánchez-Mendoza *et al.*, 2020; Enríquez del Valle *et al.*, 2018; García Martínez *et al.*, 2020). However, due to the vast diversity and lengthy growth cycles of existing species, the specific nutrient requirements for each species currently remain largely unknown (Torres *et al.*, 2016).

Conventional synthetic fertilizers are widely used to provide nutrients to agave plants; however, they often result in significant losses due to leaching, volatilization, and fixation, leading to serious environmental pollution issues and health in humans. A promising alternative to replace conventional fertilizers is slow-release fertilizers (SRF) use, which gradually release nutrients into the soil, thereby minimizing losses and reducing environmental pollution and health risks (Kiplangat *et al.*, 2019). Much of the research on the effectiveness of SRF in promoting plant growth has been conducted with forest species, demonstrating their efficiency and the potential for single-application use (Aguilera *et al.*, 2016).

In addition to SRF, exploring supplementary alternatives to enhance growth and sugar accumulation in *Agave* genus plants is crucial. One worthwhile option is the growth regulators (GRs) use (auxins, gibberellins, and cytokinins), which are synthetic or naturally derived compounds that regulate plant growth and biochemical activities, thus, currently the GRs use has increased significantly, although research on species within the *Agave* genus remains limited (Alcántara-Cortes *et al.*, 2019; Borjas-Ventura *et al.*, 2020). Sánchez-Mendoza & Bautista-Cruz (2022) evaluated the effects of two GRs (Biozyme TF® and Agromil Plus®) on *A. angustifolia* Haw. plants and found that these treatments did not yield a positive response in terms of growth or stem sugar content. Other studies have primarily focused on evaluating the effects of GRs on agave plants cultured *in vitro* (Barreto *et al.*, 2010; Cancino-García *et al.*, 2020; Reyes-Zambrano *et al.*, 2016).

The study of sugar content in the de-leafed stems, or “piñas”, of agave is crucial since the alcohol content produced during fermentation directly correlates with the amount of reducing sugars present. In the tequila and mezcal production, two primary analyses are conducted: the measurement of degrees Brix (°Bx) and the determination of reducing sugars using Fehling’s reagent. Degrees Brix measures the sugar solutions density, indicating the proportion by weight of soluble solids, predominantly sugars, in a sample. For instance, *A. tequilana* piñas must contain at least 24 % total sugars to be considered high-quality (Bautista-Justo *et al.*, 2001).

Given this background, this research aimed to evaluate the effect of two doses of SRF and GRs on the growth and accumulation of total soluble solids (TSS) in the stems of *A. potatorum* Zucc plants under nursery conditions.

Material and Methods

Study area

The study was conducted in Ocotlán de Morelos (16° 48' N, 96° 40' W), Oaxaca, Mexico, at an altitude of 1 523 m, mean annual temperature of 20.5 °C and mean annual precipitation of 695 mm. Seeds of *A. potatorum* Zucc were collected in the Santa Catarina Minas municipality, belonging to the Ocotlán de Morelos district. Healthy plants with the greatest height and stem diameter were selected. Vigorous and pest-free fruits (capsules) were collected, and healthy seeds were selected from each fruit and stored in 30.5 × 13 cm kraft paper bags (60 g caliber).

Sowing, fertilization, and growth regulators application

The seeds were placed in 200-cavity polystyrene germination trays and cosmopeat® was used as substrate. Irrigations were applied at two to four days intervals, during which the substrate visibly decreased its moisture content. After two months of growth the agave seedlings in germination trays in a nursery (25 % shade netting) of 8 × 20 m, were transplanted into 20 × 25 cm polyethylene bags, which were filled with 9 kg of soil; the physical and chemical characteristics of the used soil are shown in Tables 1 and 2.

Table 1. Physical and chemical properties of the soil in which the experiment was conducted.

| Texture | FC | PWP | SP | TC | OM | BD (g cm ⁻³) | pH |
|-----------------|-------|-------|-------|------|------|-----------------------------|-----|
| Sand: 55.48 %. | | | | | | | |
| Silt: 17.28 %. | | | | | | | |
| Clay: 27.24 %. | 42.40 | 22.10 | 56.20 | 0.70 | 2.37 | 1.0 | 6.9 |
| Sandy clay-loam | | | | | | | |

FC, field capacity; PWP, permanent wilting point; SP, saturation point; TC, total carbonates; OM, organic matter; BD, bulk density.

Table 2. Nutritional content of the soil where the experiment was established.

| N | P | K | Ca | Mg | Na | Fe | Zn | Mn | Cu |
|-----------------|------|--------|---------|--------|-------|-------|------|-------|------|
| ----- ppm ----- | | | | | | | | | |
| 125.99 | 7.45 | 127.08 | 2848.00 | 310.80 | 70.22 | 61.00 | 3.44 | 64.33 | 6.89 |

N, inorganic nitrogen; P, available phosphorus (Bray); K, available potassium; Ca, available calcium; Mg, available magnesium; Na, available sodium; Fe, iron; Zn, zinc; Mn, manganese; Cu, copper.

Fertilization was performed 45 days after transplanting to ensure the plants were free of transplanting stress and well-adapted to the soil-filled containers. The used SRF was ICL brand Osmocote Plus® (OS) (15 % N, 9 % P₂O₅, 12 % K₂O, 6 % SO₄, 0.02 % B, 0.05 % Cu, 0.46 % Fe, 0.06 % Mn, 0.02 % Mo, 0.05 % Zn) with a release period of 8-9 months. The doses evaluated for SRF were based on Sánchez-Mendoza & Bautista-Cruz (2022) recommendation for *A. angustifolia* Haw. plants, where they applied 19 g of Osmocote Plus per plant. However, to reduce costs and consider the smaller size of the species evaluated in this experiment, the doses were reduced to 5 and 10 g per plant. The fertilizer was applied circularly, 5 cm away from the stem and 5 cm deep. The GRs evaluated were: 1) Biozyme TF® (BI) (plant extracts and biologically active phytohormones 78.87, gibberellins 32.2 ppm, indoleacetic acid 32.2 ppm, zeatin 83.2 ppm, 0.14 % Mg, 0.44 % S, 0.30 % B, 0.49 % Fe, 0.12 % Mn, 0.37 % Zn, diluent and conditioners 19.27 %) and 2) Agromil Plus® (AG) [cytokinins 0.204 % w/v, diluents and conditioners to complete one liter of product 100 % w/v]. Since the commercial GRs do not provide technical recommendations for Agavaceae, the dose suggested for perennial species with crassulacean acid metabolism was used. The evaluated dose for both products was 2.5 ml L⁻¹, and 25 mL per plant was applied by spraying onto the foliage. The commercial surfactant Prolux® was added to each dilution at a dose of 0.75 ml L⁻¹ for greater efficiency. Weekly irrigations were applied, with each plant receiving 1 L of water. As there was no specific information on the frequency of phytohormone application, three foliar applications were conducted during the evaluation period. The first application was made three months after transplanting, when the plants were adapted and developing new leaves; the second and third applications were made at six and nine months, respectively.

Experimental design

The experiment was established under a completely randomized design with a bifactorial arrangement where the factors evaluated were: 1) doses of Osmocote Plus® (0, 5, and 10 g plant⁻¹) and 2) GRs (Biozyme TF® and Agromil Plus®). A control without the application of GR (NGR) was also included, resulting in a total of 9 treatments, each with 10 replicates. Since agave is a perennial species, a pot with one agave plant was used as the experimental unit (Martínez-Ramírez *et al.*, 2012). The evaluated treatments were: 1) without SRF (NSRF) + NGR, 2) NSRF +

BI, 3) NSRF + AG, 4) OS 5 g + NGR, 5) OS 5 g + BI, 6) OS 5 g + AG, 7) OS 10 g + NGR, 8) OS 10 g + BI, 9) OS 10 g + AG. After 12 months of evaluation under nursery conditions and according to Enriquez del Valle *et al.* (2018), 7 plants were randomly harvested from the 10 included in each treatment, totaling 63 plants. Measurements taken included plant height (PH); unfolded leaves number (ULN); stem diameter (SD) using a Maxwell digital caliper; root length (RL), determined with a measuring tape by measuring the longest root; root volume (RV), measured by introducing roots into a 250 mL test tube with a known volume of water and recording the volume of water displaced; root density (RD) determined by the mass-to-volume ratio; fresh weight of leaves (FWL), stem (FWS), and roots (FWR); and TSS content, determined with a portable refractometer RHB-32 ATC.

Statistical analysis

The data for each variable were subjected to the Kolmogorov-Smirnov normality test (Moraguez-Iglesias *et al.*, 2017), and homogeneity of variance was also determined using Bartlett's test (Bartlett, 1937). Variables that did not meet the assumptions of normality and homogeneity were transformed to $\log_{10}(x)$. Finally, a two-way ANOVA and Tukey's multiple mean separation tests with significance level $p \leq 0.05$ were performed using SAS v. 9.1 statistical software (SAS Institute, 2004).

Results and Discussion

The SRF and GR application, either individually or in combination, promoted the growth of *A. potatorum* Zucc. plants. Compared to control plants, the application of OS 10 g increased PH by 40.1 %, SD by 17.7 %, FWL by 58.8 %, FWS by 43.2 %, FWR by 43.7 %, RD by 33.3 %, and TSS by 25.3 %. The application of OS 5 g increased PH by 24.4 %, SD by 19.3 %, FWL by 49.0 %, FWS by 61.1 %, and TSS by 22.6 % (Table 3). Biozyme TF[®] increased PH by 9.7 %, SD by 19.6 %, FWS by 77.3 %, FWR by 50.0 %, RV by 72.2 %, and TSS by 18.6 %. Agromil Plus[®] increased ULN by 23.8 %, SD by 24.5 %, and FWS by 101.7 % (Table 3). OS 5 g + BI increased PH by 43.0 %, SD by 81.6 %, FWL by 144.2 %, FWS by 332.2 %, RV by 137.1 %, and TSS by 75.8 % (Table 3). OS 5 g + AG increased ULN by 52.3 % and RV by 136.1 %. OS 10 g + NGR increased FWL by 141.1 %. OS 10 g + BI increased PH by 43.7 %, FWR by 129.8 %, and TSS by 75.0 % (Table 4). OS 10 g + AG increased RD by 46.1 % (Table 4). Although the interaction between SRF and GR generated a positive response in growth and TSS accumulation parameters, the individual factors (SRF and GR) produced the greatest variation in these variables, surpassing the variability levels generated by the interaction (Table 5).

Obtained data show the positive effect of OS on agave plants. Most studies on agave nutrition focus on conventional synthetic fertilization. Similar data was reported by Sánchez-Mendoza *et al.* (2020), who found that field-grown *A. angustifolia* Haw. plants fertilized with SRF Multigro 6[®] showed significant increases in RL, FWL, FWS, and SD compared to control plants. However, they did not observe a significant increase in TSS content in the stems of the plants, a result that contrasts with this study where OS application increased TSS content in *A. potatorum*

Zucc stems. The agave piña functions as the main reserve organ, where the greatest amount of carbohydrates is accumulated (Montañez-Soto *et al.*, 2011). According to Téllez (1998), the reducing sugars present in the piña of an adult agave plant constitute between 20 and 30 % of its fresh weight. Sugar content in agave plants can vary due to extrinsic and intrinsic factors. Extrinsic factors include the physiological plant stage (Arrizon *et al.*, 2010), species type, crop management, and environmental conditions (Cruz-García *et al.*, 2013). Intrinsic factors include the portion of the agave used for analysis (either leaves or stems, and even fractions of these) and the method used for carbohydrate quantification (Cruz-García *et al.*, 2013). For example, young *A. tequilana* plants have higher levels of monosaccharides (e.g., glucose, fructose) compared to adult plants, which accumulate fructans starting from 8-10 years of age (Cedeño, 1995). The relationship between the fresh weight of the piña and the reduced sugar concentration is considered one of the main indicators of biomass accumulation in agaves, an aspect of great relevance for producers. Harvesting heavier piñas could increase the amount of available reducing sugars, which is crucial for mezcal production (Cruz-García *et al.*, 2013).

Sánchez-Mendoza & Bautista-Cruz (2022) indicated that the application of OS Plus® promoted increases in the ULN, PH, SD, FWL, and FWS of *A. angustifolia* Haw. plants under nursery conditions. However, they did not find a significant increase in TSS content. These results agree with those obtained in this study for PH, SD, FWL, and FWS, but not for ULN or TSS.

Castillejos-Reyes *et al.* (2023) evaluated the application effect of SRF OS Plus® (15-09-12) and Multicote Agri® (18-06-12) on agave coyote (*Agave spp.*) plants under field conditions and found that, compared to control plants, PH increased by 21.2 %, ULN by 28.4 %, FWL by 77.0 %, and FWS by 62.8 % with the application of OS. Multicote Agri® increased PH by 15.3 %. Except for ULN, the results agree with those shown in this study.

Martínez-Ramírez *et al.* (2013) evaluated the influence of four doses of conventional synthetic fertilization (0-0-0, 30-20-15, 60-40-30, and 90-60-45 kg N-P-K ha⁻¹) on the growth and biomass production of *A. angustifolia* Haw. and *A. potatorum* Zucc. seedlings in a greenhouse. These authors found that, compared to control seedlings, seedlings fertilized with any of the N-P-K doses increased the agave rosette diameter, ULN, and biomass accumulation, but not in PH. These results coincide with those obtained in this study for biomass accumulation variables (FWL, FWS, and FWR), PH, and SD. Díaz *et al.* (2011) reported increased ULN in *A. cocuy* plants fertilized with 0.5 g L⁻¹ of ammonium nitrate. In contrast, in this study, the applied doses of OS did not generate a statistically significant response in ULN.

Zúñiga-Estrada *et al.* (2018) indicated that *A. tequilana* plants with base fertilization (162-150-250 kg ha⁻¹ of N, P, and K) combined with fertigation (315.3 g of N; 179.9 g of P₂O₅; 353.4 g of K₂O; 111 g of CaO and 89.1 g of MgO) showed an increase of 18.8 % in PH, 201.4 % in the ULN, 406.4 % in fresh plant weight, and 471.6 % in dry biomass. Enríquez del Valle *et al.* (2013) evaluated the growth response of *A. americana* var. Oaxacensis plants fertigated with Steiner nutrient solution at different concentrations: 1 %, 25 %, 50 %, 75 %, and 100 %. The Steiner nutrient solution at 100 % promoted the greatest increase in ULN, PH, SD, leaf area, number of

primary roots, and RV. Except for the results for ULN, these findings are consistent with those obtained in this study, where the addition of OS promoted growth in *A. potatorum* Zucc. plants.

Enríquez del Valle *et al.* (2016) evaluated the response of different concentrations of Steiner nutrient solution (1, 20, 40, 60, 80, and 100 %) on micropropagated plants of *A. potatorum* Zucc. Their results revealed that plants fertigated with 1 and 100 % Steiner nutrient solution showed an increase in ULN, leaf area, SD, RV, and leaf dry weight. Similarly, the OS addition increased the SD in *A. potatorum* Zucc. plants. Garcia-Martinez *et al.* (2020) evaluated the effect of different doses of phosphorus (P) (0, 14.4, 29.0, and 43.5 mg kg⁻¹) on growth and TSS content in stems of *A. potatorum* Zucc. and *Agave* spp. (agave coyote) and found that, compared to control plants, the dose of 43.5 mg kg⁻¹ of P in *A. potatorum* Zucc. increased PH by 13.2 %, FWL by 34.9 %, FWS by 36.1 %, SD by 21.5 %, and RV by 20.4 %. In agave coyote, the dose of 29.0 mg kg⁻¹ of P increased PH by 16.4 %, FWS by 44.4 %, and SD by 18.6 %; the TSS content increased by 40.0 % with 43.5 mg kg⁻¹ of P, and the FWL increased by 51.0 % with 14.4 mg kg⁻¹ of P. Similar results were obtained in this study in *A. potatorum* Zucc. plants fertilized with OS for PH, FWL, FWS, SD, and TSS.

Table 3. Mean value ± standard error of unfolded leaves number (ULN), plant height (PH), root length (RL), stem diameter (SD), fresh weight of leaves (FWL), fresh weight of stems (FWS), fresh weight of roots (FWR), root volume (RV), root density (RD) and total soluble solids in the stem (TSS) in response to the application of different doses of slow-release fertilizer (SRF) and growth regulators (GR) in *Agave potatorum* Zucc plants under nursery conditions.

| Factor | ULN | PH | RL | SD | FWL | FWS | SWR |
|---|------------|----------------|-------------|----------|---------------|-------------|------------|
| | | ----- cm ----- | | | ----- g ----- | | |
| SRF Osmocote plus® (g plant ⁻¹) | | | | | | | |
| 0 | 17.7±0.8a | 14.7±0.4c | 132.6±10.3a | 6.2±0.2b | 641.19±60.3b | 119.7±13.1b | 52.6±6.2b |
| 5 | 18.7±1.1a | 18.3±0.7b | 135.3±7.3a | 7.4±0.2a | 955.9±75.7a | 192.9±19.7a | 65.4±6.0ab |
| 10 | 20.5±0.8a | 20.6±0.5a | 125.1±7.5a | 7.3±0.2a | 1018.8±60.0a | 171.5±16.9a | 75.6±7.4a |
| GR | | | | | | | |
| NGR | 16.8±0.8b | 17.4±0.7ab | 128.2±7.7a | 6.1±0.2b | 786.1±83.7a | 100.9±11.4b | 53.2±5.0b |
| Biozyme TF® | 19.3±0.9ab | 19.1±1.0a | 138.7±9.4a | 7.3±0.3a | 960.3±76.9a | 178.9±18.3a | 79.8±8.3a |
| Agromil plus® | 20.8±0.9a | 17.1±0.5b | 126.3±8.3a | 7.6±0.2a | 869.8±58.4a | 203.6±15.2a | 61.3±5.7ab |

Continuation Table 3.

| Factor | RV cm ³ | RD g cm ⁻³ | TSS °Brix |
|---|-----------------------|--------------------------|--------------|
| SRF Osmocote plus® (g plant ⁻¹) | | | |
| 0 | 42.5±5.2a | 1.2±0.04b | 15.0±0.7b |
| 5 | 56.3±5.8a | 1.2±0.09b | 18.4±0.9a |
| 10 | 47.7±6.2a | 1.6±0.1a | 18.8±0.9a |
| GR | | | |
| NGR | 36.4±3.3b | 1.4±0.08a | 16.6±0.9b |
| Biozyme TF® | 62.7±7.3a | 1.3±0.08a | 19.7±0.9a |
| Agromil plus® | 47.7±5.0ab | 1.3±0.11a | 16.0±0.5b |

SRF, slow-release fertilizer; GR, growth regulator Biozyme TF® and Agromil plus®; NGR, without growth regulator. Means with the same letter in each column are not statistically different (Tukey ≤ 0.05).

Table 4. Mean value \pm standard error of number of unfolded leaves (ULN), plant height (PH), root length (RL), stem diameter (SD), fresh weight of leaves (FWL), fresh weight of stem (FWS), fresh weight of roots (FWR), root density (RD), root volume (RV) and total soluble solids in the stem (TSS) in response to the interaction of different doses of slow-release fertilizer and growth regulators in *Agave potatorum* Zucc plants under nursery conditions.

| Treatment | ULN | PH | RL | SD | FWL | FWS | FWR |
|----------------------|------------|----------------|-------------|-----------|----------------|---------------|-------------|
| | | ----- cm ----- | | | ----- g ----- | | |
| NSRF + NGR (control) | 14.7±1.2b | 15.1±0.8cd | 114.6±18.3a | 4.9±0.4d | 493.3±98.5c | 64.2±18.0d | 40.5±7.2b |
| NSRF + BI | 18.7±1.0ab | 14.4±1.1d | 137.6±19.9a | 6.5±0.2cd | 664.9±84.6bc | 132.2±12.2cd | 66.5±13.4ab |
| NSRF + GR | 19.7±1.4ab | 14.6±0.6d | 145.4±15.8a | 7.2±0.3bc | 765.4±115.1abc | 162.5±20.1bc | 50.8±9.6ab |
| OS 5 g + NGR | 15.4±1.3b | 16.3±0.8of | 131.8±12.5a | 6.3±0.1cd | 675.6±107.0bc | 113.0±11.7cd | 50.4±7.5ab |
| OS 5 g + BI | 18.1±2.2ab | 21.6±1.4a | 155.7±12.5a | 8.9±0.3a | 1204.7±115.0a | 277.5±28.4a | 79.8±13.9ab |
| OS 5 g + AG | 22.4±1.3a | 17.4±0.7bcd | 121.5±11.2a | 7.2±0.3bc | 1023.0±91.4ab | 200.4±27.0abc | 68.1±7.8ab |
| OS 10 g + NGR | 20.2±1.0ab | 21.0±0.6ab | 138.2±7.4a | 7.2±0.3bc | 1189.6±73.8a | 125.4±22.2cd | 68.7±8.7ab |
| OS 10 g + BI | 21.0±1.5ab | 21.7±0.9a | 125.2±15.2a | 6.6±0.3c | 1046.3±112.1ab | 141.1±17.8cd | 93.1±15.9a |
| OS 10 g + AG | 20.2±1.9ab | 19.2±0.8abc | 112.0±14.8a | 8.3±0.3ab | 821.0±80.4abc | 248.0±24.1ab | 65.1±12.1ab |

Continuation Table 4.

| Treatment | RD g cm ⁻³ | RV cm ³ | TSS °Brix |
|----------------------|--------------------------|-----------------------|--------------|
| NSRF + NGR (control) | 1.3±0.07abc | 28.8±4.3b | 12.4±1.2c |
| NSRF + BI | 1.1±0.06bc | 56.2±11.5ab | 15.8±1.1bc |
| NSRF + GR | 1.1±0.06bc | 42.4±7.7ab | 17.0±0.9abc |
| OS 5 g + NGR | 1.5±1.2abc | 34.2±5.9ab | 17.0±2.0abc |
| OS 5 g + BI | 1.1±0.09bc | 68.3±10.5a | 21.8±1.2a |
| OS 5 g + AG | 1.0±0.02c | 68.0±8.2a | 16.8±0.8abc |
| OS 10 g + NGR | 1.4±0.08abc | 46.2±5.6ab | 20.4±0.5ab |
| OS 10 g + BI | 1.6±0.1ab | 64.2±16.2ab | 21.7±1.6a |
| OS 10 g + AG | 1.9±0.2a | 32.7±3.5ab | 14.2±0.8c |

NSRF, without slow-release fertilizer; NGR, without growth regulator; OS 0 g, OS 5 g, OS 10 g, grams of Osmocote plus® applied per plant; BI, Biozyme TF®; AG, Agromil plus®; Means with the same letter in each column are not statistically different (Tukey ≤ 0.05).

Table 5. Mean squares and significance level of the variables of unfolded leaves number (ULN), plant height (PH), root length (RL), stem diameter (SD), fresh weight of leaves (FWL), fresh weight of stems (FWS), fresh weight of roots (FWR), root density (RD), root volume (RV) and total soluble solids in the stem (TSS) in response to the interaction of different doses of slow-release fertilizer and growth regulators in *Agave potatorum* Zucc plants under nursery conditions.

| S.V. | D.F. | ULN | PH | RL | SD | FWL | FWS | FWR | RD | RV | TSS |
|----------|------|--------|---------|---------|--------|------------|-----------|----------|-------|----------|--------|
| SRF | 2 | 42.66 | 185.25* | 575.34 | 9.22* | 856450.94* | 29267.86* | 2788.40* | 1.30* | 987.31 | 86.92* |
| GR | 2 | 85.87* | 24.69* | 944.15 | 12.41* | 163142.95 | 60849.22* | 3806.81* | 0.13 | 3647.14* | 80.58* |
| SRF × GR | 4 | 25.63* | 18.90* | 2011.75 | 6.89* | 343756.97* | 16018.41* | 227.45* | 0.46* | 1017.47* | 59.75* |
| Error | 53 | 15.19 | 5.74 | 1497.80 | 0.81 | 66496.07 | 2956.76 | 844.58 | 0.13 | 558.84 | 10.80 |
| C.V. (%) | | 20.53 | 13.39 | 29.54 | 12.84 | 29.61 | 33.79 | 45.00 | 25.98 | 48.5 | 18.86 |

S.V., sources of variation; SRF, slow-release fertilizer; GR, growth regulator; C.V., coefficient of variation; D.F., degrees of freedom; *, Significant ($p \leq 0.05$).

Although few studies have evaluated the SRF OS Plus in agave plants, its positive effects have been demonstrated in other plant species. For example, Escamilla-Hernández *et al.* (2015) evaluated the effect of three substrates (vermiculite, agrolite, and peat moss) mixed with the SRF Basacote® (16 N-8 P-12 K, 9-month release period), Osmocote® (15 N-9 P-12 K, 12-month release period), and Multicote® (18 N-6 P-12 K, 8-month release period) at three doses each: 10 (low), 20 (medium), and 30 kg m⁻³ (high), plus a control without fertilization, on the growth of teak (*Tectona grandis* L. f.) grown in expanded polyethylene tubing. These authors found that plants fertilized with OS at medium and high doses showed the greatest increase in all growth variables evaluated (SD, collar height, aerial biomass, and root biomass). Currently, few studies evaluate the simultaneous SRFs and GRs application or the individual effect of GRs on the growth of agaves in nursery conditions. Most studies on the effect of GRs on agaves focus on *in vitro* propagation trials (Barreto *et al.*, 2010; Cancino-García *et al.*, 2020; Reyes-Zambrano *et al.*, 2016). Sánchez-Mendoza & Bautista-Cruz (2022) evaluated the combined effect of OS Plus® and Basacote Plus® with the commercial GRs Biozyme TF® and Agromil Plus® on *A. angustifolia* Haw. plants and found a positive response in PH and FWL variables with the combination of OS Plus and Biozyme TF®. These results align with those obtained in this study, where the combination of OS Plus and Biozyme TF® increased PH, ULN, SD, FWL, FWS, FWR, RV, and TSS in *A. potatorum* Zucc. plants. Garnica-García *et al.* (2020) evaluated the growth of apomictic tillers of *A. angustifolia* Haw. grown in different substrate mixtures made with sand, soil, and bovine manure. They also evaluated different types of irrigation: a) tap water, b) 50 % Steiner nutrient solution, and c) 50 % Steiner nutrient solution + 25 mg L⁻¹ of benzylaminopurine (a commercial cytokinin). After 14 months of evaluation, they found that plants irrigated with 50 % Steiner nutrient solution and 50 % Steiner nutrient solution + 25 mg L⁻¹ of benzylaminopurine showed increased leaf length, ULN, and SD. These results are consistent with those obtained in this study, where the combinations of OS + BI and OS + AG increased PH and SD, and OS 5 g + AG favored the ULN in *A. potatorum* Zucc. plants.

The results show that regardless of the adaptations that agaves have to grow in soils that are poor in nutrients and organic matter, they can respond positively to the SRF and GR application. According to NOM-021-RECNAT-2000 (SEMARNAT, 2000), the soil analysis results indicate very high N, Fe, Mn, and Cu contents, moderately high Ca and Zn contents, medium Mg contents, low P and K contents, and a moderately high amount of organic matter (Table 2). The OS addition likely increased the content of P, K, and some micronutrients such as S, B, Cu, Fe, Mn, Mo, and Zn in the soil, thereby generating a better nutritional balance. This improved balance favored the synthesis of structural and metabolic compounds (amino acids, proteins, nucleic acids, chlorophyll, phospholipids, ATP, among others), resulting in greater growth in agave plants (Enriquez del Valle *et al.*, 2013). Nobel *et al.* (1989) mentioned that adequate nutrition in agave plants improves their physiological condition by increasing CO₂ fixation efficiency, biomass accumulation, and productivity. Although SRFs contain a wide variety of nutrients in their chemical composition, the highest concentrations correspond to primary macronutrients such as N, P, and K, which are most demanded by the plant. Previous studies on species of the *Agave* genus have found that the addition of these nutrients favors their growth and TSS content (García-Martínez *et al.*, 2020; Martínez-Ramírez *et al.*, 2013; Martínez-Ramírez *et al.*, 2012; Díaz *et al.*, 2011). In fact, Zhong *et al.* (2017) mentioned that plants with good N availability have a high capacity to synthesize

amino acids, proteins, chlorophyll, and nucleic acids. Furthermore, the photosynthetic capacity of leaves is related to N content, mainly because Calvin cycle proteins and thylakoids account for the majority of leaf N (Latsague *et al.*, 2014). In addition to an adequate N supply, it is essential to ensure a good P supply to the plant because if this nutrient is deficient, it limits crop growth and productivity. This is because inorganic P (Pi) modulates many enzymatic reactions that regulate metabolic processes such as photosynthesis (Salinas *et al.*, 2013; Salinas *et al.*, 2012). P is also a constituent of proteins, a structural component of phosphoproteins, phospholipids, and nucleic acids. It participates in the breakdown of sugars and the transfer of energy and nutrients (Lynch and Brown, 2008). P deficiency is manifested by reduced leaf number and loss of photosynthetic efficiency (Salinas *et al.*, 2012). K is the third essential macronutrient required for plant growth, so its limitation significantly affects crop production (Parmar & Sindhu, 2013; Gouda *et al.*, 2018). In plants, K plays a very important role in processes such as photosynthesis, where it regulates stomatal opening and closing and thus CO₂ absorption. It is also involved in enzyme activation, protein synthesis, maintenance of cell turgor, reduction of respiration, transport of sugars, and N uptake, making it vital for better plant development (Ahmad & Zargar, 2017).

The SRFs and GRs application also promoted the growth of *A. potatorum* Zucc. This positive effect on the growth of agave plants is possibly attributed to the ability of auxins to promote cell division, elongation, and differentiation processes (Garay-Arroyo *et al.*, 2014). George *et al.* (2008) considers auxins as a type of morphogen (an organic molecule produced and secreted by a group of cells that can diffuse and act favoring the development of cells, tissues, and organs) capable of inducing the differentiation of organs such as roots, stems, and leaves, and promoting their development. Like auxins, gibberellins were possibly involved in the growth and development of *A. potatorum* Zucc plants because they can stimulate root elongation and the formation of young leaves. Additionally, gibberellins play an important role in the elongation of nodal segments by stimulating cell elongation (Gupta *et al.*, 2013). Finally, cytokinins can stimulate and induce high cell proliferation and division. Cytokinins are usually accompanied by the presence of auxins due to their high complementarity. Together, they can stimulate an increase in root production and induce a higher production of plant shoots. In a suitable substrate, they could improve and accelerate plant growth (Salazar-Cerezo *et al.*, 2018; Ferraro, 2014).

Conclusions

Agave potatorum Zucc plants responded positively to the individual and combined addition of SRF and GR under nursery conditions. The application of 5 g of Osmocote Plus® increased SD, FWS, and TSS. With 10 g of Osmocote Plus®, PH, FWL, FWR, RD, and TSS increased. The application of Biozyme favored PH, SD, FWS, FWR, RV, and TSS. Agromil Plus increased the ULN, SD, and FWS. The application of 5 g Osmocote Plus® + Biozyme promoted higher PH, SD, FWL, FWS, RV, and TSS. The combination of 5 g Osmocote Plus® + Agromil Plus® increased ULN and RV. Additionally, 10 g Osmocote Plus® + Biozyme increased PH, FWR, and TSS. Finally, 10 g Osmocote Plus® + Agromil Plus® increased RD.

The evaluation of more doses of SRF (doses lower than 5 g and intermediate doses between 5 and 10 g) should be considered to have a greater certainty of the adequate dose for *A. potatorum* Zucc plants in nursery conditions. It is also important to evaluate the application of GR directly to the rhizosphere, considering that the root is an organ with greater specialization in absorption processes. Finally, it is suggested to continue evaluating the application of SRF and GR in more advanced stages of growth of *A. potatorum* Zucc under open field conditions.

Author contribution

Work conceptualization, fund acquisition, development of methodology, analysis of results, writing, and preparation of the manuscript, S.S.M. Development of methodology, analysis of results, writing, and preparation of the manuscript, A.B.C.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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