

Reproductive evaluation of

(*Capra hircus* L.)

# bucks

with usual management in  
herds from Benito Juarez,  
Guerrero, Mexico

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# Phenology of Four Varieties of Gooseberry (*Physalis peruviana* L.) in Greenhouses and Hydroponics for its Commercial Production in Mexico

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## ABSTRACT

**Objective:** To measure the phenology, morphology and development of four varieties of *Physalis peruviana* L. under greenhouse and hydroponics conditions, by effect of the ionic strength of the Steiner nutrient solution for cultivation in Mexico.

**Design/Methodology/Approach:** The research was conducted under a completely random design with factorial arrangement, the treatments evaluated resulted from the combination of the levels of the variety factor (Colombia, Sacha, Chiclayo and Modified) with the levels of the ionic strength of the Steiner nutrient solution (50, 100 and 150%), and the experimental unit was a potted plant. For phenology each phase was recorded in the plants, SPAD readings were taken in the vegetative stage, for diameter and height several samples were taken, and pH and electrical conductivity were accomplished on stems and petioles of shoots of each treatment. An analysis of variance and the comparison of means per Tukey ( $p \leq 0.05$ ) were implemented with the SAS 9.2 program.

**Results:** The varieties under study showed differences in the time of occurrence of the phenological stages. SPAD readings, basal diameter, pH and electrical conductivity in sap were affected by each factor except for the interaction. Meanwhile, plant height was significantly affected in the first 24 days after transplant (dat) by the combination of the two factors.

**Study Limitations/Implications:** The results obtained are limited to the varieties, the environmental conditions, and the time when the varieties were evaluated.

**Conclusions:** The phenology of each variety was not expressed at the same time in any concentration, as an early variety was given to Chiclayo, Colombia and Sacha, which are varieties that in the concentrations 50 and 150% of the nutrient solution started harvesting at 99 dat.

**Keywords:** gooseberry, phenology, morphology, hydroponics.

## INTRODUCTION

**Gooseberry** (*Physalis peruviana* L.) is a fruit species native to the South American Andes, known for being tolerant to different environmental conditions and able to adapt to different types of soil (Góngora and Rojas, 2006).

In Mexico, this species is known as Peruvian cherry or "uchuva" (Morton, 1987); however, there are very few studies focused on its adaptation to distinct climates, soils, plagues and diseases (Gastelum, 2012). There is only the study by Mora *et al.* (2006), which characterized six species of *P. peruviana*. These researchers found significant genotypic differences between fruits collected in terms of fruit yield, fresh fruit weight with and without calyx, growth habit, height of first bifurcation, stem diameter, and number of leaves.

The available published information only focuses on the agronomic management of the Colombia variety; for example nourishment with different concentrations of Steiner solution (Gastelum *et al.*, 2013), application of different proportions of ammonium and nitrate in plants grown from seed, regrowth and cutting (Antúnez *et al.*, 2016), and phenological behaviour in the field and in greenhouses (Sabino *et al.*, 2016). Due to the aforementioned, it is important to evaluate the productivity of other varieties, since there are excellent opportunities in select markets. Therefore, the objective of this study was to measure the phenology, morphology and development of varieties of *Physalis peruviana* L. under greenhouse and hydroponic conditions, resulting from different percentage concentrations of Steiner nutrient solution.

## METHODS AND MATERIALS

### Experiment location

The study was carried out in a greenhouse from the Plant Nutrition Area of Colegio de Postgraduados in the Montecillo campus, located at kilometer 36.5 of the Mexico-Texcoco highway in Montecillo, Texcoco, in Estado de México from May until December 2019. The study was developed in a 50 m<sup>2</sup> greenhouse with overhead ventilation and side curtains, with a galvanized steel structure covered in UVII-720 polyethylene. It is located on coordinates 19° 27' 41.0" North latitude and 98° 54' 32.2" West latitude, at an altitude of 2,243 m.

### Plant Material

In order to carry out this experiment, the Sacha and Modified variety were brought in from Ecuador, the Chiclayo variety from Peru and the Colombia variety is the one that has been used in the institution, with a germination rate of 80%. The seeds were sown in styrofoam trays with 200 cavities filled with peat moss as a substrate, placing one seed per cavity and moistened each day with tap water.

### Substrate Preparation and Bag Filling

Red *tezontle* was sifted in order to obtain particle size between 5 to 7 mm, which was then disinfected with sodium hypochlorite (Cloralex®) in a 1:10 proportion; requiring three rinses with tap water. Afterwards, 40×40 cm black polyethylene bags were filled, which were previously perforated in order to allow water to drain.

**Transplant.** This step was carried out when the plants reached a height of 10 cm and a good amount of roots, which occurred 56 days after sowing (das).

**Pest Control.** Prepared solutions were sprayed containing garlic extract at a dose of 75 mL 15 L<sup>-1</sup> of water, for cabbage looper pupa (*Trichoplusia ni* Hubner).

### Treatment and Experimental Design

The experiment was established on July 26, 2019. It was carried out in a completely randomized design (CRD) with factorial arrangement, the treatments evaluated were the combination of the variety factor (Colombia, Sacha, Chiclayo and Modified) with the levels of Steiner nutrient solution concentration (50, 100 and 150%), and the experiment unit was one plant per pot.

### Nutrient Solution

In order to implement the treatments, three 1000 L capacity tanks were used, in which universal Steiner nutrient solution (1984) was prepared in three different concentrations, adjusting pH levels between 5.5 and 6.5 and using sulfuric acid. Irrigation with the nutrient solution was carried out six times a day, with duration of 10 minutes each, by means of drip irrigation.

### Study Variables

#### Phenology Registry

The phenology registry is described next. **Days to flowering:** the number of days elapsed between emergence of seedlings until the release of the first

flower were counted. **Days to anchoring:** the days since emergence until the recently formed fruit were counted. **Days to physiological maturity:** counted from the emergence of the plant until the fruit turns yellow color. **Days to production:** counted from the emergence until the fruit harvest from each plant began.

**SPAD Readings.** Using a portable SPAD-502 Minolta® meter, the leaves greenness index was determined at 46 das; during each treatment repetition, a SPAD reading was performed in each cardinal point in the medial stratum of the plant and the average per plant was measured.

**Electrical Conductivity and Sap pH.** Stems and petioles were cut from buds from each treatment, and then they were chopped as finely as possible in order to be placed into a blender and ground at low speed. Afterwards it was passed through an anti-aphids mesh and manual pressure was applied in order to obtain 40 mL of sap, of which samples were taken.

**Stem Basal Diameter.** A digital Vernier was used to measure the stems thickness at 52 days after transplant (dat), which coincides with the plants' vegetative and reproductive stages.

**Plant Height.** A metric tape was used to measure, using the neck of the stem as the base and up to the longest measurable branch that it was possible to measure.

**Statistical Analyses.** An analysis of variance and Tukey's means comparison test ( $p \leq 0.05$ ) were used for each of the response variables, using the statistical software SAS (SAS Institute, 2002).

## RESULTS AND DISCUSSION

### Morphology

Of the four varieties evaluated, three had different morphology in some of their organs when compared to **Colombia**, which is the variety that all previous studies have been done on. Subsequently, the differences among the varieties are described. **Chiclayo:** Calyx with five ribs and an elongated shape, with fruit with a diameter between 17-22 mm. **Sacha:** Heavily veined calyx with 6-7 ribs, fruit with a diameter between 17-25 mm. **Modified:** Very pubescent stems with a purple color with short internodes, pubescent leaves ranging in size from 5-12 cm long and 6-10 cm wide, a brown color calyx in ripening with fruits of 10-18 cm diameter, with a greenish brown color, with similar flavor to shell tomatoes (*Physalis ixocarpa* Brot.).

### Phenology

The different varieties in the study showed differences in the timing of phenological stages. The phenology of the Chiclayo variety (Figure 1) in the vegetative and reproductive stages showed the same tendencies with the three solutions, the phases were expressed almost simultaneously, yet the production phase was out of synch with the Steiner solution at 100%. The fruits of these plants treated ripened on average 104 days after transplant (dat).

With Steiner solution at 100% the Colombia variety (Figure 2) went ahead of the rest in the productive stage in 7 days, which means it was the most precocious. Concentration at 150% showed a similar ripening for Chiclayo (Figure 1), Colombia (Figure 2) and Sacha varieties (Figure 3) which on average ripened at 99 dat.

Steiner's nutrient solution concentration (%)	Vegetative stage			Reproductive stage			Fruiting stage
	Germination (das)	Transplant (das)	Preflowering (ddt)	Flowering (dat)	Fruit set (dat)	Ripening (dat)	Fruiting (dat)
50	22	56	23	31	41	92	99
100	22	56	26	33	44	93	104
150	22	56	25	30	41	91	99

**Figure 1.** Phenological stages in function of the concentration of Steiner solution in the Chiclayo variety.

Colombia Variety							
Steiner's nutrient solution concentration (%)	Vegetative stage			Reproductive stage			Fruiting stage
	Germination (das)	Transplant (das)	Preflowering (ddt)	Flowering (dat)	Fruit set (dat)	Ripening (dat)	Fruiting (dat)
50	22	56	24	29	39	93	100
100	22	56	21	26	37	87	93
150	22	56	24	28	39	90	99

Figure 2. Phenological stages in function of the concentration of Steiner solution in the Colombia variety.

Sacha Variety							
Steiner's nutrient solution concentration (%)	Vegetative stage			Reproductive stage			Fruiting stage
	Germination (das)	Transplant (das)	Preflowering (ddt)	Flowering (dat)	Fruit set (dat)	Ripening (dat)	Fruiting (dat)
50	11	56	22	30	41	87	99
100	11	56	22	30	41	90	102
150	11	56	19	26	36	85	97

Figure 3. Phenological stages in function of the concentration of Steiner solution in the Sacha variety.

For the Modified variety, none of the three solution concentrations showed any advantage in terms of the productive stage when compared to the rest, with a difference of 10 to 15 dat for fruit ripening (Figure 4). The contrasting result for this variety compared to the others could be due to genetic variability. This is attributed to the significant difference in this variety when compared to the rest, with its morphology more similar to shell tomatoes (*Physalis ixocarpa* Brot.).

For their part, Mora et al. (2006) found that flowering, development and physiological fruit ripening in some wild *P. peruviana* initiated at 42, 52 and 102 dat, which does not coincide with that found in these varieties collected from greenhouses. The varieties studied were more precocious in every stage. Other authors indicate that the start of flowering began at 60 dat and fruit took around 120 dat to fully ripen (Ali and Singh, 2014). These variations depend on environmental factors where the studies were carried out.

### SPAD Readings

The analyses of variance showed significant differences (Tukey,  $P \leq 0.05$ ) for the SPAD readings, which were detected by the variety and the nutrient solution, while for the interaction between variety and solution the SPAD readings were not affected (Figure 5). For their part, the variety with the highest SPAD readings was Sacha (52.69) and the lowest was Modified (49.78 b), while the nutrient solution at 150% reported the highest readings (54.06 a) and the lowest with 50% (49.78 b). The SPAD readings were 52.23 a, 52.24 a, 49.78 b and 52.69 for Chiclayo, Colombia, Modified and Sacha respectively with the means test (Tukey,  $p < 0.05$ ). Regarding the concentration of the solution shown in Figure 5, it is evident that it is directly proportional to the SPAD readings. Sánchez (2019) evaluated the effect of the three concentrations of Steiner nutrient solution (25, 50 and 75%) on gooseberry plants and found that the highest SPAD readings correspond to plants with the highest concentration of nutrient solution.

Steiner's nutrient solution concentration (%)	Vegetative stage			Reproductive stage			Fruiting stage
	Germination (das)	Transplant (das)	Preflowering (ddt)	Flowering (dat)	Fruit set (dat)	Ripening (dat)	Fruiting (dat)
50	22	56	24	29	38	105	116
100	22	56	23	27	38	101	109
150	22	56	24	27	38	103	111

**Figure 4.** Phenological stages in function of the concentration of Steiner solution in the Modified variety.

Antúnez-Ocampo et al. (2016) also evaluated the effect of the  $\text{NH}_4^+ : \text{NO}_3^-$  rate in the Colombia variety of *Physalis peruviana* L. on SPAD readings during the entire crop cycle, and they did not find significant differences, although the highest readings (52.93) were found with nitrate (Steiner 50%) at 75 dat; after 90 dat they observed lower readings, which is attributed to the N demands by the fruits. It should be mentioned that these readings are higher than those with treatments at 50% in all the varieties in this experiment.

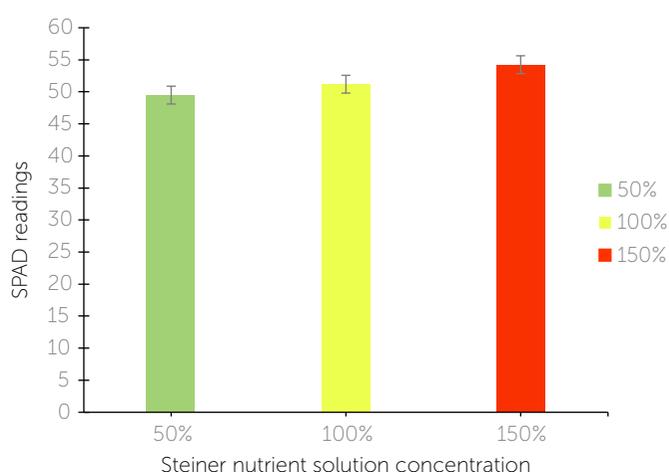
#### Electric Conductivity (EC) and Sap pH

The variance analysis for the variables pH and conductivity measured in sap extracted from stems and leaf petioles showed significant differences (Table 1) in terms of variety and solution, while the interaction of both did not have the same effect.

The variety factor affected both variables; the Modified variety registered the highest levels of pH (5.85) and sap conductivity (7.27), while the rest are statistically equal. On the other hand, the solution with the highest concentration reported the highest readings for both variables (Table 1).

The response of the Modified variety can be explained because it is morphologically different from the rest, it is most likely also genetically different, and therefore should be further studied. From the beginning, this variety showed growth and vegetative development similar to shell tomatoes (*Physalis ixocarpa* Brot.).

Although no studies have been carried out for these variables in sap from *Physalis peruviana*, pH is at a



**Figure 5.** SPAD readings and their relationship with the concentration of the Steiner's nutrient solution.

**Table 1.** Analysis of variance and mean separation test of the pH and electrical conductivity (EC) in sap of leaf petioles and stems.

Source of variation	DF	pH in sap	EC in sap ( $\text{dS m}^{-1}$ )
<b>Variety</b>	3	0.0326*	0.0346*
Chiclayo		5.73 a	6.83 b
Colombia		5.73 a	6.89 a
Modified		5.85 a	7.27 a
Sacha		5.72 b	6.86 a
<b>Solution (%)</b>	2	0.0009*	0.0001*
50		5.66 b	6.44 b
100		5.79 a	7.07 a
150		5.82 a	7.38 a
<b>Var*SN</b>	6	0.3538 ns	0.1179 ns
<b>CV</b>		2.05	5.78

CV: coefficient of variation \*  $P \leq 0.05$ ; NS:  $P > 0.05$ ; DF: degrees of freedom. Medias with different letter in the same row are statistically different (Tukey,  $p < 0.05$ ).

medial level for the 100 and 150% concentrations, while at 50% concentration there is a possible imbalance between cations. On the other hand, the electrical conductivity for sap from *Physalis peruviana* showed levels lower than those reported in the literature, where the optimal range reported should be between 12 and 15 dS m<sup>-1</sup>. This means that further studies are needed for this variable in order to have a reference value for other research.

### Plant Height and Base Diameter

The variance analysis for plant height showed significant differences based on variety, solution and the interaction of both up to 24 dat (Table 2). At 31 dat only the nutrient solution's concentration showed any effect and at 52 dat no significant difference was seen in any factor.

The Sacha and Modified varieties at 4, 15, and 24 dat showed greater height, while the plants that achieved greatest height at 30 dat were with nutrient solution at 100 and 150%. In posterior samples no factor affected plant height (Table 2). These data are superior to those reported by Mora *et al.* (2006) when six wild harvests of *Physalis peruviana* in hydroponics and fertigation were evaluated, where at 40 dat there were means of 34 and 43 cm, and 75 and 80 cm at 64 dat.

On the other hand, after 30 dat the results were similar to those found by Antúnez (2013) in gooseberry, who reported that NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> did not influence

consistently the plants' height, which means that the concentration of nutrients available is not important in terms of height.

The Chiclayo variety had a smaller base diameter at 4, 15, 24 and 31 dat, unlike the Modified variety, which had a thick stem; however at 52 dat there were no significant differences. Concerning the effect of concentration of the nutrient solution, after 24 dat the diameter was smaller with 50% solution and there were no differences at 100 and 150%, and there is no interaction. These reports are superior to those by Mora *et al.* (2006) when evaluating six wild harvests of *Physalis peruviana* in hydroponics and fertigation; they reported means between 0.86 and 0.95 cm at 40 dat, and at 64 dat diameters varied between 1.30 and 1.33 cm. On the other hand, Antúnez (2013) at 135 dat registered diameters between 14.52 and 16.59 mm in plants originating from seeds.

### CONCLUSIONS

The phenology of each variety was not expressed at the same time at any concentration, and as precocious varieties there are Chiclayo, Columbia and Sacha, which at 50 and 150% concentrations of nutrient solution had an average harvest at 99 dat. At 100% concentration the Colombia variety was the most precocious with production at 93 dat, while the other two started 10 days later. The Modified variety was the latest variety, starting production at 112 dat.

**Table 2.** Analysis of variance and mean separation test of the plant height.

Source of variation	Plant height (cm)				
	Days after transplant				
	4	15	24	31	52
<b>Variety</b>	0.0001*	0.0001*	0.0175*	0.7149 ns	0.1189 ns
Chiclayo	3.65 c	10.00 c	23.25 b	40.75 a	100.75 a
Colombia	5.11 b	11.77 b	25.92 a	42.00 a	99.42 a
Modified	5.68 b	12.54 a	25.90 a	42.00 a	100.92 a
Sacha	6.78 a	13.07 a	25.75 a	41.00 a	94.83 a
<b>Solution (%)</b>	0.0333*	0.0001*	0.0001*	0.0001*	0.6314 ns
50	4.86 b	10.76 b	22.50 b	37.94 b	97.75 a
100	5.71 a	12.68 a	27.11 a	43.44 a	99.16 a
150	5.34 a	12.10 a	26.00 a	42.94 a	100.06 a
<b>Var*SN</b>	0.0138*	0.0008*	0.0262*	0.2156 ns	0.9407 ns
<b>CV</b>	16.56	9.01	9.15	8.14	6.89

CV: coefficient of variation \* P≤0.05; NS: P>0.05; DF: degrees of freedom. Medias with different letter in the same row are statistically different (Tukey, p<0.05).

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# Characterization in Clonal Selections of *Citrus × latifolia* Tanaka ex Q. Jiménez

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## ABSTRACT

**Objective:** To physically and chemically characterize clonal selections of Persian lime (*Citrus × latifolia* Tanaka ex Q. Jiménez).

**Design/Methodology/Approach:** The principal components analysis was employed, using a mixed data factorial analysis model. Genotype distribution was graphed using principal components with the k-medoids method, while a Gower's dissimilarity matrix was determined for the conglomerate analysis and a dendrogram was developed using Ward's minimum variance cluster method. For the morphological characterization of the fruits, the study considered the following trees: *Citrus volkameriana*, *Citrus macrophylla*, *Citrus paradisi* × *Poncirus trifoliata*, × *Citroncirus* spp., and *Citrus × aurantium*. The fruit's diameter, length, weight, color, and shape were analyzed, in addition to its base shape, tip shape, surface texture, albedo adherence, number of seeds, ripening rate, juice weight, juice yield, pH, °Brix, and titratable acidity. Data were analyzed using R software and the factoextra and FactoMineR packages.

**Results:** The physical and chemical traits of Persian lime fruit vary due to the correlations between the types of rootstock that are cultivated in the citrus zone studied.

**Study Limitations/Implications:** Farmers do not know which clone or type of plant material they propagate; they simply select clones that show outstanding morpho-agronomical traits.

**Findings/Conclusions:** The morphological diversity and quality of the fruit is related to the type of rootstock used in its propagation, in addition to internal and external traits in *Citrus macrophylla* standing out in fruit quality.

**Key words:** Persian lime, graft, rootstock, shoot, accessions.

## INTRODUCTION

Consumption of Persian lime (*Citrus × latifolia* Tanaka ex Q. Jiménez) is on the rise globally, due to it being a source of antioxidants favorable for human health, in addition to its multiple uses when consumed fresh or processed (Habermann and Claro, 2014). The traits that make it more attractive to consumers are its less acidic taste, higher juice content, lack of seeds, and a larger fruit size (Hassanzadeh et al., 2017).

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Mexico is among the top producers of citrus fruits in the world (FAOESTAT, 2018) and is the primary producer of Persian lime in the world (Fernandez *et al.*, 2015). According to the Ministry of Agriculture and Rural Development (Secretaría de Agricultura y Desarrollo Rural, SADER, 2018), the state of Veracruz is the top producer of Persian lime in the country. The citrus-producing zone of Cuitláhuac, Veracruz, is considered to be the zone with second-highest Persian lime production, which represents an important economic and social activity thanks to fruit exports destined mainly toward the United States (Caamal-Cauich *et al.*, 2017).

Persian lime is an acidic lime and is the only natural triploid ( $3n=27$ ) derived from a cross between *Citrus medica* as the female parent and *Citrus micrantha* (Curk *et al.*, 2016). It has non-viable pollen and ovules; therefore, its fruit are parthenocarpic or seedless (Moore, 2001), which is why there are no varieties and they are considered to be selections or clones (Santos *et al.*, 2013).

Rivera-Cabrera *et al.* (2010) stipulate that they could be "Quebra galho" (a local Brazilian selection contaminated by viroids). In contrast, Curti *et al.* (2017) affirm that the propagated clone is SRA-58, of French origin; however, the National Epidemiology, Quarantine, and Plant Sanitation Station (Estación Nacional de Epidemiología, Cuarentena y Saneamiento Vegetal, ENECuSaV) states that its origin is in the United States. Due to these varying theories, the Persian lime clones farmed in Mexico are believed to be of unknown origin.

The spontaneous mutations and variations that occur in citrus buds or budsticks have created novel phenotypic variations that result in new, superior traits, such as greater yield and fruit quality, resistance to pathogens, and tolerance to abiotic and biotic stress. Subsequently, vegetative propagation can generate a new variety. At this point, a clonal selection is established (Rouiss *et al.*, 2018; Thi-Lam and Ishikawa, 2018). Therefore, morphological characterization allows for defining the accession groups with outstanding agronomic traits and high genetic diversity (Moreno-Ramírez *et al.*, 2019). Thus, the present study had the objective of morphologically characterizing the potential selections of Persian lime that are propagated in the citrus-producing zone of Cuitláhuac, Veracruz.

## MATERIALS AND METHODOLOGY

Ripe Persian lime fruits were collected following the methodology described in the Mexican norm NMX-FF-077-1996, within similar agronomic-management conditions implemented in commercial orchards in the citrus-producing zone of Cuitláhuac, Veracruz, Mexico, which encompasses the municipalities of Cuitláhuac, Tierra Blanca, Tlalixcoyan, and Paso del Macho, located at altitudes between 27 and 345 m.

### Morphological Characterization

The morphological characterization of the fruits was done according to the morphological descriptors implemented by the International Plant Genetic Resources Institute (IPGRI), and these were: Rootstock 1) *Citrus volkameriana*, 2) *Citrus macrophylla*, 3) *Citrus paradisi* × *Poncirus trifoliata*, 4) × *Citroncirus* spp.), 5) *Citrus* × *aurantium*), fruit diameter and length (cm), fruit weight (g), fruit color, fruit shape (spheroid, ellipsoidal, pyriform, oblique, obloid, ovoid), fruit base shape (with neck, convex, truncate, concave, pinched concave, pinched with neck), fruit tip shape (breast-shaped, pointy, rounded, truncate, sunken), fruit surface texture (smooth, rough, papillary, pock-marked, uneven, striated), albedo adherence (weak, medium, strong), number of seeds per fruit, ripening rate, juice weight, juice yield, pH, °Brix, and titratable acidity. The fruit color determination was based on the citrus fruit color index (Jiménez *et al.*, 1981) using the CR-200 model Konica Minolta® colorimeter. The Brix degrees and juice pH were determined based on the methodologies described by the OECD (2018). Juice yield was expressed in yield percentage, and Total Soluble Solids (°Brix) were analyzed with a portable refractometer (Model HI 96801, 0 to 85% Brix). The pH was measured using potentiometry with an Oakton® potentiometer (model pH 700, USA). Titratable juice acidity was measured using 10 mL of juice and 50 mL of distilled water and was titrated with NaOH 0.1 M until reaching pH of 8.1, also measured using potentiometry, and the data were expressed in % of acidity, which are equivalents for citric acid and ripening rate.

### Experiment Design and Statistical Analysis

The data were analyzed in a single matrix. The principal components analysis (PCA) was done using a factorial analysis of mixed data model (FAMD). Genotype distribution was graphed with the first two principal components (PC) using the k-medoids method (PAM). For the cluster analysis, a Gower's dissimilarity matrix was determined and a dendrogram was made using Ward's

minimum variance cluster criterion method (Borcard et al., 2018). Data analysis was carried out using R software (version 3.6.1) with the factoextra and FactoMineR packages (Kassambara, 2017).

## RESULTS AND DISCUSSION

Significant differences were found in 58% of the morphological traits analyzed, indicating that morphological diversity exists in Persian lime, but only eight morphological traits did not present significant effects on morphological diversity, indicating that the most important and statistically significant correlations are related to the categorical traits.

The correlation of the PCA identified 11 highly discriminatory traits that explain 69.73% of the variation of five principal components (Table 1). The continuous traits of highest variability were fruit length, fruit diameter and weight, juice weight, °Brix, and ripening rate, while the categorical traits that showed the most variability were rootstock, fruit shape, fruit base and fruit tip shape, and texture.

Based on the distribution of the genotypes along the plane determined by PC1 and PC2, three groups were defined in terms of spatial distribution for the first two PC.

Group one is made up of fruits that show the highest ripening rate, °Brix and color, spheroid shape, rough texture, and a concave, pinched fruit base shape and pointy fruit tip shape. In general, the fruits were smaller in size, and the rootstock *Citrus × aurantium*, also known locally as sour orange, is present in 100% of the genotypes that make up this group.

Group two was made up of fruits with intermediate values in the quantitative traits for citric acid percentage, length, diameter, and fruit and juice weight. However, the fruit's qualitative traits presented an ellipsoidal and spheroid shape, papillary and rough texture, breast-shaped base, convex, and pinched-concave fruit base shape. In addition, this group was 75% made up of *Citrus volkameriana* (volkamer lemon), 12% of *Citrus paradisi × Poncirus trifoliata*

(C35 citrange), and 13% of *Citroncirus* spp. (swingle citrumelo) rootstocks.

Group three was made up of fruit genotypes with higher weight, diameter, length, juice weight, and citric acid percentage. With respect to the qualitative traits of the fruit, these showed truncated and convex base shapes, obloid and ellipsoid shapes, and rough and papillary texture. In the end, 100% of the genotypes conforming this group were grafted with rootstock from the alemow lemon or *Citrus macrophylla* (Figure 1).

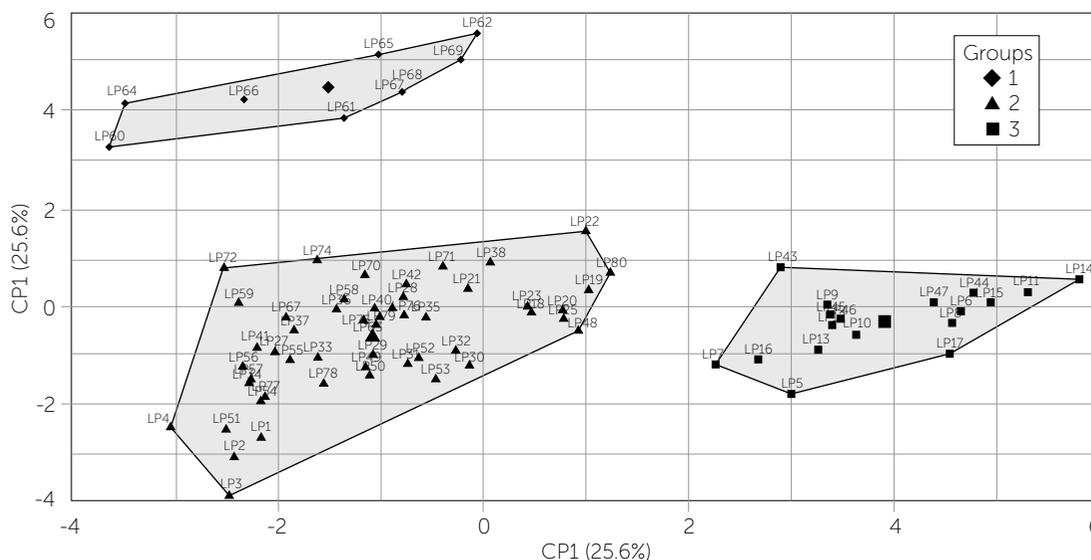
The morphological variability of the Persian lime exhibits diverse quantitative and qualitative morphological traits, although this variability in morphological traits is correlated with the rootstock used. These results coincide with those reported by Stenzel and Neves (2004), who evaluated the IAC-5 clone of Persian lime that was grafted on different rootstocks, and showed that the fruit presents diversity in quality and characteristics depending on the type of rootstock utilized.

In the dendrogram shown in Figure 2, five groups are formed according to Ward's method, and these groups showed a similar relationship with the groups formed in the principal components analysis, which is why the association shown by the groups is determined by the type of rootstock and by characteristics in fruit size. Group I was observed to consist of fruits with

**Table 1.** Eigenvalues of the first five principal components derived from 11 Persian lemon characters.

Character	Eigenvalues				
	CP1	CP2	CP3	CP4	CP5
Fruit diameter (cm)	0.62	0.03	0.032	0.058	0.000
Fruit length (cm)	0.63	0.02	0.047	0.082	0.041
Fruit weight (g)	0.72	0.02	0.015	0.054	0.022
Juice weight (g)	0.56	0.13	0.007	0.129	0.008
Brix grade	0.005	0.34	0.154	0.040	0.019
Maturity index	0.09	0.35	0.118	0.184	0.022
Rootstock	0.79	0.73	0.300	0.520	0.760
Fruit shape	0.55	0.44	0.186	0.265	0.085
Fruit base shape	0.79	0.01	0.603	0.231	0.144
Fruit apex shape	0.05	0.72	0.001	0.120	0.001
Fruit texture	0.20	0.23	0.243	0.060	0.009
Eigenvalues	5.62	3.45	2.67	2.17	1.37
Variation explained (%)	25.55	15.68	12.14	9.88	6.27
Accumulated variation (%)	25.55	41.24	53.38	63.26	69.53

CP: principal component.



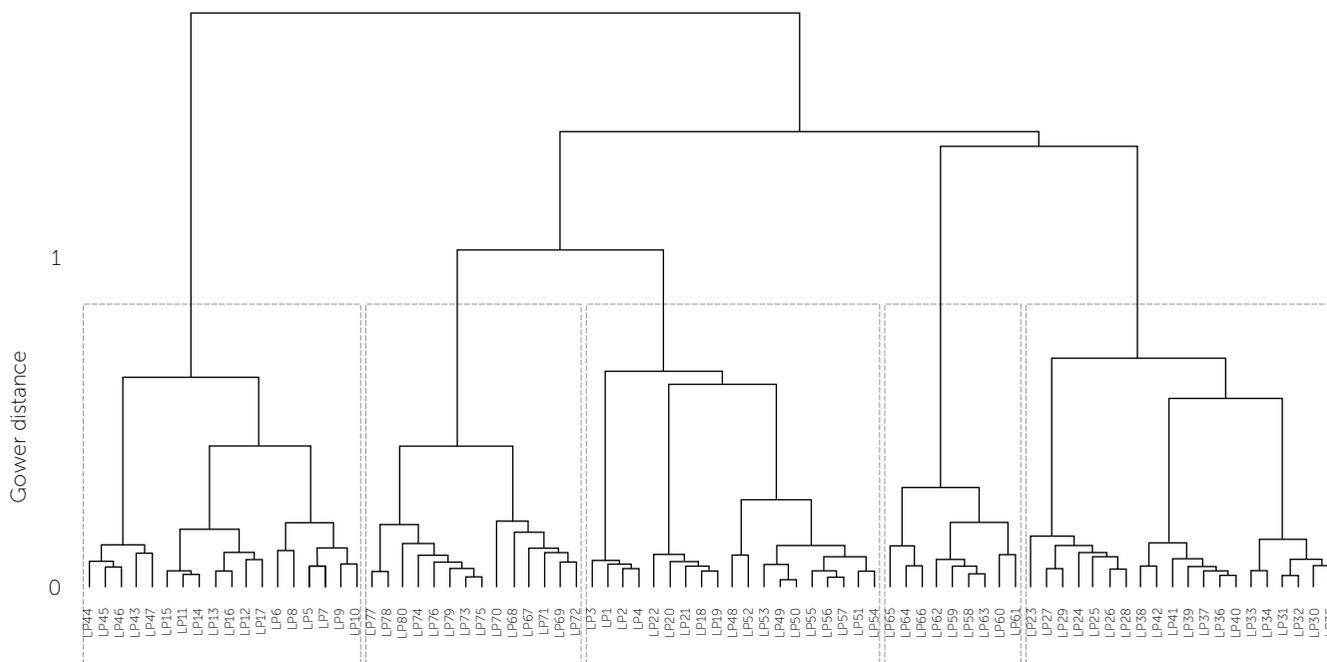
**Figure 1.** Dispersion of 80 Persian lemon genotypes, based on the first two main components of the analysis of 11 morphological characters.

the greatest diameter, length, and juice weight, as well as a truncated obloid and ellipsoid shape, rough texture, and with rootstock from *Citrus macrophylla*. In addition, the group showed a similarity distance of 0.4, while groups II and III consisted of fruits showing similar characteristics, only differing in the base shape. Group IV was made up of fruits with the smallest size,

but they showed an intense green color and higher amount of °Brix, with *Citrus × aurantium* rootstock. Finally, group V presented genotypes that were grafted with *Citrus paradisi × Poncirus trifoliata* and *Citroncirus* spp.

García-López *et al.* (2017) determined that the parameters that ensure quality in the Persian

lime fruit with respect to pH should be between 2.0 and 2.3, and values between 7.4 and 7.7 °Brix; thus, these results coincide with those reported by Zandkarimi *et al.* (2011), who mention that in limes and lemons, the most important morphological traits are fruit weight and length, in addition to their physicochemical characteristics. Authors like Grüneberg *et al.* (2009)



**Figure 2.** Dendrogram obtained by Ward's method of 80 Persian lemon genotypes based on 19 morphological characters.

indicate that in clonal crops, yield is determined by epistasis effects that cause a rise in morphological variation. Likewise, Medina-Urrutia et al. (2009) link the behavior of rootstocks to different types of soil on which adaptability to the environment depends, resulting in homogeneous production; in turn, Espinoza-Núñez et al. (2011) indicate that the alemow lemon presents high yields under irrigation conditions, while Khankahdani et al. (2019) indicate that *C. volkameriana* functions as rootstock in Persian lime from the first stages of grafting by producing vigorous plants with vegetative growth and greater absorption of iron, copper, zinc, and manganese. This relationship is of great importance due to the graft-rootstock interaction, which determines the optimal growth of the graft. Tietel et al. (2020) found that the rootstock affects yield and biochemical quality in mandarins (sugar and acidity), such that *C. volkameriana* did not increase the total sugar index, however, sour orange did increase the sugar content.

## CONCLUSIONS

The morphological diversity and fruit quality of Persian lime is related to the type of rootstock used for its propagation. Based on the morphological traits evaluated, *Citrus macrophylla* provides the best physical and chemical characteristics of lime fruits.

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# Sex Identification of *in vitro* Plants of *Carica papaya* L. MSXJ Hybrid through Molecular Markers

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## ABSTRACT

**Objective:** To identify the sex of *in vitro* plants of papaya (*Carica papaya* L.) MSXJ hybrid obtained via somatic organogenesis, through SCAR type molecular markers.

**Design/Methodology/Approach:** Eight-month old MSXJ papaya hybrid plants in the fructification stage were collected in Cotaxtla, Veracruz, Mexico. They were superficially disinfected with abundant running water, detergent during 30 min, and then alcohol at 70% was added for one minute, commercial chlorine at 30% for 30 min, and they were rinsed with sterile distilled water; then the meristems were cultivated in MS medium and after 30 d a subculture was made. The DNA extraction was made with the CTAB method, and the DNA PCR was done with the Deputy *et al.* (2002) method, and the primers T1, T12 and W11 were used.

**Results:** The T1 primer was the positive control and the T12 and W11 primers allowed the amplification of fragments that identify hermaphrodite, feminine and male plants, while the T12 and W11 primers were specific for hermaphrodite plants.

**Study Limitations/Implications:** It is required to standardize the method for it to be inexpensive.

**Findings/Conclusions:** The sexuality of papaya plants can be differentiated until the stage of flowering, which is why the implementation of molecular markers would facilitate plant selection if it is implemented at a large scale. Costs, maintenance time and elimination of plants of unwanted sex are reduced this way.

**Keywords:** *Carica papaya*, plant-sex, SCAR, *in vitro* plants.

## INTRODUCTION

# Mexico

exported 131,391 tons of papaya (*Carica papaya* L.) in 2014, with a value of 87 million dollars, and it was ranked as the top global exporter of this fruit. The volume increased 14% and the sales escalated 30.4% to inter-annual rates, marking a record in the value (SAGARPA, 2017).

Papaya is a plant with dicotyledonous and polygamous diploid characteristics (Liu *et al.*, 2004) (Caricaceae) (Badillo, 2002). Papaya cultivation presented considerable phenotypical variations with horticultural traits of great importance, which include physical and chemical characteristics such as size, shape, color, sugar content that influence its flavor (Kim *et al.*, 2002; Oliveira *et al.*, 2007). According to Ming *et al.* (2001), papaya is an excellent model for genomic and genetic research, which allows the possibility for genetic transformation, efficiency in breeding, high production of offspring, and continuous flowering.

The MSXJ papaya hybrid was developed by the National Institute of Forestry, Agriculture and Livestock Research (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, INIFAP) and it represents an alternative since one of its most outstanding characteristics is its tolerance to heat; it does not present abscission of flowers and fruits, and produces elongated fruits without deformations even when the environmental temperature exceeds 35 °C (Mirafuentes and Santamaría, 2014). However, the visual determination of sex in papaya plants in the vegetative state is not possible, since there are no embryological or morphological differences between the three sexual types. Therefore, sex determination is not carried out until flowering, which takes place four to six months after sowing. Therefore, sex determination has become a limitation for papaya growers since it implies higher costs and difficulties in planning the cultivation, especially in plantations where only hermaphrodite plants are required, since the best fruits are obtained from them (Hueso, 2015). Therefore, identifying the plant sex before flowering prevents additional costs and, because of this, sex identification before flowering through molecular markers represents an advantage.

The implementation of SCAR (Sequence Characterized Amplified Region) molecular markers (Parasnis *et al.*, 2000; Deputy *et al.*, 2002; Urasaki *et al.*, 2002; Chaves-Bedoya and Núñez, 2007) allows discriminating the sex in papaya plants and therefore can be used in its prompt determination. The importance of the use of tools from molecular biology for sex identification before flowering, specifically the SCAR molecular markers that use specific primers developed from mapped or characterized sequences from other markers, has allowed sex identification in papaya plants (Deputy *et al.*, 2002; Aspetia *et al.*, 2014). These authors have used specific

indicators such as T1, T12 and W11. Deputy *et al.* (2002) found that T1 serves as positive control, since it amplifies for female, male and hermaphrodite plants; T12 and W11 synthesize products for hermaphrodite and male plants, rarely for female plants. Aspetia *et al.* (2014) tested three sets of primers for SCAR markers: T1, T12 and W11. SCAR T1 generated bands of 800 pb for hermaphrodite plants; T12 and W11 amplified 100% of hermaphrodite plants and null in feminine plants. The objective was to differentiate the sex of *in vitro* plants of the papaya (*Carica papaya* L.) MSXJ hybrid obtained via somatic organogenesis, through SCAR type molecular markers.

## MATERIALS AND METHODS

This study began in the year 2016 and included two stages: 1) Development of seedlings of the MSXJ papaya hybrid using *in vitro* culture of apical meristems, via organogenesis and 2) Differentiation of the sex of *in vitro* plants through SCAR molecular markers.

The establishment of the *in vitro* culture of MSXJ papaya hybrid was carried out in the Agricultural Biotechnology Laboratory, of the company Agriculture and Livestock Genetic Production (Producción Genética Agropecuaria de México, PROGAMEX) located in the Innovation and Development Park of the state of Veracruz (Parque de Innovación y Desarrollo, PIDEV) in the Technological Institute and Superior Studies of Monterrey. The molecular studies were carried out in the Plant Biotechnology and Cryobiology LADISER of the Chemical Sciences School of the Universidad Veracruzana.

Explants from 20 plants of the MSXJ papaya hybrid were obtained in a plantation of the municipality of Cotaxtla, Veracruz, Mexico. The MST genotype (selection of Maradol cultivar) was used as masculine parent and J (landrace genotype) as female plants; the simple cross of these parents produced the MSXJ hybrid (Santamaría *et al.*, 2013). The material used was collected when they were eight months of age in the stage of fructification.

### Sterile Establishment

The explants were washed with abundant running water, later antibacterial soap was added, and they were placed in an orbital agitator (Ika®-Werke GmbH&Co.KG) during 30 min at 142 rpm, transferred into crystal containers with capacity for 250 mL and alcohol at 70% was added to the laminar flow bell for one minute; later they were submerged in a solution of commercial chlorine at 30% during 30 min and they were washed three times with

sterile distilled water to eliminate the remainders of chlorine.

### Extraction of the Apical Meristems

For the extractions of meristems, a stereographic microscope was used (DIGITAL MICROSCOPE) of 12VDC 4A max. 12V/15 W of transmitted light of 12V/10 W. The leaf primordia were eliminated until leaving the meristems exposed, and then a cut was made on the base of the meristem. The meristems were placed in test tubes with 20 mL of the Murashige and Skoog basal medium (1962) at 100% supplemented with 100 mg L<sup>-1</sup> of Mio-Inositol, 0.4 mg L<sup>-1</sup> of thiamine, 30 g L<sup>-1</sup> of sucrose and 2.5 g L<sup>-1</sup>, phytigel at pH 5.7±0.01, with a light period of 16 h. A subculture was carried out after one month of sowing the meristems. The meristems were transferred to 250 mL<sup>-1</sup> containers with 20 mL<sup>-1</sup> in MS basal medium at 100% supplemented with 0.5 mg L<sup>-1</sup> of BAP, 0.5 mg L<sup>-1</sup> of AIA, 10 mg L<sup>-1</sup> of adenine, 50 mg L<sup>-1</sup> ascorbic acid and 100 mg L<sup>-1</sup> citric acid, 40 mg L<sup>-1</sup> of thiamine, 100 mg L<sup>-1</sup> de Mio-Inositol and 30 g L<sup>-1</sup> sucrose, phytigel 3g L<sup>-1</sup>, pH 5.7±0.01, with a photoperiod of 16 h light.

### DNA Amplification and Visualization

For the extraction of DNA, 1 cm<sup>2</sup> of leaf lamina was selected and the CTAB method was followed (cetyltrimethylammonium bromide) (Doyle and Doyle, 1990). The amplification reaction from PCR of DNA extracted from *in vitro* plants of the MSXJ papaya hybrid was made according to the protocol described by Deputy et al. (2002). Three primers were used: T1, T12 and W11 (Table 1).

The amplification of DNA from *in vitro* plants of MSXJ papaya hybrid was carried out in volumes of 25 µL that contained 10 ng µL<sup>-1</sup> of DNA, 2.5 u of Taq-8GO (Mastermix 5xC, MP®), 5X Buffer Reaction of 25 mM (MgCl<sub>2</sub> 25 mM), dNTPs (1.25 mM each), 2.2 µL of primer R and 2.2 µL of primer F, both at a final concentration of 2 µM.

The amplification was carried out in a Thermocycler brand NYXTECHNIK with an initial cycle at 95 °C for 5 min, followed by 25 cycles at 95 °C for one minute, 58 °C for one minute, 72 °C for 2 min, and final extension

period at 72 °C for 7 min. The products from the amplification reaction were visualized in agarose gels at 1.5% supplemented with 1 µL of ethidium bromide (10 mg mL<sup>-1</sup>), the electrophoresis was carried out at 80 V during 1.5 h, and the bands were observed with UV light in a transilluminator brand UVP PHOTODOC<sup>®</sup> it Imaging System.

It was used as a marker of molecular weight 1 kb, sex identification was verified according to the number of pairs of bases. To identify hermaphrodite and feminine plants the T12 and W11 primers were used, which according to Aspetia et al. (2014) amplify for hermaphrodite plants, mark null for female plants, and with a double band of 800 pb and 1300 pb for male plants. T1 was used as positive control. To verify the association between markers and sex, a squared-Chi test was used.

## RESULTS AND DISCUSSION

### Sex Identification

Figure 1 shows the case of the T12 primer, observing amplification in 12 hermaphrodite plants (Figure 1a) and eight female plants (Figure 1b), which agrees with Aspetia et al. (2014) who obtained amplification.

The W11 primer also amplified for hermaphrodite plants. These results agree with Saalau et al. (2009), who used a multiple methodology with the T1 and W11 primers, and they obtained a double band of 800 pb (for T1) and 1300 pb (for W11) for hermaphrodite plants.

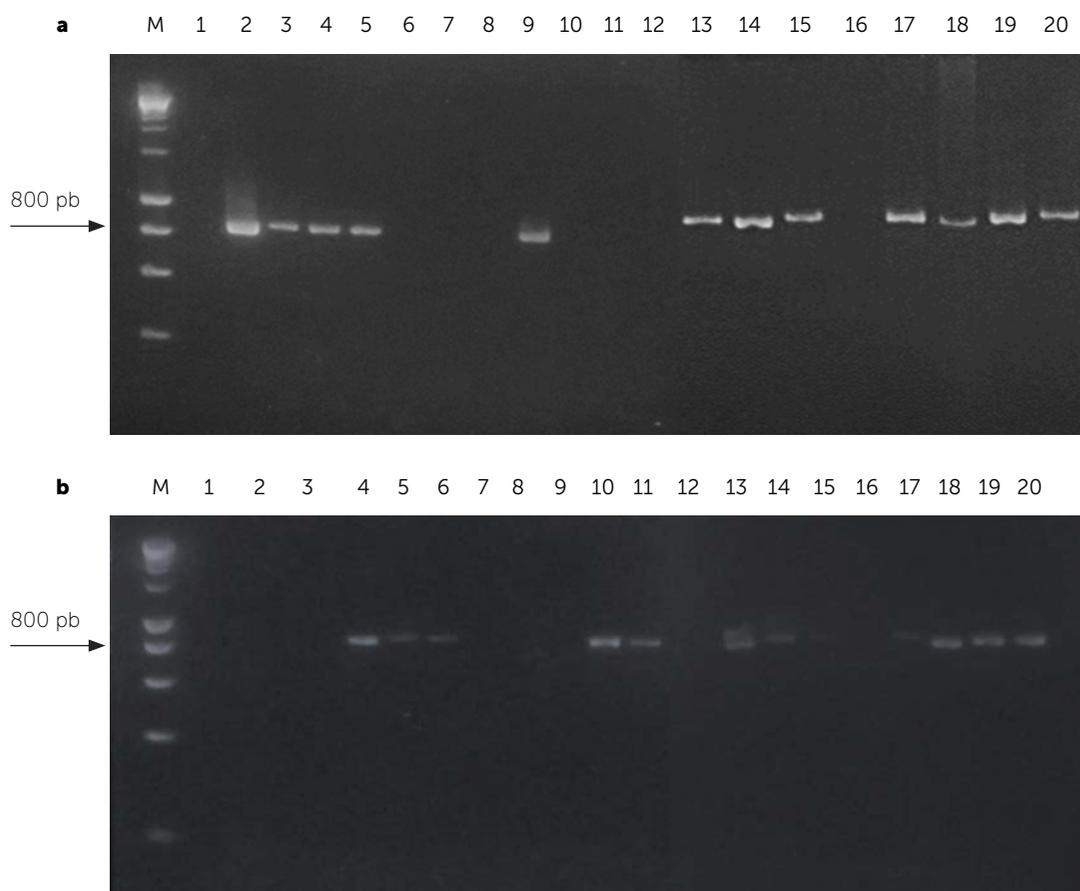
The T1 primer was used as positive control, since according to Aspetia et al. (2014), Saalau et al. (2009), and Deputy et al. (2002), it amplifies for the three sexes in papaya. In this study, although there were three samples that did not amplify for this primer, according to the

Chi<sup>2</sup> test it can be considered as positive control with 99% of probability for sex determination in the MSXJ papaya hybrid, which agrees with the authors cited.

With the T1 primer, 80% of hermaphrodite plants were observed. The T1 primer had amplification of 1300 in a female plant and 800 for hermaphrodite plants. This does not agree with Sánchez and Núñez (2008),

**Table 1.** Sequence of SCAR primers used for the identification of sex *in vitro* plants of the papaya hybrid MSXJ.

Primer	Sequence
T1-F	5'-TGCTCT12-R TTGATATGCTCTCTG-3'
T1-R	5'-TACCTTCGCTCACCTCTGCA-3'
T12-F	5'-GGGTGTGTAGGCACTCTCCTT-3'
T12-R	5'-GGGTGTGTAGCATGCATGATA-3'
W11-F	5'-CTGATGCGTGTGTGGCTCTA-3
W11-R	5'-CTGATGCGTGATCATCTACT-3'



**Figure 1.** 1a. PCR products of DNA extracted and amplified using SCAR T12 as a primer for sex identification. Bands show hermaphroditic plants, absence indicates female papaya hybrid MSXJ plants. 1b. PCR products amplified from DNA using SCAR W11 as a primer for sex identification in the MSXJ papaya hybrid. The bands show hermaphroditic plants, while their absence indicates female plants.

which obtained bands of 1300 for hermaphrodite and masculine plants in Colombian papaya cultivars.

When performing the  $\chi^2$  test to compare the results obtained with the T12 primer to identify hermaphrodite plants and T1 as positive control no significant difference was found. Therefore, it is considered that this indicator is reliable, although according to Aspeitia *et al.* (2014) it is necessary to carry out more tests to verify the results. The results also agree with these authors', who obtained bands of 800 pb for hermaphrodites and 1300 pb for females. With the W11 primer, they amplified hermaphrodite plants with fragments of 800 pb (Figure 1). Although some inconsistencies were observed, the  $\chi^2$  test was used to compare the results obtained with this primer and those obtained with the T1 and T12 primers were not significant.

## CONCLUSIONS

The primers used (T1, T12 and W11) amplified bands of 800 pb and 1300 pb for hermaphrodites and null for females, with specificity for MSXJ hybrid papaya plants. Therefore, they can be used to implement a protocol for sex identification.

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# *In vitro* Organogenesis of *Stevia rebaudiana* Bert. with Different Explants and Growth Regulators

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## ABSTRACT

**Objective:** To evaluate various explants and growth regulators in order to improve *in vitro* propagation of *Stevia rebaudiana* through organogenesis.

**Design/Methodology/Approach:** Explants and growth regulators in two different concentrations were evaluated. The explants were nodal segment, axillary bud, and apical meristem; while the growth regulators were benzylaminopurine (BAP) at 1.125 mg L<sup>-1</sup> and 3.0 mg L<sup>-1</sup>, naphthaleneacetic acid (NAA) at 1.5 mg L<sup>-1</sup> and 3.0 mg L<sup>-1</sup>, and CIDEF-4 brassinosteroids (BRs) at 1.0 mg L<sup>-1</sup> and 1.5 mg L<sup>-1</sup>. In total 18 treatments with seven repetitions. Contamination, oxidation, and survival were recorded during induction; while leaf number, regrowth height, and root presence were recorded during multiplication.

**Results:** At the induction stage there was a differential response between explants according to their ontogenetic age; during multiplication, the morphological components showed differences between concentrations of growth regulators and explants, with higher effectiveness when adding BAP to apical meristems.

**Study Limitations/Implications:** Both the origin and the age of explants can induce differential growth while interacting with growth regulators.

**Findings/Conclusions:** Apical meristem explants showed better advantages for *in vitro* reproduction of *S. rebaudiana* since they present less contamination and higher survival at the induction stage, even when exhibiting the highest oxidation among explants, which did not influence the decrease in their survival. At the multiplication stage with apical meristem, height, leaf number, and root presence were increased. Values were high when interacting with BAP.

**Keywords:** stevia, micropropagation, brassinosteroids.

## INTRODUCTION

*Stevia rebaudiana* Bert. is a native bush from Paraguay, known as sweet leaf given the presence of diterpene glycosides, such as stevioside and rebaudioside A (Oviedo *et al.*, 2015). The leaves of the plant have traditionally been used as a sweetener (Pande and Gupta, 2013). It is non-caloric and sweeter than sucrose or sugar cane (Jagatheeswari and Ranganathan, 2012). The natural sweeteners in *S. rebaudiana* are appropriate for people who should control the concentration of sugar in their

blood (Deshmukh and Ade, 2012; Jagatheeswari and Ranganathan, 2012). In addition, it is non-carcinogenic (Ramya *et al.*, 2014), an attribute distinguishing it from artificial sweeteners. This fact has increased its demand in the national and international markets, and in Mexico an increase in its crop surface has been suggested. It is considered that *S. rebaudiana* Bert. is likely to have good productivity in the Pacific Slope and some regions of the Gulf of Mexico (Ramírez *et al.*, 2011).

Conventionally, the plant is propagated through cuttings, and this traditional method only allows for the reproduction of few plants. Obtaining the plant via sexual reproduction is also limited due to the scarce seed production of *S. rebaudiana* and its low percentage of germination (Jagatheeswari and Ranganathan, 2012). Faced with this situation, *in vitro* reproduction poses an alternative in order to increase multiplication rates (Das, *et al.*, 2011).

Different studies on *S. rebaudiana* micropropagation have been carried out with various explants, such as leaves (Karimi *et al.* 2014), internodal segments (Singh *et al.*, 2014), and apexes (Das *et al.*, 2011), and different growth regulators have been tested as well (Singh *et al.*, 2014). However, changes in the cells can be induced with some regulators (Izquierdo *et al.*, 2012), such as cytokinins in high concentrations (Orbovic *et al.*, 2008). Through *in vitro* reproduction, the absence or concentration of both the medium's compounds and the growth regulators trigger several responses in explant development. Brassinosteroid or homobrassinolide (BR) analogues, which are non-traditional growth regulators, can be used as substitutes for auxins and cytokinins in biotechnological processes (Izquierdo *et al.*, 2012). These are metabolites that are capable of stimulating plant growth through cell division and elongation of shoots and roots (Sirhindi *et al.*, 2011; Vleeschauwer *et al.*, 2012) in segments of different organs and explants (Salgado *et al.*, 2008). In addition, their application positively influences against biotic and abiotic stress during development of the plants (Bajguz, 2010). With that aim, various explants and growth regulators were evaluated in order to improve *in vitro* propagation of *Stevia rebaudiana* through organogenesis.

## MATERIALS AND METHODS

The experiment was carried out at the Biotechnology Laboratory of the School of Agricultural Sciences, Campus IV, at the Autonomous University of Chiapas

(Universidad Autónoma de Chiapas, UNACH), located in the municipality of Huehuetán, Chiapas (15° 00' 25.02" N and 92° 23' 59.06" W, at 44 meters in altitude). Two stages were considered: the first was induction, 11 weeks in duration, the second multiplication, which lasted for 17 weeks.

Biological material was obtained from *S. rebaudiana* var. Morita 2 established in the greenhouse of the Experimental Field at the School of Agricultural Sciences (UNACH). The explants –axillary bud, apical meristem, and nodal segment– were obtained after a two-month growth period. At the greenhouse, the plant was sprayed with Amistar® (azoxystrobin 1.5 g L<sup>-1</sup>) fungicide during three days before getting the explants. Once removed from the plant, the explants were taken to the laboratory and washed with soap and water. Then, they were kept in agitation for 10 minutes in an azoxystrobin (1.5 g L<sup>-1</sup>) solution, to which a drop of Tween® 20 (polyoxyethylene sorbitan monolaurate) was added; likewise, sodium hypochlorite (NaClO) solution at 2.0% was added for 15 minutes in the laminar flow chamber. Subsequently, the explants were rinsed three times with sterile distilled water and 1.5 cm-long nodal segments, axillary buds between 2.0 and 3.0 mm in diameter, and 1.5 cm-long apical meristems were obtained from the plants' branches. Before planting, the explants were placed in an antioxidant solution, composed of citric acid (0.1 g), ascorbic acid (0.15 g), and sucrose (30 g).

The Yasuda *et al.* (1985) semisolid medium was employed, modified by adding vitamins and the following growth regulators: BAP (6-benzylaminopurine), NAA (naphthaleneacetic acid) and Br (brassinosteroid), each one in two different concentrations. Brassinosteroid (BR) CIDEF-4 is a homobrassinolide, produced in Mexico by the Natura del Desierto, S.A. de C.V., company which has 80% steroidal content and 10% active with a non-toxic soluble form presentation, compatible with fertilizers, insecticides, and fungicides. The medium was sterilized by autoclave at 15 PSI and 120 °C for 20 minutes.

The explants were placed in test tubes with 10 mL medium. Once the planting was carried out in each treatment, they were placed in the incubation room at temperature of 26±1 °C, 60% relative humidity, and 45 mE m<sup>2</sup> s<sup>-1</sup> light intensity for a period of 16 h of darkness and 8 h of light.

The treatments were generated with the combination of these factors: a) explants (nodal segments, axillary buds, and apical meristems), and b) growth regulators in two concentrations: BAP ( $1.125 \text{ mg L}^{-1}$  and  $3 \text{ mg L}^{-1}$ ), NAA ( $1.5 \text{ mg L}^{-1}$  and  $3 \text{ mg L}^{-1}$ ), and BR ( $1 \text{ mg L}^{-1}$  and  $1.5 \text{ mg L}^{-1}$ ). The complete factorial ( $3 \times 3 \times 2$ ) produced 18 treatments with seven repetitions, distributed in a completely random experimental design. Each explant represented one experimental unit.

After evaluating the induction stage, regrowths from the explants were transplanted for their multiplication and activated carbon (1 g) was added to the cultivation medium in order to stimulate rhizogenesis, and medium changes were carried out every 20 days.

The response variables during the induction stage resulted from the average of contamination, oxidation, and survival percentages, evaluated each week. At the multiplication stage, regrowth height, leaf number in each regrowth, and percentage of roots were evaluated. The mean results of the variables from the induction stage were graphed using Sigma Plot (V. 11.0) software by Jandel Scientific. Data of the variables from the multiplication stage were analyzed with SAS for Windows Ver. 8.1 (1999-2000) software, and the means comparison between treatments was performed with Tukey's test ( $P \leq 0.05$ ).

## RESULTS AND DISCUSSION

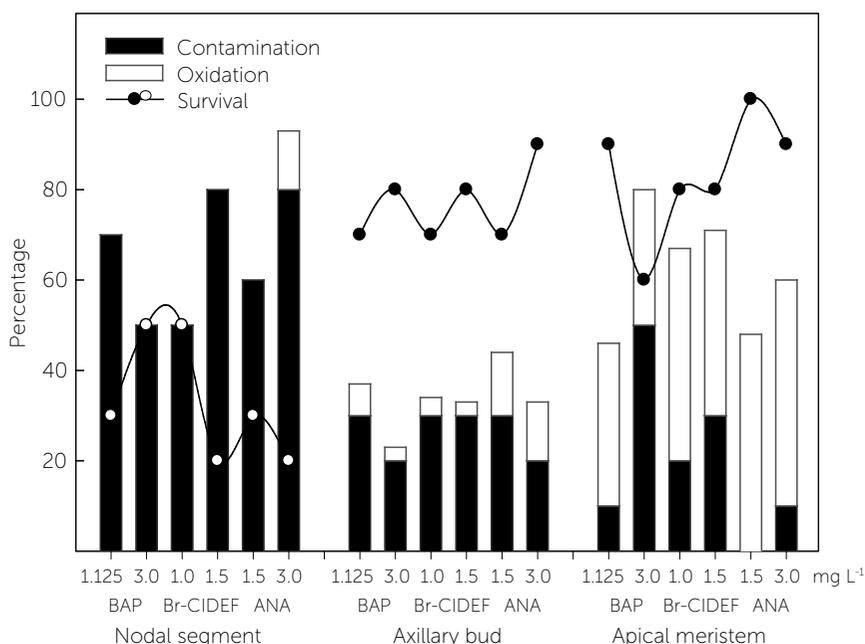
### Induction Stage; Contamination, Oxidation and Survival

The explants showed lower survival percentages as medium contamination increased. During week 11 of evaluation, average contamination was 65% in nodal segments, 27% in axillary buds, and 20% in apical meristems (Figure 1). In general, contamination in apical meristems decreased when interacting with the lowest doses of all growth regulators. When adding the lowest concentration of NAA ( $1.5 \text{ mg L}^{-1}$ ) to the medium, the same explant did not show contamination, but when concentration increased to  $3.0 \text{ mg L}^{-1}$ , 10% showed contamination (Figure 1).

Higher contamination was recorded in nodal segment and axillary bud

explants, which have older ontogenetic ages, when interacting with growth regulators. With regards to NAA application, a contracting effect was present in contamination; that is, contamination increased in nodal segments with the highest concentration ( $3.0 \text{ mg L}^{-1}$ ), while an increase in contamination occurred in axillary buds with the lowest dose ( $1.5 \text{ mg L}^{-1}$ ). When adding BAP to the cultivation medium, contamination increased in both nodal segments and axillary buds with the lowest dose ( $1.125 \text{ mg L}^{-1}$ ). When using Br in the medium, no differences in contamination figures of axillary buds were detected in the two concentrations evaluated; however, contamination was higher with the highest doses in both nodal segment and apical meristem explants (Figure 1).

The increase in contamination in explants that are older is described by López-Gómez et al. (2010) in *Coffea* spp. leaves, while Martínez et al. (2016) add that explants do not always respond to the procedures employed in the initial decontamination of the material, especially fungi and bacteria. It is worth mentioning that some plants have various particular morphological attributes that hinder the elimination of contaminants, such as presence of epicuticular waxes, and increase in trichome type, form, and density; or else when a plant is exposed longer to the environment, favoring fungal invasion of the stomatic complex.



**Figure 1.** Contamination, Oxidation and Survival of *Stevia rebaudiana* explants in Yasuda medium with different concentrations of growth regulators. Values are average of seven repetitions.

Concerning oxidation or tissue darkening, it seems to be more closely related to the explant's age. Apical meristem, which is the young tissue, showed the highest oxidation average (42%), and decreased to 7% in axillary buds and to 2% in nodal segments, which are older tissues. The increase in oxidation in apical meristem was found when NAA was added to the medium. In this regard, the increase in oxidation of young tissue can be caused by the tissue's oxidative stress, resulting from deficiencies in antioxidant defenses (Turrens, 2003), or else by the presence of phenols in the tissues as a result of their reactions to polyphenol oxidases (Scherer *et al.* 2006), which are exuded into the medium through wounds in the tissue. This problem tends to stop or decrease when the explant starts growing, as was observed in *Gossypium hirsutum* L (Ozyigit *et al.* 2007). Oxidation in some species has resolved using polyvinylpyrrolidone (PVP) (Méndez-Alvarez and Abdelnour-Esquivel, 2014). However, Aguirre-Medina *et al.* (2018) used PVP combined with growth regulators, such as BAP, NAA, and IAA, in *S. rebaudiana* explants of leaves, longitudinally cut stems, and apical meristem, and they indicate that there was no influence in the percentages for oxidation averages.

Among explants, the highest survival of seedlings with different concentrations of growth regulators was observed in apical meristem (82%) and axillary bud (75%), while the lowest survival corresponds to the nodal segment (33%). In *Heliconia bihai* (L.) cv. Lobster Salmón, Marulanda-Angel *et al.* (2011) account for lower survival on the basis of floral meristems treated with BAP (2 mg L<sup>-1</sup>). In our case, survival was high in apical meristem with 1.25 mg L<sup>-1</sup> concentration, and it decreased in axillary bud and nodal segments when increasing concentration to 3.0 mg L<sup>-1</sup>.

### Multiplication Stage: Regrowth Height and Leaf Number

The higher average increase in regrowth height among the explants was found in apical meristems (2.26 cm), and it amounted to 10 and 36% more growth in relation to nodal segments and axillary buds. Apical meristems (3.45) also presented the highest number of leaves per regrowth on average, and it amounted to 22 and 66% more leaves when compared to nodal segments and axillary buds. Likewise, it was statistically different ( $P \leq 0.05$ ) (Table 1).

The regrowth height and the leaf number in explants with growth regulators showed the highest increase when adding BAP in the highest concentration (3.0 mg L<sup>-1</sup>) and were also statistically different ( $P \leq 0.05$ ) from other regulator concentrations. In this respect, Hassanem and Khalil (2013) mention the same response when applying higher concentrations of BAP, but to MS medium. On the contrary, lower height appeared with Br at a 1.5 mg L<sup>-1</sup> concentration, and represented 5% less when compared to a Br concentration of 1.0 mg L<sup>-1</sup>, although the leaf number increased by 4% with the highest Br concentration when compared to the lowest one (Table 1). This outcome is probably linked to its increase in the plant's apexes. The Br is not subjected to active transport inside the plant (Hategan *et al.*, 2013), since its metabolism interacts with different enzymes in the cell's organelles and is capable of exhibiting one of the many physiological activities of Br, such as cell division and elongation (Sirhindi, 2013). Induction in plant growth depends on Br concentration, treatment duration, and age when the treatment was introduced, as happened when applying different concentrations of 24-epibrassinolide and 28-homoethylcastasterone to *Vigna irradian* L. and *Brassica juncea* L., which improved photosynthetic rate, total chlorophyll, chlorophylls a and b, carotenoids, shoot length, and fresh and dry weight (Fariduddin *et al.*, 2011; Sirhindi *et al.*, 2011).

Regarding the interaction between concentrations of growth regulator and explants, the greatest increase in height occurred for apical meristems and nodal

**Table 1.** Comparisons of explant and growth regulator factors in *Stevia rebaudiana* Bert.

Explant	Height (cm regrowth <sup>-1</sup> )	Number of leaves regrowth <sup>-1</sup>
Nodal segment	2.03 b*	2.66 b
Apical meristem	2.26 a	3.45 a
Axillary bud	1.43 c	1.14 c
Growth regulators (mgL <sup>-1</sup> )		
BAP 1.125	1.91 c	2.71 b
BAP 3.0	2.01 a	3.14 a
Br 1.0	1.86 e	2.42 d
Br 1.5	1.77 f	2.52 c
ANA 1.5	1.98 b	1.42 f
ANA 3.0	1.91 d	2.28 e

\*Different letter values, within columns and factor, are statistically different (Tukey,  $p \leq 0.05$ ). BAP (6-benzyl amino purine), Br (Brassinosteroid), ANA (Naphthaleneacetic Acid).

segments. The first increased with the two concentrations of NAA, plus BAP at 3.0 (mg L<sup>-1</sup>), and in nodal segments all treatments were included within the first statistical group.

With regards to the interaction of both factors, explants and growth regulators, the number of leaves per regrowth increased by 5.0 in apical meristems and the lowest BAP dose (1.125 mg L<sup>-1</sup>), and was statistically superior to the rest of the treatments ( $P \leq 0.05$ ), followed by the induction of 4.4 leaves with BAP at 3.0 mg L<sup>-1</sup> (Table 2). This result agrees with what was reported by Jagatheeswari and Ranganathan (2012), who mention that high BAP doses delay growth in *S. rebaudiana*. Other authors recount similar results when adding BAP to MS medium in the same species (Khalil et al., 2014; Jagatheeswari and Ranganathan, 2012).

The highest leaf number per regrowth (5.0) was achieved in epical meristems interacting with BAP at 1.125 mg L<sup>-1</sup>, and in nodal segments (4.0) with inclusion of BAP. In this respect, the addition of BAP to MS medium has conveyed this same result in *Stevia* (Abd-Alhady, 2011; Rangappa and Aind, 2013). In relation to concentrations, there is evidence that adding 3.0 mg L<sup>-1</sup> fosters regrowth (Abd-Alhady, 2011; Fatima and Khan, 2011; Hassanen and Khalil, 2013), but also the 1.0 mg L<sup>-1</sup> dose (Thiyagarajan and Venkatachalam, 2012) in MS medium. Intermediate values of 2.7 leaves per regrowth were obtained with Br in nodal segments. Br (28-homoethylcastasterone) was applied to *Mallus prunifolia* (Willd.) Borkh. apex, which increased the number of shoots when benzyladenine was added to the medium (Schaefer, 2002).

In axillary buds, BAP and NAA induced lower regrowth, one regrowth on average, and just two regrowths when adding Br in its lowest concentration (1.0 mg L<sup>-1</sup>). Other authors have combined BAP and naphthaleneacetic acid (in a 2 to 1 proportion) in *Stevia* axillary bud and MS medium, in order to increase the number of regrowths (Rangappa and Aind, 2013). In other results for nodal segment and axillary

bud, it was found that there was better regrowth induction when adding low concentrations, BAP from 1.0 to 1.5 mg L<sup>-1</sup> plus kinetin in more than 0.5 mg L<sup>-1</sup>, to MS medium (Aamir et al., 2010; Fatima and Khan, 2011). On the contrary, Khalil et al. (2014) indicate that adding 2,4-D to the medium, in combination with BAP and NAA or indolebutyric acid, on its own or combined with NAA, significantly inhibits the number of stems per explant in *S. rebaudiana*.

The presence of roots was apparent in the apical meristem explant while interacting with the three growth regulators added to the medium. This result suggests the importance of both the explant and its age in order to favor organ development in plants under *in vitro* conditions. Several authors report greater radical growth when applying NAA to *S. rebaudiana* explants (Shatnawi et al., 2011; Sikdar et al., 2012).

## CONCLUSIONS

The apical meristem explant shows better advantages for *in vitro* reproduction of *Stevia rebaudiana* given that it shows less contamination and higher survival at the induction stage. Even when it did show the highest

**Table 2.** Morphological components in explants of *Stevia rebaudiana* Bert. in interaction with different concentrations of growth regulators during the multiplication stage.

Explant	Treatment (mgL <sup>-1</sup> )	Height (cm regrowth <sup>-1</sup> )	Number of leaves regrowth <sup>-1</sup>	Roots (%)
Nodal Segment	BAP 1.125	1.80 bcde*	2.28 de	20
	BAP 3.0	2.21 ab	4.00 bc	0
	Br 1.0	2.11 abc	2.71 de	0
	Br 1.5	1.42 de	2.71 de	10
	ANA 1.5	2.42 a	1.14 fg	0
	ANA 3.0	2.21 ab	3.14 cd	0
Axillary bud	BAP 1.125	1.50 cde	1.00 g	0
	BAP 3.0	1.44 de	1.00 g	0
	Br 1.0	1.54 cde	2.00 ef	0
	Br 1.5	1.50 cde	1.00 g	0
	ANA 1.5	1.34 de	1.00 g	0
	ANA 3.0	1.25 e	1.00 g	0
Apical meristem	BAP 1.125	2.45 a	5.00 a	20
	BAP 3.0	2.31 ab	4.42 ab	20
	Br 1.0	1.92 abcd	2.57 de	20
	Br 1.5	2.40 ab	3.85 bc	10
	ANA 1.5	2.18 ab	2.14 e	20
	ANA 3.0	2.25 ab	2.71 de	10

\* Different letter values, within columns and factor, are statistically different (Tukey,  $p \leq 0.05$ ). BAP (6-benzyl amino purine), Br (Brassinosteroid), ANA (Naphthaleneacetic Acid).

oxidation among explants, this did not influence on the decrease of their survival. Height, leaf number, and root presence were increased at the multiplication stage with apical meristem. Values were outstanding when interacting with BAP.

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# Genetic Uniformity of the MSXJ papaya hybrid (*Carica papaya* L.) during Micropropagation

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## ABSTRACT

**Objective:** To analyze the genetic uniformity of MSXJ hybrid papaya *in vitro* plants, obtained by direct organogenesis.

**Design/Methodology/Approach:** The MSXJ papaya hybrid demonstrates quality characteristics for the national and exports market. *In vitro* culture of plant tissues represents a useful tool for their multiplication and conservation, but somaclonal variation can diminish their genetic and agronomic uniformity. In order to analyze the genetic uniformity of *in vitro* plants of this hybrid, ten ISSR primers were used for *in vitro* plants micropropagated during nine subcultures. DNA was extracted using the CTAB method. Data were analyzed using the program PopGene v 1.3.1.

**Results:** Eighty-five loci of 200 to up to 2000 pb were generated, with 37 polymorphic loci. In the cluster analysis, three groups were observed which separate subculture one, subcultures two to eight, and subculture nine; the Gst value of 0.87 indicated genetic uniformity as far as subculture eight.

**Study Limitations/Implications:** Papaya is one of the most important tropical fruits worldwide; however, these plants need to be healthy and genetically uniform to guarantee commercial success. *In vitro* propagation allows obtaining healthy and uniform plants, but it is necessary to study genetic uniformity during their micropropagation.

**Findings/Conclusions:** The *in vitro* multiplication of the MSXJ papaya hybrid permitted the regeneration of vigorous plants in 30 d. Molecular profiles indicate that as far as subculture eight, there is genetic uniformity. As such, no more than eight subcultures are recommended during micropropagation.

**Keywords:** *In vitro* plants, genetic uniformity, papaya, ISSR.

## INTRODUCTION

**Papaya** (*Carica papaya* L.), along with mango (*Mangifera indica* L.), avocado (*Persea americana* L.) and pineapple (*Ananas comosus* (L.) Merr), is one of four tropical fruits with the highest volume in exports. Globally, it is among the most well-known and consumed fruits (Altendorf, 2018). It is cultivated extensively in more than 60 countries in the tropics and subtropics (FAO, 2017). In 2017, Mexico positioned itself as the third top producer in the world and the fifth top exporter with 961,768 tons (SIAP, 2018).



The cultivation of papaya faces significant problems, such as the ringspot virus (Valencia *et al.*, 2017), which affects its production and productivity (Vincent *et al.*, 2019). For this reason, the development of cultivars with better characteristics is of great importance. The MSXJ papaya hybrid developed in the Experimental Camp of Huimanguillo, Tabasco (INIFAP) demonstrates quality characteristics for the national and exports market: it has red flesh and is tolerant to high temperatures during its flowering and fruiting stage (Mirafuentes and Santamaría, 2014). However, efficient propagation methods are needed to ensure its genetic characteristics, sex, safety, and agronomic uniformity. One of these methods is *in vitro* cultivation, which is useful for obtaining plants with high genetic and phytosanitary quality, as well as facilitating their use in plant-breeding efforts (Olivera, 2009). In the case of the papaya, there are various reports on its *in vitro* propagation (Ascencio *et al.*, 2008; Setargie *et al.*, 2015; Teixeira, 2016; Bindu and Bindu, 2017; Solórzano *et al.*, 2018; Chaudhary and Prakash, 2019). Posada *et al.* (2004) reported a propagation protocol using apices and Solis *et al.* (2011) using meristems. Recently, Al-Shara *et al.* (2018) published a review of the methods and limitations in papaya micropropagation where they refer to the difficulties they have found in commercial *in vitro* propagation of papaya; among these, they cite the small margin of certainty in reproducing and ensuring the production of healthy plants without genetic changes. Regarding this, it is necessary to confirm the genetic uniformity of the plants obtained using *in vitro* cultivation techniques (Posada, 2005). Genetic uniformity studies constitute a valid and fast technique for the detection of variability, since it allows distinguishing genotypes (Dávila and Castillo, 2007). Genetic markers can be successfully employed to search for differences in the expression patterns between cultivars, varieties, and plants. The ISSR markers are dominant and highly reproducible, therefore very useful for DNA profiling (González and Aguirre, 2007). Thus, the genetic uniformity of *in vitro* plants of the MSXJ papaya hybrid obtained via direct organogenesis was analyzed using ten ISSR primers during nine subcultures.

## MATERIALS AND METHODOLOGY

The *in vitro* plants used were MSXJ papaya hybrids, developed in the Agricultural Biotechnology Laboratory of the Innovation and Development Park in the state Veracruz, located in the Monterrey Institute of Technology and Higher Education (ITESM) facilities. The *in vitro*

plants were obtained from two hermaphrodite plants of the MSXJ hybrid, eight months old (P2 and P3). The samples were collected in Cotaxtla, Veracruz, Mexico. They were then regenerated via direct organogenesis from apical meristems, in a Murashige and Skoog (1962) basal medium at 100%, supplemented with 100 mg L<sup>-1</sup> of myo-inositol, 0.4 mg L<sup>-1</sup> of thiamine, 30 g L<sup>-1</sup> of sucrose, and 2.5 g L<sup>-1</sup> Phytigel at pH 5.7±0.01, with a photoperiod of 16 h light.

**Multiplication of MSXJ hybrid.** The *in vitro* plants were multiplied in the Agricultural Biotechnology Laboratory, and sub-cultured every 30 d nine times. For the *in vitro* multiplication of the MSXJ papaya hybrid, a Murashige and Skoog 100% solid mineral salts medium was used, supplemented with adenine 10 mg L<sup>-1</sup>, thiamine 40 mg L<sup>-1</sup>, myo-inositol 100 mg L<sup>-1</sup>, ascorbic acid 100 mg L<sup>-1</sup>, sucrose 30 g L<sup>-1</sup>, and Phytigel 3 g L<sup>-1</sup>. The growth regulators used were naphthalene-acetic acid (NAA) 0.05 mg L<sup>-1</sup>, benzyl aminopurine (BAP) 0.5 mg L<sup>-1</sup>, and gibberellic acid (GA3) 0.1 mg L<sup>-1</sup> with a pH of 5.8. Jars with 250 mL capacity with 30 mL of culture medium were sterilized in an autoclave (Felisa) at 120° C for 20 min and were left to rest for 24 h so that it could solidify.

Six shoots of MSXJ hybrid *in vitro* plants were placed in each jar with culture medium and kept at a controlled temperature (24 to 26 °C) for 30 d. After this time, each shoot was individualized and sub-cultured again in fresh culture medium. Additionally, remains of the gelling agent and callus that could accumulate on the seedlings were removed. A small sample of plant tissue was collected from each sub-culture of the *in vitro* plants and placed in an Eppendorf tube; the samples were frozen in a CRIOTEC freezer at -50 °C. This was carried out for each of the nine sub-cultures of the MSXJ hybrid micropropagated to analyze its uniformity.

**Analysis of genetic uniformity.** The genomic DNA extraction was done in the Teaching, Research and Services Laboratory (LADISER) of Plant Biotechnology and Cryobiology in the Molecular Biology department of the Faculty of Chemical Sciences at Universidad Veracruzana. The CTAB 2% method was used (Murray and Thomson, 1980) with modifications. Two samples of *in vitro* plants were used for each one of the nine sub-cultures, resulting in a total of 18 samples. The purity and amount of DNA extracted from each of the samples was quantified and confirmed by UV spectrophotometry;

purity was determined through optical density (OD 260 nm/OD 280 nm), and DNA integrity and quality were determined with 1.5% agarose gels.

DNA was amplified with the polymerase chain reaction (PCR) technique in an AXYGEN MAXYGENE II thermocycler. A preliminary evaluation was carried out of 20 ISSR (Inter Simple Sequence Repeats) reported by Sudha et al. (2013). Only the primers with the greatest number of the clearest bands were selected. The reaction mixture final volume was 25  $\mu\text{L}$ , which included 2  $\mu\text{L}$  of genomic DNA of the MSXJ hybrid, 5  $\mu\text{L}$  of 5x My Taq Reaction Buffer, 2  $\mu\text{L}$  of the primer, 0.2  $\mu\text{L}$  of My Taq DNA Polymerase, and 15.8  $\mu\text{L}$  of ultrapure water. The DNA amplification program consisted of an initial denaturation at 94 °C for 5 min, followed by 45 one-minute denaturation cycles at a temperature of 52 °C, extension at 72 °C for 2 min, and final extension at 72 °C for 7 min. The amplified products were separated by electrophoresis in 3% agarose gel using 2  $\mu\text{L}$  of Tri-color Buffer (5x DNA Loading), Gel Red Nucleic Acid Stain, and 8  $\mu\text{L}$  of each PCR sample; 3  $\mu\text{L}$  of MPM Hyper Ladder 1Kb molecular weight marker was used, suspended in TBE 1x (Tris/Borate/EDTA) at 90 V for 1:30 h. Finally, the amplified products were read in a UV transilluminator (Benchtop UV), capturing images for their subsequent analysis.

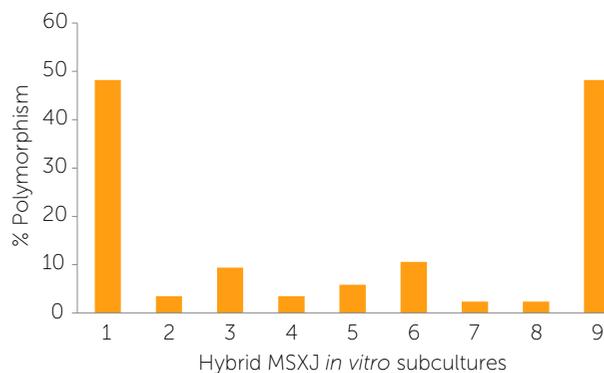
**Data analysis.** The number of bands per primer were calculated, as well as the percentage of polymorphism, genetic identity and distance, according to Nei (1972). In addition, a cluster analysis was carried out with the unweighted pair group method with arithmetic mean (UPGMA) using the PopGene program v. 1.3.1.

## RESULTS AND DISCUSSION

**Micropropagation.** The *in vitro* propagation protocol for the MSXJ papaya hybrid allowed obtaining vigorous seedlings free of diseases, with enough shoots for their multiplication. This coincides with that reported by Solís et al. (2011), since when propagating *in vitro* *Carica papaya* var. PTM-331 from apical meristems at 35 d they obtained large seedlings with a multiplication coefficient of 3.42.

**Molecular analysis.** The first critical step to carry out a molecular study in plants is to obtain good-quality DNA (Castro-Gómez et al., 2012). The purity of extracted genomic DNA, determined by absorbency proportion at 260/280 nm, was 1.8 on average; which, according to Alejos-Velázquez (2014), is accepted as pure DNA. In addition, the integrity gels showed appropriate quality for their amplification with ISSR primers. The genomic DNA concentration of the 18 MSXJ hybrid samples was 30.9 ng  $\mu\text{L}^{-1}$  on average, allowing the amplification of clear DNA bands.

**Genetic uniformity analysis.** Once the preliminary analysis of 20 ISSR primers for the genetic uniformity analysis of *in vitro* plants of the MSXJ hybrid micropropagated for nine subcultures was completed, ten ISSR primers were selected: UBC- 857-ACA CAC ACA CAC ACA CYC; T05- CGT TGT GTG TGT GTG TGT; ICL3- DBD ACA CAC ACA CAC ACA; UBC 841- GAG AGA GAG AGA GAG AYC; UBC 836- AGA GAG AGA GAG AYA; ICL16- GAG AGA GAG AGA GAG AYG; UBC 807- AGA GAG AGA GAG AGA GT; UBC 842- GAG AGA GAG AGA GAG AG; UBC835- AGA GAG AGA GAG AGA GYC; UBC825- ACA CAC ACA CAC ACA CT. These primers amplified 7 to 12 bands in 3% agarose gel with an average of 8.5 bands (Figure 1).



**Figure 1.** Percentage of polymorphism for ten ISSR primers, in nine *in vitro* subcultures of *Carica papaya* MSXJ hybrid.

The amplifications generated 85 loci with a molecular weight of 200 to 2000 pb, and 37 polymorphic loci. The primers showing higher polymorphism were UBC841 with 12 bands, followed by UBC807 with 11, and UBC842 with six bands. The consistent and well-defined profiles of these ISSR primers showed that they can be used to determine the genetic uniformity of the MSXJ papaya hybrid during its *in vitro* micropropagation.

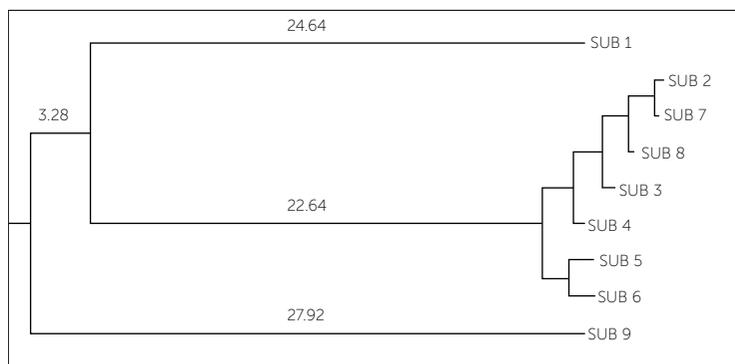
In the genetic uniformity analysis of the MSXJ papaya hybrid subcultures micropropagated *in vitro*, a 9.41% polymorphism was observed in subculture one. This polymorphism was greater than that of the next subcultures, and this can be explained by high heterozygosity due to the plant's cross-pollination. In subcultures two to eight, polymorphisms from 1.18 to 9.41 were present. The plants regenerated in these

subcultures, though not identical, are considered similar amongst themselves since the G<sub>st</sub> value was 0.87, which indicates high genetic identity between subcultures. Because *in vitro* cultivation is a cloning technique, the variability in polymorphism in these subcultures is explained by the high heterozygosity that is reflected in variability among explants. This had already been reported by Rani and Raina (1998), who cited that the variability in the subcultures of plants regenerated using *in vitro* processes does not necessarily indicate that they are different from their phenotype. Additionally, López *et al.* (2006), after studying the genetic uniformity of papaya plants micropropagated from the proliferation of axillary buds and meristems, demonstrated that their morphological, agronomic, industrial, and molecular characteristics are stable.

The above is corroborated with the cluster analysis of the MSXJ papaya hybrid (Figure 2), stemming from the ISSR profiles obtained through Nei's (1972) similarity algorithm and the UPGMA grouping algorithm, which shows one group that includes subcultures two to eight and the separation of subcultures one and nine. Subculture one presented the highest values in genetic identity (0.6342 to 0.66001) according to Nei. In subcultures two to eight it decreases slightly (0.5122 to 0.5983), and the lowest value was observed in subculture nine. Subculture nine presented the highest values in genetic distance (0.5158 a 0.6690) from the other subcultures, representing the greatest distance from subculture one. These data explain the separation of subcultures one and nine. In addition, the decrease of the genetic identity when increasing the number of subcultures coincides with that reported on the increase in frequency of the appearance of somaclonal variants as propagation cycles increase. This study recommends not exceeding eight subcultures, with the aim of minimizing the risk of genetic variability in the propagated material.

## CONCLUSIONS

The *in vitro* multiplication of MSXJ hybrid papaya plants permitted the regeneration of vigorous plants in 30 d, useful for establishing commercial orchards. The DNA obtained has sufficient quality to analyze genetic uniformity with ISSR primers in *in vitro* MSXJ hybrid papaya plants. The molecular profiles obtained indicate that genetic uniformity exists as far as the eighth subculture. Therefore, to reduce the risk of



**Figure 2.** UPGMA dendrogram of nine *in vitro* subcultures for *Carica papaya* MSXJ hybrid, from DNA amplified with ISSR primers based on Nei's genetic distance.

genetic variability in the propagated material, it is recommended that no more than eight subcultures during micropropagation.

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# Effect of Irrigation Volume on Biomass and Nutritional Value of *Zea mays* L. as Green Hydroponic Forage

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## ABSTRACT

**Objective:** To evaluate the effect of irrigation water volume on biomass production and to quantify the nutritional value of green hydroponic maize forage (GHMF) for sheep.

**Design/Methodology/Approach:** Three treatments were used to evaluate water irrigation volume: T1) 9.48 L m<sup>2</sup> day<sup>-1</sup>; T2) 18.95 L m<sup>2</sup> day<sup>-1</sup>; and T3) 28.43 L m<sup>2</sup> day<sup>-1</sup>, employing a completely randomized statistical block design. To determine nutritional value, four inclusion levels of GHMF were used (0, 20, 40, 60% DM) in the diet of 16 sheep. A completely randomized statistical design was used as well as a linear regression model.

**Results:** A greater weight in fresh biomass of GHMF was observed with T3 (P<0.01). The values of apparent digestibility of DM, OM, CP, NDF, and ADF of GHMF oscillate between 80 and 89%. The estimated digestible energy was 3.9 megacalories/kg DM.

**Study Limitations/Implications:** Forage production in the dry tropics is characterized by being markedly seasonal; however, GHMF represents a viable alternative for the rapid and sustainable production of forage with high nutritional value for animals.

**Findings/Conclusions:** The greatest yields in fresh biomass of green hydroponic maize forage are obtained by using a greater volume of irrigation water. Likewise, the forage has high nutritional value for sheep, with considerable delivery of digestible energy, thus it can be used as an excellent source of forage in animal feeding.

**Keywords:** Forage, hydroponic, maize, biomass, digestibility.

## INTRODUCTION

Green hydroponic maize (*Zea mays* L.) forage (GHMF) is a technology for producing plant biomass from the growth of seedlings from viable seeds, cultivated under controlled environmental conditions (light, temperature, and humidity) in the absence of soil (FAO, 2002). It is a fast-paced (10 to 15 days) forage production system with high safety and nutritional quality, and it can be employed any time of year and in any geographic location, as long as the conditions for it are established (Juárez *et al.*, 2013). It has been shown to be an efficient production system because it saves and controls water usage and nutrients, and because of the minimum space requirements from cultivation in vertical modules, it optimizes usable space (Müller *et al.*, 2006; Salazar *et al.*, 2014; Zagal *et al.*, 2016).

Forage production in the dry tropics is characterized by being markedly seasonal, such that the highest production and the best quality are obtained during the rainy season (Muñoz *et al.*, 2016; Merlo *et al.*, 2017). This variability in forage quantity and quality throughout the year causes grazing animals to gain and lose weight (Castro *et al.*, 2017; Coleman *et al.*, 2018), which results in economic losses for farmers. Therefore, GHMF represents a viable alternative for rapid production of clean and sustainable plant biomass with nutritional quality for animal feeding. Some authors, such as Herrera *et al.* (2007) and Acosta *et al.* (2016), mention that it is a food with high protein and energy that can be used for grazing animals in substitution of concentrated feed. However, little is known about the production conditions and nutritional quality of hydroponic forage in the Mexican tropic. Based on the former, this study evaluated the effect of irrigation water volume on the production of biomass using green hydroponic maize forage for sheep, quantifying its nutritional value.

## MATERIALS AND METHODOLOGY

The study was conducted in Chiná, Campeche, Mexico (19° 44' N and 90° 26' W, at 15 m altitude). The climate is Aw subhumid tropical according to the Köppen classification modified by García (1973), with 1200 mm in annual precipitation distributed between June and November (Duch, 2002). The maximum, mean, and minimum temperatures are, respectively, 36, 26, and 18 °C. The photoperiod is less than 11 h in December and above 13 h in July (UNAM, 1991).

### Evaluation of Irrigation Water Volume

Forage production was carried out in 13 d cycles in a production module located within a greenhouse equipped with metal racks and tray holders, and completely protected by a bicolor, anti-aphid mesh. Maize seeds (*Zea mays* L.) were used with a 95% germination rate, free of impurities and agrochemicals. The seeds underwent a pre-germination stage, they were washed and disinfected with 3% NaClO for 20 min and soaked in water for 24 h. Later, they were placed in plastic trays measuring 60×37×7 cm, with a planting density of 45 kg m<sup>-2</sup>. For germination and to inhibit the development of fungi and phytopathogens, the trays were left for 48 h in a completely sealed dark chamber or area. Once the seeds germinated, the trays were fitted into the metal racks two levels high (top and bottom), with a distance of 0.50 cm between each level. Irrigation was carried out using an automated system equipped with sprayers and a potential output of 31.8 L h<sup>-1</sup>. To evaluate the volume of irrigation water, three treatments were used: T1) 9.48 L m<sup>2</sup> day<sup>-1</sup>; T2) 18.95 L m<sup>2</sup> day<sup>-1</sup>, and T3) 28.43 L m<sup>2</sup> day<sup>-1</sup>. Each treatment was applied in five waterings per day with a duration of two to three minutes and with two-hour intervals, from 9:00 a.m. to 5:00 p.m., for a period of 8 d starting on day four of plant development. Per treatment, 24 repetitions were carried out, where each tray represented one experimental unit. The plants were fertilized on days 7, 9, and 11 of the cycle in a single morning application, using a solution of 0.1 mg KNO<sub>3</sub>, 0.2 mg phosphonitrate (33-3-0), and 0.02 mL H<sub>3</sub>PO<sub>4</sub>/L water using a Venturi system. Starting on day five, daily recordings were made of the plant's root layer height, plant height (from the root neck), and fresh biomass weight (seeds, roots, and plants). The

plants were harvested on day 13 of the production cycle. A completely randomized statistical block design was applied (Montgomery, 2004), using the level of the tray holder in the rack as the block criterion, and the results were analyzed using a linear model with the Proc GLM procedure of the SAS statistical package (SAS Inst. Inc., 2003).

### Determining Nutritional Value of GHMF

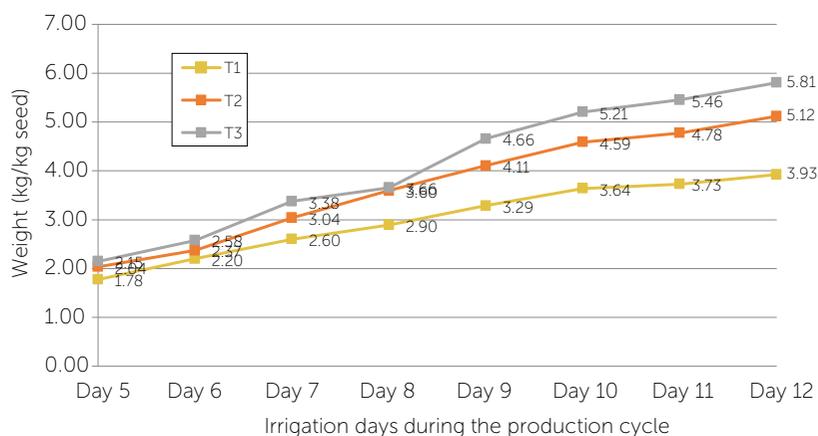
At the end of the production cycle (day 13) of the GHMF, samples of approximately 500 g were taken to determine its dry material content (DM), organic material content (OM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF), according to the procedures recommended by the AOAC (2016). The nutritional value of the GHMF was estimated by the *in vivo* digestibility technique (Rodríguez and Llamas, 1990) using 16 adult male Pelibuey sheep with an average live weight ± standard deviation (DE) of 35.2±4 kg. They were placed in individual metabolic cages made of wood and equipped with a feeder, water dispenser, and collector of feces and urine. The animals were assigned, through a completely randomized statistical design (Montgomery, 2004), four treatments that consisted of different GHMF inclusion levels (0, 20, 40 and 60% BS) in their diet based on commercial feed with 15% crude protein. Each treatment had four repetitions and each of these consisted of one animal in a metabolic cage. Before the start of the trial, the animals were treated for parasites internally (Ivermectin™) and given a 14 d period to adapt to the diets and cages. The commercial feed was provided first in the morning, and

the GHMF was provided after 12:00 p.m. A 7 d period of measurements followed, in which the total quantity of feces produced per day was recorded, as well as food and GHMF consumption, weighing daily the amounts offered and rejected. Once the total production of feces was determined, feces samples (10%) were collected as well as samples of feed and GHMF offered and rejected daily, in order to have compound samples at the end of the measurement period. These were preserved frozen at minus 20 °C until their DM, OM, CP, NDF, and ADF content was determined in a laboratory according to procedures described by the AOAC (2016). The apparent digestibility of the different diets was determined and a linear regression analysis was carried out with the different levels of GHMF inclusion in the diet. Based on the presented regression model, an equation was calculated for each of the variables studied to estimate the total apparent digestibility of the GHMF. The results were analyzed using a repeated measurements and linear regression model, using the Proc Mixed and Proc Reg procedures from the SAS statistical package (SAS Inst. Inc., 2003).

## RESULTS AND DISCUSSION

### Effect of Irrigation Water Volume

The irrigation water volume was observed to have a significant effect on the different evaluated variables (Table 1). The highest weight of fresh biomass (5.81 kg for each kg of maize seed) was obtained from the treatment with the most irrigation water volume (T3), higher by 1.9 and 0.7 kg of biomass for each kg of seed in comparison to T1 and T2, respectively ( $P < 0.01$ ). This greater production of fresh forage was linearly maintained during crop development, becoming more evident starting on day eight of the production cycle (Figure 1). High quantities of water and high watering frequencies have been reported to improve the agronomic characteristics



**Figure 1.** Effect of irrigation water volume on biomass production during the cycle of green hidroponic maize forage (GHMF). (T1=9.48 L/m<sup>2</sup> day<sup>-1</sup>; T2=18.95 L/m<sup>2</sup> day<sup>-1</sup>; T3=28.43 L/m<sup>2</sup> day<sup>-1</sup>).

of the maize plants (Jahanzad et al. 2013). Other authors, like Zagal et al. (2016), report lower total yields of GHMF (3.5 kg of biomass for every kg of seed) harvested at 13 d. This could be because fertilizer was not used in the crop and they used a conventional irrigation system, at a rate of one liter of water kg<sup>-1</sup> of maize every 24 h. Authors like Vargas et al. (2008) mention that with an irrigation and fertilization system similar to that used in this study, they observed GHMF yields of 4.3 kg of biomass per kg of seed at 10 d of cultivation, which explains in part the differences observed due to reduced time in the production cycle. The differences in the reports found can be attributed also to the quality and variety of the utilized seed, since these are the principal factors that affect maize forage production (Pérez et al., 2006; Salas et al., 2010). Greater root layer and plant height was detected in T2 and T3 compared to T1 ( $P < 0.01$ ).

No significant differences were found for these variables between T2 and T3 ( $P > 0.05$ ). The plants that were on the bottom rack level registered significantly greater ( $P < 0.01$ ) height (23, 85 cm) and fresh biomass weight (5.31 kg of biomass per kg of seed), compared to those that were located on the top level (19.70 cm and 4.6 kg of biomass per kg of seed for both variables,

**Table 1.** Effect of irrigation water volume on growth and biomass production of green hidroponic maize forage (GHMF).

Variable	Treatments			P Value	SEM
	T1	T2	T3		
Root layer height (cm)	4.437a	5.738b	5.925b	0.0001	0.573
Plant height (cm)	18.067a	22.583b	24.675b	0.0001	5.141
Total weight of fresh biomass (kg/kg seed)	3.930a	5.123b	5.809c	0.0001	0.636

Different letters in the same row indicate statistical difference ( $P \leq 0.01$ ).

SEM=Standart error of the mean. T1=9.48 L/m<sup>2</sup> día<sup>-1</sup>; T2=18.95 L/m<sup>2</sup> día<sup>-1</sup>; T3=28.43 L/m<sup>2</sup> día<sup>-1</sup>.



respectively). This is due to the fact that the bottom rack level has less sun exposure, which causes more competition and stimulates the vertical development of the plant by effect of cell wall elongation (Montemayor *et al.*, 2006; Lambers *et al.*, 2008), thus increasing biomass production.

**Nutritional Value of GHMF**

The linear regression equations obtained in order to estimate the apparent digestibility of the different GHMF components are shown in Table 2. The slopes of the straight line for DM, OM, and CP had negative values, that is, for each 1% increment of GHMF in the diet, digestibility decreased 0.03, 0.03 and 0.04% for these components, respectively. In contrast, an increase of 0.05% in the digestibility of NDF and ADF was observed for each unit of change in the ration’s GHMF level. Correlation coefficients above  $r=0.50$  were observed in the majority of the components evaluated, with the exception of CP digestibility, which had  $r=0.48$ , considered to be moderate ( $P>0.05$ ). The residual values (RMS) ranged between 2 and 5.7%, which indicated low data variability.

The estimated digestibility percentages of GHMF (Table 3) showed 89% digestibility for DM and OM, while CP, NDF and ADF had percentages above 80%. Other authors (Herrera *et al.*, 2007), using *in vivo* digestibility samples in sheep, observed digestibility values for the DM of GHMF of 56%, which is below that reported in this study. However, Acosta *et al.* (2016), using the same method in goats, reported very similar digestibility for DM (93%), OM (85%), and CP (80%) of the GHMF compared to those in this study. It is important to note that the low digestibility percentages of DM obtained by Herrera *et al.* (2007) could be due to the experimental conditions in which the study was conducted and to the characteristics of the plant material used. The digestibility values for NDF and ADF were above those obtained with other conventional forages (Naranjo and Cuartas, 2011; Coblenz *et al.*, 2019). This could be because of the physical characteristics of the GHMF, since it is a seedling composed mainly of young leaves with more digestible cell walls. Based on the digestibility of DM, the digestible energy of the GHMF was estimated (NRC, 1984), resulting in a value of 3.9 megacalories  $kg^{-1}$  of DM, this being very similar to that reported for maize grain (NRC, 1985).

**Table 2.** Regression equations for estimating the apparent digestibility of green hidroponic maize forage (GHMF).

Component	Regression equations	r	RMS
Dry matter	DDM=92.144-0.0315*X	0.53	2.56
Organic matter	DOM=91.729-0.0292*X	0.54	2.06
Crude protein	DCP=83.074-0.0413*X	0.48	6.33
Neutral detergent fiber	DNDF=78.5057+0.0549*X	0.59	5.41
Acid detergent fiber	DADF=77.6158+0.0464*X	0.54	5.75

RMS=Residual mean square.

**CONCLUSIONS**

The highest yields of fresh biomass in green hidroponic maize forage are obtained by using greater volumes of irrigation water. The forage shows highly nutritional values for sheep, with a considerable availability of digestible energy, thus it can be used as a source of quality forage in animal feeding.

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**Table 3.** Estimated apparent digestibility values of green hidroponic maize forage (GHMF).

Component	Digestibility (%)
Dry matter	88.99
Organic matter	88.81
Crude protein	79.94
Neutral detergente fiber	83.99
Acid detergent fiber	82.25
Energy (Mcal/kg DM) *	3.91

\*Estimated based on the NRC (1984): Digestibility=4.4 (Dry matter digestibility %)/ 100. Mcal=Megacalories.

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# Vermicompost and Mycorrhizae Use on *Cedrela odorata* L. Growth in Nursery Conditions

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## ABSTRACT

**Objective:** To evaluate the effect of mycorrhizal inoculation and the use of vermicompost on the growth of red cedar (*Cedrela odorata* L.) seedlings under nursery conditions.

**Materials and Methods:** The treatments were with and without a *Glomus intraradices* inoculum, combined with different amounts of vermicompost in the growth substrate (0, 10, 20 and 30%), in a completely randomized block design. The variables evaluated were plant height (PH), stem diameter (SD), number of leaves (NL), dry weight (DW), mycorrhizal colonization (MC) and number of spores (NS).

**Results:** The addition of vermicompost to the growth substrate improved the growth of *C. odorata* seedlings, the best treatment being 30% of vermicompost without mycorrhizae. The percentage of mycorrhizal colonization and number of spores per 100 g of soil decreased when increasing the amount of vermicompost in the substrate.

**Study Limitations/Implications:** None.

**Findings/Conclusions:** Vermicompost was the leading promoter of *C. odorata* plant growth under nursery conditions.

**Keywords:** substrate, arbuscular mycorrhizal fungi, colonization.

## INTRODUCTION

The quality of the substrate used in nurseries is one of the factors that influence the growth of plants, which means it is important to have organic and microbiological alternatives for the sustainable production of *Cedrela odorata* plants (Oros-Ortega *et al.*, 2015). Vermicompost is characterized by being made up of finely divided, highly porous, aerated, drainable, moisture retaining materials. These materials have reduced quantities of soluble salts, higher cation exchange capacity (CEC) and a growing number of total humic acids. It is an organic substrate with high content of nitrogen, potassium, phosphorus, magnesium and micronutrients (Ruiz, 2011); it contains biologically active substances that regulate vegetative growth (Atiyeh *et al.*, 2000c).





In addition, vermicompost has a large surface area, which permits it to adsorb and strongly retain the nutritional elements, which are easily assimilable by plants (Atiyeh *et al.*, 2000a; Atiyeh *et al.*, 2000c), besides having a high capacity to be mixed with the soil in order to slowly liberate nutritional elements and accelerate plant growth (Capistran *et al.*, 1999).

Another important biological element to be taken into account in the substrate is the use of arbuscular mycorrhizal fungi (AMF) which has become a viable alternative in order to reduce losses in multiplication, acclimatization and adaptation processes of different plant species in diverse agro ecological conditions.

Due to the low quality of *C. odorata* seedlings grown in nurseries, the use of AMF has been proposed, which may improve development and growth of seedlings during their first months of life, considering that some species of the *Cedrela* genus can potentially form mycorrhizae with *Glomus* and *Acualospora* genera (Souza *et al.*, 2006). The symbiosis between arbuscular mycorrhizal fungi and plants plays an important role in the adaptation of plants in terrestrial ecosystems (Honrubia, 2009). One of the benefits of this symbiosis is related to plant nutrition in environments with nutrient restrictions, where the plant supplies carbohydrates to the fungi for its metabolism and the fungus aids in intake and transport of the nutrients the plant requires (Smith, 2013). The symbiosis favors the plant's tolerance to biotic and abiotic stress, improves the physical characteristics of the soil, and favors the diversification of plant species in ecosystems (Smith and Read, 2008).

*C. odorata* seedlings with high mycorrhizal colonization have been obtained when the seedlings were inoculated with spores native to high evergreen and medium sub-evergreen forests (Méndez-Cortés *et al.*, 2013). A positive effect has also been observed with the inoculation of *Glomus* spp. in terms of plant height, stem diameter and number of leaves 30, 60 and 90 days after sowing in Antillean mahogany (*Swietenia micorrmahagoni* (L.) Jacq.) in nurseries (Bango-Folgoso *et al.*, 2013).

Similar results were found for plant height, stem diameter and number of leaves by Falcon-Oconor *et al.* (2013) in *Swietenia mahagoni* and *Lisyloma latisiliquum* inoculated with *Glomus hoi* like and *Glomus intraradices*. When Oros-Ortega *et al.* (2015) evaluated *C. odorata* seedlings inoculated with *Rhizophagus intraradices* in nurseries,

they found larger and more vigorous plants in terms of plant height, stem diameter, root dry weight, plant dry weight, and mycorrhizal colonization of up to 50%.

Similar results were found by Méndez-Cortés *et al.* (2013) in *C. odorata*, obtaining greater height, diameter, number of folioles, aerial and radicular dry biomass, number of spores and colonization percentage (50 to 80%). These results make sense with that cited by Moína-Quimí *et al.* (2018) who determined that the percentage of colonization of tropical fungi species is higher than 50%.

Accordingly, and in the interest of generating knowledge about the use of vermicompost and the application of mycorrhizal fungi, the effect of vermicompost and mycorrhization of the roots of *C. odorata* on the growth of seedlings under nursery conditions, was evaluated.

## MATERIALS AND METHODS

The study was carried out in nurseries in Campeche, Mexico. The predominant climate is warm humid, with summer rains (Awo) according to the Köppen climate classification modified by García (2004), with an average annual precipitation of 1290 mm and mean temperature of 22 °C.

For this study *C. odorata* seeds were used which were obtained from fruits collected from trees in Campeche communities, making sure to use intact unopened capsules without any insect or fungal damage. The fruits (capsule) were dried by solar dehydration for 8 h during 4 d, afterwards they were disinfected with 5% sodium hypochlorite during 7 min. Then they were rinsed with sterile water and stored in glass jars in a dry and cool place. In order to obtain seedlings, seeds were germinated in plastic trays with 72 cavities previously washed and sterilized, using Peat-Moss<sup>®</sup> as substrate, placing one seed per cavity at a depth of 2 cm. Once the seedlings had been in the trays for 95 days after sowing (DAS), they were transplanted to black polyethylene bags with 2.1 kg capacity using sterilized black forest soil with methyl bromide as substrate. The vermicompost used as part of the study's treatment was sterilized with a 100 mg dose of Basamid<sup>®</sup> on a surface of 1 m<sup>2</sup> per 30 cm of depth; afterwards it was covered with polyethylene plastic during seven days, and then aerated for three days. When the seedlings were transplanted, they were inoculated with the mycorrhizal fungus *Glomus intraradices* at a rate of 10 g per plant. The colonization percentages of

roots used as inoculum for *G. intrarradices* were 90% colonization with 1200 spores per 100 g of soil. The experimental design was a randomized block design with eight treatments and 40 repetitions per treatment, considering one plant per bag as an experimental unit. The treatments were 0-0, 10-0, 20-0, 30-0, 0-M, 10-M, 20-0 and 30-0, considering the first number as the percentage of vermicompost in the substrate and the second as the inoculation with arbuscular mycorrhizal fungi (AMF) *G. intrarradices*.

The experimental unit consisted of 40 plants lined up at a distance of 1.5 m between lines and 40 cm between plants. The study variables were plant height (PH), stem diameter (SD), number of leaves (NL), leaf area (LA), mycorrhizal colonization (MC), and number of spores (NS). Measurements were taken every 30 d, with a total of five measurements. The quantification of dry weight was calculated by placing leaves, stems and roots into brown paper bags which were then dried in an oven at 80 °C during 48 hours. In order to determine the presence of the mycorrhizal fungus and to quantify colonization, a technique was used to color the fungal structures in the root and then to count the infection by observing through a microscope using the Phillips and Hayman method (1970). The infection percentage was determined based on the total segments colonized by the fungus and the segments that had arbuscules and vesicles, depending on the case. The infection percentage was calculated using the following equation:

$$\% \text{infection} = \frac{\text{No. of colonized segments}}{\text{No. of observed segments}} \times 100$$

Separating and counting spores was carried out using the Nicolson (1963) method, mentioned by Ferrera-Cerrato and González-Chávez (1993) as a sieving and decanting method, which requires the observation and evaluation of spores through a stereoscopic microscope or, sometimes, an optic microscope. The number of spores was obtained by counting the amount found on filter paper with the use of a stereoscopic microscope. The result was expressed in number of spores per 100 g of dry soil using the following formula:

$$\text{Spores in 100 g of dry soil} = \frac{(\text{No. of counted spores})(\text{grams of dry soil})}{100 \text{ g humid soil}} (100)$$

The data obtained was subjected to a variance analysis and Tukey's multiple comparison of means test (Tukey,  $\alpha=0.05$ ) using the statistics program SAS (Statistical Analysis System Institute, 2004).

## RESULTS AND DISCUSSION

### Plant Height and Stem Diameter

The results showed a greater effect with vermicompost in substrate than those inoculated with AMF. At a higher concentration of vermicompost in the substrate, the plant's height and diameter were greater, showing significant differences between treatments during the dates of measurement (Table 1).

The greatest height (28.65 cm) was obtained at 150 days after transplant (DAT) using the substrate with 30% vermicompost without AMF inoculation, while the best inoculation treatment was with AMF and 10% vermicompost (22.61 cm). These results indicate the feasibility of the use of vermicompost in the production of nursery seedlings which contribute to the development of *C. odorata* plants.

The effect of the AMF was not observed in the growth of the seedlings due to the substrate enriched with vermicompost, which made nutritional elements available through this organic component, as is the case with phosphorus that reduces the capacity of AMF for the extraction and transport of available chemical elements to the plant. With respect to mycorrhizal inoculation the results are not in accordance with what was found in previous studies carried out with *C. odorata* where mycorrhizal inoculation favored an increase in plants' height and diameter (Falcon-Omar et al., 2013; Bango-Folgoso et al., 2013; Oros-Ortega et al., 2015). However, the results obtained by Moreno-Reséndez et al. (2014) with *Acacia pennatula* (Schlttdl. and Cham.) Benth. determined that the use of 10% vermicompost and 90% natural soil obtained the best results at 240 days after transplant (DAT). It is also important to consider that the results obtained could be due to factors such as the type of mycorrhizal fungus, the amount of inoculum, the organic material content in the substrate, as well as the plant species being studied.

In terms of the number of leaves, the results showed a similar trend to those observed for height and

**Table 1.** Height and diameter stem per plant average of *Cedrela odorata* with different levels of vermicompost and mycorrhizal fungi.

Treatment	Height plant (cm)		Stem diameter (cm)	
Days after trasplant	90	150	60	150
00	9.96 c	10.71 f	0.70 c	1.08 d
10-0	12.40 b	18.98 d	0.77 abc	1.18 c
20-0	13.46 a	25.51 b	0.92 ab	1.25 b
30-0	13.98 a	28.65 a	0.94 a	1.30 a
0M	13.43 a	14.52 e	0.75 abc	1.24 b
10M	12.18 b	22.61 c	0.74 abc	1.31 a
20M	10.61 c	11.93 f	0.71 c	1.09 d
30M	10.61c	11.65 f	0.73 bc	1.09 d
MSD	0.73	1.30	0.20	0.03

Treatments with the same letter are statistically the same (Tukey, 0.05). MSD=Minimum significant difference. Results of two samplings of the five carried out.

stem diameter, where the higher number of leaves was due to the use of vermicompost in the substrate and not because of mycorrhizal inoculation (Figure 1).

The effect of vermicompost on the growth of *C. odorata* plants was attributed to higher availability of nutrients in the substrate, the improvement of its physical properties, and higher moisture availability (Costa and Martin, 2018). A higher number of leaves was observed with the 30V-M treatment (66.75 leaves), and a similar trend was found with the 10V-M treatment (56.57 leaves).

The number of leaves obtained (66.07) at 90 d with *Glomus intraradices* inoculation without the use of vermicompost is similar to that found by Bango-Folgozo *et al.* (2013) in mahogany plants (*Swietenia mahagoni*) who obtained 9.03 leaves on average per plant with an inoculation with the *Glomus* genus. On the other hand, Méndez-Cortés *et al.* (2013) found that during the initial development of *C. odorata* there were plants with a higher number of leaves using different sources of mycorrhizal inoculum, with the results varying depending on the type of soil and the relation with the organic matter present.

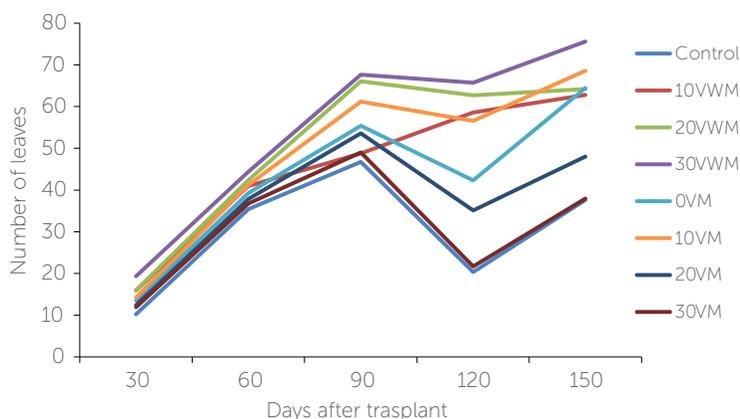
The dry weight increased with 30% vermicompost application without AMF inoculation, with each section of the plants weighing 4.26 g (leaves), 10.94 g (stem) and 14.93 g (roots). In terms of the mycorrhizal inoculation treatments, 0V-M and 10V-M were the most noteworthy. All of the treatments

with vermicompost with or without mycorrhizal inoculation exceeded the control treatment in terms of weight of leaves, stem and root.

The results suggest that the addition of vermicompost improved the availability of nutrients and improved the physical properties of the substrate, which could be interpreted as improved photosynthesis activity in the plant. This confirms an overall improved plant quality for future establishment in the field, which agrees to that established by Costa and Martín (2018). Although the mycorrhizal effect was overshadowed by the use of vermicompost, it is important to consider its subsequent effect for the survival of *C. odorata* plants when they are taken into the field where agro-climatic conditions could be more limited. In the field it is important to evaluate the relationship of foliage weight and dry root weight, in which the best treatments were those inoculated with *G. intraradices* based on the results in Table 2. Those in the nursery business prefer plants that are not too big and with abundant roots; roots with sufficient anchoring capacity, but especially the capacity to explore and aid in the extraction of the field's nutrients.

### Mycorrhizal Colonization and Number of Spores

The percentage of mycorrhizal colonization (56.6%) and the number of spores per 100 g of soil (51.40) were higher without vermicompost in the substrate, and decreased as the amount of organic component increased (Figure 2) to values of 50.8% 42.8%, 32.2% for 10, 20 and 30% of vermicompost, respectively. A similar trend was seen with the number of spores per 100 g of soil with values



**Figure 1.** Growth kinetics average per plant of *Cedrella odorata* by vermicompost and mycorrhizal inoculation use (*Glomus intraradices*). 10VWM: 10 g of vermicompost without mycorrhiza, 20VWM: 20 g of vermicompost without mycorrhiza, 30VWM: 30 g of vermicompost without mycorrhiza, 0VM: 0 g of vermicompost with mycorrhiza, 10VM: 10 g of vermicompost with mycorrhiza, 20VM: 20 g of vermicompost with mycorrhiza, 30VM: 30 g of vermicompost with mycorrhiza.

**Table 2.** Dry weight average of leaves, stem, and root average of *Cedrela odorata* plants growing at different levels of vermicompost and inoculation with *Glomus intraradices* as substrate.

Treatment	Dry weight (g)			DWL / DWR
	Leaf	stem	root	
00	0.81 c	3.69 c	4.50 d	1.00
10-0	1.56 bc	7.61 bc	7.61 bcd	1.20
20-0	3.07 ab	10.04 ab	10.95 a	1.20
30-0	4.26 a	10.94 ab	14.93 a	1.02
0M	1.88 bc	12.73 a	10.05 ab	1.45
10M	2.80 abc	10.85 ab	14.04 a	0.97
20M	1.24 bc	5.25 c	9.13 bc	0.71
30M	0.82 c	4.01 c	5.68 cd	0.85
MSD	2.14	3.98	3.50	

Treatments with the same letter are statistically the same (Tukey, 0.05). MSD=Minimum significant difference.

at 44.20, 39.40 and 29.60 spores per 100 g of soil. The results are attributed to the enriched organic substrate which reduced the functionality of the AMF.

The value found for mycorrhizal colonization without the use of vermicompost was 55.60%, within the values found by Oros-Ortega *et al.* (2015) at 50%, Moína-Quimí *et al.* (2018) at 50%, and Méndez-Cortés *et al.* (2013) from 50 to 80%. Authors such as Oros-Ortega *et al.* (2015), when working with red cedar, found that inoculation produced high levels of mycorrhization in roots by the sixth month. Moreno-Resendez *et al.* (2014) considered that timely inoculation occurs in the early stages of plant growth and increases as new roots are formed. The number of spores found was good compared to those reported on mahogany grown in nurseries by Guetra-González (2009) at 12 for *Caulospora* spp., 3 for *Glomus* spp., and 6 for *Entropospora* spp.

In their study using native mycorrhizal fungal species, Mendes-Cortez *et al.* (2013) found an average number of 30.38 spores per 100 g of soil, amount lower than that found in the present study with *Glomus* spp. of 51.40 100 g<sup>-1</sup> of soil.

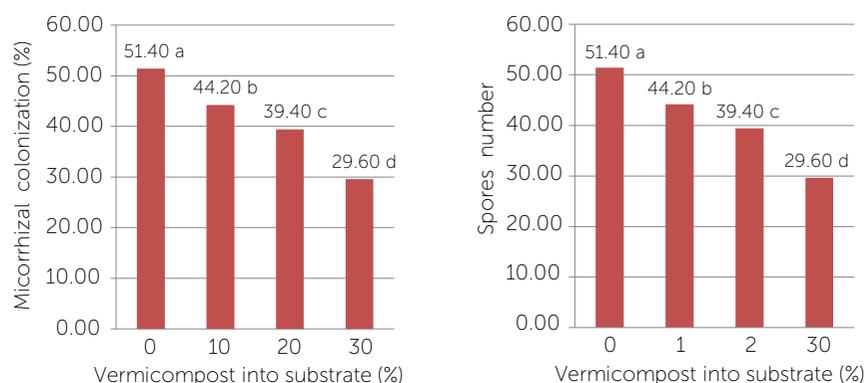
## CONCLUSIONS

The use of organic fertilizers could be a sustainable alternative for the production of red cedar (*C. odorata*) in nurseries. The use of vermicompost at a 30% dose as

part of the substrate promoted a more vigorous growth in the plants. The effect of biofertilizer with arbuscular mycorrhizal fungi (*Glomus intraradices*) favored better root development, which is a favorable effect in field conditions.

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**Figure 2.** Mycorrhizal colonization (left) and number of spores (right) percentage at different levels of vermicompost into the growth substrate.

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# Sustainability of Four Agroecosystems in the State of Veracruz, Mexico

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## ABSTRACT

**Objective:** To evaluate the sustainability of four agroecosystems in the state of Veracruz, Mexico: sugarcane, maize grain, orange and moringa.

**Design/Methodology/Approach:** Producers that provided information about the crops' management were located. Semi-structured interviews were conducted to identify critical points affecting sustainability, and the indicators were weighted using the bottom-up criterion and the PSR model.

**Results:** The most sustainable crop was moringa, and the least sustainable was maize for grain. Conventional crops are characterized by being monoculture plantations and demanding large amounts of non-renewable external inputs that undermine their sustainability. Lack of technical training for producers was identified as a critical point.

**Study Limitations/Implications:** The results obtained are limited to the analysis of four production systems and their environmental, social and economic dimensions.

**Findings/Conclusions:** Moringa is presented as an alternative crop with low environmental impact that generates employment and strengthens social capital.

**Keywords:** moringa, sustainable agriculture, sustainability indicators.

## INTRODUCTION

Current agricultural practices of crop management under the principles of the Green Revolution favor high production in the short term; however, they have generated an environmental cost that compromises the long term (Gliessman, 2003). As a result, natural resources have been degraded, global ecological processes have been altered, the social conditions for their preservation have been weakened and in extreme cases they have been dismantled (Gliessman, 2003).

In conventional crops in the state of Veracruz, Mexico, these principles have traditionally been applied in terms of management and technology. Therefore, it is necessary to implement more sustainable forms of agriculture based on



a better understanding of ecological processes. Likewise, the modification of consumption patterns in favor of the long-term preservation of soil productivity, and the promotion of greater equity that favors each link in the agrifood chain, from the farmer to the final consumer (Gliessman, 2013) (Gliessman, Engles, & Krieger, 1998).

Sustainable agriculture is characterized by long-term sustainability, with the ability to permanently harvest biomass from an agricultural system that is capable of renewing itself without putting itself at risk (Masera, Astier, & López-Ridaura, 2000).

Assessing agricultural sustainability involves recognizing that the pressure to increase yields conflicts with the long-term requirements of sustainable development (Abbona, Sarandón, & Marasas, 2006). Conventionally, agricultural systems have been evaluated by means of cost-benefit analysis to determine their economic profitability; however, this approach is not functional in the long term because it sidesteps the social and environmental dimensions that are becoming increasingly relevant (Sarandón *et al.*, 2006).

In contrast to the conventional cost-benefit analysis, Sarandón (2002) considers that a sustainable agroecosystem must meet four requirements, it should be: 1) sufficiently productive, 2) ecologically sound (conserving the natural resource base and preserving the integrity of the environment at the local, regional and global levels), 3) economically viable, and 4) culturally and socially acceptable.

The objective of this research was to evaluate the sustainability levels of four agroecosystems in the state of Veracruz, Mexico, the three conventional agroecosystems with highest economic value and *Moringa oleifera* Lam. (moringa), a recently introduced crop for which there are still no records in the state,

through the use of indicators for the characterization of sustainability and identification of critical points that affect agroecosystems according to Sarandón and Flores (2009).

## MATERIALS AND METHODS

The study was conducted in the state of Veracruz, Mexico (22° 28' N, 17° 09' S, 93° 36' E, 98° 39' W), and with an altitude from zero to 5,610 m, (SEDECOP, 2020). The average annual precipitation is 1500 mm, and the mean annual temperature is 23 °C (INAFED, 2020). The research included the following stages:

1. The three crops of highest economic value in the state of Veracruz were identified. The most recent economic value was obtained from the state's System of Agrifood and Fishery Information (Sistema de Información Agroalimentaria y Pesquera, SIAP, 2018), where sugarcane (*Saccharum* spp.), maize grain (*Zea mays* L.) and orange (*Citrus sinensis* L.) were identified as the three crops with the highest commercial value.
2. Characterization of the agroecological spatial scope. Nineteen cooperating production sites were located to provide information on crop management through semi-structured interviews (Table 1). The information obtained was organized in a database for tabulation and evaluation.
3. Selection of indicators and evaluation. To identify the critical points affecting sustainability, indicators were selected based on the bottom-up criterion (Astier, Mazera, & Galván-Miyoshi, 2008) and the PSR (Pressure, State, Response) model, which explains the cause and effect of management practices on agroecosystem components (Abbona *et al.*, 2006) applied by Sarandón and Flores (Sarandón & Flores, 2009).

**Table 1.** Characteristics of the crops and average data of the producers.

N ° producers	Culture	Floor	Weather	Average extension (ha)	Average production value (Mx)	Average age	Average schooling (years)
6	Sugar cane	Phaeozem, andosol	Warm sub humid, temperate humid	1.00	50.165	44.6	8.67
6	Grain corn	Phaeozem	Warm sub humid	33.33	292.25	56.8	8
3	Moringa	Vertisol, leptosol y phaeozem	Warm sub humid	0.50	45.00	47.5	17
4	Orange	Phaeozem	Warm sub humid	20.00	553.85	44.25	10.2

MX: total production value of producers in thousands of Mexican pesos.

In this context, the pressure indicators are constituted by crop diversification, yield and commercialization; status indicators include cultivated area, availability of workforce and access to basic services; finally, the response indicators included soil preparation, fertilization, and pest, disease and weed control. The indicators were ordered according to their environmental, economic, and social performance (Table 2).

In order to compare the four agroecosystems and ease the evaluation of the three dimensions of sustainability, the indicators were standardized and weighted. A scale of zero to four was created for standardization purposes, where four represents the value closest to sustainability and zero the value furthest from it. The weighting factor depends on the importance of the indicator for the system's operation, ranging from one to two. Indicators that contribute the most to sustainability were assigned a value of two, and those that contribute the least were assigned a value of one. The weighting value was determined by the product value of standardization\* weighting factor. The weighting factor was estimated as the average of the responses by producers associated with each crop.

The overall sustainability of the agrosystems was obtained by averaging the values obtained for the three dimensions as shown in the formulas (Table 3). The value of each indicator is a quotient whose numerator is the weighted sum of indicators and sub-indicators considered, and the denominator is the number of variables considering their weighting (Sarandón & Flores, 2009).

Representation of Indicators. The results obtained from Tables 2 and 3 were represented graphically in order to identify the critical points of each agroecosystem. The critical points refer to the management of the

agroecosystem as a variable of improvement or deterioration of sustainability.

## RESULTS AND DISCUSSION

Figure 1 shows the three crops of highest economic value in Veracruz according to their value: industrial sugarcane (\$50,165), orange (\$27,693), and white maize grain (\$8,768). Industrial sugarcane places Veracruz as the national leader with a yield of 69.85 t ha<sup>-1</sup>. The state's harvest in the 2018 cycle made possible a sales flow of 15 billion 249 million pesos with a yield of 73.11 t ha<sup>-1</sup> (SIAP, 2019). Regarding orange, Veracruz is also the national leader with an indicator of 14.87 t ha<sup>-1</sup>, and a yield above the national average of 13.95 t ha<sup>-1</sup> (SIAP, 2019).

White grain maize is a crop of high economic and social importance, due to the surface sown, the value of production, and from occupying 20% of the economically active population (INIFAP, 2017). On the national scale Veracruz ranks eighth in production, with yields of 2.25 t ha<sup>-1</sup> (SIAP, 2018), lower than the national mean which is 3.2 t ha<sup>-1</sup> (AgroDer, 2012).

### Characterization of the Agroecological Spatial Environment

**Social Dimension.** Moringa producers had the highest

**Table 3.** Mathematical formulas used to estimate sustainability by dimensions and in general of Veracruz crops.

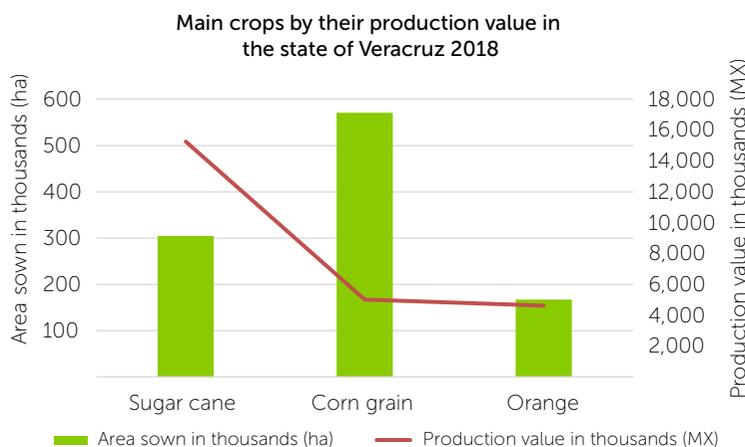
Dimensions	Model
Environmental	$ES = ((a1+2a2+a3+(2(b1+b2+b3+b4))/4))/7$
Economical	$ES = (2(c1+c2)/2)+d1+d2+d3/5$
Social	$SS = (f1+(2(f2+f3))/2)+g1+h1+i1/6$
General	$GS = (SA+SK+SS)/3$

ES: Environmental sustainability; ES: Economic Sustainability; SS: Social Sustainability; GS: General Sustainability.

**Table 2.** Sustainability indicators for four Veracruz agroecosystems selected under the Bottom-up criterion and the PER model proposed by Sarandón and Flores (2009).

CV	Environmental	WF	KI	Economical	WF	KI	Social	WF
a1	Crop diversification	1	c1	Value (\$/t)	1	f1	Scholarship	1
a2	Land preparation	2	c2	Yield (t/ha)	2	f2	Access to education	2
a3	Tillage type	1	d1	Commercialization	1	f3	Basic services	2
b1	Chemical fertilization	2	d2	External origin of inputs	1	g1	Training	1
b2	Control of pests and diseases	2	d3	Harvest destination	1	h1	Family involvement	1
b3	Weed control (chemical pesticide)	2				i1	Form of organization	1
b4	Packaging destination	2						

KI: Key of the indicator, WF: Weighting Factor.



**Figure 1.** Conventional crops with higher production value in the state of Veracruz (SIAP, 2018).

level of schooling and training (Figure 2A), sugarcane and orange growers have the greatest access to basic services, while maize growers do not stand out in any area; it should be noted that most producers, except for moringa growers, have little or no training. In this sense, education and participation is an important aspect for social sustainability, since it is built from a local vision and requires the cooperation of the whole society (Masera *et al.*, 2000).

**Economic dimension.** The highest unitary value per ton of biomass was for moringa followed by orange. The highest yield per ha is for sugarcane and orange followed by maize grain (Figure 2B). On the other hand, crops are highly dependent on non-renewable external resources, an aspect which makes them less sustainable. However, moringa has greater control over its value chain by transforming the biomass into finished product for delivery to the final customer, unlike sugarcane and maize grain agroecosystems that market their product without added value. On the other hand, when comparing the

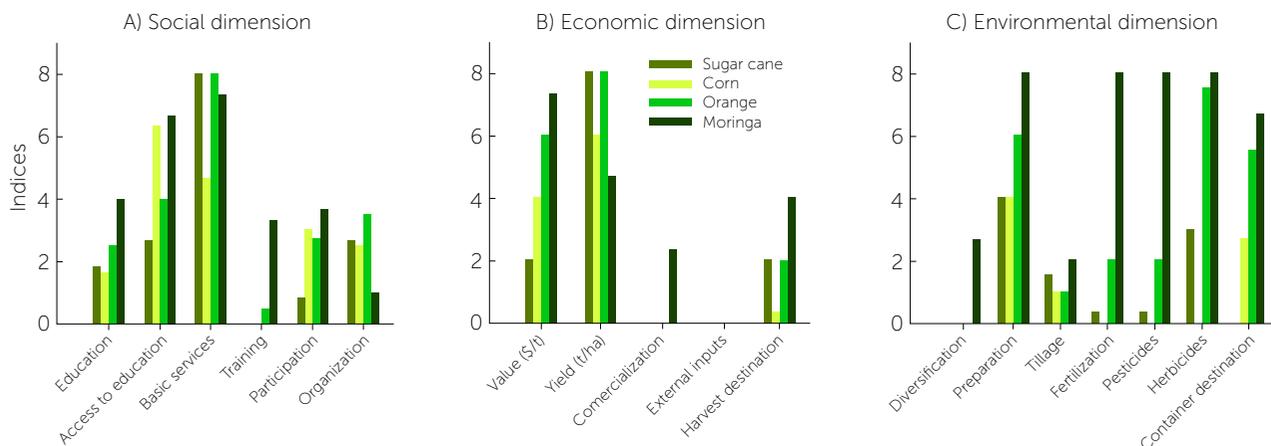
prices of traditional crops with respect to moringa, the low selling prices of sugarcane contrast with the high selling prices of moringa that allow very attractive yields to be obtained (Valdés-Rodríguez *et al.*, 2014). In this sense, moringa presents an alternative for the primary sector as a high-value economic activity that promotes the reduction of working poverty.

**Environmental dimension.** Moringa shows high productive diversification; however, the other agroecosystems show a strong preference for monoculture, with an absence of diversification (Figure 2C). Fertilization, pest and disease control, and agrochemical container management are high impact actions that drive sugarcane, maize grain, and orange agroecosystems away from sustainability (Inés *et al.*, 2013). This prolonged use of conventional practices implies greater dependence on external inputs. At the same time, intensive farming and monoculture degrade the soil, which increases dependence on fertilizers (Gliessman, 2003).

In contrast, moringa showed greater orientation towards sustainability, being a perennial tree that increases productive diversification, where biofertilizers and bioinsecticides are applied, as the leaves are the harvest product and for human consumption (Mota-Fernández *et al.*, 2019). In addition, moringa does not require high application of agricultural inputs, compared to the high input demand of sugarcane, maize and citrus crops (Mrini *et al.*, 2001).

**Evaluation of Crop Sustainability**

The results obtained from the evaluation of the indicators are presented in Figure 3. According to the findings on



**Figure 2.** Indices of environmental sustainability of the four crops evaluated.

Critical points and sustainability of agroecosystems

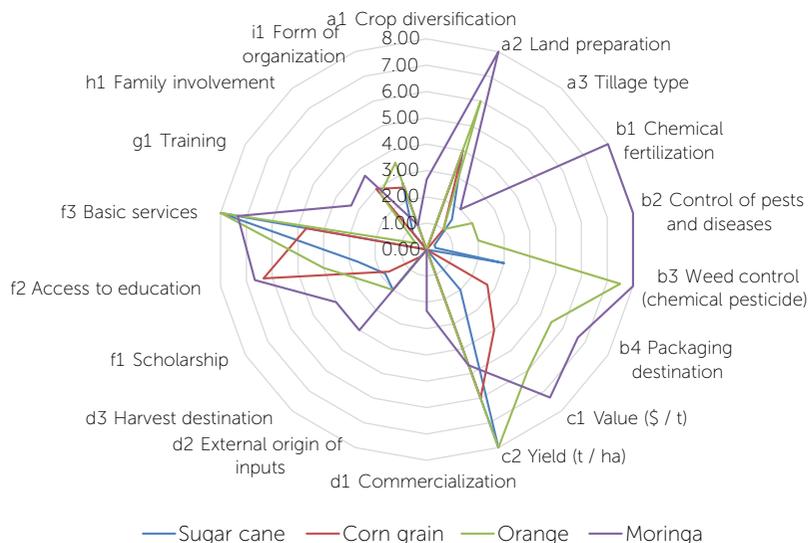


Figure 3. General sustainability index of the evaluated crops.

sustainability, the following critical points were identified that put it at risk:

**Environmental dimension.** Sugarcane, maize and orange crops generate practices with high negative impact on resources: land, water, air and soil. The presence of pests is recurrent due to monoculture. There is an excessive application of agrochemicals with negative impact on biodiversity, soil and water. The pressure they exert on natural resources causes them to move away from the best sustainable practices (Gómez-Limón & Arriaza Balmón, 2011).

**Economic dimension.** External dependence on non-renewable inputs was high in all four crops. In addition, however, sugarcane and maize grain lack a transformation process of fresh product that allows for added value; and there is a high degree of intermediaries. The economic sustainability of crops is associated with the benefits perceived by producers in the long term; it is important not to associate it with monetary retribution, but with management motivations and decision-making capacity to manage local resources efficiently (Meza & Julca, 2015).

**Social dimension.** For sugarcane, maize and orange producers, there was a lack of training and organization of producers, lack of standardization of production processes, and low educational level of producers. In order to aspire to social sustainability, there must be a starting point, such

as the measurement of the social dimension that seeks to evaluate the quality of life, social integration and producer satisfaction (Sarandón et al., 2006).

**General Sustainability**

The overall evaluation shows that moringa stands out in each of the dimensions by obtaining the highest sustainability value, followed by oranges, while the farthest crop was maize (Figure 4).

**CONCLUSIONS**

According to the Sarandón and Flores (2009) model, moringa had the highest level of sustainability because of its low environmental impact due to the minimal use of agrochemicals, good solid waste management, high market value per ton processed, producer training, and community integration. White maize had the lowest level of sustainability due to its high environmental impact and loss of biodiversity, followed by sugarcane and orange.

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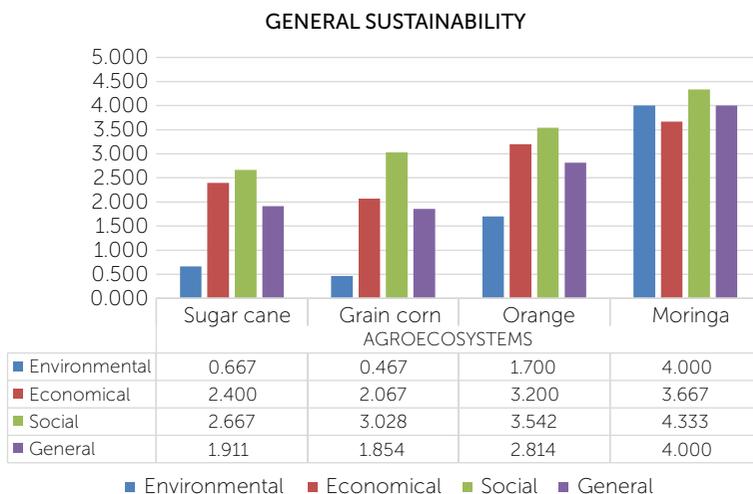


Figure 4. General sustainability of the evaluated crops.

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# Socioeconomic and competitive positioning of livestock chains in Zacatecas, Mexico

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## ABSTRACT

**Objective:** To determine the positioning of the most important livestock production chains in Zacatecas, Mexico, within a matrix of socioeconomic importance and market competitiveness, and to identify research and technology transfer needs.

**Design/Methodology/Approach:** Arranging livestock production chains into a hierarchy was carried out based on the methodology proposed by the International Service for National Agricultural Research (ISNAR), taking as analysis axis the dimensions of socioeconomic importance and competitiveness. The six most outstanding livestock production chains in the state were selected.

**Results:** The results placed the beef cattle chains as sustainable, the goat and sheep chains were placed as vulnerable, pork and honey were located in the retraction quadrant due to their low socioeconomic importance and competitiveness, and dairy cattle was placed in the strained quadrant.

**Study Limitations/Implications:** It is important to take into account other links.

**Findings/Conclusions:** Strategies for livestock production chains involve making the production more efficient through sustainable practices, providing technical assistance to producers, conducting research to generate technology, investing in infrastructure, and generating products with added value that meet the needs of consumers.

**Keywords:** Production chains, indicators, technologies.

## INTRODUCTION

Zacatecas, Mexico, is considered a potentially rich state for livestock production, as it has more than 5 million hectares dedicated to grazing, with soils conducive to the development of good quality pastures and native grasses (Poaceae) of high forage value (Sánchez *et al.*, 2015). The livestock sector in the state of Zacatecas generated a production value equal to 5.6 million pesos during 2017, within which beef cattle production accounted for 56% of the state production value with a contribution of \$3.1 million pesos, through the production of 45,501 tons of meat. The second place was occupied by the dairy subsector, with 19.4% which generated \$1.1 million pesos from the commercialization of 186,483 of liters of milk (SIAP, 2017).

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Livestock activities in the state represent a source of employment and income for the rural population. However, in order to improve productivity and quality to meet the demands of domestic and international markets, it is imperative to adapt and adopt technological changes and, therefore, optimize competitiveness. To achieve competitiveness in agrifood chains, the sector must design sectoral regulations that take into account not only the links involved in production, but also the options for specialization and interactions between production and the environment (Díaz and Hartley, 2006). To study the differentiated competitiveness of agricultural systems-products, the analysis of agricultural chains has been suggested (Antúnez and Ferrer, 2016).

Research on agro competitiveness and socioeconomic importance has been conducted in the state of Zacatecas (Sánchez *et al.*, 2013; Rincón *et al.*, 2004). However, the dynamism of production chains requires continuous monitoring, and previous studies have not focused on livestock production chains. Therefore, the objective of this study was to determine the positioning of the most important livestock production chains in Zacatecas, within a matrix of socioeconomic importance and market competitiveness, and to identify research and technology transfer needs. The information generated by this type of study will help decision-makers to efficiently direct financial, technological and research resources to each of the production chains.

## MATERIALS AND METHODS

The study was carried out in 2019 in Zacatecas, Mexico, with the search of secondary information through the Service for Agrifood and Fishery Information (Servicio de Información Agroalimentaria y Pesquera, SIAP, 2017). A total of six livestock production chains were included, which were defined according to their economic and social importance in the state. The methodology does not consider a static point, but rather the trend over the last five years.

The methodology proposed by the International Service for National Agricultural Research (ISNAR) (Pardey and Roseboom, 2004) was used to rank the livestock production chains in Zacatecas according to weighted criteria, in order to identify strategic production chains through dimensionless values. Two guidelines were considered for this study: a) the socioeconomic relevance of the production chains; in other words, this axis considered attributes that justified the productive

activity of each chain, due to their relevance; and b) competitiveness, which is an axis that explains the capacity of those involved in a production chain to face the challenges of change and their ability to adapt and overcome them.

To analyze socioeconomic importance, the following indicators were considered: a) Size: this concept refers to the dimension of the livestock production chain in terms of production value and contribution to the state's economy; the area occupied by the number of heads of livestock species and the number of day laborers required for the activity were estimated; b) Dynamism: it represents the trend of production value and was presented with a simple linear regression for each chain. The slope indicated the rate of change of prices per unit of time. This section also evaluated the evolution of real prices by calculating the mean and standard deviation of the last five years, as well as the slope of the jobs produced in the state by the production chain; c) Specialization: it identified the level of specialization and economic concentration.

Competitiveness was studied using the following parameters: a) Productivity: this was based on three items: 1) relative productivity:  $[(\text{state yield per chain}) / (\text{national yield per chain})]$  and 2) labor productivity, which was estimated by dividing the cost invested in wages by gross income; b) Sustainability: it considers soil erosion estimated from the universal soil loss equation (USLE) (Wischmeier and Smith, 1978), in this case the mean state erosion value per chain was used. Similarly, water efficiency and contamination from the use of fertilizers were analyzed, and for these sections a group of expert researchers (18 individuals) was formed to evaluate both aspects; c) Commercial performance: this variable was estimated based on the trend of real prices, which were obtained through the slope of the simple linear regression model of the price values (this value was considered as the exchange rate per unit of time).

All criteria or variables were standardized to zero mean and standard deviation of one, in order to have variables with equal magnitude and units (standard deviations) (Sánchez *et al.*, 2013). Thus, the positioning matrix with the two reference axes, weighted and accumulated, was obtained. The results generated by the standardized matrix were presented as an interaction between the axes of a graph with four quadrants that indicate the positioning of each of the production chains (Jolalpa *et*

al., 2010). The data were analyzed with the Microsoft Excel software.

## RESULTS AND DISCUSSION

It was found that there are six major livestock production chains in Zacatecas, so they were prioritized. Analysis of the information made it possible to rank the livestock chains according to the sum of all their indicators, their relative value and their hierarchy. The results indicated that the most important production chain was dairy cattle, followed by beef cattle, sheep, goats, honey and pork (Table 1).

Integration of the information made it possible to locate the livestock production chains in the positioning matrix, which formed four groups according to the socioeconomic relevance and competitiveness of each chain in the state of Zacatecas (Figure 1).

In 2013, the beef cattle chain ranked 0.7 in competitiveness and 1.7 in socioeconomic importance; six years later, this chain ranked 1.1 and 0.4 in competitiveness and socioeconomic importance, respectively. This showed that beef cattle decreased in competitiveness, but increased its socioeconomic importance. Nevertheless, this chain obtained the greatest socioeconomic and competitive importance (Figure 1, Quadrant I). This was sustained in the 2015-2016 cycle, where 31,769 head were exported; with this, the state ranked sixth nationally in live cattle exports to

**Table 1.** Dimensionless scores for the livestock production chains in the State of Zacatecas, Mexico. Experimental Station of Zacatecas, 2019.

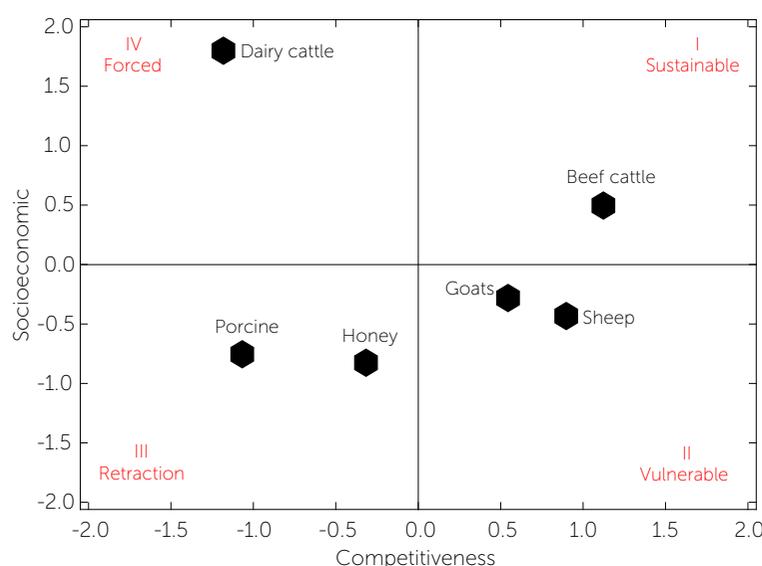
Chains	Socioeconomic	Competitiveness	Sum	Order
Dairy cattle	148.4	19.8	168.3	1
Beef cattle	86.8	68.8	155.7	2
Sheep	42.8	64.0	106.9	3
Goats	50.0	56.5	106.6	4
Honey	24.2	38.2	62.5	5
Porcine	27.6	22.2	49.8	6

the United States (FAOSTAT, 2017). However, for this chain to continue to be sustainable, it is imperative to guarantee safety and to produce excellent quality meat in the shortest possible time, in order for the livestock enterprise to be efficient and profitable. In terms of the international market, quality is considered to be that which contains high muscle content and sufficient intramuscular fat (marbling) to satisfy the organoleptic requirements of the consumer (Monsón et al., 2005).

At the same time, all market segments must be taken into account, for which one of the options is to opt for the minimum processing of the product (from live animals to meat cuts). With the minimum process, the profitability of the farmer increases, since the price received per standing calf is \$28 per kg and up to \$40 per kg of bull calf; while the average price per cut varies between \$90 and \$140 per kg of meat (SNIIM, 2019), which means 250% increase. This explains that much of the price is generated within the commercialization process through minimal processing of the product (from live animals to meat cuts), transportation (from the production area to the consumption centers), and from there to international distribution.

It is also necessary for farmers to become involved in the commercial process in order to reduce the number of intermediaries involved in the chain. Studies indicate that, from calf collection to the final consumer, there are between 5 and 10 links in the chain; and, depending on the flowchart of the chain, the participation time for each agent varies from one day (stockers, wholesalers and slaughterers) to four to eighteen months (fatteners and breeders) (Licea et al., 2015).

Market opportunities for this chain in Mexico are high, despite the fact that beef consumption has declined, but its nutritional and economic



**Figure 1.** Bi-dimensional positioning for the livestock production chains. Experimental Station of Zacatecas, 2019.

importance is high; in 2000 more than 22 kg per capita were consumed, but in 2016 there was a significant decrease (14.9 kg *per capita*) (SIAP, 2017). This reduction in consumption was due to the sustained increase in per capita consumption of chicken meat (29.2 *per capita*) (SIAP, 2017), and the recovery in pork consumption (16.6 kg *per capita*) (SIAP, 2017). Consequently, marketing strategies must be implemented to maintain and promote the positioning of the beef cattle production chain.

At the reproductive level, farmers in the region must pay attention to improving the genetic quality of cattle to obtain better yields. Genetic improvement through the acquisition of registered sires is vital to increase the quality and productivity of cattle herds and, therefore, to achieve a significant improvement in the technical and economic parameters of the activity (Carrera, 2008).

The goat and sheep chains are in the vulnerable quadrant (Figure 1, Quadrant II). In 2019, these production chains were competitive, but they require greater social and economic importance. The negative values in the socioeconomic axis are due to the low number of heads per herd, the decrease in the production value, and the few day laborers used to carry out productive activities. Low precipitation in Zacatecas (less than 400 mm in some areas) has reduced crop productivity and the availability of grass forage which has led to a decrease in the herd (Echavarría *et al.*, 2014). Despite this, the momentum of these chains is preponderant as it contributes to the survival of low-income producers, providing food and income (Echavarría *et al.*, 2015). Therefore, in order to move these production chains from a state of impulse to one of high strategic priority, technology transfer projects must be implemented to reduce the seasonality of reproduction, since the offspring are born in late winter and early spring, when the highest end-of-year market demand has already passed; this decreases profits and is the longest period of drought, which reduces the availability of grasses, leading to increased mortality rates or low development rates. Thus, it is necessary to breed at times that guarantee births at the beginning of the rainy season, that is, to change the reproductive cycle (López *et al.*, 2011).

Likewise, it is necessary to develop abilities in farmers to improve management practices (Salinas and Rumayor, 1999) and reduce animal health problems (Echavarría *et al.*, 2010). Similarly, it is imperative to add value to primary production and to focus products on

specialized segments. Segmentation allows producers to avoid head-on competition in the market by differentiating offers, not only on the basis of price but also in terms of product characteristics, advertising messages, and distribution methods (Fernández and Aqueveque, 2001).

In 2019, the pork and honey production chains were positioned in quadrant III, with low socioeconomic importance and low competitiveness in the state context. These chains have been introduced recently to the State, and were characterized by low dynamism and innovation, low pay for workers, and a marked inability to generate jobs. From 2013 to 2019 these chains dropped from quadrant II to quadrant III (Figure 1). The change in dynamism of the pork and beekeeping sectors (vulnerable quadrant to retraction quadrant) in this region was due to a combination of various factors specific to the state and the macroeconomic and sectoral policies adopted by Mexico as a result of trade liberalization. This had an impact on the development of the agricultural sector, especially the pork production sector. The withdrawal of government subsidies to pig farmers caused a decrease in the activity by consolidating the most efficient companies and eliminating the semi-technified ones (García *et al.*, 2004). This event generated variations in pork production growth rates and different effects among the country's regions. Thus, the pork production chain in the state remained a slow-growing activity, because of inefficient use of resources, low productivity and a low degree of technification; this led to it being classified as a lagging region (Rebollar *et al.*, 2015). The strategy should be to increase technical assistance in terms of health, nutrition, vaccination and biosecurity programs, and to improve infrastructure and seek alternative marketing channels.

The beekeeping chain in Zacatecas has low production (2,077 t), but stands out nationally for its quality and amber color. The honey commercialization process in the state is carried out directly by the beekeeper or family members and is packaged in containers with many presentations. Among the factors that influenced its low production are the indiscriminate use of pesticides, high feed costs, poor genetic quality of queen bees and weather conditions, particularly the drought in 2011, which worsened in 2012, resulting in low flora, water scarcity and death due to hypothermia and diseases that attack the insect (Secretaría del Campo, 2018).

The importance of pollinating insects in global food production is undisputed, and it can be argued that it will become increasingly important in a context of increasing food production needs and declining pollinators, especially the domestic honeybee (Miñarro et al., 2018).

Sustainable agricultural practices, and in particular agroecology, can help protect bees by reducing exposure to pesticides and helping to diversify agricultural landscapes (FAO, 2017). Consequently, the strategy for this chain includes projects that promote bee health, technology transfer to increase productivity and safety, pesticides and practices that help reduce the exposure of pollinators, as well as research and investment in infrastructure.

The dairy cattle chain in 2013 was positioned at  $-1.9$  and  $0.5$  in competitive and socioeconomic circumstances, and six years later this chain is positioned at  $-1.1$  and  $1.7$  respectively. Consequently, this chain improved in terms of socioeconomic importance, but declined  $0.8$  in competitiveness. The dairy cattle chain is in a strained state (Figure 1, Quadrant IV), that is, it has high socioeconomic importance but lacks competitiveness in the state context. The weaknesses faced by the dairy cattle chain are reduced sustainability due to the amount of water for fodder production, commercial performance and labor productivity. The strength of this chain is its dynamism and specialization, in addition to the fact that it is the chain with greatest social weight (high number of rural production units, production value and jobs generated).

The permanence of the dairy cattle chain in the region is explained by the dynamics of intensive livestock production in the Comarca Lagunera (Coahuila and Durango). However, milk production in Zacatecas is losing importance, mainly due to the effects of recurrent drought in recent years, which has made forage more expensive, making the activity less profitable and leading producers to sell or slaughter their cattle (Sánchez et al., 2015). Therefore, in order for this chain to change from a state of maintenance to one of high strategic priority, projects aimed at promoting the organization of production, financing, investment in technology, efficient commercialization channels and technical assistance must be implemented. In addition, the availability of forage alternatives that allow increasing productivity in the use of water; that is, reducing the amount of water to produce one kg of forage with

high nutritional value. Support for small producers by government institutions is necessary because it has been concentrated in vertically integrated producer groups that collect, add value, and market both milk and its byproducts. However, it is important to mention that, in the state of Zacatecas, production is located in the semi-arid-temperate region, and is characterized as a family dairy system.

Innovations related to feeding, milk quality, genetics and technical assistance are the most relevant in the family dairy production system. In family systems, feeding is the main cost of production, but at the same time, if it is done properly, it represents an opportunity; silage feeding allows sustaining livestock production throughout the year, avoids the seasonality of milk production, reduces losses caused by poor feeding, and increases milk production and profitability (Raymond et al., 1989).

## CONCLUSIONS

The strategic positioning of the chains indicated that the beef cattle chain was sustainable, the goat and sheep chains were vulnerable, the pork and honey chains were in retraction due to their low socioeconomic importance and competitiveness, and the dairy cattle chain was a strained chain. The strategies for livestock production chains are to be efficient in production through sustainable practices, to provide technical assistance to producers, to conduct research to generate technology, to invest in infrastructure, to generate value-added products that meet the needs of consumers, and to seek short commercialization channels.

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# Erosion and control options in the La Ciénega Microbasin in Malinaltepec, Guerrero, Mexico

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## ABSTRACT

**Objective:** To estimate the water erosion rate in the La Ciénega Microbasin in Malinaltepec, Guerrero, Mexico, and to evaluate control options.

**Design/methodology/approach:** Potential erosion rate, actual erosion rate, and erosion control were estimated using the Universal Soil Loss Equation (USLE) under three conservation practices: contour plowing, successive land terracing, and live terracing with vetiver grass (*Chrysopogon zizanioides* (L.) Roberty).

**Results:** High erosion potential is shown by 99.18% of the microbasin surface area. Due to vegetation, only 41% of the surface area demonstrates high or very high actual erosion, 53.6% light, and 4.6% moderate. A living barrier using vetiver is the only conservation practice, of those assessed, that effectively diminished erosion.

**Study limitations/implications:** The lack of accurate data on rain-induced erosion was a limiting factor in this study.

**Findings/conclusions:** The La Ciénega Microbasin has a high risk of erosion and current erosion is a problem on agricultural land. The most effective option is living barriers using vetiver.

**Keywords:** Soil loss, USLE, living barriers, Poaceae.

## INTRODUCCIÓN

In the Montaña region in Guerrero, Mexico, agricultural soils are susceptible to water erosion due to mountainous conditions and the frequency of torrential rains. Studies for quantifying erosion in this region are nonexistent, although evidence of its significance can be seen in the high concentration of sediments found in runoffs caused by rain.

To estimate erosion, the Universal Soil Loss Equation (USLE) was used, a model widely tested on a global level for diverse environmental and management conditions (Alewell *et al.*, 2019; Mancino *et al.*, 2016; Lin *et al.*, 2016), with reliable results for Mexico (Prado-Hernández *et al.*, 2017). The Universal Soil Loss Equation is used to estimate soil loss and to support in planning agricultural production (Renard *et al.*, 1997). It estimates average soil loss from erosion per unit of surface and time. It uses physical and management parameters, expressed numerically for a specific site and multiplied to estimate the quantity of soil lost. The equation is expressed as:  $A=R K L S C P$  (Devata *et al.*, 2015).  $A$  is soil loss ( $t ha^{-1}$ );  $R$  is the erosivity factor (dependent on the rainfall's characteristics);  $K$  is the soil's erodability (dependent on the soil's characteristics);  $L S$  is the slope length and angle;  $C$  is the factor for vegetation cover; and  $P$  is the factor for management practices. In recent decades, to estimate parameters for the Universal Soil Loss Equation, Geographic Information Systems are widely used (Flores *et al.*, 2003; Mihaiescu, 2013) through interpolation maps, mainly with the Kriging method, in order to apply the equation in the geographic information system (Tesfaye *et al.*, 2018). This helps improve the planning process for soil conservation practices because they can be previously evaluated considering specific scenarios for plots and basins (Bravo *et al.*, 2009). This study had the objective of estimating the water erosion rate in the La Ciénega Microbasin in Malinaltepec, Guerrero, Mexico, and assessing options for its control.

### MATERIALS AND METHODOS

The study was carried out in the La Ciénega Microbasin (Figure 1), in Malinaltepec, Guerrero, Mexico. The microbasin has an extension of  $91.75 km^2$  ( $17^{\circ} 9' 30''$  and  $17^{\circ} 18' 30''$  N, and  $98^{\circ} 35' 30''$  and  $98^{\circ} 44' W$ ). Climate varies from temperate [C(w2)(w)] in the northern zone to semi-warm and warm [A(C)w2(w) and Aw2(w)] in the southwestern zone. The existing soils are: eutric regosol, lithosol, dystric cambisol, and haplic phaeozem, with the first two predominating. Vegetation is pine-oak, oak-pine, oak, and mountain mesophyll forests, with induced pasture areas and agricultural plots.

To estimate average annual erosion, the Universal Soil Loss Equation (USLE) was used:

$$A=R K L S C P$$

Where:  $A$ =Soil loss ( $ton*ha^{-1}*year^{-1}$ );  $R$ =Rainfall erosivity factor ( $MJ mm*ha^{-1}*h^{-1}*year^{-1}$ );  $K$ =Soil erodability factor ( $t*ha^{-1}*h$ ) ( $MJ mm*ha^{-1}$ );  $L$ =Slope length (dimensionless);  $S$ =Slope angle (dimensionless);  $C$ =Crop or vegetation cover factor (dimensionless); and  $P$ =Management practices (dimensionless).

The potential erosion was estimated considering only the  $R K L S$  parameters of the USLE. To estimate the rainfall's erosivity (R Factor), a regression model was obtained for average annual precipitation ( $Pm$ ) and altitude ( $H$ ), with data from 10 weather stations and a Digital Elevation Model with a 15 m resolution. The resulting equation was:

$$Pm=1375.163+0.410H R^2=0.791, p=0.001$$

With the Raster Calculator tool from the ArcMap 10.3 program, rainfall erosivity and average annual precipitation per pixel were estimated using the equation reported by Cortés (1991) for the V zone:

$$R=3.4880P-0.000188P^2$$

( $R$  is the annual EI30, in  $MJ mm/ha hr$ ;  $P$  is annual rain, in  $mm$ ). Erodability (K Factor) was obtained from morphological, physical, and chemical characteristics of the soils in each soil unit (INEGI, 2014), considering the values reported by the FAO (1980). In the vector soil mapping, a field with the K value was added and it was converted to raster format with a 15 m pixel resolution.

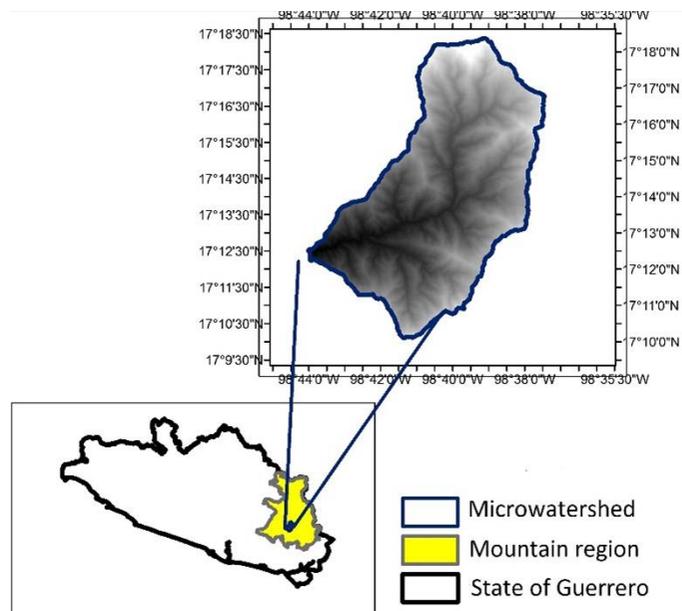


Figure 1. Location of the La Ciénega Microbasin.

The  $LS$  factor was estimated based on a digital elevation model with a 15 m resolution. The  $L$  factor was calculated using the following formulas, according to Pastrana (2014):

$$L = (l / 22.3)^m; m = F / (1 + F); F = (Senb / 0.0896) / [3(senb)^{0.08} + 0.56]; L_{(i,j)} = \left[ \left( A_{(i,j)} + D^2 \right)^{m+1} - A_{(i,j)}^{m+1} \right] / \left[ X^m * D^{m+2} * (22.3)^m \right]$$

Where:  $L$  is the slope length factor;  $l$  is the slope length in meters;  $m$  is a parameter for the slope's steepness;  $A_{(i,j)}$  [ $m$ ] is the unit catchment area at a cell's opening;  $D$  is the cell or pixel size, in this case with a 15 m resolution;  $X$  is the form correction factor.

In ArcGis 10.3, the Raster Calculator tool was used by applying the previous formulas (Pastrana, 2014):

$$F = ((\sin(\text{Slope} * 0.01745) / 0.0896) / (3 * \text{Power}(\sin(\text{Slope} * 0.01745), 0.8) + 0.56))$$

To estimate  $m$ , the formula  $F/(1+F)$  was used, and the following expression was used for  $L$ :

$$\frac{(\text{Power}(\text{Flow}_{acc} + 625), (m + 1)) - \text{Power}(\text{Flow}_{acc}, (m + 1))}{\text{Power}(25, (m + 2) * \text{Power}(22.13, m))}$$

The  $S$  factor was estimated considering the following formulas:  $S_{(i,j)} = 10.8 \text{ Sen } b_{(i,j)}$  if  $b_{(i,j)}$  is less than 0.09;  $S_{(i,j)} = 16.8 \text{ Sen } b_{(i,j)}$  if  $b_{(i,j)}$  is more than 0.09. The  $S$  factor was estimated as:

$$\text{Con}((\tan(\text{Slope} * 0.01745) < 0.09), (10.08 * \sin(\text{Slope} * 0.01745) + 0.03), (16.8 * \sin(\text{Slope} * 0.01745) - 0.5))$$

To estimate the  $C$  factor (vegetation cover), six types of soil use were digitalized using Spot 5 images from Google Earth with a spatial pixel resolution of 2.5 m. A  $C$  value was assigned for each type of soil (human settlements, pastures, natural forests, landslides, secondary vegetation, and croplands).

The scenarios considered for conservation and erosion control practices were: 1) Contour plowing, 2) Successive land terracing, and 3) Live terracing with vetiver grass (*Chrysopogon zizanioides*), assigning them  $P$  values of 0.8, 0.6, and 0.01, respectively.

## RESULTS AND DISCUSSION

### Erosion potential of the microbasin

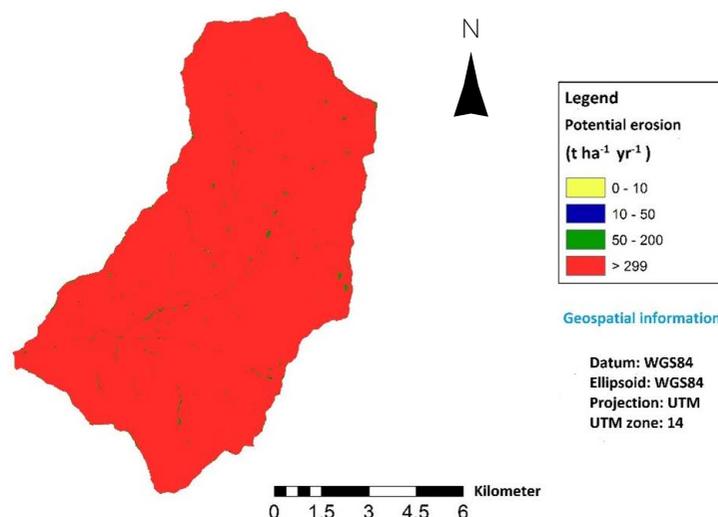
The microbasin's erosion potential is high (Figure 2; Table 1). The majority of the surface area has an erosion potential greater than 299 t ha<sup>-1</sup> year<sup>-1</sup>, which is characteristic for the vast majority of the national territory (Montes-León et al., 2011), in contrast to countries like Argentina where only 9.1% of its territory presents erosion potential levels greater than 200 t ha<sup>-1</sup> year<sup>-1</sup> (Gaitán et al., 2017). This indicates a high risk of erosion in the whole microbasin if the vegetation is removed or if adequate soil conservation practices are not used in agricultural lands.

### Actual Erosion

Of the microbasin's surface area, 53% has light erosion (Figure 3; Table 2). This is associated with the presence of forests and other types of vegetation in more than half of the microbasin's area. However, strong erosion is

**Table 1.** Potential erosion.

Potential erosion (t ha <sup>-1</sup> yr <sup>-1</sup> )	Level	km <sup>2</sup>	hectare	%
0 - 10	Low	0.010575	1.0575	0.01
10 - 50	Moderate	0.039825	3.9825	0.04
50 - 200	High	0.7029	70.29	0.77
> 200	Very high	91.006875	9100.6875	99.18



**Figure 2.** Erosion potential of the La Ciénega Microbasin.

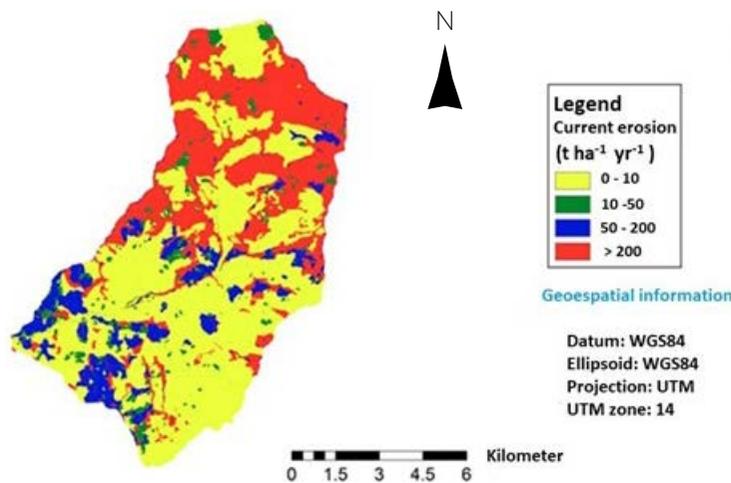


Figure 3. Actual erosion in the microbasin (2020).

Table 2. Actual erosion in the La Ciénega Microbasin (2020).

Current erosion (t ha <sup>-1</sup> yr <sup>-1</sup> )	Level	km <sup>2</sup>	hectare	%
0 - 10	Low	49.15395	4915.4	53.6
10 - 50	Moderate	4.244175	424.4	4.6
50 - 200	High	8.991225	899.1	9.8
>200	Very high	29.29635	2929.6	32.0

present in 32% of the microbasin, in lands of agricultural use and much degraded zones, with erosion rates higher than 200 t ha<sup>-1</sup> year<sup>-1</sup>. This constitutes a significant area where soil conservation practices should be promoted.

**Erosion control options**

Contour plowing and terracing practices, considered to be mechanical practices, have a very low impact on erosion reduction (Figure 4, Table 3). Incidence on the erosion rate of zones of high and very high actual erosion is low. Living barriers using vetiver will significantly reduce erosion in agricultural lands with high and very high actual erosion (Figure 5). The effectiveness of live vetiver hedges has been documented, reporting reductions of 56.2 to 87.9 % in steep terrain (Donjadee *et al.*, 2010).

Vetiver is currently being tested in the microbasin (Figure 6). The plant can adapt to a diverse range of environmental conditions and because of its growth type, it does not compete with crops and does not become a weed (National Research Council, 1993). Live vetiver hedges accumulate soil and avoid the loss of soil productivity and the decrease of crop yields due to soil erosion (Oshunsanya, 2013). This suggests that it can be accepted by rural producers in the microbasin.

**CONCLUSIONS**

The erosion potential in the La Ciénega Microbasin is high, with potential erosion rates greater than 299 t ha<sup>-1</sup> year<sup>-1</sup>. Actual erosion in almost half of the microbasin surface area (53.6%) is light, due to the presence of primary forest. Meanwhile, very high erosion (32 % of the microbasin area)

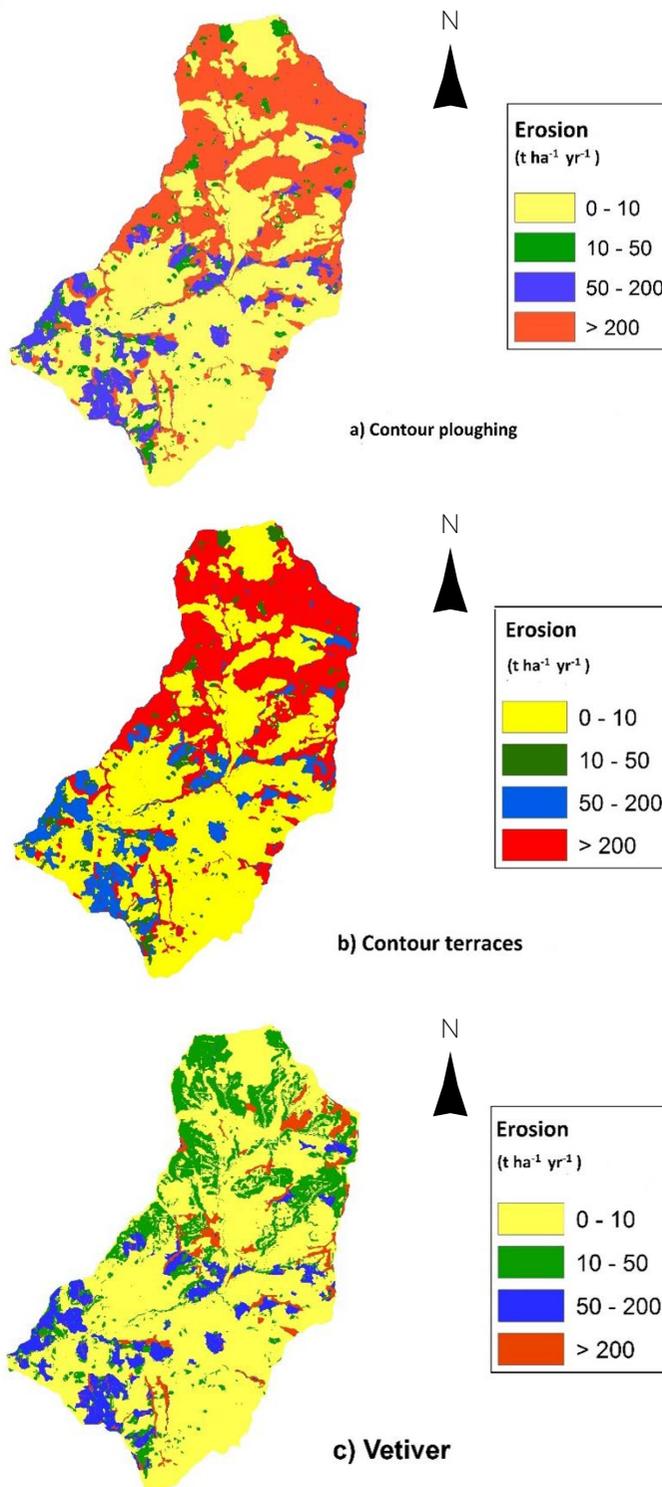
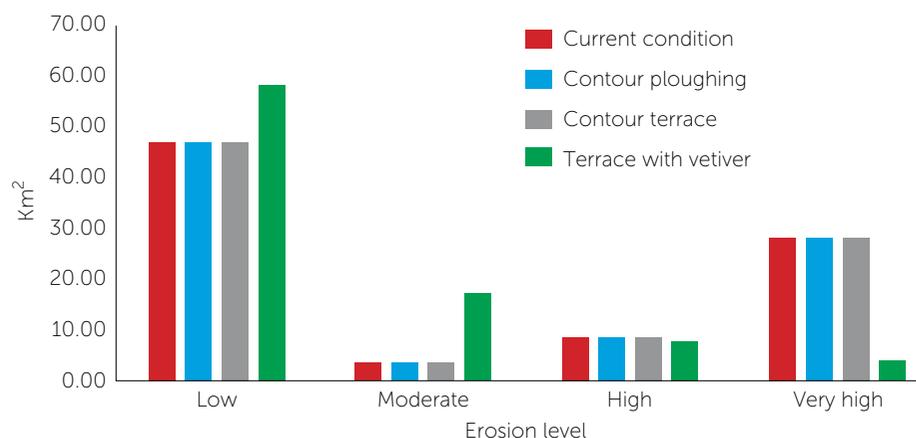


Figure 4. Maps of estimated erosion using contour plowing practices, contour terracing, and live vetiver barriers.

**Table 3.** Effects of conservation practices on surfaces with erosion levels.

Erosion t ha <sup>-1</sup> yr <sup>-1</sup>	Level	Current condition		Contour ploughing		Contour terrace		Terrace with vetiver	
		km <sup>2</sup>	%	km <sup>2</sup>	%	km <sup>2</sup>	%	km <sup>2</sup>	%
0 - 10	Low	49.15	53.6	49.15	53.6	49.15	53.6	60.83	66.3
10 - 50	Moderate	4.24	4.6	4.27	4.7	4.31	4.7	17.73	19.3
50 - 200	High	8.99	9.8	9.12	9.9	9.36	10.2	8.67	9.5
>200	very high	29.30	32	29.14	31.8	28.86	31.5	4.46	4.9

**Figure 5.** Microbasin area and degree of erosion for scenarios of conservation practices.**Figure 6.** Vetiver plant (*Chrysopogon zizanioides* (L.) Roberty) in a nursery and in lands in the microbasin.

is associated with agricultural activities and degraded areas. Of the three management scenarios evaluated for water erosion control, both contour ploughing and successive land terracing do not result in reducing soil loss at an acceptable level. On the other hand, the use of live barriers with vetiver hedges (*Chrysopogon zizanioides*) in agricultural lands and degraded zones results in a 27.1% reduction in erosion within the very high to moderate and light categories.

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# Evaluation of producer inflation, subsidies and profitability of vegetables and grains in Sinaloa, 2018-2019

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## ABSTRACT

**Objective:** To evaluate producer inflation, equity in PROCAMPO subsidy distribution, as well as profitability of eight agricultural products in the state of Sinaloa, 2018-2019 cycle.

**Design/Methodology/Approach:** First, inflation is estimated in the value of agricultural production, using the agricultural producer price index (INPP) base 2019. Second, the inequality in the allocation of PROCAMPO is calculated with Lorenz curves. Third, the internal rate of return (IRR) is estimated for the eight products and compared with the 28-day yield of the treasury certificates (CETES).

**Results:** The current values generated show growth in cereals (corn, wheat), and vegetables (tomato, chili peppers), with downward inflationary gaps in the period 2000-2019. There is a concentration of the PROCAMPO allocation in producers with high income deciles. The IRR is high in vegetables, and low in corn and beans.

**Study Limitations/Implications:** This study does not specify the size of the productive unit and only the data is generalized. It does not address marketing channels and their destinations.

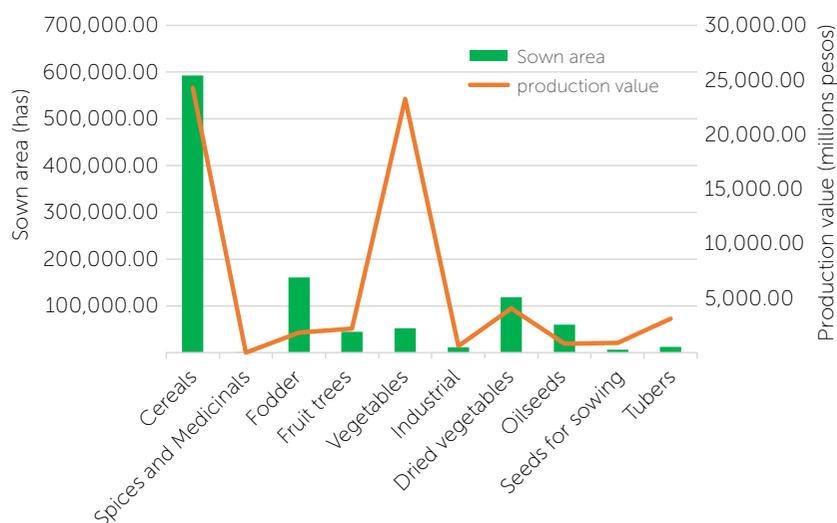
**Findings/Conclusions:** The producer is assuming the inflationary increase. Income transfers via PROCAMPO are inequitable. The IRR in corn and beans is sometimes less profitable than CETES.

## INTRODUCTION

In Mexico, agriculture and livestock productive units confront increasingly complex competitive environments, such as: new agreements in the USMCA, uncertainty of prices for their products, variability of production costs, climate risks, impact from pests and diseases, among other aspects.

The agriculture and livestock sector, according to INEGI (2021), has had a participation in the GDP of 3%. According to SIAP (2020), the average value between 2011 and 2019 was \$895 billion MXN, and the participations per subsector are: agriculture 55%, livestock 42%, and fishing 3%. Although the three subsectors show a growing trend, agriculture shows the best performance with a growth higher than 8% (see Table 1).

According to SIACON (2020), for the year 2019 the agricultural value reached \$675,368 million MXN, with nearly a third of this distributed among the states of Michoacán, Jalisco and Sinaloa. Taking as example the state of Sinaloa, one of these large agrifood producers, Figure 1 shows that slightly over one million hectares were sown, of which more than 500 thousand ha are for cereals that generate more than \$24 billion MXN; whereas vegetables, with 51 thousand hectares, produce more than \$23 billion MXN.



**Figure 1.** Sinaloa: planted area and production value, according to crop groups, 2019. Source: SIACON. <https://www.gob.mx/siap/documentos/siacon-ng-161430>

Table 2 shows that, in cereals, maize represents 56% of the surface planted and 38.5% of the production value. In vegetables, tomato with slightly over 1% of the surface sown generates more than 12% of the state agriculture value. In turn, the accident rate, as approximate indicator of the risk level, shows with high indicators the following: tomato 15.2%, maize and green chili pepper 13.5%.

As complex systems, agrifood supply chains face multiple sources of uncertainty that can cause a significant imbalance between offer and demand in terms of varieties of products, amounts, qualities, client requirements, times and prices. All of these complicate their management (Alemany, et. al, 2021), which forces agricultural producers to confront various structural problems that cause increase in prices of agricultural and food products (Keskin, 2020). Within this scope, the PROCAMPO subsidy did not support technological

impetus, as Zarazúa et al. (2011) described. And, according to FAO-SAGARPA (2015), its distribution was unequal, since in the states of northwestern Mexico, the support was concentrated in commercial production units.

This study suggests three of the aspects that are crucial in the decisions made by producers: input prices (producer inflation); equity in the subsidy distribution; and financial profitability of the crop. The objective of this study is to evaluate the producer inflation, the equity in PROCAMPO distribution, as well as to compare the profitability of eight agricultural products with high accident rate with regard to a low-risk financial instrument.

### MATERIALS AND METHODS

In the first section, based on the current value SIACON (2020) and the price index of agricultural producer at constant value from 2019 INEGI (2021b), the producer inflation that the producer “absorbs” is estimated and which is not transferred to the consumer. According to Sidaoui et al. (2009), the producer prices have causality in the consumer

**Table 1.** Production value of the agricultural and fishing sector in Mexico, 2000-2019. (In millions of nominal pesos).

Year	Agriculture	Livestock	Fishing	Sector total
2011	354,657	264,245	17,786	636,687
2012	410,160	286,571	19,022	715,753
2013	395,508	323,433	19,855	738,796
2014	417,347	356,168	24,110	797,624
2015	444,138	382,462	31,490	858,090
2016	513,936	394,417	35,664	944,017
2017	587,233	423,065	39,781	1,050,078
2018	641,026	451,566	41,728	1,134,321
2019	675,368	479,960	28,679	1,184,008
Average value	493,264	373,543	28,679	895,486
Percentage participation %	55%	42%	3%	100%
AAGR	8.4%	7.7%	6.2%	

Source: SIACON, SIAP, CONAPESCA, SAGARPA, 2020. <https://www.gob.mx/siap/documentos/siacon-ng-161430>

Notes: the value of the fishing sector for 2019 is estimated as an average for the period, since SIACON does not report the data for that year.

AAGR=Annual Average Growth Rate

**Table 2.** Sinaloa: Production structure for selected products, 2019.

Cultivated product	Sown area	Harvested area	Damaged surface	Production value
Green chile	0.49	0.42	13.50	3.55
Beans	6.42	6.49	7.12	4.46
Chickpea	4.76	4.84	0.00	2.21
Corn	52.64	53.43	13.57	38.53
Potatoe	1.15	1.17	0.00	5.09
Sorghum	8.83	8.97	0.00	1.73
Tomatoes	1.16	1.09	15.22	12.18
Wheat	3.37	3.43	0.00	1.23
Total Sinaloa	100	100	100	100

Source: Own elaboration based on: SIACON 2020.

prices. In this sense, the percentage breach between the current value and the deflated value approximates as proxy indicator of inflation that the agricultural producer assumes without this increasing the consumer prices. Next, two equations on which this percentage is based are presented.

$$VAC = \left( \frac{VA}{innpa} \right) * 100 \quad (1)$$

$$D = (VAC - VA) / VA \quad (2)$$

Where:  $VAC$ =agricultural value at constant prices from 2019=100;  $innpa$ =national price index to the agricultural producer;  $VA$ =agricultural value at current prices;  $D$ =percentage variation of the difference between current agricultural and constant value.

In the second section, based on the National Survey of Household Income Expense (*Encuesta Nacional Ingreso Gasto de los Hogares*, ENIGH), 2016 and 2018, through the SPSS version 14 software. The level of equity in the distribution of PROCAMPO subsidies.

The level of dispersion or concentration in the distribution of the subsidy is exhibited through the representation of Lorenz curves, according to the levels of income of the producer who receives them, as expressed in Figure 2, which connects the proportion of the producer's income with the proportion of subsidy accumulated. In this representation, the straight line of 45° reflects an equal relation between the subsidy granted and the producer's income. A curve above

implies progressive concentration, where those of low income participate more in the subsidy. For a regressive curve, the subsidies are concentrated in higher income deciles.

In the third part, based on the data from the Agro-costs portal by FIRA (2021), the production costs, yield, probable price, utility, and equilibrium points were obtained, for crops selected in the 2018-2019 cycle. Then, according to Mete (2014) and Molina, P.O. (2017), the IRT from each crop selected were estimated, and compared with the yields from a low-risk instrument, such as CETES at 29 days from BANXICO (2020). Where:

$$IP = Y * P \quad (3)$$

$$CU = CT / Y \quad (4)$$

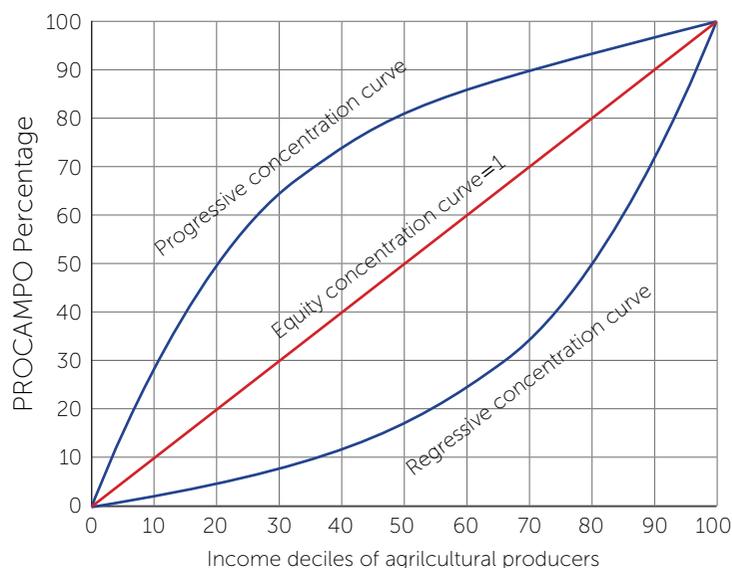
$$TIR = IP / CU \quad (5)$$

Where:  $IP$ =probable income;  $Y$ =yield per ha;  $P$ =price;  $CU$ =unitary cost per ha by t;  $IRR$ =internal rate of return.

## RESULTS AND DISCUSSION

### Sinaloa: Product Inflation

The producer price is explained as the price fixed by him on the first instance of product purchasing. If the producer price index is applied to current agricultural

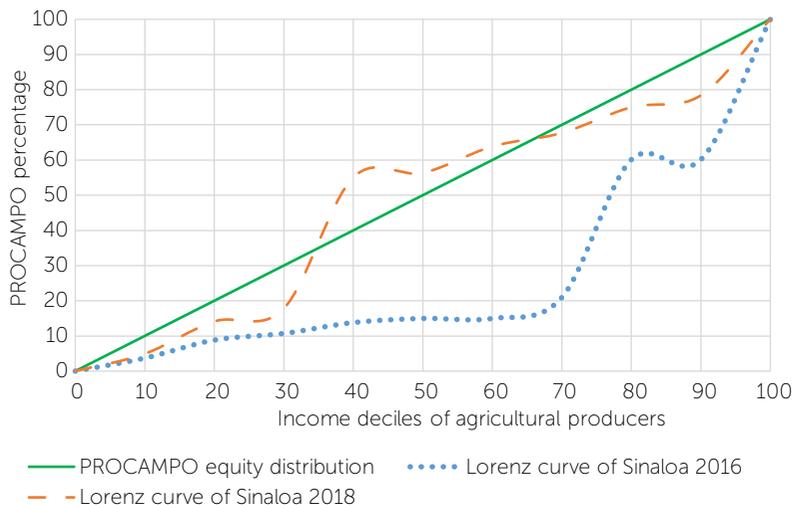


**Figure 2.** Lorenz curves, equity concentrations, progressive and regressive  
Source: Own elaboration based on: (Morales-Novelo et al., 2018, p. 5).

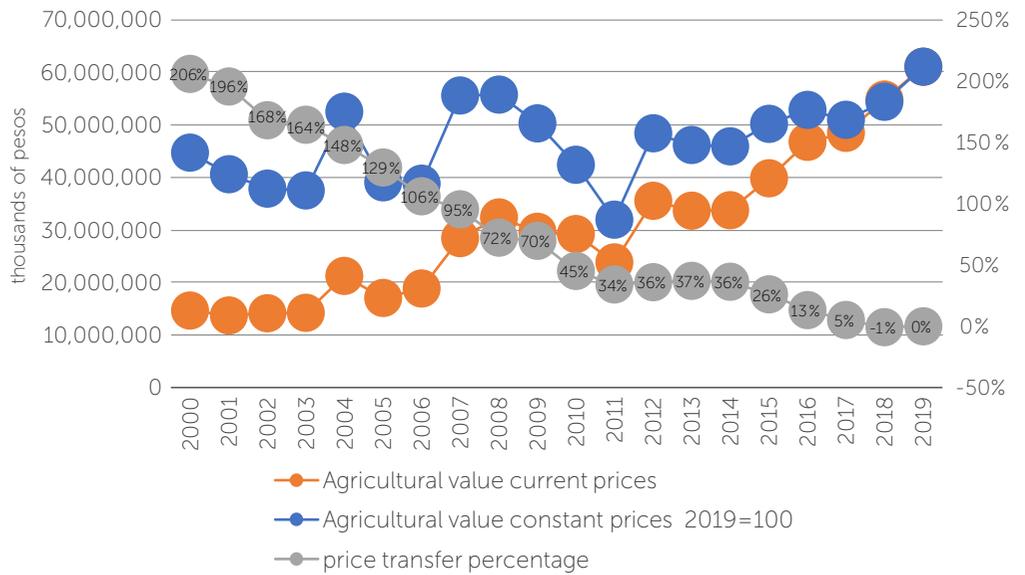
value, a deflated series is obtained, where the current prices are transferred into constant prices 2019=100. Estimation of D from equation 2 allows estimating the percentage variation of the difference between the current agricultural value and the constant value. Figure 3 expresses that in year 2000 it was 206%, the highest of the period. Since that year and until 2017, this difference decreases consistently until reaching 0% in 2019. This gives evidence of how inflation has decreased in the agricultural sector in Sinaloa. Likewise, that the producers absorb inflation coming from the increase in prices of its inputs without transferring it to the consumers.

**Sinaloa: The Subsidy Effect**

According to Sadoulet (Sadoulet *et al.*, 2001), as a consequence of the approval of NAFTA in 1994, a compensation program to transfer compensatory income to producers of basic crops was introduced, known as PROCAMPO.



**Figure 4.** Sinaloa: Equity and inequality in the distribution of resources from procampo 2016 and 2018. Source: Own elaboration based on ENIGH 2016 and 2018.



**Figure 3.** Evolution of the Agricultural Value in Sinaloa, 2011-2018. Source: SIACON. <https://www.gob.mx/siap/documentos/siacon-ng-161430>

The effects of this compensatory measure, according to García *et al.* (2011), have stimulated the production of some products such as maize. In 2013, the program was restructured and since its implementation and up until 2018, it gradually reduced its coverage. From 1994 to 2018, on average \$11.45 billion MXN were assigned to it annually. For the case of the year 2018, the subsidy was concentrated in states such as: Chiapas, Zacatecas, Oaxaca, among others. For that same year, the state of Sinaloa received 4.3% of participations nationally, based on CEDRSSA with data from Noriega and Hidalgo (2019), FAO (2019) and SIAP (2019).

For the year 2016, Figure 4 shows that the subsidy is regressive, since the producers from high deciles (8, 9 and 10) obtain between 60% and 90% of the subsidy. Meanwhile, the producers from low-income deciles (1, 2 and 3), obtain less than 15% of resources. In turn, for 2018, a progressive effect is observed in deciles 4, 5 and 6 (medium producers). However, the deciles 8, 9 and 10 continue to accumulate more than 75% and deciles 1 to 3 less than 15% of the PROCAMPO resources.

**Agricultural Production Costs in Sinaloa.**

Referring to the production costs, in this section the production indicators of the 2018-2019 agricultural cycle in Sinaloa: production, value, yield, probable income price, total cost, net utility, unitary cost, point of equilibrium, and internal rate of return (IRR). Table 3 shows that regarding the production in tons, the crop that generates most production is maize with more than five billion tons, while that of lowest production is bean with 173,992 t. According to the value generated, maize shows \$22,431,170 MXN and wheat \$1,103,360 MXN. For the yield,

**Table 3.** Sinaloa: Production costs, yield, probable price, profit and balance points for selected crops in the 2018-2019 cycle.

Concepts	Corn	Beans	Wheat	Sorghum	Chickpea	Tomatoes	Potatoe	Jalapeno pepper
Production in t	5,818,056	173,992	241,522	325,873	193,078	1,088,252	375,821	234,813
Value (in Mexican pesos)	22,431,170	2,534,612	1,103,360	1,121,143	3,357,446	7,024,020	2,462,575	1,405,789
Yield (t/ha)	11	2	5	8	2	130	30	50
Likely price (\$/t)	3,960	16,000	5,010	3,564	16,000	5,500	7,000	7,000
Probable income (\$/ha)..(A)	43,560	28,800	25,050	28,512	32,000	715,000	210,000	350,000
Total cost (in Mexican pesos)	35,211	27,094	23,871	22,378	25,648	473,692	187,833	258,424
Net profit (pesos/ha)	8,349	1,706	1,179	6,134	6,352	241,308	22,167	91,576
Unit cost (pesos/t/ha)...(B)	3,201	15,052	4,774	2,797	12,824	3,644	6,261	5,168
Balance point (t/ha)	8.89	1.69	4.76	6.28	1.6	86.13	26.83	36.92
Internal rate of return (A/B)	1.24	1.06	1.05	1.27	1.25	1.51	1.12	1.35

Source: AGROCOSTOS FIRA. 2018-2019.

they take it as an indicator of the technology used in the productive process. Tomato, which leads the figure with 130 t/ha, while both chickpeas and bean only produces 2 t/ha, respectively. Regarding the production costs, tomato is the one that shows highest cost with \$473,692/ha and the crop with lowest cost is sorghum with \$ 22,378/ha. If they compare utilities, tomato stands out with more than \$241,000/ha, and wheat has the lowest utility with \$1,179/ha.

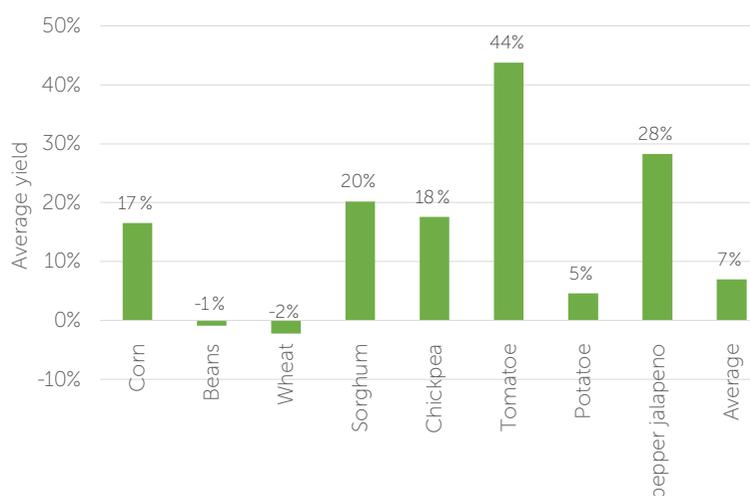
Although these indicators can be a valid parameter for the producer to choose his best option, extra-economic aspects intervene such as: productive tradition, climate, contracts, among others. Next, two parameters are analyzed that are revealing about financial profitability, such as the business criterion. As Table 3 shows, the analysis of IRR as the financial indicator par excellence, that is, is the percentage of gain or loss that an investment will have, where IRR higher than one means investment where no losses are generated. In this case, following the analysis of the products mentioned above.

The IRR for each crop are the following: maize 1.24, bean 1.06, wheat 1.05, sorghum 1.27, chickpea 1.25, tomato 1.51, potato 1.12, and jalapeño chili pepper 1.35. Examined this way, all crops present favorable profitability, that is  $IRR > 1$ . When comparing the financial yield as such from crop sowing, with the yield of CETES (treasury certificates at 28 days). That is, suggesting a comparative scenario, on which would be the best yield for an investor: CETES or sowing. In this case, according to Figure 5, bean and wheat

show lower yields than CETES: bean  $IRR 1% < CETES$ ; wheat  $IRR 2% < CETES$ .

## CONCLUSIONS

There is a tendency to decrease the transfer of prices from the producer to the consumer; that is, the producer has taken on the increase of prices from its inputs without transferring them to the consumer. The distribution of PROCAMPO subsidies is concentrated in the producers with deciles of higher income, but in the intermediate deciles it has been progressive. However, all the producers selected show a positive IRR, higher than one. When compared to the yield rate of CETES, as reference of a low-risk instrument in the market, wheat and bean exhibit lower yield rates. In sum, agriculture in Sinaloa transits as a productive activity where producers



**Figure 5.** Financial Performance of the crop VS CETES at 28 days average 2018.

Source: Own elaboration based on SIACON and BANXICO

<https://www.banxico.org.mx/tipcamb/llevarTasasInteresAction.do?idioma=sp>

do not transfer their inflationary impacts; there is still concentration of the subsidies in high-income producers, and in traditional producers such as wheat and bean, sometimes more risks are taken than if they invested on low-risk instruments.

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# Effect of activated carbon on the nitrogen balance in broilers, soil and corn forage fertilized with the excreta of the broilers

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## ABSTRACT

**Objective:** To evaluate the contribution and nitrogen retention in broilers supplemented with activated carbon (AcCa), as well as in soils and corn forage fertilized with the excreta of chickens that consumed AcCa.

**Design/methodology/approach:** Chickens individually housed received diets with four levels of AcCa: 0, 0.15, 0.30 and 0.45% and their nitrogen balance was determined using the total collection of excreta. 200 g of the chicken excreta were taken and mixed with 2 kg of soil (S+E) in plastic trays and watered every 15 d. On day 1 and 60, samples were taken to perform the nitrogen balance assessment. The S+E mixtures were added to 17 kg of agricultural soil in pots to produce corn forage for 100 d, their yield and composition were recorded. The results were analyzed with an ANOVA and linear regression.

**Findings/conclusion:** In chickens, nitrogen retention showed a quadratic response ( $P < 0.05$ ); In the S+E mixtures, the percentage ( $P < 0.01$ ) and final nitrogen content ( $P < 0.05$ ) had also quadratic responses, and in the forage, the nitrogen percentage showed a cubic response ( $P < 0.05$ ) respect to the increases in the addition of AcCa in the chicken's diet. AcCa can be used to improve the nitrogen efficiency in chickens and for nitrogen recycling through the integration of excreta in agricultural soils and its extraction in corn forage.

**Keywords:** Fattening, nutrients retention, excreta, soil, forages.

## INTRODUCTION

**Nitrogen** (N) is the most limiting essential nutrient in human nutrition, although it is the most abundant element in the Earth's atmosphere, it is also the least available, because it is found almost entirely in a non-usable form for most organisms due to the presence of a strong triple bond in its molecule. To break this bond, large amounts of energy are needed, which can be generated at high temperatures or by an enzymatic process by a small number of specialized microbes (Galloway *et al.*, 2004). The global deficiency of N can be aggravated due to the exponential growth of the human population, whose protein needs must be covered with

products of high nutritional quality such as meat, milk and eggs (Gerber *et al.*, 2013). Unfortunately, of the 100% of the N that animals consume, only 40% is retained in edible products under adequate production conditions (Gómez and Angeles, 2012; Gómez *et al.*, 2014). This means that animals eliminate approximately 60% of the consumed N (around 15% in the feces and 45% in the urine) which is lost in up to 70% as ammonia (NH<sub>3</sub>) in untreated excreta, depending on the environmental conditions, such as temperature and humidity.

NH<sub>3</sub> is the main air pollutant in livestock farms. It is a gas with toxic effects on living beings. NH<sub>3</sub> excesses in the broiler housing environment can reduce their weight gain and increase the feed conversion (Beker *et al.*, 2004; Miles *et al.*, 2004); It can also cause a reduction in the height of the villi and depth of the crypt, damage the mucus flow, ciliary action and mucous membranes in the respiratory tract, as well as reduction of specific antibody titers and other immune functions (Zhang *et al.*, 2015). Among the evaluated strategies to reduce NH<sub>3</sub> emissions is activated carbon (AcCa), which is produced by organic matter carbonization under anaerobic conditions. AcCa has been reported to act by adsorbing gases such as hydrogen sulfide and NH<sub>3</sub>, bacterial toxins and mycotoxins in the gastrointestinal tract of chickens; By binding to NH<sub>3</sub>, AcCa protects the intestine against alkalization, prevents infections and stops diarrhea, adsorbing and eliminating bacteria in the feces (Majewska *et al.*, 2011). There are suggestions that the addition of AcCa to the animal feed could reduce ammonia emissions inside closed housing, which could reduce disease risks for animals and people.

AcCa is normally used to remove metals from wastewater due to its adsorbing properties. There are reports of the AcCa high effectiveness of removing metals, due to 50-55% metals precipitation in the form of hydroxides, and the adsorption of 15-20% of cations on the surface of negatively charged AcCa (Lyubchik *et al.*, 2008). Also, benefits have been observed in the production of corn biomass in soils supplied with AcCa, attributed to the release of N during the organic fertilizers decomposition (Clemente *et al.*, 2007; Sabir *et al.*, 2013). It has also been shown that AcCa applied to the surface of cultivation soils or mixed with chicken excreta reduces NH<sub>3</sub> emissions by 20-64% (Doydora *et al.*, 2011). However, there is no published information on the effect AcCa has on animals and fertilized soils with the manure of those animals that consumed it. Due to the above, the objective of this

research was to evaluate the contribution and retention of nitrogen in broilers supplemented with AcCa, as well as the effect on soils and corn forage production fertilized with the excreta of the chickens that consumed AcCa.

## MATERIALS AND METHODS

The study was divided into three phases. In the first, 72 chickens 25 to 45 days old were individually housed in cages fitted with trays for the excreta recollection. At the beginning, the chickens were weighed and randomly assigned to four diets that contained increasing concentrations of AcCa: 0, 0.15, 0.30 and 0.45%. The diets were formulated with ground corn and soybean paste, balanced to meet the requirements of the lineage according to their age. During the first 15 days of the test, water and feed were offered *ad libitum*. During the last five days, 90% of the amount of feed previously consumed was offered and divided into two parts that were served during the morning and in the afternoon to reduce feed waste. The total collection of excreta was carried out, during four consecutive days with 24 h intervals, using plastic bags in the recollection trays. After collecting each day, the excreta were weighed and frozen. At the end of the last harvest, the chickens were again weighed. A record of the offered and rejected feed was kept to estimate the feed consumption by their difference.

Chickens were weighed at the beginning and end of the test to calculate their daily weight gain. The feed conversion was obtained by dividing feed intake by gained weight. AcCa added to feed was a commercial product used in water decontamination in fish tanks, it is prepared from coconut shell and steam activated.

During phase two, 200 g of dehydrated excreta were taken from the chickens in the balance test and homogeneously mixed with 2 kg of agricultural soil (S+E mixture) and placed in plastic trays in a greenhouse. For each level of AcCa received by the chickens, there were six repetitions (trays) per treatment. During days 0, 15, 30 and 45, 1 L of water was added to the S+E mixtures to favor NH<sub>3</sub> production and N loss by volatilization. At the beginning and on day 60, the trays were weighed, and samples of the mixtures were taken to a laboratory for processing.

In phase three, the tray's contents from phase two were homogeneously mixed with 17 kg of agricultural soil and

transferred to 20 kg capacity pots. Also, pots with agricultural soil without excreta were the control with their weight adjusted to the weight of the pots where the S+E mixture was added. The pots were placed in a greenhouse, in each one, three corn plants (*Zea mays* L.) were planted and watered for 100 d. The corn forage was harvested and evaluated in a laboratory.

In the nutrition laboratory, the excreta from phase 1 were thawed, lyophilized and grounded. In the excreta and feed the dry matter, ash, N and energy were determined. All laboratory determinations were carried out following the AOAC (2019) recommendations. With the results and feed consumption data, the balance of dry matter, ash, N and energy (consumption, excretion and retention) was estimated. Also, the apparent metabolizable energy corrected to a zero N retention (EMAn) was estimated. In the S+E mixtures taken at the beginning and end of phase two, the content of dry matter, ash and N was analyzed in the laboratory, their content of organic matter and organic carbon was also estimated. With these results and the initial and final weight of the mixtures, the dry matter, ash, organic matter, the organic carbon and initial and final N contained in each tray were calculated. The corn forage from phase three was weighed fresh, oven-dried and grounded. In the samples, the dry matter content, ash, and N were determined and the yield of dry matter and the amount of ash and N recovered in the forage was estimated.

At the beginning of phase three, a representative sample of the soil from the pots was taken to assess the percentage of N and calculate the amount of N provided by the soils. The N provided by the S+E mixtures from phase two was added to the N in the soil. Additionally, the N efficiency use from the soil of the pots was calculated for the extraction of N through the forage with the formula:

$$\text{Total N Efficiency use} = \frac{\text{Total N content in pots}}{\text{Total N content in forage}} \times 100$$

Additionally, the amount of N obtained in the forage of the pots that only had soil was subtracted from the N obtained from the pots where the S+E mixture was added to estimate the N coming only from the added mixture. This result was used to calculate the N use efficiency from the S+E mixture with respect to the extraction of N through the forage with the formula:

$$\text{Efficiency of use of the N from S+E} = \frac{\text{N contributed by the S+E mixture}}{\text{N from the S + E mixture in the forage}} \times 100$$

All the results were subjected to an analysis of variance and a simple linear regression (SAS, 2002) to test for a response pattern of the evaluated variables to the AcCa supplementation. The analysis of the first derivative was used, from the obtained regression equations to estimate the inflection point of the curve and define the optimal level of inclusion of AcCa depending on the affected variables. The percentage values were arcsine transformed before

analysis. In phase one (balance test with chickens) there were 18 repetitions per treatment, in phases two and three six repetitions per treatment.

## RESULTS AND DISCUSSION

In phase 1, there were no differences in the weight gain and feed intake in the chickens fed with different levels of AcCa (Table 1). The feed conversion showed ( $P < 0.05$ ) a quadratic type response in relation to the increase in the AcCa addition in the diet, since at level 0 it was higher, it decreased between the 0.15-0.30% level and slightly rose at 0.45% AcCa level. The resulting equation was:

$$y = 2.2831 - 1.4848x + 3.0309x^2 \\ R^2 = 0.96$$

The equation was derived and determined by estimating the inflection point of the curve where the optimum level of AcCa inclusion to minimize the feed conversion was 0.24%. A lower feed conversion means that less feed is required for each kg of weight produced, so the meat production cost is lower.

The consumption, excretion and retention of dry matter, ash and energy were similar between treatments (Table 1). There were no differences between treatments in the N consumption, but quadratic type responses were observed in the N excretion ( $y = 1.5229 - 1.5908x + 2.4571x^2$ ;  $R^2 = 0.95$ ;  $P < 0.01$ ), the grams of N retained (and  $= 1.9147 + 1.7691x - 3.0073x^2$ ;  $R^2 = 0.96$ ;  $P < 0.05$ ) and the N percentage retained ( $y = 55.318 + 49.563x - 79.88x^2$ ;  $R^2 = 0.95$ ;  $P < 0.01$ ) respect the increasing AcCa levels in the diet of the chickens. The equations

were derived, and it was found that the optimal level of AcCa inclusion to reduce the excretion and increase the N retention in grams and percentage was 0.32, 0.29 and 0.31%, respectively. The quadratic responses of the feed conversion, excretion and retention of N due to the inclusion of AcCa in the diet were probably due to the adsorption effect of AcCa on NH<sub>3</sub> inside the intestines.

It has been shown that excess NH<sub>3</sub> inside chicken houses causes different negative effects on the bird's physiology, including damage to the digestion surface and absorption of the digestive mucosa (Zhang *et al.*, 2015); for example, a reduction in the height of the villi and the depth of the crypts that line the mucosa has been observed. By binding to the NH<sub>3</sub>, AcCa protects the intestine against alkalization, prevents intestinal infections, and stops diarrhea, helping to eliminate bacteria in the stools (Majewska *et al.*, 2011). Besides, due to the high adsorption properties of AcCa, it acts

curatively in the digestive system by adsorbing other gases such as hydrogen sulfur, bacterial toxins and mycotoxins (Edrington *et al.*, 1997; Shareef *et al.*, 1998). The reduction of bacterial and exposure to toxins in chickens supplied with AcCa probably caused better digestion and greater absorption of N in the digestive system and better metabolic use, which was reflected in the greater N retention.

It has also been observed that AcCa contains insoluble minerals that dissociate in the presence of hydrochloric acid secreted in the stomach and converted into colloids, soluble and active (Scott *et al.*, 1976); In this way, they contribute to regulating metabolic processes, maintaining the necessary osmotic potential in body fluids, activating enzymes, hormones and antibodies. AcCa minerals form bases with water, reducing surface tension in the digestion and emulsify fats, improving their digestion. Probably, in the present work, the combination of these effects caused that the addition

of AcCa in the chicken diet better intestinal health and better N use (Beker *et al.*, 2004; Miles *et al.*, 2004). In other studies, benefits have been observed in the production and reduction of mortality in chickens supplied with other AcCa sources (Majewska *et al.*, 2009; Majewska *et al.*, 2011).

In phase two, the weight of the S+E mixtures, percentage and weight of the initial and final dry matter were similar between treatments (Table 2). Quadratic responses were observed in the initial ash weight ( $y=1699.6-334.98x+597.64x^2$ ;  $R^2=0.99$ ;  $P<0.05$ ), organic matter ( $y=404.81+282.87x-526.98x^2$ ;  $R^2=0.99$ ;  $P<0.01$ ) and organic carbon ( $y=224.9+157.15x-292.77x^2$ ;  $R^2=0.99$ ;  $P<0.01$ ) respect the increase in AcCa concentrations in the chickens diet. The equations were derived, and it was found that the optimal level of inclusion of AcCa in the chicken's diet to reduce the ash content and increase the matter and organic carbon in the soils was 0.28, 0.27 and 0.27%, respectively. The excreta that contained AcCa Probably conserved a greater quantity of organic compounds during the recollection,

**Table 1.** Productive variables and nitrogen balance in chickens (Phase 1).

	Concentration of activated carbon (%)				SEM <sup>a</sup>
	0	0.15	0.3	0.45	
<b>Productive variables</b>					
Feed consumption, g/d	128.7	132.7	136.5	139.2	4.087
Weight gain, g/d	62.3	68.2	66.9	66.0	2.411
Feed conversion	2.29 <sup>b</sup>	2.11 <sup>c</sup>	2.13 <sup>c</sup>	2.22 <sup>b</sup>	0.062
<b>Dry matter</b>					
Consumption, g/d	141.8	144.0	143.8	143.8	1.540
Excretion, g/d	39.2	40.0	39.4	39.5	1.800
Retention, %	72.4	72.2	72.6	72.5	1.107
<b>Ashes</b>					
Consumption, g/d	7.14	7.23	7.28	7.44	
Excretion, g/d	6.05	6.07	6.24	6.09	
Retention, %	15.30	13.53	13.57	14.72	
<b>Nitrogen</b>					
Consumption, g/d	3.44	3.45	3.44	3.41	0.038
Excretion, g/d	1.51 <sup>e</sup>	1.37 <sup>ef</sup>	1.24 <sup>f</sup>	1.31 <sup>f</sup>	0.060
Retention, g	1.92 <sup>b</sup>	2.08 <sup>cd</sup>	2.20 <sup>d</sup>	2.09 <sup>cd</sup>	0.057
Retention, %	55.59 <sup>e</sup>	60.13 <sup>f</sup>	63.82 <sup>g</sup>	61.17 <sup>f</sup>	1.580
<b>Energy</b>					
Consumption, kcal/d	575.0	583.5	581.0	581.4	6.236
Excretion, kcal/d	138.0	141.6	138.6	139.9	6.506
Retention, %	75.9	75.6	76.0	75.8	0.989
AMEn, Kcal/kg of feed	2958	2945	2948	2944	37.254

<sup>a</sup> Standard error of the mean.  
<sup>b-d</sup> Quadratic effect of AcCa,  $P<0.05$ .  
<sup>e-g</sup> Quadratic effect of AcCa,  $P<0.01$ .

**Table 2.** Initial and final composition of soils in trays fertilized with chicken excreta (Phase 2).

	Concentration of activated carbon (%)				
	0	0.15	0.3	0.45	SEM <sup>a</sup>
Initial weight, g	2228.8	2229.2	2228.0	2225.6	1.873
Initial dry matter, %	94.4	94.2	93.9	94.2	0.203
Initial dry matter, g	2103.7	2100.6	2092.8	2096.1	5.111
Final weight, g	2120.0	2103.2	2099.6	2101.3	6.823
Final dry matter, %	96.2	96.5	96.5	96.5	0.316
Final dry matter, g	2040.3	2029.4	2025.5	2028.8	9.527
Initial ashes, %	80.1	79.1	78.7	79.5	0.423
Initial ashes, g	1699.2 <sup>b</sup>	1664.3 <sup>c</sup>	1651.5 <sup>c</sup>	1670.4 <sup>bc</sup>	9.753
Final ashes, %	84.9	84.8	85.2	85.4	0.381
Final ashes, g	1799.7	1783.4	1789.1	1794.5	10.173
Initial organic matter, g	404.5 <sup>e</sup>	436.4 <sup>f</sup>	441.3 <sup>f</sup>	425.7 <sup>ef</sup>	7.421
Final organic matter, g	308.3	308.5	299.5	296.2	4.123
Initial organic carbon, g	224.7 <sup>e</sup>	242.4 <sup>f</sup>	245.1 <sup>f</sup>	236.5 <sup>ef</sup>	3.547
Final organic carbon, g	171.3	171.4	166.4	164.5	1.970
Initial N, %	0.49	0.52	0.51	0.51	0.012
Initial N, g	10.29	10.98	10.68	10.61	0.252
Final N, %	0.43 <sup>e</sup>	0.47 <sup>f</sup>	0.46 <sup>f</sup>	0.45 <sup>ef</sup>	0.010
Final N, g	8.75 <sup>b</sup>	9.60 <sup>d</sup>	9.22 <sup>cd</sup>	9.09 <sup>bc</sup>	0.177

<sup>a</sup> Standard error of the mean.  
<sup>b-d</sup> Quadratic effect of AcCa, P<0.05.  
<sup>e-f</sup> Quadratic effect of AcCa, P<0.01.

milling and processing in the laboratory, which reflected in a higher content of matter and organic carbon in the initial S+E mixtures. These differences disappear over time since the final weight of the ash, organic matter and organic carbon were similar between treatments.

There were no differences in the percentage and amount of initial N of the soils. Quadratic patterns were found in the final N percentage ( $y=0.4323+0.2834x-0.5692x^2$ ;  $R^2=0.74$ ;  $P<0.01$ ) and in the final N content ( $y=8.8197+5.3316x-10.874x^2$ ;  $R^2=0.70$ ;  $P<0.05$ ) in the S+E mixtures from chickens that consumed diets supplemented with increasing concentrations of AcCa. The inflection point that represented the optimal level of inclusion of AcCa in the diet to maximize the percentage and amount of N recovered in the trays was 0.25 and 0.24% respectively. The foregoing concurs with a previous report, where a reduction in the N loss in the form of NH<sub>3</sub> was found in soils when directly applying AcCa or in soils fertilized with excreta that contained AcCa (Doydora *et al.*, 2011).

Lower NH<sub>3</sub> emissions have also been observed in chicken excreta added with AcCa and composted (Steiner *et al.*, 2010).

In phase three, a lower yield of fresh and dry biomass was found. A lower ash content and amount of N ( $P<0.05$ ) as well as a lower percentage of N ( $P<0.01$ ) in the corn forage obtained from non-fertilized soil compared to the forage harvested from pots fertilized with the excreta of chickens that consumed increasing levels of AcCa was also found (Table 3).

The yield of the fresh and dry biomass and the ash content and amount of N were similar between the treatments with 0 to 0.45% addition of AcCa in the chickens. Only the N percentage showed a cubic pattern between the AcCa levels, the response being lower with the 0% AcCa level, intermediate at the 0.30% AcCa level and high at the 0.15 and 0.45% AcCa levels. ( $y=0.6912+0.5262x-2.8539x^2+4.0158x^3$ ;  $R^2=0.99$ ). The total N content in the pots that only had soil was lower ( $P<0.05$ ) compared to that of

the pots added with the S+E mixtures, but there was no difference in the total N content between the pots that received the S+E mixtures with different AcCa content. The efficiency of total N use, as well as the contribution of N to the pots and forage and the efficiency of N from the S+E mixtures were similar between AcCa levels.

The highest percentage of N obtained in the forage fertilized with excreta from chickens that consumed AcCa coincides with the results of Doydora *et al.* (2011) and Steiner *et al.* (2010), probably due to greater NH<sub>3</sub> to AcCa adsorption, making it later and gradually available to the roots of the plants. In the present study, no differences were found in the yield of green and dry forage due to the addition of AcCa, which does not coincide with other studies where increases in the biomass and root growth have been reported in corn plants produced in soils supplied with AcCa (Clemente *et al.*, 2007; Sabir *et al.*, 2013). However, it should be noted that in phase two there was a greater recovery of N in the S+E mixtures of chickens supplemented with AcCa, with

**Table 3.** Yield and composition of corn forage fertilized with chicken excreta and N use efficiency (Phase 3).

	Soil	Concentration of activated carbon (%)				
		0	0.15	0.3	0.45	SEM <sup>a</sup>
Fresh yield, g	220.0 <sup>b</sup>	326.7 <sup>c</sup>	356.7 <sup>c</sup>	338.3 <sup>c</sup>	306.7 <sup>c</sup>	29.275
Dry matter, %	90.90	90.85	90.96	90.81	91.08	0.115
Dry yield, g	199.9 <sup>b</sup>	296.1 <sup>c</sup>	324.3 <sup>c</sup>	306.5 <sup>c</sup>	279.5 <sup>c</sup>	26.529
Ashes, %	11.23	11.08	10.87	11.46	11.48	0.206
Ashes weight, g	24.73 <sup>b</sup>	36.25 <sup>c</sup>	38.84 <sup>c</sup>	36.74 <sup>c</sup>	34.42 <sup>c</sup>	3.328
Nitrogen, % *	0.65 <sup>b</sup>	0.67 <sup>bc</sup>	0.72 <sup>d</sup>	0.70 <sup>cd</sup>	0.72 <sup>d</sup>	0.017
Nitrogen weight, g	1.31 <sup>e</sup>	2.01 <sup>f</sup>	2.33 <sup>f</sup>	2.11 <sup>f</sup>	2.02 <sup>f</sup>	0.191
<b>Efficiency of nitrogen use</b>						
Total N in soil, g	19.92 <sup>b</sup>	26.37 <sup>c</sup>	27.11 <sup>c</sup>	26.80 <sup>c</sup>	26.67 <sup>c</sup>	0.178
Total N in soil/total N in forage, %	6.57	7.73	8.61	7.57	7.34	0.671
N contributed by the S+E mixture, g	0.00	8.78	8.99	9.10	9.13	0.166
N contributed by the S+E mixture to the forage, g	0.00	0.73	1.03	0.72	0.65	0.180
N contributed by S+E to the soil/ N contributed by S+E to the forage, %	0.00	8.33	11.59	7.91	7.08	2.066

<sup>a</sup> Standard error of the mean.

<sup>b-d</sup> Effect of AcCa vs Soil, P<0.05; \*Cubic effect of AcCa, P<0.05.

<sup>e-f</sup> Effect of AcCa vs Soil, P<0.01.

an inflection point of 0.24%, and in phase three there were increases in the N concentration in soils fertilized with the excreta that contained AcCa. This suggests greater fertility in these soils and greater availability of N over time so that in the long term a greater forage yield would be expected.

The total N balance, taking into account the three evaluated phases, indicates that there is a positive effect on the efficiency of N use considering the integrated animal-soil-plant system, which results in the reduction of the environmental impact caused by the emissions associated with N such as NH<sub>3</sub> and NO<sub>2</sub> through the use of AcCa. However, the high cost of the AcCa source used in the present study is the main limitation for using it as an animal feed. So, it is recommended to look for alternative sources of AcCa. A product with characteristics similar AcCa content is known as biochar

(BC). In the case of BC obtained from chicken excreta, high content of nutrients, especially N and P, compared to other sources of BC has been reported (Agbede *et al.*, 2020; Chan *et al.*, 2008; Gaskin *et al.*, 2008); When the excreta are processed at temperatures of 400 °C, a greater cation exchange capacity has been observed, and with this, greater potential to maintain a neutral pH in the soil, (Gaskin *et al.*, 2008); and greater ability to sequester metal ions (Lima *et al.*, 2009). In the future, it is advisable to carry out more studies evaluating cheaper AcCa sources to optimize the N use in agricultural production systems.

## CONCLUSIONS

Optimal AcCa inclusion levels to maximize feed conversion and N retention in chickens were 0.24 and 0.33%. Additionally, it was possible to retain a greater amount of N in corn forage fertilized with chicken excreta that consumed diets with increasing levels of AcCa, especially, with the level of 0.24%. AcCa can be used to improve the efficiency of nitrogen use in chickens and for nitrogen recycling through the integration of excreta in agricultural soils and its extraction in corn forage.

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# Reproductive evaluation of bucks (*Capra hircus* L.) with usual management in herds from Benito Juarez, Guerrero, Mexico

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## ABSTRACT

**Objective:** To evaluate the reproductive characteristics of male goats (*Capra hircus* L.) with habitual management on their herds at Benito Juarez municipality, Guerrero, Mexico.

**Design/methodology/approach:** Ten male Creole goats from seven herds were evaluated, aged between 2 to 7 years and 2 to 3 body condition (BC). The males were evaluated regarding their sexual behavior, sperm quality, physical examination and reproductive clinical evaluation.

**Results:** All the assessed male Creole goats displayed sexual behavior ( $P < 0.05$ ). However, for the males of three of the evaluated herds the sexual behavior was lower ( $P < 0.05$ ). Of the ten electro-ejaculated males, 20% ejaculated and 80% did not ( $P < 0.05$ ). From the males that ejaculated, a sperm concentration of  $224 \times 10^6$  and  $16 \times 10^6$  sperm  $\text{mL}^{-1}$  was recorded for the first and second male. Finally, the physical and clinical reproductive evaluation concurred with the established parameters for the species.

**Limitations of the study/implications:** Under the conditions in which the study was carried out, it was not possible to evaluate males in the same age conditions and reproductive characteristics. However, this allowed collecting information from field conditions so that producers can apply it for herd improvement.

**Findings/conclusions:** The male Creole goats of the evaluated herds showed intense sexual behavior with low sperm characteristics.

**Keywords:** sexual behavior, sperm quality, clinical examination, herd management.

## INTRODUCTION

Currently, in Mexico, there are around 9 million heads of goat cattle (*Capra hircus* L.), which produces 44 thousand tons of goat meat in carcasses (SIAP, 2018). In this context, the state of Guerrero, Mexico, is the fifth producer of goat cattle for meat with 655,055 heads (SIAP, 2018; Mellado *et al.*, 2012). Goats in their different gestation stages may suffer alterations in the uterine environment that could be due to

undernourishment (Laporte-Broux *et al.*, 2011). Consequently, malnourished females modify their metabolism to survive and distribute nutrients to the placenta, causing the uterine environment in which the embryo and fetus develop to be nutrient deficient (Laporte-Broux *et al.*, 2011). Animals that developing in restricted growth conditions during gestation are at increased risk of health complications, metabolic and reproductive problems (Caton *et al.*, 2019). This phenomenon is known as maternal malnutrition or "fetal programming" (Barker and Osmond, 1986). In sheep and goats, it is known that gene expression related to the growth of the offspring occurs during gestation thanks to the "fetal programming" (Macías-Cruz *et al.*, 2017). Showing damage to the metabolism and the offspring reproduction, in females (primordial follicles/oocytes) and males (Sertoli cells, sperm and testicular size in adulthood) affecting cell proliferation, the reproductive axis and hormonal control (Pedrana *et al.*, 2020).

Consequently, the sexual behavior displayed by males during their adult life directly influences the reproduction of goat herds (Fernández *et al.*, 2011). Indeed, the sexual behavior displayed by males during adult life is important for the reproductive future of the herd (Lacuesta *et al.*, 2018), even more so, if their production system is extensive, where both nutritional and seasonal factors influence (Espinoza-Flores *et al.*, 2020). In the Costa Grande region of Guerrero, there are problems regarding the reproductive, genetic and sanitary management in goat herds. The advice of veterinarians is necessary before bucks' acquisition, to ensure the adequate genetics of the offspring and in this way select bucks which exhibit minimum physical (phenotypic characteristics, jaws, poise and vision) and reproductive (reproductive system, testicles, libido and sperm quality) characteristics.

Also, the knowledge of the place of origin of the bucks, allows one to know the nutritional effects it might have and that may affect their sexual performance. Due to the aforementioned the reproductive characteristics of male Creole goats with habitual management in herds at the municipality of Benito Juarez, in the Costa Grande region of Guerrero, Mexico, were evaluated.

## MATERIALS AND METHODS

All procedures, methods, and animal handling in this research were done following the accepted guidelines for ethical use, care and welfare of research animals at the national (NAM, 2002) and international (FASS, 2010) levels. The study took place during October 2019 at San Jeronimo de Juarez community, Benito Juarez, Guerrero, Mexico (17° 08' 09" N and 100° 28' 08" W). The mean temperature is 26 °C (range 20-32 °C) and 89.93% ambient humidity, registering their highest during the summer months, and lowest during winter months (24 to 30 °C). Precipitation occurs from June to September (1,236 mm) (Uribe and Vázquez-Zavaleta *et al.*, 2017).

### Description of a characteristic herd

In this region, particularly in the Benito Juarez municipality, subsistence goat farming is practiced. Local goat herds have between 25 to 100 animals: including multiparous females (pregnant and empty), bucks, replacement primal females, pubescent males for sale, and kids of both sexes at different

ages. The animal breed is Creole for meat, their main market is local consumers. These herds graze and remain in the field from 12:00 to 19:30 h, during the evening they are kept in open pens until taken to graze again, this practice is carried out throughout the year including the dry season. Goats receive no food supplementation, although some producers provide corn stubble to females that with low weight and low body condition, but never done in pregnant females that are key for good offspring. The pens are built with wood and cyclonic mesh, they have feeders and drinkers out of plastic tubs. Regarding preventive and curative medicine, they are generally dewormed every six months with ivermectin (gastroenteric parasitosis: 1 mL per 24 kg, Ivermectin, Sanfer Laboratory, Mexico City). Additionally, when animals become ill, they receive treatment with broad-spectrum and anti-inflammatory antibiotics. The goat farmers usually have no medical advice. The most common diseases are diarrhea, respiratory problems, pododermatitis, among others.

### Animals and measurements

Ten male Creole goats were assessed in this study, with an age between 2 to 7 years and body condition of 2 to 3 (scale 1 to 4, with increments between units of 0.5; Walkden-Brown *et al.*, 1997). Seven herds of goat cattle were evaluated (R-1, R-2, R-3, R-4, R-5, R-6 and R-7), herds 2, 6 and 7 had two bucks, the rest only one. In male goats, the genital organs (testes, foreskin and penis) were anatomically evaluated. Also, the general appearance of the scrotum for lacerations, wounds, or discoloration was evaluated. Likewise, testicular symmetry was

determined according to the parameters indicated for bovines (Boligon *et al.*, 2010); scrotal circumference was measured from the widest part of the testicles, using a graduated tape measure in centimeters. Subsequently, the sexual behavior was evaluated in a pen constructed of cyclonic mesh at more than 300 m from the herd corral. For this, an estrogenized goat was used, the female was introduced to the pen where the male was, the development of sexual behavior was assessed; Nudging, ano-genital sniffing, flehmen, vocalizations, mounting attempts and mounts with intromission (Ponce *et al.*, 2014).

The evaluations of sexual behavior were made between 8:00 and 9:00 h by two persons previously trained in the evaluation criteria. The goats used to stimulate the male goats were synchronized with CIDR for seven days, 24 h before removing the device, 200 IU of eCG were injected intramuscularly (García y González *et al.*, 2018). Sperm quality was also evaluated: sperm concentration, latency to ejaculate and semen pH; technique described by Jeyendran *et al.* (1984). Additionally, other physiological constants (rectal temperature, heart rate, respiratory rate, capillary return time, ruminal movements and mucous coloration) were measured in the males. Likewise, weight and body condition, state of the locomotor system (displacement-lameness or pain), vision (eyeball injury) and teeth (prognathism and agnathism) were evaluated. Finally, the FAMACHA<sup>®</sup> method was used to compare the mucous membrane color and obtain an estimate of the parasite load.

### Electroejaculation and sperm count

In the evaluated male goats, the semen was collected via electroejaculation following Abril-Sánchez *et al.* (2017). Semen sampling was considered not possible when the male did not ejaculate after ten 3 V pulses followed by ten 4 V pulses, each pulse of a three-second period and rest periods of the same duration. The above, so that the buck was not stressed, since it was the first time, they received this type of management. To calculate the sperm count, a Neubauer chamber was used after the sperm mass was added to 1.0 mL of diluent. The percentage of motile spermatozoa and the quality of the motility were qualitatively evaluated using a 400-x phase-contrast microscope. Additionally, the sperm pH was measured with test strips (range: 6.5 to 10.0) after depositing a drop on the strip, after a ten-second period their color was compared with the scale.

### Feeding and accommodation

The plants that the animals consume in the field are plum (*Spondias purpurea*), hawthorn (*Crataegus*), vines (*Cissus verticillata*) and seasonal fruits such as coconut (*Cocos nucifera*) and mango (*Mangifera indica*).

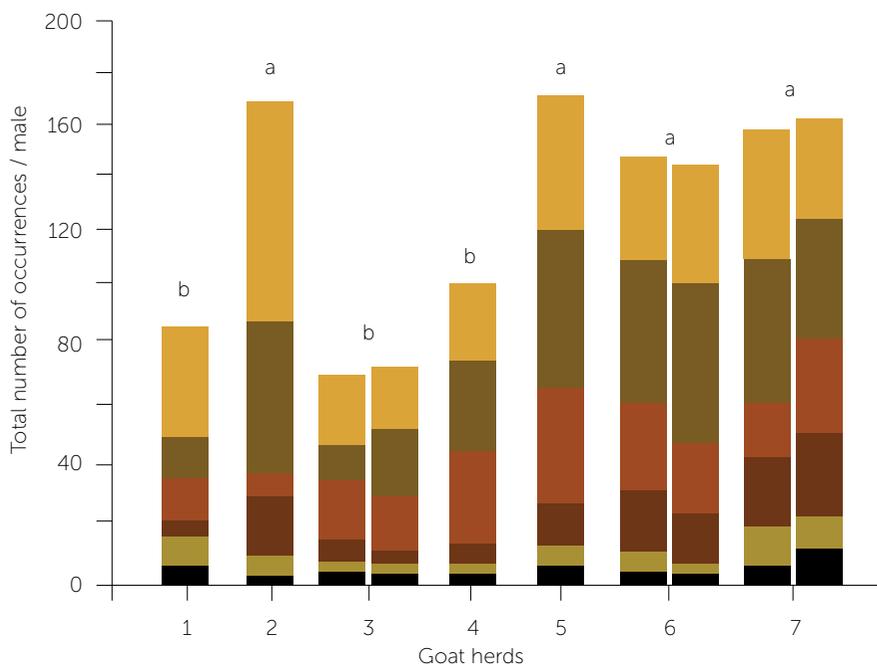
### Statistical analysis and definitions

The data of the sexual behavior of the males: Nudging, flehmen, vocalizations, mounting attempts and mounts with intromission were analyzed with the Friedman test for non-parametric statistics. Subsequently, a Wilcoxon test was used to compare two by two. Likewise, descriptive statistics (percentages, means and  $\pm$  standard error from the mean) were used to analyze the data on the sperm quality, physical and clinical reproductive diagnosis of the bucks. All data were analyzed with the SAS (2004) statistical software. The abbreviations of concepts and variables used in the study are also described: international units (IU), hydrogen potential (pH), standard error from the mean (sem), centimeters (cm), body condition (BC), testicular circumference (TC), right testicular depth (RTD), left testicular depth (LTD), foreskin length (FL), respiratory rate (RR), heart rate (HR), rectal temperature (RT), capillary return (CR), breaths per minute (BPM) and beats per minute (bpm).

## RESULTS AND DISCUSSION

All the evaluated male goats presented sexual behavior: Nudging, flehmen, vocalizations, ano-genital sniffing, mounting attempts and mounts with intromission ( $P < 0.05$ ). However, 40% of the evaluated male goats (4 males from 3 herds: R-1, R-3 and R-4) presented low sexual behavior ( $P < 0.05$ ; Figure 1).

There are available studies in the literature regard male goats from tropical regions. These mentions that they present sexual activity throughout the year (De-Combellas, 1993; Ahmed *et al.*, 1997). Other studies affirm that male goats from tropical regions such as those evaluated here have a period in which their sexual behavior decreases, this phenomenon observed when the environmental temperatures increase, there is the presence of rains and a decrease in forage availability (Chemineau *et al.*, 1986; Ponce *et al.*, 2019). Similar events to that observed in males originated or adapted to temperate and subtropical regions, where these animals have a sexual rest period due to the photoperiod (Zamiri and Haidari, 2006).



**Figure 1.** Sexual behavior of male goats: Nudging (■), ano-genital sniffing (■), flehmen (■), vocalizations (■), mounting attempts (■) and mounts with intromission (■) (different colour within bar implies each behaviour). The "Y" axis represents the scale of the total number of occurrences per male and the "X" axis the number of herds where the males came from.

In the present study, all males exhibited sexual behavior; however, males from three herds showed low sexual behavior. This was due to the fact that the males presented claudication problems due to interdigital pododermatitis and the pain possibly prevented them to display sexual behavior like the rest of the males.

From the total number of electro-ejaculated male goats, 20% ejaculated, and 80% did not. Of the males that ejaculated (volume of 1 mL per male), a sperm concentration of  $224 \times 10^6 \text{ mL}^{-1}$  was found in the first male and  $16 \times 10^6$  in the second, the latency to ejaculate was 5 min. Sperm motility was 80% in the first male and 40% in the second. Finally, the semen pH was seven in one male and eight in the other. The ejaculation of the evaluated males was carried out in October when at the end of the rainy season and the beginning of the forage availability; however, the sperm quality of the males was lower ( $224 \times 10^6$  sperm  $\text{mL}^{-1}$  and  $16 \times 10^6$  sperm  $\text{mL}^{-1}$ ) than that established for this species ( $2,500$  to  $3,000 \times 10^6$  sperm  $\text{mL}^{-1}$ ) (Hernández-Corredor *et al.*, 2018). This can be explained in the following way (first) the males were recovering from the dry season where there was little available food, followed by the moment when the males were ejaculated, who had finished the riding period, and finally, for a male to ejaculate, previous

training is needed to reduce their stress level.

It is to be noted that the present study took place under normal management conditions in local goat herds, so it is not possible to control when they mate, and if there are some diseases such as interdigital pododermatitis, which are common during the rainy season, they affect their health and impact their reproductive performance; However, some recommendations may be offered to goat farmers to improve the habitual management of their herds, to improve the reproductive and productive performance of females and male goats. The reproductive evaluation of the ten male goats: TC ( $26.5 \pm 8.7$  cm), LTD ( $19.7 \pm 0.9$  cm), RTD ( $20 \pm 0.7$  cm) and FL ( $16.6 \pm 0.7$  cm) were similar between the evaluated males according to the species parameters (Table 1).

In this regard, a RR ( $22.2 \pm 3.7$  rpm), HR ( $60.7 \pm 9.3$  bpm) and RT ( $38.3 \pm 0.4$  °C) were within normal physiological parameters. The mucous membranes: bulbar and palpebral, buccal, nasal; were found to be normal among the evaluated males and there were no alterations in capillary return (Table 2).

According to the literature for small ruminants and cattle, the physical examination and reproductive evaluation include the external genitalia: testicles, foreskin, glans; in the present study, normal values were found to what was established for the species (Espitia-Pacheco *et al.*, 2018). However, some bodily characteristics such as the general condition of the animals: dull and bristly hair, watery eyes, presence of mucus in the nasal cavity, dirt in

**Table 1.** Body condition and reproductive variables of male goats under grazing conditions (mean  $\pm$  standard error from the mean).

Males	BC	TC	RTD	LTD	FL
10	$3.3 \pm 0.8$	$26.5 \pm 8.7$	$20 \pm 0.7$	$19.7 \pm 0.9$	$16.6 \pm 0.7$

Body condition (BC): scale 1 to 4. Testicular circumference: TC (centimeters). Right testicular depth: RTD (centimeters). Left testicular depth: LTD (centimeters). Foreskin length: FL (centimeters).

the hairs of the foreskin can alter the physical and reproductive health of the animals (De Celis et al., 1996). Therefore, even when the physiological constants and reproductive variables evaluated were normal, this situation could have adverse effects and consequently on the reproductive performance of the bucks and affect their herds. This situation provides the guideline for technical advice to the goat farmers. Also, it was recorded that some pregnant females had low weight and body condition, also, some offspring, especially primal females, presented general deterioration, watery eyes and shaggy hair. This is most likely because pregnant females are not supplemented to obtain the nutritional requirements according to their gestation stage, leading to health and reproductive problems in the offspring.

## CONCLUSIONS

The male Creole goats from herds at Benito Juarez municipality extensively grazed showed intense sexual behavior, although this was lower in the males of three of the assessed herds. Of all the males, only two were able to ejaculate and their sperm concentration was low. All males had normal physiological constants and in the reproductive clinical evaluation were found within the established normal parameters for this species.

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**Table 2.** The FAMACHA® technique and the physiological variables in male goats (mean  $\pm$  s.d.m).

Males	Value	FAMACHA®	RT	HR	TR	CR
10	Normal <sup>1</sup>	1 a 5	12 a 20	70 a 80	38.6	2 a 3
	Registered	4.0 $\pm$ 0.18	22.2 $\pm$ 3.7	60.7 $\pm$ 9.3	38.3 $\pm$ 0.4	2.3 $\pm$ 0.48

Respiratory rate: RT/min. Heart rate: HR/min. Rectal temperature: RT/min. Capillary return: CR/sec. FAMACHA®: 1 to 5 points. <sup>1</sup>DERBYSHIRE JB. 1967. The goat "*Capra hircus* L." In: The UFAU Handbook on the care and management of laboratory animals. 3rd Ed. E.S. Livingstone Ltd. Edinburg and London.

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# Analysis of the beef supply in Veracruz, Jalisco and Chiapas states, Mexico, 2000-2019

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## ABSTRACT

**Objective:** to determine the effect of the variables that impact the supply of beef in Veracruz, Jalisco and Chiapas states, Mexico, from 2000 to 2019.

**Methodological design/approach:** a multiple linear regression model was used; where the supply was the dependent variable and the price of beef, corn price and annual rainfall were the explanatory variables.

**Results:** the dynamics of the beef production in Veracruz, Jalisco and Chiapas were directly and inelastically explained by its price with a value of 0.89, 0.13 and 0.49; inversely and inelastically by the price of corn ( $-0.05$ ,  $-0.005$  and  $-0.05$ ) and directly and inelastically by the state annual precipitation (0.16, 0.01 and 0.21).

**Study limitations/implications:** it is suggested to test the statistical and economic significance with the Cobb-Douglas supply models to contrast their elasticities.

**Findings/conclusions:** the variable that explained the dynamics of bovine production in these Mexican states was the price of the product, while the price of corn was the one with the least impact.

**Keywords:** cattle, elasticities, price, econometric model, production

## INTRODUCTION

**Protein** of animal origin is basic in people's diets; the main sources are poultry, beef and pork. In 2019, Mexico produced 7.22 million tons (mt) in carcass of these species, 48.11% poultry meat, 28.06% cattle, 22.14% to swine and the rest 1.69% to sheep, goats and turkeys (SIAP, 2020). Beef meat is the muscle tissue, accompanied or not by connective tissue, bone and fat, as well as nerve fibers, lymphatic vessels, which come from slaughter animals (CIMA, 2018).

In Mexico, beef is one of the most significant consumed meat due to its high protein value and its social and economic importance (Puebla *et al.*, 2018) and is considered the second most important productive activity, from the productive point of view (SIAP, 2020); however, the productive structure in this sector has undergone substantive modifications at the regional level. From 2000 to 2019, the national volume of beef registered an average annual growth rate (TCMA) of 2.06%, going from 1.40 mt in 2000 to 2.02 mt in 2019 (SIAP, 2020).

(BP) for heteroscedasticity, which showed the results output of each of the models.

The economic significance was carried out taking into account the signs and magnitude of the coefficients of the fixed variables of the estimated models, interpreted with respect to the micro economic theory; that is, the relation between the supplied quantity, and the beef carcass price, as well as the supply with rainfall, which must be direct; while with the price of corn, the relationship should be inverse.

In some regions, the corn price and precipitation variables lagged one or two years because the producers do not immediately react to a change in prices or fluctuations in the rainfall in the study areas, by decreasing or increase production. Nonetheless, also to factors such as the duration of the productive cycle of the animals, the degree of investment, the production volume and financial situation of the trade, so this procedure is different in the different producing areas of the country (Puebla *et al.*, 2018), because in the market, in this case, the response of supply to changes in its determining factors is rarely instantaneous.

This is even more evident for the case of the supply of agricultural products, in which, due to the biological process, they need a period for their production. They often respond after a certain time, a period that called "lag" or "delay" (Gujarati and Porter, 2010). The cited model assumed that some of the exogenous variables are influenced by one or two lag periods, which was statistically justified based on its individual significance.

The dynamism of bovine production in Mexico showed disparities among the States that comprise it (Puebla *et al.*, 2018). In 2019, the region (entities) of Veracruz, participated with 13.02% (208.13 thousand t); Jalisco contributed 11.82% (188.94 thousand t) and Chiapas contributed 5.23% (83.60 thousand t); together, these three regions contributed 30.08% of the national production (SIAP, 2020).

Therefore, the beef production behavior among the aforementioned entities in Mexico was different. The objective here was to determine the effect of the variables that impacted on the beef supply in Veracruz, Jalisco and Chiapas states, México, during the year 2000 to 2019, in order to generate recommendations that allow the design of strategies to support beef production in a regional scope. The main hypothesis indicates that the supply of beef in the three states of Mexico responds positive and inelastically to the price of the product, is negative and inelastic to the price of its input, and positive and inelastic to rainfall.

## MATERIALS AND METHODS

The supply of carcass beef was analysed in the states of Veracruz (Ver), Jalisco (Jal) and Chiapas (Chis), Mexico, from 2000 to 2019, as these are the most important entities to produce this meat, which represent little more than 30% of the national volume.

For this, a multiple linear regression econometric model (MLR) was specified for each region in which three fixed, independent or predetermined variables were included as determinants for the supply of this meat product, in addition to the delayed dependent variable as conclusive or exogenous of the current offer for the case of Jal and Chis states.

In the estimation of the model, cross-sectional secondary information was used from official sources in Mexico, such as SIAP (2020), Fideicomisos Instituidos en Relación a la Agricultura (FIRA) (FIRA, 2020) and the Sistema Nacional de Información e Integración de Mercados (SNIIM) (SNIIM, 2020) (SNIIM, 2020).

The monetary variables were deflated with the Índice Nacional de Precios al Productor (INPP) base 2019=100 (INEGI, 2020).

The estimation of the value of the model parameters associated with the supply function was performed *via* the Ordinary Least Squares (OLS) method (Gujarati and Porter, 2010) in the SAS statistical software (Statistical Analysis System) version 9.1.3 (SAS, 2003).

The statistical congruence of the supply models was determined with the adjusted  $R^2$ -coefficient of determination. The global statistical significance of the models was assessed with the value of the calculated-F and the individual significance of each coefficient linked to each predetermined variable performed with the Student's t test or the "t ratio". Results of the statistical tests of the Durbin-Watson (DW) were added for the autocorrelation degree, White for multicollinearity and Breusch-Pagan

The economic elasticities of each of the explanatory variables that determined the beef supply in each of the studied regions were also calculated, through the observed average of the period and evaluated according to the sign and magnitude of their coefficients (Nicholson and Snyder, 2015; Parkin and Loria 2015; Rebollar et al., 2019). The statistical specification of the econometric models to determine the behavior of the beef supply in the evaluated states was:

$$BMO_t = \beta_{11} + \beta_{12} BRP_t + \beta_{13} PRM_t + \beta_{14} MP_{t-1} + E_t \text{ (Veracruz)}$$

$$BMO_t = \beta_{21} + \beta_{22} BRP_t + \beta_{23} PRM_t + \beta_{24} MP_{t-1} + \beta_{25} BMO_{t+1} + E_t \text{ (Jalisco)}$$

$$BMO_t = \beta_{31} + \beta_{32} BRP_t + \beta_{33} PRM_{t-2} + \beta_{34} MP_{t-2} + \beta_{35} BMO_{t+1} + E_t \text{ (Chiapas)}$$

Where  $BMO_t$ : Supply of beef carcass during the study period, approximate to the state production of this product, figures in tons (t);  $BRP_t$ : real price of beef carcass, in pesos per ton (\$/t);  $BRP_{t-2}$ : real price of beef carcass, with a two-year lag in pesos per ton (\$/t);  $MP_t$ : mean rainfall in millimeters during the current period (millimeters);  $MP_{t-1}$ : one-year lag rainfall in millimeters (thousand);  $MP_{t-2}$ : two-year lag of rainfall in millimeters (thousand);  $BMO_{t-1}$ : one year lag state beef supply, figures in tons.

Regarding the prices of beef and corn, the first was determined through the real price of the product in carcass. For the price of feed, the real price of corn (grain) was considered as the main component in the diet of cattle (Puebla et al., 2018).

To calculate the elasticity of supply value related to each of the explanatory variables, the coefficient of the partial derivative of the estimated model was multiplied by the average observed value of each of the independent variables with respect to the supplied quantities (Gujarati and Porter, 2010; Guzmán et al., 2012; Rebollar et al., 2019). Considering that the linear supply function has a variable elasticity in its estimation range; For this reason, it was determined for the average of the analyzed period (Sheperd, 2006; Puebla et al. 2018; Rebollar et al., 2019); and with the above, the effects established in the functional relationships were quantified.

## RESULTS AND DISCUSSION

The results obtained from the supply models estimated in their linear form in each of the three states (entities) of Mexico, are presented in Table 1.

The MLR models estimated for the supply of beef in Ver, Jal and Chis, period 2000-2019, were:

$$BMO_t = -24.764 + 1.179 BRP_t - 0.940 PRM_t + 8.80 MP_{t-1} \text{ (Veracruz)}$$

$$BMO_t = -9.613 + 0.372 BRP_t - 0.191 PRM_t + 3.296 MP_t + 0.795 PB_{t-1} \text{ (Jalisco)}$$

$$BMO_t = -24.907 + 0.717 BRP_t - 0.932 PRM_{t-2} + 8.664 MP_{t-2} + 0.270 PB_{t-1} \text{ (Chiapas)}$$

The goodness of fit, given by the adjusted coefficient of determination  $R^2$ , was 0.86 (Ver), 0.98 (Jal) and 0.88 (Chis); which means that, of all the source of total variation of the estimated models, 86%, 98% and 88%, was explained by the exogenous variables included in them. The difference to the 100% was due to other variables that were not included in this research.

With respect to the value of the calculated-F statistic ( $F_c$ ), for a total of  $n=20$  observations, the models were statistically significant with values of 39.03, 179.40 and 30.75, (Table 1). Therefore, statistically, there was no beta equal to zero when performing the hypothesis test at any level of reliability. As a whole, all the exogenous variables have the capacity to explain the behavior of the dependent or explained variable (beef supply in Ver, Jal and Chis, México).

In all the explanatory variables, the value of the calculated t ( $t_c$ ), associated with each estimator, was greater than the unit, a scenario that indicates that the value of the estimated parameter is greater than twice its standard error (Brigham and Pappas, 1992; Pérez et al., 2010); favorable situation from the point of view of the efficiency of the results that emerge from it.

Under this argument, all the coefficients of the independent variables were statistically significant and their signs and congruent with the microeconomic theory in terms of the law of supply (the direct relationship with the price of the product, inverse with the price of the input and direct with rainfall).



**Table 1.** Estimated coefficients for the supply of beef carcass in Veracruz, Jalisco and Chiapas states, Mexico, 2000-2019.

Region	Dependent variable	Intercept	Explanatory variables				R <sup>2</sup> adj	Prob>F
Veracruz	OCB <sub>t</sub>		PRB <sub>t</sub>	PRM <sub>t</sub>	PP <sub>t-1</sub>		0.86	0.0001
	Coefficient	-24.764	1.179	-0.940	8.809			
	SE	10.444	0.368	0.269	3.764			
	t value	-2.370	3.210	-3.500	2.340			
	DW	1.775						
	BP	4.420						
	White	5.030						
	F value	39.030						
Jalisco	OCB <sub>t</sub>		PRB <sub>t</sub>	PRM <sub>t</sub>	PP <sub>t</sub>	OCB <sub>t-1</sub>	0.98	0.0001
	Coefficient	-9.613	0.372	-0.191	3.296	0.795		
	SE	3.382	0.104	0.097	1.208	0.101		
	t value	-2.840	3.570	-1.970	2.730	7.840		
	DW	2.008						
	BP	6.000						
	White	6.620						
	F value	179.400						
Chiapas	OCB <sub>t</sub>		PRB <sub>t</sub>	PRM <sub>t-2</sub>	PP <sub>t-2</sub>	PB <sub>t-1</sub>	0.88	0.0001
	Coefficient	-24.907	0.717	-0.932	8.664	0.270		
	SE	6.905	0.294	0.249	2.405	0.178		
	t value	-3.610	2.440	-3.740	3.300	1.510		
	DW	2.388						
	BP	4.710						
	White	9.690						
	F value	30.750						

Source: own elaboration, based on SAS output. SE: standar error. BP: Breusch-Pagan. DW: Durbin-Watson.

The DW statistic indicated a low level of autocorrelation between the time series, due to the nature from which the information came; while the result of the BP and White calculated in the three models indicated the absence of heteroscedasticity between the explanatory variables (Gujarati and Porter, 2010), and based on the results of the three models in terms of the adjusted R<sup>2</sup>, the Fc, the value of the standard error and the tc was indicative of no evidence of multicollinearity between the series (Gujarati and Porter, 2010), so the unbiased and efficiency properties of the OLS estimators are present.

Under the microeconomic theory approach, the estimated models were significant, because the expected signs in each of the estimators were congruent. In other words, a positive sign was expected in the price of beef and precipitation and a negative sign for the price of corn (input price).

Thus, for the assessed period, in Veracruz, Jalisco and Chiapas, the supply of beef was explained both by the behavior of the price received by the producer, as well as by the behavior of the price of corn grain and the annual rainfall.

**Economic analysis: elasticities**

The supply of any good, is overall, a function of the price of the product, the inputs price, the climate, as well as the technology (Salvatore, 1977). In each of the three estimated MLR models, the elasticity value related to its explanatory variable (Table 2), required the usage of the series' average; for example, for Jalisco, the beef carcass price was \$64,869.61/t (Mexican pesos per ton); for corn grain \$4,409.25/t and rainfall of 823.90 millimeters (thousand), when replacing these values in the estimated supply model, the result was 182,030.64 t of beef carcass.

With the above information, for Jalisco (Jal) the price elasticity of the beef supply was:

$$\varepsilon_p^o = (0.372) \left( \frac{64869.61}{182030.64} \right) = 0.132$$

Similarly, for the same variable in Ver and Chis, and the fixed of corn price, rainfall, and production delay variables.

For the 2000-2019 period, the beef supply in Ver, Jal and Chis, México, was explained by the behavior that the price received by the meat producers of this species, due to the price of feed (corn grain), rainfall and the delayed production variable in the case of Jal and Chis.

In the LRM model, for Ver, the intercept term ( $-24,764$ ) lacks of economic interpretation or is of secondary importance (Gujarati and Porter, 2010), as in Jal and Chis, because it is not possible to understand the supply of beef in such a quantity (in this case negative supply associated with the intercept with a negative sign), when all the explanatory variables occupy a value of zero; analogous to that reported by Rebollar et al. (2008) on a response function in sheep from the south Estado de Mexico, México; however, 1,179 (Table 1), which is the assigned coefficient to the price of beef in Ver, expresses that during the analysis period, for every thousand Mexican pesos, as a monetary unit (US\$50.00), in increase price for the meat producer (*ceteris paribus*=everything else constant), the quantity of supplied beef is expected to increase by 1,179 t.

Consequently, for every thousand pesos in which the price of a ton of corn (food price) increases (*ceteris paribus*), it is expected that the state supply of beef will decrease by 940 t. Additionally, for each increase unit in rainfall (*ceteris paribus*), it is expected that the quantity of offered beef will be reflected in an increase of 8,809 t of that meat.

In the case of Jal, for every thousand pesos increase in the price for the beef producers, the supply in that entity is expected to increase by 372 t and an inverse effect on the price

of corn (feed price) (*ceteris paribus*), the supply of beef is expected to distance itself by 191 t. Likewise, for each unit of increase in rainfall (*ceteris paribus*), the quantity of offered beef shows a 795 t increase.

Regarding Chis, for every thousand pesos of increase in the price of the product, the quantity of beef offered will increase by 717 t; whereas, due to the increase of one thousand pesos in the corn price, the supply will decrease by 932 t and, as rainfall increases by one unit, the quantity of supplied beef will be increase in 8.66 t (Table 1). Elasticity, by itself, does not help (Nicholson and Snyder 2015; Parkin and Loría 2015), but when relating it to variables that explain a certain market, then it has attractive interpretations (Vázquez and Martínez, 2015).

In its theoretical form, the term elasticity states how sensitive a dependent variable is to unit percentage variations of the predetermined variable. Consequently, the price elasticity of supply; in this case, for beef in Ver, Jal and Chis, México, can be elastic (greater than unity), inelastic (whose value is between zero and one) or unitary (equal to one). When the elasticity is unitary, it means that the percentage increases in prices of the good generate percentage increases in the supplied quantities of this in the same proportion. If the magnitude is less than 1.0, the elasticity is said to be inelastic and its effect is that the quantities supplied react little to changes in the price of the product. The values of the elasticities of the price of supply of a greater than one magnitude reveal that the percentage changes in prices have a more than proportional impact on the good's supply; in this case it is stated that the elasticities are high, and the supply is elastic.

**Table 2.** Calculated elasticities for beef carcass in Veracruz, Jalisco and Chiapas, México, 2000-2019.

Elasticity	Regions		
	Ver	Jal	Chis
Supply			
PRB <sub>t</sub>	0.895	0.132	0.496
PRM <sub>t</sub>	-0.057	-0.005	-
PRM <sub>t-2</sub>	-	-	-0.053
PP <sub>t</sub>	-	0.015	-
PP <sub>t-1</sub>	0.163	-	-
PP <sub>t-2</sub>	-	-	0.212
PB <sub>t-1</sub>	-	0.857	0.349

Source: calculations based on the results of the estimated model.

When analyzing the information in Table 2, the price to the beef producer in Ver, Jal and Chis, México, had an inelastic effect during the evaluated period, because increases of 1% in the price of this meat increase the quantity offered of the meat product by less than 1% (0.89, 0.13 and 0.49); the quantity of beef offered responds less than proportionally to an unit increase in its price; However, the greatest effect of the changes in the price of the product was in Ver (0.89) and the least impact in Jal



(0.13). Research related to this finding was not found sufficiently; however, such a result is consistent with those reported by other researchers such as Castro *et al.* (2019) (0.38); Puebla *et al.* (2018) (0.06); Vázquez and Martínez (2015) (0.67); Cruz and García (2014) (0.34); Ramírez *et al.* (2011) (0.03); Benítez *et al.* (2010) (0.12), confirmed positive and inelastic values of the beef supply related to its price. The response of beef with respect to the expected price of corn (as the main feed component) responded in an inverse and inelastic way. Although it is an important input for meat production of this livestock species, the dynamics in its price does not significantly affect its production in the aforementioned entities; however, the results are similar to those reported by Castro *et al.* (2019) (-1.47), Puebla *et al.* (2018) (-0.07), and Cruz and García (2014) (-0.33). However, the elasticity magnitude varies between the different studies because they evaluate different periods; however, the reverse character remains.

The response of beef in the three evaluated states to the effect of their mean annual precipitation was direct and inelastic (0.16, 0.01 and 0.21); therefore, their unit percentage increases would mean increases of less than one percentage unit in the beef supply. Such results concur with those by Puebla *et al.* (2018), where during 1994-2013 period, obtained an elasticity for the supply of beef in different regions of Mexico in relation to the precipitation of 0.06.

The response to the supply of beef in Jal and Chis, regarding the supply delay in one period, was positive and inelastic; that is to say, knowing the behavior of the supply of the previous year has tangible effects of less than one percentage unit on the current supply of beef.

## CONCLUSIONS

The elasticities of the supply of carcass meat in Ver, Jal and Chis, Mexico, in relation to each of the explanatory variables, showed that the factor that most influenced the growth of bovine production was the price of the product, with highest effect in Ver, followed by the annual state precipitation, accentuated in the state of Chiapas. The price of corn in the production of the evaluated species was not appreciable.

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# Analysis of the invasion of water lilies (*Eichhornia crassipes* (Mart) Solms) in the Cointzio dam, Michoacán, Mexico

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## ABSTRACT

**Objective:** to analyze the growth dynamics of the water lily (*Eichhornia crassipes*) in the Cointzio dam due to the water availability.

**Design/Methodology/Approach:** a monthly series of 45 Sentinel 2 and Landsat satellite images were used, with which the percentage of invasion of the water lily in the total area of the reservoir was calculated. The relative coverage of the lily was analyzed looking for growth patterns over time, as well as the precipitation data, total reservoir area over time, and water storage data for the 2010-2020 period to broaden its context.

**Results:** Three ascending and two descending patterns of the water lily coverage were identified; a 15-month upward growth pattern with slight inland slope changes; decreasing patterns are associated with decreased water storage. The largest lily covers were found in critical storage stages during the 2010-2020 period.

**Study limitations/implications:** faced with climate change conditions, the filling pattern of the dam could change, aggravating problems related to the water supply.

**Conclusions:** containment/mitigation efforts have a reduced effect because the lily easily recovers the covered area, growing about 400% between October 2017 and June 2018, therefore it is necessary to implement a containment strategy using the biophysical interactions of the basin in conjunction with social, political, economic and governance interactions.

**Keywords:** water management, social-ecological systems, available water, remote sensing, socio-hydrology.

## INTRODUCTION

The growth of large population centers requires actions to provide basic services, so guaranteeing access to freshwater is one of humanity's greatest challenges (Mekonnen & Hoekstra, 2016; Vollmer *et al.*, 2018). In Mexico, the most frequent water sources for domestic use are deep wells (Ríos, Prado-Hernández, Romero-Bautista, Reyes-López, & Pascual-Ramírez, 2019), to a lesser extent surface

water is used through the construction of warehousing structures. Surface water reservoirs face problems due to the degradation of their catchment basins, which facilitates the spread of aquatic plant species that alter the functioning of the ecosystems; One of these species is the water lily (*Eichhornia crassipes*), which easily adapts to various environmental conditions due to its capacity to absorb nutrients (Ting, Tan, Salleh, & Wahab, 2018). This causes damages such as water loss, flow retardation, obstruction of pipeline infrastructure, health hazards due to accumulation of human disease vectors and alteration of the physical-chemical characteristics of water and hydraulic soil (Ali & El-Din Khedr, 2018); Furthermore, it decreases the available oxygen in the water and reduces sunlight passage which that causes the death of organisms in the aquatic ecosystems (Keller & Lodge, 2009).

The water lily reproduces both, sexually and asexually. The first has high success levels, generates up to 2,534 seeds per m<sup>2</sup> and their germination rate is 54.17% in a maximum period of three days (Gunnarsson & Petersen, 2007; Thamaga & Dube, 2018), even despite the high flowers proportion lost by the species that inhabit the environment in which it develops (Albano Pérez, Coetzee, Ruiz Téllez, & Hill, 2011). Regard their asexual reproduction, it can double its biomass within two weeks; Thus, 10 plants can in eight months produce 655,360 new plants, reaching a surface area of half a hectare (Thamaga & Dube, 2018). Therefore, the objective of this work was to analyze the growth dynamics of the water lily in the Cointzio dam in Michoacán, Mexico, whose use is to supply water for agricultural and domestic use in the city of Morelia.

### MATERIALS AND METHODS

The research was carried out in the Cointzio dam, north of the state of Michoacán (19° 24' 30" and 19° 38' 30" N and 101° 08' 30" and 101° 33' 00" W). The dam has a 300 m long crown 8 m wide, 46 m high at the curtain, with an water mirror extension of 500 ha, and a useful storage volume of 65.61 h m<sup>3-1</sup> (CONAGUA, 2018). The meteorological data from the observatory 16081 'Morelia' of the National Meteorological Service (SMN, 2020), reports annual precipitation of 795.6 mm, mean annual temperature

of 19 °C, mean minimum temperature of 10.4 °C and average maximum temperature of 27.5 °C. A set of 45 satellite images from November 2016 to July 2020 (Table 1) were used, these were obtained by the Sentinel-2B (MSI sensor) and the Landsat 8 (OLI sensor) satellites. The images from February 22 and August 17, 2017, as well as February 24, August 23 and December 13, 2019, were Landsat (Table 1).

The satellite images, with radiometric and geometric corrections, were downloaded from their websites. The QGIS 3 software was used to delimit the dam reservoir based on the water area. The Semi-Automatic Classification Plugin tool classified the areas of water and aquatic vegetation, these were rectified using spectral patterns associated with water and vegetation (Pascual Ramírez *et al.*, 2010). After classification, the data matrices were converted to vector format to calculate the area and percentage of invasion of the water lily in the reservoir.

Using linear regression techniques, explanatory patterns of the progress of lily coverage in the dam were sought, using the determination coefficient and the slope of the line, as fit descriptors. The total coverage area of the dam over time and the reported precipitation values were analyzed; The data of the storage volumes and water extraction of the dam during the 2010-2020 period were also used (CONAGUA, 2020).

### RESULTS AND DISCUSSION

The minimum coverage of the lily in the dam vessel was during November 2016 (Table 2), a similar value was recorded during April 2017 (2.15%). It is worrying the number of months during which the lily coverage exceeded 50%, such as the period from June to August 2018 and, during 2019, March, July, August and October;

**Table 1.** Satellite images used.

Date	Date	Date	Date	Date
11/11/2016	17/08/2017	30/05/2018	24/02/2019	16/11/2019
21/12/2016	12/09/2017	24/06/2018	26/03/2019	13/12/2019
10/01/2017	27/10/2017	19/07/2018	20/04/2019	25/01/2020
22/02/2017	16/11/2017	18/08/2018	15/05/2019	24/02/2020
10/03/2017	26/12/2017	22/09/2018	14/06/2019	25/03/2020
30/04/2017	15/01/2018	07/10/2018	09/07/2019	24/04/2020
15/05/2017	04/02/2018	28/11/2018	16/08/2019	25/05/2020
09/06/2017	16/03/2018	21/12/2018	27/09/2019	15/06/2020
19/07/2017	30/04/2018	20/01/2019	07/10/2019	13/07/2020

**Table 2.** Relative lily coverage in the dam.

Month	2016	2017	2018	2019	2020
January		10.36	36.12	43.12	35.63
February		16.16	42.61	45.56	39.27
March		10.39	35.77	50.61	35.00
April		2.15	34.83	49.17	32.26
May		9.56	46.39	43.94	25.00
June		5.46	56.19	42.85	20.52
July		4.42	50.00	50.00	33.05
August		15.50	51.21	51.00	
September		13.19	37.08	49.50	
October		12.00	46.76	50.00	
November	2.10	19.41	43.57	49.00	
December	5.56	34.32	44.31	46.68	

From all cases, the highest was during June 2018, when the lily coverage reached 56.19% of the reservoir surface. If coverage is considered greater than or equal to 40%, then, the entire 2019 was above this threshold; while, during 2018, only three months registered values below 40% (March, April and September), with values (35%) very close to the proposed threshold.

In fact, the annual average coverage during 2018 and 2019, exceeded 40%, which in ecological terms is worrying, since it damage the freshwater fauna by limiting oxygen availability for vital activities (Keller & Lodge, 2009), which affects fishing and recreational activities.

The results of the relative lily coverage were plotted on a continuum throughout the 45 months of the analysis, together with the precipitation data from the beginning of the assessment until September 2019 (Figure 1). Three growth regions two of decreased coverage were located, the first growth series began in November 2016 and lasted until February 2017, where a linear adjustment was made, with an  $R^2$  of 0.99 and a slope of 4.7. As of February, a decrease was observed, due to containment and mitigation practices that led, together with the decrease in storage volume, to a minimum, observed in April.

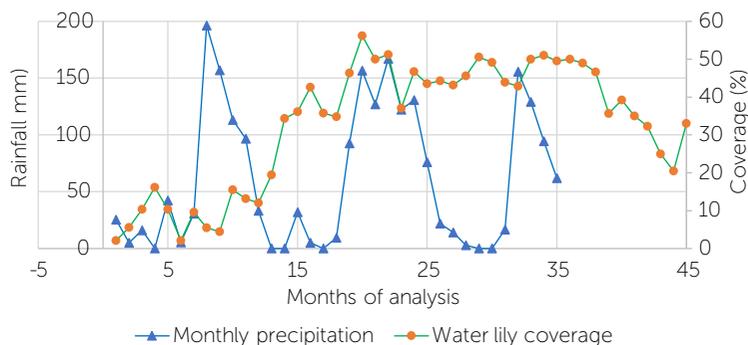
The second growth series is the most alarming, it began in October 2017 and lasts until February 2018, these five months of uninterrupted growth caused high levels of coverage and that stabilized in their upper part, with a linear  $R^2$  adjustment of 0.94 and a 7.79 slope, which denotes the intense growth in the area occupied by lily pads during that

period. There is another growth period over the previous one from March to June 2018, which adjusts to a line with an  $R^2$  0.87 and slope of 7.28. These two growth periods can be coupled in one, from October 2017 to June 2018, adjusted to a line with  $R^2$  of 0.79 and slope of 4.31. A period of intense growth that goes from April 2017 to June 2018 is noticeable, with slight changes in its slope between July and October 2017, and April 2018; This prolonged growth from the beginning to the end of the analysis period can be adjusted to a line with  $R^2$  of 0.9 and slope of 0.65. The stated above highlights the importance of the mitigation and containment practices of the lily in the dam, since, when left, in short time it reaches high coverage rates due to its reproductive success, both sexual and asexual (Gunnarsson & Petersen, 2007; Thamaga & Dube, 2018).

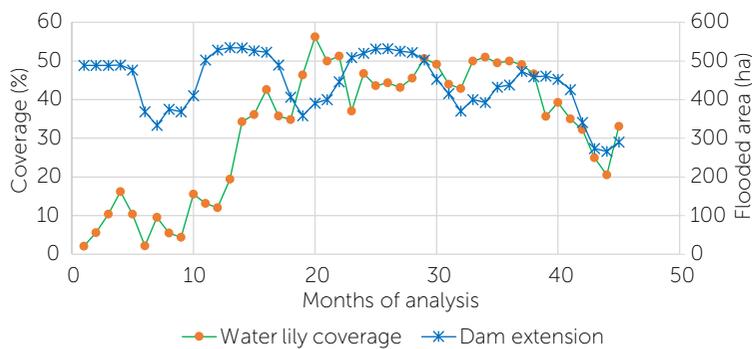
As shown in Figure 1, there are periods in which the lily cover dynamics on the dam is associated with precipitation presence / absence. For example, during the March-April-May period, low lily covers were present while the highest growth rates were observed from May-June, the period with the highest rainfall in each of the analyzed years.

There are two important decline periods, the first of which goes from June to September 2018, which was adjusted to a line with  $R^2$  of 0.79 and slope of  $-5.61$ ; the second decline period goes from February to June 2020, which was adjusted to a line with  $R^2$  of 0.98 and slope of  $-4.75$ .

The reservoir area depends of the water inlets and outlets in the system during May, which recorded the minimum occupied area in the dam during 2017 and 2018, and changed to June in 2019 and 2020 (Figure 2). The dam was full during September 2017 and 2018, occupying areas greater than 500 ha; However, in 2019, the storage of the dam decreased, since its occupied area was



**Figure 1.** Time series of the coverage and precipitation.



**Figure 2.** Time series of lily coverage and precipitation.

432 ha during September and its maximum reached in November, with 473 ha.

It is interesting to observe that as the area of the dam decreased, the relative lily cover also decreased; In fact, as of November 2019, a downward trend is observed until June 2020, a behavior coupled with an area decrease (and consequently storage) and a decrease in the percentage of lily coverage in the body of water.

By the end of the analysis period, between November 2019 and June 2020, the relation between coverage and area occupied by the dam has a linear behavior with an  $R^2$  of 0.81 and slope of 8.06, which leads to deduce that the relative coverage is associated with the area covered by the body of water. It has been observed that as the water level in the dam decreases, the lily accumulates on the periphery during the dry season months (mid-February to mid-May), when the second moment of intense water extraction occurs. As it accumulates on the walls, several specimens die, at that moment local inhabitants take chance to carry out mitigation works by setting the dry lily on fire; However, their seeds remain in the surrounding environment and due to their longevity (latency greater than five years), the persistence of the seed is a factor that influences the long-term control and eradication of it (Albano Pérez *et al.*, 2011). As soon as the first rains occur (May-June), the storage volume increases, and the coverage increases, thus achieving the conditions for the germination of the seeds that remain on the edges of the dam.

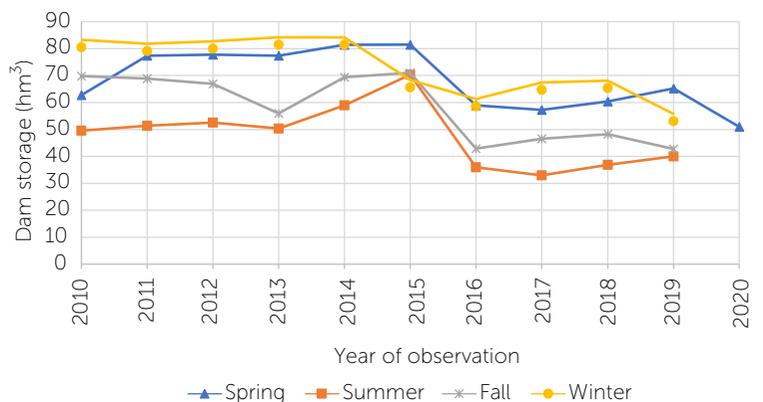
From what is shown by Figure 1 and Figure 2, during 2018, there was little attention to the water lily and, therefore, it had a long upward curve, regardless of the intensity of the rain events, extraction or filling of the dam. This situation may be aggravated by

the climate change effects, since the incidence of atypical rains during the dry season can generate intense periods of heatwave (Maass *et al.*, 2018) which forces the use of extraordinary volumes of water in the filling of the dam, so water availability will decrease in the short term.

The projected water demand for 2030 for the city of Morelia, which is currently  $4.1 \text{ m}^3/\text{s}$ , is expected to increase to  $4.3 \text{ m}^3/\text{s}$  (CONAGUA, 2010); With the enormous pressure in the water resource and changes in land use, it is estimated that the La Mintzita spring will decrease its water supply; while the abatement of deep wells will create greater dependence on the dam's water.

In the analysis of the monthly storage volume during the 2010-2020 period (2020 only January-March), a downward trend was observed, although an explanatory line cannot be adjusted, the observed slope (data not shown) was negative. To facilitate the analysis, the reported monthly volumes were grouped and averaged by season of the year (Figure 3); In all cases, storage had a decreasing trend, with a peak in 2015 (except in winter) and a sharp decrease in the 2016-2019 period; on the contrary, in the 2010-2015 period, there was a stable slightly upward trend. During the 2010-2015 period, the average storage in all stations was above  $50 \text{ h m}^3$ , even during the winter it was above  $80 \text{ h m}^3$ . During the 2016-2019 period, storage during summer and autumn was between  $30 \text{ h m}^3$  and  $70 \text{ h m}^3$ , the minimum values of the previous period were close to the maximum records in this period, which shows a strong water availability deficit in the period from 2016 to 2020.

The growth of the lily occurred in one of the most sensitive periods for the storage in the dam (2017-



**Figure 3.** Average water storage by season.

2018), which marks the importance of containing it and other threats to the water sustainability of the storage system. The intensity of the lily coverage was particularly intense during the period in which the lowest storages of the 2010-2020 series were reported. The potential influence of climate change on world food production and access to water for domestic use has made climate variables the main concern of many studies (Yang et al., 2020); However, the role played by the invasion of aquatic species in bodies of water in which they cause significant losses due to evapotranspiration should not be overlooked (Ali & El-Din Khedr, 2018; Anda, Simon, Soos, Teixeira da Silva, & Kucserka, 2016), since the water lily can reach evapotranspiration levels in reservoirs 2.13 times higher than in conditions without it.

Although mitigation efforts through the extraction of the lily during the dry season contribute to reduce its impact, it is important to undertake actions that consider the basin as a fundamental work unit. Beyond this, it would be important to analyze the problem of the lily invasion in the reservoir considering the biophysical system with human interactions derived from the analysis of socio-ecological systems, since water dynamics is a key component in the ecosystems management, and human activities have an effect and are affected by the response of the ecosystem to these interventions (Maass, 2017). Due to this, any action to mitigate the damage caused by the water lily proliferation must be fully agreed with the actors involved in the management process, both internally and externally, throughout the basin and its users (Figure 4).

## CONCLUSIONS

The water lily has high propagation rates in the Cointzio dam, the highest incidence was recorded during the

period of least storage. Although mitigation work is carried out, its effect is marginal, since the plant easily recovers given the incidence of rains and the germination of surrounding seeds; This leads the lily to double the area occupied from one month to the next, the highest growth rate was from October 2017 to June 2018, where it grew at a rate of 400%, coincidentally the time of least water storage in the dam, the 2010-2020 period. The reduction of the affected area occurs during the dry season; however, the rainy season encourages spread. To improve water availability, it is urgent to address the problem from a comprehensive perspective, implementing a containment strategies based on biophysical interactions in conjunction with social, political, economic and governance interactions, considering local, internal and external actors to the Basin.

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**Figure 4.** Water lily and invasion overview (*Eichhornia crassipes* (Mart) Solms) in the Cointzio dam, Michoacán, Mexico.

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# Forage yield of *Urochloa brizantha* [(Hochst. Ex A. Rich.) R.D.] cv. Insurgente at different cutting heights

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## ABSTRACT

**Objective:** Evaluate the forage yield of *Urochloa brizantha* cv. Insurgente at different cutting heights.

**Design/methodology/approach:** the experiment was carried out at the Universidad del Papaloapan, Loma Bonita, Oaxaca, Mexico. Four cutting heights were evaluated (5, 10, 15, and 20 cm) during the rainy, norther, and dry seasons. The experiment followed a randomized block design with four replicates. We evaluated plant height (PH), green matter yield (GMY), dry matter yield (DMY), growth rate (GR), and morphological components, such as leaf yield (LY), sheath yield (SY), and stem yield (StY).

**Results:** the cutting height and season interaction was significant for all the evaluated variables ( $P \leq 0.01$ ). The highest PH (42 cm) was obtained with a cutting height of 20 cm during the rainy season. The highest GMY and DMY (2,484 and 606 kg ha<sup>-1</sup>, respectively) were obtained with cutting heights of 15 cm during the rainy season. These values were similar ( $P > 0.05$ ) to those obtained at 20 cm (2,410 and 582 kg ha<sup>-1</sup>, respectively). The highest LY, SY, and StY values were obtained with cutting heights of 15 and 20 cm during the rainy season. The highest GR (31 kg MS ha<sup>-1</sup> day<sup>-1</sup>) was observed during the rainy season, regardless of cutting height.

**Findings/conclusions:** for each of the evaluated seasons, cutting heights of 15 and 20 cm resulted in the highest forage yields of *U. brizantha* cv. Insurgente.

**Keywords:** *Urochloa brizantha*, Insurgent grass, forage production, cutting height.

## INTRODUCTION

In the tropical wet climate of Oaxaca, cattle production is the main economic activity. Like other regions in the country, livestock is an extensive activity, where native grasses are the primary feed source. However, these grasses have low forage yields, and their seasonal production is an important problem in animal productivity; during the rainy season, there is a forage surplus, the contrary occurs

during the dry season due to water shortage (Hernández *et al.*, 2002). Therefore, new forage management strategies are necessary to increase forage availability and secure its constant production throughout the year (Araya and Boschini, 2005). *Urochloa brizantha* is a grass species with high dry matter yields that adapts to acidic soils with medium soil fertility, seasonal floods, and moderate droughts (Lascano *et al.*, 2002). However, the quality and yield of this grass depend on several factors, such as cultivar, climatic conditions, and pasture management (Juárez and Bolaños, 2007). Within pasture management, defoliation intensity is an important factor to consider. After defoliation, high forage yields are obtained if there is a sufficient carbohydrate reserve, with an adequate number of leaves and growth rate (Beltrán *et al.*, 2002). Thus, each forage species must be harvested at an optimal height to obtain the maximum forage yield. Dos Santos *et al.* (2008) concluded that the best harvesting height for *U. brizantha* cv. Marandu was 15 cm. Similarly, Cruz *et al.* (2017) observed maximum dry matter yields at cutting heights ranging from 13 to 15 cm, every 28 days, in *U. humidicola* cv. Chetumal. Rojas *et al.* (2018) reported the highest dry matter yield in hybrid *Urochloa* cv. Cobra at 15 cm. At this height, assuming that the plant has significant reserves, growth is faster, consequently increasing forage production.

Although there are studies regarding pasture management in Mexico, the information on harvest height in Insurgente grass is still deficient, so it is necessary to reinforce this information for said tropical grass.

Therefore, this study aimed to evaluate the effect of the cutting height on the productive behavior of *U. brizantha* cv. Insurgente.

## MATERIALS AND METHODS

### Localization of the experimental area

The study was performed, under rainfed conditions, from July to May, in the Experimental Field of the Universidad del Papaloapan, Loma Bonita Campus, Oaxaca, Mexico (18° 01' 19" N, 95° 51' 33" W, and 26 m of altitude). This region has a tropical climate with rainfall during summer and an annual mean temperature and precipitation of 25 °C and 1,845 mm. The average monthly temperature and total monthly precipitation throughout the study were 24.2 °C and 1,038 mm, respectively (FAM, 2011). Based on a fertility analysis, the soil had a sandy loam texture with a pH of 4.9, 1.8% of organic matter, and 14.8, 23.5, 37.0, 241.0, and 42.3 mg kg<sup>-1</sup> of N, P, K, Ca, and Fe, respectively.

### Treatments and experimental design

Four cutting heights were evaluated: 5, 10, 15, and 20 cm above the soil surface during the rainy (June-October), norther (November-February), and dry (March-May) seasons. Treatments were distributed in a randomized complete block design with four replicates. The experimental plot consisted of four 4.8 m-long furrows spaced 80 cm apart; Insurgente grass shrubs were spaced 80 cm apart. Thus, the experimental plot size was 4.8×3.2 m, with a total area of 15.36 m<sup>2</sup> and an available area of 5.12 m<sup>2</sup> located in the two central furrows, leaving the last grass shrub at each end.

### Experiment establishment and management

For this study, the vegetative material (stumps with three to five stems) of an Insurgente grass pasture, sown in November 2006, was used. At the beginning of each season of the year, forage was manually harvested at the height of 10 cm. Subsequently, at 30 days of regrowth, forage was subjected to the different experimental cutting heights to evaluate forage production. During the dry season (March-May), forage can only be harvested two times. Therefore, the same number of harvests was carried out in the three different seasons. Harvests were carried out at four, five, and six weeks after regrowth for the rainy, norther, and dry seasons, respectively. On August 1, 2009, during the rainy season, the pasture was subjected to the different experimental cutting heights. The first forage harvest occurred on August 29, the second on September 26. On December 1, during the norther season, the pasture was subjected to the different experimental cutting heights. The first forage harvest occurred on January 5 (2010), the second on February 9. On March 5, during the dry season, the pasture was subjected to the different experimental cutting heights. The first forage harvest occurred on April 16, the second on May 28. The pasture was not fertilized, but weeds were controlled with the herbicide 2,4-D amine at 15 days of regrowth and subsequent manual weeding.

### Evaluated variables

We evaluated plant height (PH, cm), green matter yield (GMY, kg GM ha<sup>-1</sup>), dry matter yield (DMY, kg DM ha<sup>-1</sup>), growth rate (GR, kg DM ha<sup>-1</sup>).

day<sup>-1</sup>), and the yield of morphological components: leaf yield (LY), sheath yield (SY), and stem yield (StY).

PH was measured in four shrubs per plot, from ground level to the upper end of the plant, without stretching the leaves. GMY was determined by cutting and weighing the fresh forage obtained from four randomly selected shrubs per plot based on plant density. DMY was determined from a subsample of 150 g obtained from the total fresh forage collected. This subsample was separated into its morphological components: leaf, sheath, and stem. Subsequently, each component was dried in a forced-air oven at 65 °C for 48 h. The dry matter yield of each morphological component was calculated using the following equation:

$$DMY=(FW \times dw)/fw$$

where *DMY*=dry matter yield, *FW*=fresh weight of the sample, *dw*=dry weight of the subsample, and *fw*=fresh weight of the subsample. The total forage yield (kg DM ha<sup>-1</sup>) for each season was obtained by adding the yield of the two harvests carried out. GR was calculated using the following equation:

$$GR=HF/t$$

where *GR*=growth rate (kg DM ha<sup>-1</sup> day<sup>-1</sup>); *HF*=harvested forage (kg DM ha<sup>-1</sup>), and *t*=days elapsed between treatment and harvest.

### Statistical analysis

Data were subjected to an analysis of variance based on the randomized complete block experimental design with four replicates. Means were compared using the Tukey test with a significance level of 5% (SAS, 2011).

## RESULTS AND DISCUSSION

### Plant height and forage yield

Results show a significant effect of the interaction between cutting height and season on PH, GMY, and DMY ( $P < 0.01$ ). The tallest plants (36 and 42 cm) were obtained during the rainy season at cutting heights of 15 and 20 cm. During the norther season, the tallest plants (27 and 31 cm) were obtained at 15 and 20 cm, respectively. Similar heights (25 and 31 cm) were observed during the dry season at cutting heights of 15 and 20 cm, respectively (Table 1).

Joaquín et al. (2019) reported similar results in *U. brizantha* cv. Insurgente. They obtained plant heights of 43, 29, and 19 cm with a cutting height of 15 cm during the rainy, norther, and dry seasons, respectively. Additionally, Martínez et al. (2008) observed the greatest forage yield of *U. humidicola* cv. CIAT 6133 at the harvesting height of 15 cm.

In this study, the highest plant heights obtained at 15 and 20 cm, regardless of the season, were attributed to a higher growth rate (Table 2).

These results indicate that with higher cutting heights, the plant remains with a greater number of leaves and reserve substances that influence plant growth and, consequently, result in higher plant heights (Beltrán et al., 2002).

Regarding GMY, the highest values (2,484 and 2,410 kg GM ha<sup>-1</sup>) were obtained during the rainy season with cutting heights of 15 and 20 cm, respectively. These values ( $P > 0.05$ ) were similar to those obtained at 5 and 10 cm (1,875 and 2,293 kg GM ha<sup>-1</sup>, respectively); however, they were significantly different and higher ( $P \leq 0.05$ ) to those obtained during the norther and dry seasons, with average values of 1,038 and 1,018 kg GM ha<sup>-1</sup>, respectively (Table 1). DMY behaved similarly; the higher values (606 and 582 kg DM ha<sup>-1</sup>) were observed during the rainy season at 15 and 20 cm, respectively. These values were similar ( $P > 0.05$ ) to those obtained

**Table 1.** Plant height and green and dry forage yield of *Urochloa brizantha* cv. Insurgente at four different cutting heights and in three seasons of the year.

Season of the year	Cutting height (cm)			
	5	10	15	20
<b>Plant height (cm)</b>				
Rainy	24 <sup>cd</sup>	31 <sup>bc</sup>	36 <sup>ab</sup>	42 <sup>a</sup>
North winds	14 <sup>e</sup>	21 <sup>de</sup>	27 <sup>cd</sup>	31 <sup>bc</sup>
Dry	13 <sup>e</sup>	20 <sup>de</sup>	25 <sup>cd</sup>	31 <sup>bc</sup>
<b>Fresh matter yield (kg FM ha<sup>-1</sup>)</b>				
Rainy	1,875 <sup>a</sup>	2,293 <sup>a</sup>	2,484 <sup>a</sup>	2,410 <sup>a</sup>
North winds	943 <sup>b</sup>	1,012 <sup>b</sup>	1,128 <sup>b</sup>	1,067 <sup>b</sup>
Dry	942 <sup>b</sup>	920 <sup>b</sup>	1,026 <sup>b</sup>	1,185 <sup>b</sup>
<b>Dry matter yield (kg DM ha<sup>-1</sup>)</b>				
Rainy	387 <sup>b</sup>	525 <sup>a</sup>	606 <sup>a</sup>	582 <sup>a</sup>
North winds	212 <sup>e</sup>	226 <sup>de</sup>	255 <sup>cde</sup>	256 <sup>cde</sup>
Dry	316 <sup>bcd</sup>	326 <sup>bc</sup>	362 <sup>b</sup>	408 <sup>b</sup>

abcde Values with different letters within each variable, regardless of the row and column, indicate a significant difference ( $P < 0.05$ ).

at 10 cm (525 kg DM ha<sup>-1</sup>) but differed and were greater ( $P \leq 0.05$ ) than those obtained at 5 cm (387 kg DM ha<sup>-1</sup>). During the norther season, the highest dry matter values (255 and 256 kg DM ha<sup>-1</sup>) were observed at cutting heights of 15 and 20 cm, respectively. The same occurred during the dry season, where the highest values (362 and 408 kg DM ha<sup>-1</sup>) were obtained at 15 and 20 cm (Table 1). Similar results were previously reported for *U. humidicola* cv. CIAT 6133, where the highest forage yield (11,154 kg DM ha<sup>-1</sup> year<sup>-1</sup>) was obtained with a cutting height of 15 cm (Martínez *et al.*, 2008). In *U. humidicola* cv. Chetumal, the highest forage production was obtained with harvesting heights of 13 to 15 cm every 28 days (Cruz *et al.*, 2017). Similarly, in hybrid *Urochloa* cv. Cobra, the highest amount of forage was obtained after harvesting at 15 cm every 35 days after regrowth (Rojas *et al.*, 2018).

The highest amount of green and dry matter forage obtained with 15 and 20 cm cutting heights during the rainy season was attributed to the precipitation recorded during August and September (250 and 580 mm, respectively), which influenced plant growth, and the higher number of remaining leaves, which resulted in a faster recovery of the photosynthetic capacity of the plant and, consequently, a higher forage yield (Dos Santos *et al.*, 2011).

In this study, the average distribution of forage yield was 48, 21, and 31% during the rainy, norther, and dry seasons, respectively. However, for this same species, cultivar, and study location, Joaquín *et al.* (2019) reported a forage yield distribution of 65, 30, and 5% during the rainy, norther, and dry seasons, respectively. Furthermore, a previous study in *U. humidicola* cv. Chetumal reported a forage yield distribution of 66, 26, and 7% during the rainy, norther, and dry seasons, respectively (Cruz *et al.*, 2017). While the forage yield of hybrid *Urochloa* cv. Mulato II, at a cutting height of 15 cm and harvesting at 28 days, was 48.8, 29.5, and 21.6% during the rainy, norther, and dry seasons, respectively (Cruz *et al.*, 2018). This difference in forage yield distribution could be attributed to the different climatic conditions, precipitation and temperature, and pasture management practices during the study.

### Morphological composition

The interaction between the cutting height and season of the year was significant for LY, SY, and StY ( $P \leq 0.05$ ; Table 2). The highest LY values (554 and 534 kg DM ha<sup>-1</sup>) were observed during the rainy season with cutting heights of 15 and 20 cm, respectively. The average distribution for the rainy, norther, and dry seasons was 48, 22, and 30%, respectively. LY contributed 90% to the total forage yield. Similarly, during the norther season, the highest LY values (254 and 252 kg DM ha<sup>-1</sup>) were observed at cutting heights of

**Table 2.** Growth rate and leaf, sheath, and stem yields of *Urochloa brizantha* cv. Insurgente at four different cutting heights and in three seasons of the year.

Season of the year	Cutting height (cm)			
	5	10	15	20
<b>Lamina yield (LY; kg DM ha<sup>-1</sup>)</b>				
Rainy	371 <sup>b</sup>	476 <sup>b</sup>	554 <sup>a</sup>	534 <sup>a</sup>
North winds	204 <sup>f</sup>	219 <sup>e</sup>	254 <sup>de</sup>	252 <sup>def</sup>
Dry	265 <sup>de</sup>	278 <sup>d</sup>	320 <sup>cd</sup>	366 <sup>bc</sup>
<b>Sheath yield (SY; kg DM ha<sup>-1</sup>)</b>				
Rainy	30 <sup>b</sup>	45 <sup>a</sup>	50 <sup>a</sup>	46 <sup>a</sup>
North winds	6 <sup>e</sup>	5 <sup>e</sup>	4 <sup>e</sup>	3 <sup>e</sup>
Dry	12 <sup>d</sup>	13 <sup>cd</sup>	14 <sup>cd</sup>	19 <sup>c</sup>
<b>Stem yield (SY; kg DM ha<sup>-1</sup>)</b>				
Rainy	0.3 <sup>c</sup>	2.0 <sup>a</sup>	0.7 <sup>b</sup>	0.4 <sup>c</sup>
North winds	0.0 <sup>c</sup>	0.0 <sup>c</sup>	0.1 <sup>c</sup>	0.1 <sup>c</sup>
Dry	0.0 <sup>c</sup>	0.0 <sup>c</sup>	0.0 <sup>c</sup>	0.0 <sup>c</sup>
<b>Growth rate (GR; kg DM ha<sup>-1</sup> d<sup>-1</sup>)</b>				
Rainy	27 <sup>ab</sup>	33 <sup>a</sup>	31 <sup>a</sup>	33 <sup>a</sup>
North winds	14 <sup>c</sup>	16 <sup>bc</sup>	19 <sup>bc</sup>	17 <sup>bc</sup>
Dry	13 <sup>c</sup>	15 <sup>c</sup>	15 <sup>c</sup>	17 <sup>bc</sup>

a,b,c,d,e,f Values with different letter within each variable, regardless of row or column, indicate significant difference ( $P \leq 0.05$ ).

15 and 20 cm, respectively. The same occurred during the dry season, where the maximum values (320 and 366 kg DM ha<sup>-1</sup>) were recorded at 15 and 20 cm. SY behaved similarly. The highest values (45, 50, and 46 kg DM ha<sup>-1</sup>) were observed during the rainy season with cutting heights of 10, 15, and 20 cm. However, stems were only observed during the rainy season; the highest value (2 kg DM ha<sup>-1</sup>) was obtained with a cutting height of 10 cm (Table 2).

These results confirmed the previous observations of Joaquín *et al.* (2019), who reported, for this same species and cultivar, that the highest leaf ratio was obtained during the rainy season, followed by the norther and dry season, where leaf yield contributed 80% to the total forage yield, regardless of the season of the year. In *U. humidicola* cv. Chetumal, a

previous study reported a leaf yield distribution of 86, 20, and 30% during the rainy, norther, and dry seasons, respectively (Cruz *et al.*, 2017). Moreover, in hybrid *Urochloa* cv. Cobra, Rojas *et al.* (2018) observed the highest leaf yield value with the cutting height of 15 cm, 35 days after regrowth. Dos Santos *et al.* (2011) reported that pastures have good energy reserves and significant amounts of leaves when harvesting at mild intensities. However, with moderate to severe defoliation, the availability of stem photosynthates decreases, and, consequently, leaf yield also decreases. The low leaf yield observed in this study resulted from the low plant density (15,525 plants ha<sup>-1</sup>; datum not included), since it has been indicated that the distribution of forage yield components is influenced by the season and plant density (Rojas *et al.*, 2016).

### Growth rate

The height and season interaction was significant for GR ( $P \leq 0.01$ ). The highest values were obtained during the rainy season, regardless of the cutting height, with an average of 31 kg DM ha<sup>-1</sup> day<sup>-1</sup>. Similar behavior was observed during the norther and dry seasons, with averages of 16.5 and 15.0 kg DM ha<sup>-1</sup> day<sup>-1</sup>, respectively. Cruz *et al.* (2018), in a previous study with hybrid *Urochloa* cv. Mulato II, also reported the highest growth rate during the rainy season, followed by the norther and dry seasons. Furthermore, Garay *et al.* (2019) observed the highest growth rate (136 kg DM ha<sup>-1</sup> day<sup>-1</sup>) in *Pennisetum ciliare* cv. H-17 at four weeks of age, during the rainy season. The growth rate during the dry season was 3.4 kg DM ha<sup>-1</sup> day<sup>-1</sup>.

The highest growth rate was observed during the rainy season due to the higher precipitation and temperature (810 mm and 26 °C). During the dry and norther seasons, the growth rate decreased due to low precipitation (96 and 15 mm, respectively). According to Lemaire *et al.* (2000), humidity deficit limits grass growth during the dry season, which results in low growth rates. Furthermore, low temperatures decrease growth rate by inhibiting leaf growth, decreasing canopy development, leaf area index, and photosynthetic capacity.

### CONCLUSIONS

This study suggests that the best cutting height for *Urochloa brizantha* cv. Insurgente ranges between 15 and 20 cm for each of the evaluated seasons. However, further research is required to determine more precisely the best cutting height for Insurgente grass.

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