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Evaluation of the Plant Growth Regulator SPGP4 in Agricultural Crops: A Case Study in Oaxaca, México

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Abstract: The search for new plant growth regulators is a cornerstone of agricultural research; however, laboratory studies rarely go on to be evaluated in the field. This is because greater production is required, as well as longer studies. Particularly, brassinosteroids present these difficulties, and although they have been evaluated in crops with good results, their high production cost gives rise to the search for new alternatives. 22-Oxocholestanes such as SPGP4, previously used in silico and in vitro studies, have shown great potential, so their evaluation in crops grown from native seeds from the study region becomes of interest. Based on these data, SPGP4 was evaluated under crop conditions in three agricultural plots located on the Isthmus of Tehuantepec region, Oaxaca, México. The seeds were treated with a 0.5 mg/L aqueous solution of the 22-Oxocholestane compound SPGP4 by imbibition one night before sown. Later, 45 days after sowing, a solution of 0.5 mg/L at a rate of 200 L per hectare was applied. At the production level, the bean harvest showed an increase in the range of 21.0–38.1%, and the corn harvest increased between 22 and 32%. In addition, the latter also demonstrated an increase in biomass production, given the increase in diameter and height observed in the corn plant. This indicates that SPGP4 functions as a regulator of plant growth at the crop level to increase both seed and biomass production.

Keywords: agricultural production; steroidal plant growth regulator; cellular elongation

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1. Introduction

The increase in the human population demands an increase in food production from agriculture. This need has resulted in a race in the search for new plant growth regulators, which can be classified into two groups. The first is the use of bacterial consortia that allow the crop to fix nutrients more efficiently [1,2]. The second group includes plant growth regulators that activate one or more pathways to increase plant production by proliferation, elongation and/or differentiation [3,4]. 3-Indole acetic acid is one of the most studied auxins. The exogenous application of a 200 ppm solution increased the total phenol content six times, and a 50 ppm solution promoted the development of the onion bulb and increased its weight [5]. The exogenous application of gibberellic acid improved resistance to temperature stress conditions of Roma and Amar genotypes of tomato; this effect, at an optimum dose of 75 mg/L, also increased the root biomass [6]. Regarding this last group, brassinosteroids stand out (for some examples, see Figure 1A). These are steroidal molecules that activate the BRI1 protein via BAK and SERK, resulting in elongation processes. These last compounds have been proven at the laboratory level to produce very good results in agricultural crops. Given the high effect of increasing the production by brassinosteroids such as Brassinolide and Castasterone, alternative steroidal plant growth promoters were investigated. So, synthesized 22-Oxocholestane compounds were evaluated at the greenhouse level to guarantee a positive effect in real culture conditions. Synthetic routes for brassinosteroids involve a high number of steps with low final yields of the order of 1.0% [7,8]. Through the structural modification of some steroids, Cuban researchers obtained the commercial Biobras-16 that tested positively in crops, finding excellent results that range from an increase of 31.7 to 61.3% in the seed mass of beans with one and two foliar sprays for the crop [9], and a 29.0% increase in the corn crop [10]. Other evaluations in agricultural bean crops demonstrated the beneficial effect of Biobras-16, not only on yield but also on height, width and number of pods [11,12]. Therefore, the search for alternatives to these has given rise to a large family of analogs. 22-Oxocholestanes (Figure 1B) as plant growth regulators are an alternative to this limitation, highlighting that these can be obtained in one or two reaction steps from a commercial spirostan with overall synthesis yields greater than 40% and are already being evaluated at in vitro and greenhouse levels [13,14].

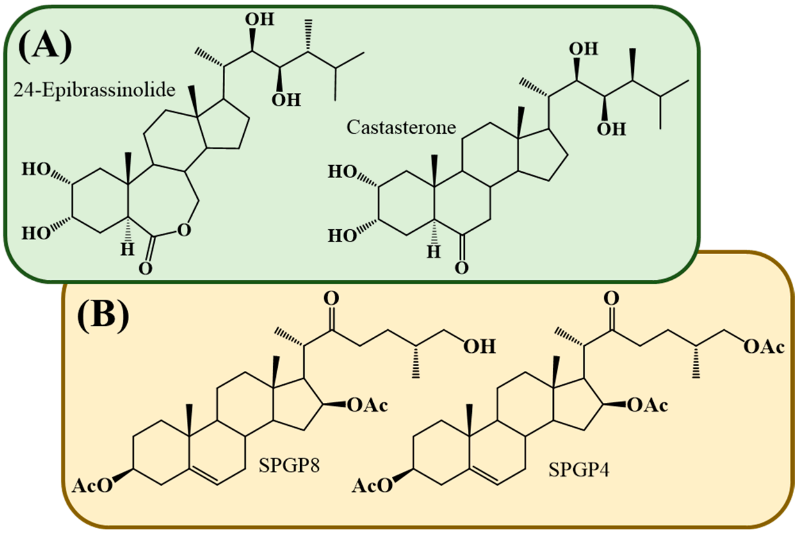

Figure 1. Examples of (**A**) natural brassinosteroids, (**B**) 22-Oxocholestane steroidal plant growth regulators.

The 22-Oxocholestane compounds, such as SPGP8 and SPGP4 (see Figure 1B), are produced through a few reaction steps from natural spirostans. These compounds have shown plant growth activity similar to that of the well-known brassinosteroids when applied at a greenhouse level [7,8]. Compound SPGP4 has recently been studied in laboratory assays and in controlled field experiments, detecting cell elongation, cell proliferation and cell differentiation. From in silico studies, in addition to the activation of the BRI1 protein, an effect on auxins and strigolactone receptors was detected and evaluated in vitro [8]. However, these studies need to be scaled up to an agricultural level to determine their behavior in an uncontrolled, real environment and to determine the relationship between these pathways and their application to increase agricultural production [15]. It is important to highlight that for most Mexican farmers, it is important to have an improvement in native grains, given that these are planted year after year. In this manuscript, the in silico-designed compound SPGP4 is reported. This compound was evaluated in vitro on beans and native corn seeds from the region of the Isthmus of Tehuantepec, Oaxaca, México, and also in agricultural lands with different soil properties and humidity, determining the effect on the production of both seeds, given the mixed cultivation carried out in the region. In addition to corn yield, the effect of morphological properties and biomass production was evaluated, given its relevance as livestock food for local families.

2. Materials and Methods

2.1. Preparation of a Solution of SPGP4

The solution of SPGP4 was prepared according to the previously reported methodology [8] and validated by NMR spectroscopy (Figure S1). The SPGP4 stock solution (10 mg/mL) in acetone was prepared and maintained at 4 °C. From the stock solution, an aliquot was taken and diluted with water to prepare a 0.5 mg/L solution for seed immersion and foliar spraying.

2.2. Seed Selection

Seeds were selected from the previous annual harvest of the native maize race (*Zea mays* L. Zapalote Chico) and beans (*Phaseolus vulgaris* L. Pinto Group) in order to homogenize their weight and texture. A viability test was carried out with a report greater than 90% in both seeds [16].

2.3. Application of SPGP4

2.3.1. Imbibed Seeds

Beans or corn seeds (1.0 kg) were placed in containers containing 3.0 L of SPGP4 aqueous solution for 4 h. Then, the seeds were separated by filtration, and the wet seeds were permitted to air dry prior to sowing [8].

2.3.2. Foliar Spraying

A solution of compound SPGP4 was foliarly applied at a rate of 200.0 L/ha 45 days after the appearance of seedlings using a 4-gallon backpack manual sprayer (solo®, Houston, TX, USA), which sprays at 90 PSI, using a 4-nozzle system [17].

2.4. Agricultural Cultivation

The test was carried out on 3 agricultural crops: (1/16°35′14.9994″, 94°44′4801″/, 2/16°34′44.0004″, 94°46′40.0002″/, 3/16°24′ 40.9998″ and 94°44′48.0012″/), located in the region of the Isthmus of Tehuantepec, Oaxaca, México (Figure S2). The plots possess specific characteristics; the first is located inside a wetland located in the vicinity of a water tributary, classified as silt loam soil. The second is on arid land dependent on seasonal rains, classified as silt soil. The third has an irrigation source from an aquifer mantle, classified as silt loam soil. Cultivation was carried out by planting both species simultaneously. The crops were sown rainfed, in the period of May–November, free of herbicide and insecticide agrochemicals. Additionally, farmers used urea as a classic fertilizer at a rate of 100 kg/ha. The climatological conditions in the 3 cultivation areas remained on average at the same level; the minimum recorded temperature was 25 °C, the maximum 32 °C and the average temperature was 28 °C during the cultivation period. The main source of irrigation was rain precipitation, obtaining an average of 78.5 mm in April (prior to sowing) but increasing in May to 180.5 mm; then, in June to 582.4 mm and a maximum peak in July of 732.8 mm, and gradually decreasing to 222.6 mm in November.

2.5. Experimental Field Crop Design

Groups of 5 rows (separated 1.0 m apart and 2.0 to 3.0 km long) were created in each plot (Figure S3). Groups of maize and beans plants were treated with SPGP4, separated by 5 control rows, where seeds and plants were treated only with water, interspersing these groups to give a total of 20 furrows with treatment and 20 furrows without treatment. Automated sowing was used; both types of seeds were planted simultaneously at a rate of 2 seeds of each species per hole at a depth of 4.0 to 6.0 cm with a separation of 30.0 cm between holes. Sections of 10.0 m^2 were used for scaling at a hectare level to determine the morphometric variables and production yield. The experiment was carried out in triplicate independently on each plot.

2.6. Statistical Analysis

Production was analyzed in terms of metric tons per hectare, resulting from a scaling factor of 10.0 m^2. The determined morphometric results are plant height (in m) and plant diameter (in cm), and the dry and fresh foliage weights were calculated from areas of 10.0 m^2 and scaled using Minitab® Statistical Software 18.0 [18]. Each set of data was analyzed for normality tests. Then, the results of the treated area were compared with those of the untreated area in each field to determine if the effect was significantly affected by the introduction of SPGP4. In order to compare the effect in each crop, the non-parametric Kruskal–Wallis test $p \leq 0.05$ was used, given that when analyzing the data using the Anderson Darling normality test, they turned out to be a non-normal test [19].

3. Results and Discussion

SPGP4 has previously shown great potential in vitro and greenhouse experiments and that prompted us to do an evaluation in field crops. An appropriate cultivation statistical model was designed for the selected cultivation region where maize and bean plants grow together (in the system usually known as "milpa"). Farmers choose the best seeds from the previous crops, promoting, in this sense, the conservation of natural genes. Cultivars of maize and beans followed the same technical treatment (irrigation, fertilization) except for some plants treated by the application of the growth promoter SPGP4. Results on production yield (see Table 1) and morphometric variables (see Table 2) for treated and untreated plants show that the 22-Oxocholestane compound is an effective plant growth promoter. The long-term effect of plant growth regulation is directly related to the signaling cascade that it triggers. In the first instance, the use of the regulator increased the germination rate and viability of the seeds in the agricultural field. However, the use of SPGP4 at an early stage (first 45 days) does not necessarily influence the final phase of production; therefore, in this experimental design, it was also decided to apply a foliar spray of SPGP4 (0.5 mg/L) at a rate of 200 L/ha to guarantee the presence of SPGP4 for the new receptors. With these two applications, the three crop fields were evaluated.

Table 1. Results of agricultural production of native corn and beans treated with SPGP4.

Crop Field/Treatment	Bean Weight (ton/ha) **	Corn Weight (ton/ha) **
1/Water	0.52	5.22
1/SPGP4	0.67 *	5.68
2/Water	0.38	4.52
2/BSS4	0.46 *	5.53 *
3/Water	0.42	5.11
3/BSS4	0.58 *	6.77 *

* Statistical difference against the target using the Kruskal–Wallis test ($p < 0.05$). ** metric tons.

Table 2. Morphological and foliage results of native corn treated with water or SPGP4.

Crop Field/Treatment	Height (m)	Diameter (cm)	Fresh Foliage Weight (Ton/ha)	Dry Foliage Weight (Ton/ha)
1/Water	1.87	7.18	12.91	4.01
1/BSS4	2.18 *	8.57 *	15.32 *	4.35
2/Water	1.61	5.32	11.18	3.05
2/BSS4	1.90 *	6.39 *	12.57 *	3.32
3/Water	1.63	7.01	13.98	4.51
3/BSS4	1.99 *	7.51 *	17.01 *	4.97 *

* Statistical difference vs control against the target using the Kruskal–Wallis test ($p < 0.05$).

3.1. Effect of SPGP4 on Agricultural Production

Table 1 shows the effects of the production of the dual system in corn and beans. In the latter, we can observe a clear increase in production in the three experimental cultivation plots. Bean production is mediated mainly by auxin-type phytohormones at a commercial level, as these promote the formation of roots that facilitate the uptake of necessary nutrients, mainly nitrogen [20]. In the first instance, we can highlight that the first plot of land, that is, characterized by being a wetland, shows the largest production, which agrees with the need for the development of bean plants, with an increase in production of 0.15 tons/ha due to the treatment with SPGP4. The second plot showed the lowest production, given the dependency only on rainwater, with an increase of 0.08 tons/ha. In the third plot of land, with an underground source of water, production increased at a similar level to that of the wetland plot, with an increase of 0.16 tons/ha., which indicates that the promoter works preferably in soils with more direct access to water sources. This is given by the high demand for water for the production of legumes, such as beans.

Corn production is mediated by different factors; it depends on good rooting (mediated by auxins) to provide nutrients and support the cob [20], good proliferation (strigolactone receptor) [21] and cell elongation (activation of the BRI1 gene) [22]. These factors fit with the previous in silico and in vitro results for SPGP4. Table 1 shows the effect on corn production per hectare; two of the three crop fields show a significant increase of 22.3 and 32.5% in production, while in Plot 1, there is a slight increase to 8.8%, denoting an increase in constant production, which suggests that it works independently of the proximity of the water source. However, at a qualitative level, the plants did present growth with a greater difference, so it is of interest to analyze the effect on morphometric variables, such as the diameter and height of the plant, as well as the effect on pasture production and humidity.

3.2. Morphological Effects and Foliage-Produced Effects

Although agricultural production is a relevant variable because of the effect at the molecular level, the production is also the result of the effect of other morphometric variables such as the height and diameter of corn plants. Height development is directly related to cell elongation and the water retention capacity of plant cells [23]. Figure 2 shows differences in corn plant development submitted to water or SPGP4 treatment. Table 2 reflects differences in the development of corn (height and diameter) in the three plots. The height of plants treated with SPGP4 is greater than those treated only with water: increases of 16.6%, 18.0% and 22.1% were obtained. In the case of the diameter of the corn stalk, directly related to cell proliferation and the formation of layers, the SPGP4 treatment generates thickening [24]; significant increases are observed in the three plots with 19.3%, 20.0% and 7.1%. These results indicate that the diameter development is not proportional to the increase in height; nonetheless, it reflects the production of plants more resistant to strong winds.

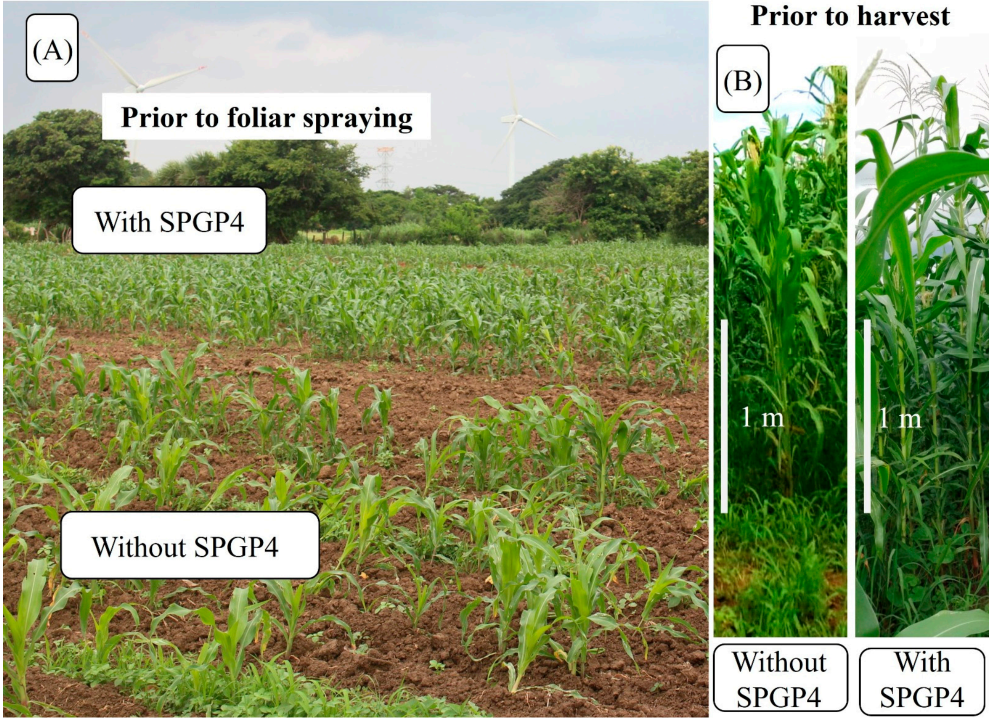

Figure 2. Representative morphological effects in corn plants (**A**) before the second treatment and (**B**) after the second treatment.

Although the main objective for corn plant development is its grain production, the increased production of the corn cane implies an increase in the production of foliage, useful for livestock feed. Therefore, it was of interest to analyze its production. Table 2 summarizes data on fresh and dried foliage. The fresh weight of foliage was notably increased in the three regions when plants were treated by SPGP4. However, when analyzing the corresponding dry weights, a significant difference is only observed in Crop 3, suggesting a water retention effect as a result of the application of SPGP4.

4. Conclusions

SPGP4 is an efficient plant growth regulator in field conditions and is easy to apply to native corn and bean crops from Oaxaca, México. SPGP4 increased bean production from 21.0 to 38.1% depending on the availability of underground water, while for corn, the increase in grain production was from 22.3 to 32.5%, without following a clear trend depending on the groundwater. However, the production of fresh pasture was directly related to the availability of water, indicating that SPGP4 increases water retention within the plant body because the dry weight decreases significantly to the same level as those without treatment. SPGP4 promises excellent potential as a positive regulator of agricultural production of corn and beans as well as pasture, however, more studies are necessary in different varieties.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/crops4020013/s1, Figure S1: ^{1}H and ^{13}C NMR at 500 and 125 MHZ respectively of SPGP4 for structure validation; Figure S2: Map of the Isthmus of Tehuantepec region of Oaxaca used as a study model for the crop field evaluation of the plant growth promoter SPGP4; Figure S3: Crops design to evaluation.

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J.S.-R.; project administration, J.S.-R., A.C.-C. and A.W.-V.; funding acquisition, J.S.-R., A.C.-C. and S.L.C.-H. All authors have read and agreed to the published version of the manuscript.

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