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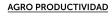
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Año 16, Volumen 16, Número 1, enero 2023, Agro productividad es una publicación mensual editada por el Colegio de Postgraduados. Carretera México-Texcoco Km. 36.5, Montecillo, Texcoco, Estado de México. CP 56264. Tel. 5959284427. www.colpos.mx. Editor responsable: Dr. Jorge Cadena Iñiguez. Reservas de Derechos al Uso Exclusivo No. 04-2017-031313492200-203. ISSN: 2594-0252, ambos otorgados por el Instituto Nacional del Derecho de Autor. Responsable de la última actualización de este número, M.C. Valeria Abigail Martínez Sias. Fecha de última modificación, 22 de febrero de 2023.

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# Inventory of tourism resources of the mountain area of Texcoco, State of Mexico

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#### ABSTRACT

**Objective**. To elaborate an inventory of tourism resources identified in the mountain area of Texcoco, which serve as an instrument for planning and organizing the local tourism system.

**Design/Methodology/Approach**. We considered the application of a survey to the auxiliary authorities, as well as to local and external actors that would allow contextualizing the tourism dynamics in the area. The task was supported by field trips applying the ethnographic method. From the collected data, eight localities were selected within the characteristics, a greater number of tourism resources were identified.

**Results**. It was determined that some of the selected localities have sufficient attributes to potentiate some of their resources as a strategy for local development.

**Limitations/Implications**. The restriction to access information in local offices of the municipal government limited the approach to some resources; thus, self-reported data had to be constructed through the perception of some individuals.

**Conclusions**. It is necessary to develop tourism land-planning instruments through mechanisms of management that include training for local actors on various topics for the sustainable use of tourism resources.

Keywords: territorial planning, tourism management, local development, sustainability, rural tourism.

Citation: Durán-Méndez, J. A., Almeraya-Quintero, S. X., Guajardo-Hernández, L. G., Rosas-Jaco, M. I., & Borja-Bravo, M. (2023). Inventory of tourism resources of the mountain area of Texcoco, State of Mexico. *Agro Productividad*. https://doi.org/10.32854/ agrop.v16i1.2344

**Academic Editors**: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: July 11, 2022. Accepted: December 19, 2022. Published on-line: February 22, 2023.

*Agro Productividad*, *16*(1). January. 2023. pp: 3-12.

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#### INTRODUCTION

Tourism is defined by the Secretaría de Turismo (SECTUR, 2021) as an activity that individuals perform during a trip outside their usual environment for less than a year through various leisure activities. According to the North American Industrial Classification System (DATATUR, 2019) such activity contributes approximately 8.7% of the Gross Domestic Product.



The tourism activity is developed through tourism resources, attractions and products. The first concept (tourism resource) is described by Navarro (2020) as the raw material of the Components of the Target Subsystem which he divides into: natural, artificial, cultural, social, economic and political. A tourism attraction is defined according to Núñez (2004) as any activity that arises from a tourism product, incorporating facilities, equipment and services. As for tourism product, the World Tourism Organization (WTO, 2022) indicates that it is a combination of the elements corresponding to tourism resources and attractions, which is marketed through distribution channels setting price and permanence.

Currently, as the practice of tourism intensifies, mechanisms are proposed to regulate its impact, through the use of concepts such as sustainability, which is proposed by Zarta (2018), as the production of goods and services that satisfy human needs and guarantee the quality of life by strengthening the conditions of the environment and the use of natural resources. On the other hand, sustainability, as indicated by the United Nations (UN, 2015), emphasizes singularities of development from a unique and not integral perspective, which can then be linked to other axes that form it.

According to Camara (2014), as tourism evolves, land-planning and management-planning instruments must be developed, such as inventories of tourism resources, which contribute to recording the status and potential of resources. The elaboration of an inventory of tourism resources implies a methodology based on their classification (origin), and hierarchy (level of interest), which Cárdenas (1991) lists from the highest to the lowest, with 5 as the one of greatest interest, and 1 the one of least. Likewise, criteria are included in the registry such as those integrated by Huizar (2021), such as degree of conservation (excellent, good, regular and bad), type of visitor (foreign, national, regional and local), and degree of influx to the resource on a scale of 1 to 4, with 1 being the highest level and 4 the lowest level.

Therefore, based on an analysis of existing planning instruments such as the Municipal Development Plan of Texcoco (2022-2024), and the "Pueblos Bonitos" program, it is perceived that tourism activity is not addressed as an issue of relevance for the development of the mountain area of the municipality, despite the fact that it houses tourism resources of great value. For this reason, the objective of this study is to elaborate an inventory of tourism resources that motivate to create new management mechanisms for the management- and land-planning of the local tourism system.

#### MATERIALS AND METHODS

The study was conducted in the mountain area of the Municipality of Texcoco in the State of Mexico; at an altitude of 2890 m in areas of dwellings, and up to 4125 m, at the top of mountains. It has an estimated population of approximately 54 936 inhabitants, according to data from the INEGI Population and Households Census (2020); and is composed of sixteen localities.

A classification of the sixteen localities that form the mountain area was made, based on altitude recorded in INEGI (2020), in relation to their main territorial characteristics (urban, semi-urban, rural and semi-rural). This classification was adjusted into three groups: low mountain (MB [urban and semi-urban]), medium mountain (MM [semi-

urban and semi-rural]), and high mountain (MA [semi-urban and rural]). Subsequently, a survey was applied to the auxiliary authorities of each locality, structured in four modules: I. Sociodemographic data, II. Identification of tourism resources, III. Economic and social perception, and IV. Sense of identity. The information obtained was used to select eight locations corresponding to the groups "Medium mountain and high mountain" where a greater concentration of tourism resources was observed, some of them verified by sources such as INEGI (2022) and INAFED (2021).

Field visits were made to identify, photograph and evaluate the state of tourism resources using the ethnographic method. The task was supported by different programmed activities (4 sports and 4 cultural), during which 4 interviews were applied to key informants. The collection of information was completed with a visit to the Municipal Tourism Office where information and physical material were provided that served to analyze how tourism management operates at the municipal level.

From the information obtained, the inventory of tourism resources was elaborated based on the "Components of the Target Subsystem" proposed by Navarro (2020). As for the hierarchy, the one defined by Cárdenas (1991) was used.

#### **RESULTS AND DISCUSSION**

All identified resources are listed in Table 1, by area, locality and subsystem.

#### Natural

The mountain area presents hydrological resources (bodies and streams of water) only in the MA that by means of runoff supply 6 dams distributed in the MM; the water is used for domestic use, rainy storm agriculture (MM), and irrigation (MA), which are favored by the predominance of a phaeozem type soil. The relief profile is presented in a fractured scheme with a topography of hill and mountain type, where the climate is subhumid temperate (MM and MA), and humid semi-cold (MA). These characteristics according to Cartuche (2019) favor an attractive condition as a function of environmental services. Oak and coniferous (MA) forest predominate, and arid scrubland (MM); as well as induced biotic species (MM and MA), wild and creole species were identified, the latter are bred by grazing and backyard pen for self-consumption and marketing. High mountains in the mountain area are considered protected natural areas in the category of state reserve and is part of the 13 000 hectares of forests according to the List of Priority Regions of CONABIO (2017).

#### **Artificial**

"Both adapted spaces and structures, facilities, infrastructure and artifacts are part of this subsystem" (Navarro, 2020: 23). In this sense, some of the resources stand out for their historical and cultural character that influences the local tourism dynamics. The Archaeological Zone "Cerro de Tetzcotzinco" (INAH, 2018) is paramount, located at an altitude of 2580 m (MM), which preserves ruins of the pre-Hispanic era and the Molino de Flores Nezahualcoyotl National Park (MM), as well as the Casco de la Ex Hacienda Tierra Blanca (MA). Also noteworthy are the Hacienda la Taconera and the Ejidal Nezahualcoyotl

Recreation Center (MM). The religious architecture is also notable (MM and MA), and some movable assets such as the organ inside the church of San Jerónimo (MA), and the Baroque altarpiece inside the church of the Purification (MM). Infrastructure and equipment vary in proportions by locality. Adaptations to the natural environment are observed for the offer of gastronomic services (MA).

#### Cultural

Montiel (2021) indicated that the mechanisms of cultural transmission in the mountain area are transmitted and preserved through knowledge converted into craft trades such as traditional indigenous medicine, traditional therapeutic practices, herbal medicine and processes of healing, liberation, even exorcism through religious rituals (MA). The traditions are linked to the Catholic religion, a characteristic that in the villages, according to Zurita *et al.* (2019), is usually susceptible to religious tourism. However, events of this nature do not represent market opportunities. These events are usually enlivened with wind music, an outstanding peculiarity as a cultural practice and craft from which arises one of the most valued events at the local level (MA), called "Vientos de la Montaña" Festival. Other identified scheduled events are held by thematic years (1), ephemeris (2) and business entrepreneurship (7), out of which nine corresponded to the middle mountain and one to the high mountain.

The belief system is intangible but becomes apparent through myths such as that of the "Ahuaques", which according to Montiel (2021) are spirits that reside and guard the water bodies. Like the myth of the "Witches" that is part of the local narratives, both on the high and middle mountain. On the other hand, a deep-rooted system of uses and customs (MA) was observed, such as control in the distribution of environmental services, limitations in the purchase and sale of lands, creation of local transport collectives, permanence of agrarian nuclei and relations of reciprocity that, as described by Sánchez (2012), are linked to kinship, religious beliefs and political ideologies.

#### **Social**

It was found that in some localities (MM), communication is established between internal and external actors for the use of tourism resources. Autonomous local groups (MA) were identified for political and cultural purposes, as well as external groups with activities linked to sports (mountain biking, hiking, running or jogging and camping), which visit the eight locations through different ascent dynamics (calling, quota, routes, among others). It is worth mentioning the incidence of institutional actors that develop academic and governmental programs inclined to solve the needs of the territory (MA and MM).

#### **Economic**

As for the reproduction of economic forms of subsistence, the practice of playing wind music (MA), the extraction of non-timber forest resources and vegetation (MA), and floriculture (MM), which according to Anastacio *et al.* (2015), generate a demand mainly in the metropolitan area of Mexico City. On the other hand, gastronomy (MA and MM),

emerges as a resource through characteristic meals of the region that in the mountain area, according to González *et al.* (2017), are considered traditional due to their geographical origin (location), time record, the method of elaboration, the raw material used, and the customs associated with their elaboration.

In regard to the offer of housing for tourists, two cabin-type properties (MA) were identified, and it could be evidenced that the stay in the mountain area lasts one day and the days with the highest attendance of visitors are Fridays (12.5%), Saturdays (37.5%), and Sundays (43.8%). In the case of a visit due to a scheduled event, the day and month may vary.

#### **Political**

The City Council of Texcoco does not have a solid direction that can open the way to a concrete and effective local tourism development. Therefore, it does not respond to the dynamics that emerge in an unregulated way in relation with tourism activity in the mountain area. However, it promotes a document called "Gaceta Imagen Urbana Pueblos Bonitos" through which it invites to strengthen the image of the localities but subjected to compliance of characteristics that not all possess. Within the Municipal Development Plan (2022-2024), a planning model is presented, composed of four pillars and three transversal axes where lines of action based on three of the Sustainable Development Goals established by the UN (2022) are proposed.

#### **CONCLUSIONS**

It was determined that the tourism resources with the most potential for use belong to the Natural classifications (MM and MA), likewise the tourism resources of the Natural and Cultural subsystem present a good degree of conservation within the hierarchy 1, 3 and 4, a degree of influx 3 of local and regional visitors. On the other hand, Artificial type resources have a degree of interest in hierarchy 0, 1, 2, 3 and 4, and Cultural (hierarchy 1), both with a degree of good conservation and type of local and regional visitors with an influx level of 3.

Tourism is not the main economic activity, but it is recommended to regulate it through management, planning and territorial planning mechanisms that respond to the needs of the local population and visitors, subject to what is proposed in the Municipal Development Plan (2022-2024), towards the Sustainable Development Goals. It is suggested that within those mechanisms, training shall be provided to local actors on issues as environmental awareness, construction and preservation of the natural and cultural landscapes, tourism awareness, tourism education, local community, rural enterprises, collective organization and financing.

#### **ACKNOWLEDGEMENTS**

To the Auxiliary Authorities of the localities visited. To the Municipal Tourism Office (Texcoco, Mexico). To the collectives Tlaloc Ride Girls, Trail Runners Texcoco, Ultra Ladies Runners, MIT Runners and Club de la Montaña Texcoco, for their accessibility to provide information for the construction of this document.

 Table 1. Classification of tourism resources.

	Political	псап	ment xiliary ities	ment xiliary ities	ment xiliary ities	ument xiliary ities
	Poli	roll	Local Government and Auxiliary Authorities	Local Government and Auxiliary Authorities	Local Government and Auxiliary Authorities	Local Government and Auxiliary Authorities
	T. Constant	Economic	Gastronomic products: bar- bacoa, tlacoyo, pulque: seasonal agri- culture	Gastronomic products: bar- bacoa, tlacoyo, pulque. seasonal agri- culture: Flowers, fruits	Gastronomic products: bar- bacoa, tlacoyo, pulque. seasonal agri- culture: Flowers, fruits Craft: blown glass	Gastronomic products: bar- bacoa, tlacoyo, pulque. seasonal agri- culture: Flowers, fruits
	Society	Social	Internal actors: auxiliary au- thorities External actors: collec- tive groups, academic and government in- stitutions, local government	Internal actors: auxiliary au- thorities External actors: collec- tive groups, academic and government in- stitutions, local government	Internal actors: auxiliary au- thorities External actors: collec- tive groups, academic and government in- stitutions, local government	Internal actors: auxiliary au- thorities External actors: collec- tive groups, academic and government in- stitutions, local government
	Cultural	Intangible	Scheduled events: Tlacoyo's fair, Carnival.  Myths and legends: Ahuaques Festivities: Candelarías day, All saints, Holy week Customs and habits: per- manence of agrarian nuclei, religious beliefs, idiosyncrasy	Scheduled events: Commemorative day for the birth of Nezahualeoyoul, Hot air balloon festival, Myths and legends: Ahuaques Festivities: San Nicola's day, All saints, Holy week Gulstons and habits: per- manence of agrarian nuclei, religious beliefs, idiosyncrasy	Scheduled events: Apple and flower fair, Representation of the battle of May 5, Myths and legends: Ahuaques Festivities: San Mignel's day, All saints, Holy week Gustons and habits: permanence of agrarian nuclei, religious beliefs, idiosyncrasy	Scheduled events: Apple and flower fair, Representation of the battle of May 5, Myths and legends: Ahuaques Festivities: San Dieguito's day, All saints, Holy week Customs and habits: permanence of agrarian nuclei, religious beliefs, idiosyncrasy
		Tangible			Sinaloense Folk Ballet	
Subsystem	Artificial	Immovable	Religious architecture: La Purificación de María (church) urban equipment, road, electrical, hydraulic and sanitary infrastructure	Tetzcotzinco archaeological zone Hydraulic engineer: Centro recreativo ejidal "Neza- hualcoyotl" Religious architecture: San Nicolas de Bari (church) contemporary architec- ture: La Taconera's Farm, bridge/aqueduct fragment urban equipment, road, electrical, hydraulic and sanitary infrastructure	Religious architecture: San Miguel Arcángel (church), Señor de la presa (chapel) Contemporary architecture: Molino de Flores (farm), El Breco (farmhouse) urban equipment, road, electrical, hydraulic and sanitary infrastructure	Religious architecture: San Diego de Alcalá (church) urban equipment, road, electrical, hydraulic and sanitary infrastructure
		Mobavle	Religious artwork: altarpiece inside the local parish			
	Comerato	Naturai	Physiography: Hills (Cuauhyacac's hill). Hydrography: Barranca seca creek (runoff), three waterdams. Clymatology: temperate subhumid Edaphology: Phaeozem soil Land use: rainy season agriculture Vegetation: scrub (arid zone) Induced vegetation: cacus, succulents, bougainvillea, fruit trees (apple, peach, capulin, tejocote); non-fruit trees (pirul, cedar). Fauna: rabbit, squirrel, cacomiztle, field rat, rattlesnake, swallows, sparrows, magpies, hummingbirds, pigeons, frogs, butterflies, dragonflies, bees, fireflies and native species such as: sheep, goat, cow, bull, pig, hen and turkeys.	Physiography: Hills (Tetzcotzinco hill's) Hydrography: Palmilla's creek (runoff) Clymatology: temperate subhumid Edaphology: Phaeozem soil Land use: rainy season agriculture Vegetation: scrub (arid zone) Induced vegetation (cactus, succulents, bougainvillea, fruit trees (apple, peach, capulín, tejocote), non-fruit trees (pirul, cedar) Fauna: rabbit, squirrel, cacomiztle, field rat, rattlesnake, gecko, mountain chameleon, swallows, sparrows, magpies, hummingbirds, pigeons, frogs, butterflies, dragonflies, bees, fireflies and native species such as: sheep, cows, pigs, chickens and turkeys.	Physiography: Hills (basaltic prisms, Tecluilache's hill [lookout]) Hydrography: Silverio Pérez waterdam (runoff) Clymatology: temperate subhumid Edaphology: Phaeozem soil Land use: rainy season agriculture Vegetation: scrub (arid zone) Induced vegetation: succulents, bougainvillea, fruit trees (apple, peach, tejocote, plum); non-frui trees (cedar); Fauna: squirrel, vole, geko, swallows, magpies, hummingbirds, doves, frogs, butterflies, dragonflies, bees , fireflies and native species such as: sheep, cow, pig, chicken and turkeys.	Physiography: Hills (Metecatl's hill) Hydrography: one dam (runoff) Clymatology: temperate subhumid Edaphology: Phaeozem soil Land use: rainy season agriculture Vegetation: scrub (arid zone) Induced vegetation: succulents, bougainvillea, fruit trees (apple, peach, tejocote); non-fruit trees (cedar); Fauna: field rat, swallows, magpies, hummingbirds, butterfly doves, bees and native species such as: sheep,
	um		shirəqəT nòisashiru4 s.I	San Nicolás Tlaminca	San Miguel Tlaixpan	San Dieguito Xochimanca
	əue	οZ		dle Mountain	biM	

Table 1. Continues...

Natural Physiography: Hills and sierra	ural		Mobavle	Subsystem Artificial Immovable Relicious architecture: San	Tangible	Cultural Intangible Scheduled events: obest	Social	Economic	Political [Octable]
Physiography: Hills and sierra (Tláloc mountain) Hydrography: watercourses (Los hornos river, la cantera river), springs. Clymatology: temperate subhumid and humid semicold temperate Edaphology: Phaeozem soil Land use: irrigation agriculture Vegetation: forest (oak and conifers) Induced vegetation: succulents, fruit trees (apple, peach, rejocote, pear, plum); non-fruit rees (cedar); Fauna: rabbit, hare, cacomistle, field vole, sparrows, swallows, magpies, hummingbirds, butterfly doves, bees and Creole species such as: sheep, goat, cow, bull, pig, chicken and turkeys	mountain) ss river, la can- l humid semi- ces (apple, peach, cet); cle, sparrows, lord, sparrows, ord, bull, pig,			Religious architecture: San Pablo (chapel) Material interventions in the natural space: El Cedral (ecotouristic park), two Lookout's (glass and metal structure) urban equipment, road, electrical, hydraulic and sanitary infrastructure		Scheduled events: ghost mountain Myths and legends: Ahuaques Festivities: San Pablo's day, All saints, Holy week Customs and habits: per- manence of agrarian nuclei, religious beliefs, idiosyncrasy	Internal actors: auxiliary au- thorities External actors: collec- tive groups, academic and government in- stitutions, local government	Gastronomic products: barbacoa, tlacoyo, pulque. seasonal agriculture: Flowers, fruits extraction of non-timber resources wild bark and ecitible mushrooms	Local Government and Auxliary Authorities
Physiography: Hills and sierra (Tetorre's crag) Hydrography: watercourses and springs. Clymatology: temperate subhumid and humid semicold temperate Edaphology: Phaeozem soil Land use: irrigation agriculture Vegetation: forest (oak) Induced vegetation: succulents, fruit trees (apple, peach, tejocote, pear, plum); non-fruit trees (cedar); Fauna: rabbit, hare, cacomistle, field rat, gecko, mountain chameleon, rattlesnake, sparrows, swallows magpies, hummingbirds, dragonffies, toads, frogs, doves, butterflies, bees and native species such as: sheep, goats, cows, bulls, pigs, chickens and turkeys	. n- n- ats,	Mu must musik ihu:	Musical instruments: musical organ inside the local church	Religious architecture: San Jeronimo Doctor (church) Contemporary architecture: Tierra Blanca (farm) Material interventions in the natural space: El Partidor Partido	Traditional therapeutic practices: rubbed sanatorium processes: exorcisms, evil retreats	Teaching and practice of music with wind instruments Scheduled events: Winds of the mountain festival, carnaval Myths and legends: Ahuaques Festivities: San Jeronimo's day, All saints, Holy week Customs and habits: permanence of agrarian nuclei, religious beliefs, idiosyncrasy, preservation and use of indigenous language, reci- indigenous language, reci- indigenous language, reci- procity relations, control of environmental services	Internal actors: auxiliary au- thorities External actors: collec- tive groups, academic and government in- stitutions, local government	Gastronomic products: bar-bacoa, tlacoyo, pulque. seasonal agri-culture: Flowers, fruits extraction of non-timber resources wild bark and ecfible mushrooms aquaculture: rainbow trout music as a profession	Local Government and Auxiliary Authorities
Physiography: Hills and sierra Hydrography: watercourses and springs (Tecoatita's creek) Clymatology: temperate subhumid and humid semi- cold temperate Edaphology: Phaeozem soil Land use: irrigation agriculture vegetation: forest (oak) induced vegetation: succulents, fruit trees (apple, peach, tejocote, pear, plum); non-fruit trees (cedar); Faunar rabbit, hare, cacomistle, field rat, gecko, mountain chameleon, rattlesnake, sparrows, swallows magpies, hummingbirds, dragonflies, toads, frogs, doves, butterflies, bees and native species such as: sheep, goats, cows, bulls, pigs, chickens and turkeys.	is (Tecoatitla's I humid semi- ese (apple, peach, edan); It, gecko, moun- swallows mag- frogs, doves, ings, sheep, goats,			Religious architecture: Santa María Magdalena (church) Material interventions in the natural space: Atlmeya (ecotouristic park), urban equipment, road, electrical, hydraulic and sanitary infrastructure		Teaching and practice of music with wind instruments Scheduled events: Winds of the mountain festival, carnaval Myths and legends: Ahuaques Festivities: Maria Magda- lena's day, All saints, Holy week Customs and habits: per- manence of agrarian nuclei, religious beliefs, idiosyncrasy, preservation and use of indigenous language, reci- procity relations, control of environmental services	Internal actors: auxiliary au- thorities External actors: collec- tive groups, academic and government in- stitutions, local government	Gastronomic products: bar-bacoa, dacoyo, pulque. seasonal agri-culture: Flowers, fruits extraction of non-timber resources wild bark and edible mush-rooms music as a profession	Local Government and Auxiliary Authorities

Table 1. Continues...

	D-15451	ronneal	Local	Government	and Auxiliary	Authorities												
	Economic		Gastronomic	products: bar-	bacoa, tlacoyo,	pulque.	seasonal agri-	culture: Flowers,	fruits	extraction of	non-timber	resources	wild bark and	edible mush-	rooms	music as a	profession	
	1-:3	Social	Internal actors:	auxiliary au-	thorities	External	actors: collec-	tive groups,	academic and	government in-	stitutions, local	government						
	Cultural	Intangible	Teaching and practice of mu- Internal actors:	sic with wind instruments	Scheduled events: Winds	of the mountain festival,	carnaval	Myths and legends:	Ahuaques	Festivities: Santa Catarina's	day, All saints, Holy week	Customs and habits: per-	manence of agrarian nuclei,	religious beliefs, idiosyncrasy,	preservation and use of	indigenous language, reci-	procity relations, control of	environmental services
		Tangible	Traditional	therapeutic	practices:	rubbed	Traditional	healing rem-	edies: herbal	medicine								
Subsystem	Artificial	Immovable	Religious architecture:	Santa Catarina Martir	(church),	urban equipment, road,	electrical, hydraulic and	sanitary infrastructure										
		Mobavle																
	,	Natural	Physiography: Hills and sierra	Hydrography: watercourses and springs (Tecoatitla's	creek)	Clymatology: temperate subhumid and humid semi-	cold temperate	Edaphology: Phaeozem soil	Land use: irrigation agriculture	vegetation: forest (oak)	induced vegetation: succulents, fruit trees (apple, peach,	tejocote, pear, plum); non-fruit trees (cedar);	Fauna: rabbit, hare, cacomistle, field rat, gecko, moun-	tain chameleon, rattlesnake, sparrows, swallows mag-	pies, hummingbirds, dragonflies, toads, frogs, doves,	butterflies, bees and native species such as: sheep, goats,	cows, bulls, pigs, chickens and turkeys.	
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### Linkage to the market of jalapeño pepper producers through supplier development in Quintana Roo

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#### **ABSTRACT**

**Objective**: To analyze the jalapeño pepper characteristics and demand of the restaurant sector in Quintana Roo, in order to identify the supply requirements of this sector.

**Design/Methodology/Approach**: A random sample of n=73 restaurants were surveyed online.

**Results**: Most of the restaurants (87.5%) have been in business for less than six years and —although most of them sell Mexican food—only 41.6% are interested in purchasing jalapeño peppers from the producers.

**Study Limitations/Implications**: Producers must invest their social capital in order to firmly establish a supply strategy.

**Findings/Conclusions**: The restaurants that purchase jalapeño pepper demand a constant delivery frequency, size, pungency level, and specific color.

Keywords: quality, commercialization, strategy, jalapeño.

#### Toledano, B. I., Góngora-Pérez, R. D., Borja-Bravo, M., & Sánchez-Gómez, J. (2023). Linkage to the market of jalapeño pepper producers through supplier development in Quintana Roo. *Agro Productividad*. https://doi.org/

Citation: Cuevas-Reyes, V., Sánchez-

10.32854/agrop.v15i11.2351

Academic Editors: Jorge Cadena

Iñiguez and Libia Iris Trejo Téllez

Received: July 25, 2022.
Accepted: December 23, 2022.
Published on-line: February 22, 2023.

Agro Productividad, 16(1). January. 2023. pp: 13-21.

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#### INTRODUCTION

In Mexico, 8.7% of the producers meet the demands of 74.2% of the agricultural market, while 73% of the farmers (who engage in small family agriculture) only have a 7.5% of the market share (FAO-SAGARPA, 2012). Caicedo (2013) pointed out that the scarce bargaining power in the local market shows the high level of exposure of the rural community to market intervention processes carried out by external agents. Consequently, small fruits and vegetables producers face more difficulties finding established markets and, therefore, they usually develop marketing systems adapted to their particular situations (Dunn *et* 



al., 2015). Small producers face different challenges regarding the commercialization of their harvests. According to Rodríguez and Riveros (2016), the major challenges are: the organization of producers, the differentiation of the products to be commercialized, the distance between the producer and the final consumer, and the quality of the relationship between the participants of the purchase-sale process, including the degree of formality of the agreements they establish.

Agricultural commercialization is understood as the process that takes the products from the agricultural exploitation to the consumer, while the market is the physical place where the transactions between sellers and buyers take place (Caldentey, 1992). The commercialization process requires that the products reach the market. Consequently, certain utilities—such as ownership, place, time, and form— must be added to this process (McCarthy and Perreault, 1994). Consequently, once they have been produced, several products require intermediation, in order to easily reach the consumers (Belleflamme and Peitz, 2010).

Rodríguez and Riveros (2016) have proposed some alternatives: short circuits, productive linkages, and commercial linkages of differentiated products. Recent studies about the commercialization process between the traditional and modern supply chains of fruits and vegetables point out that traditional chains —which include commercialization intermediaries and longer supply chains, as well as physical loss of the product and lack of integration between producers— are less efficient than short commercialization chains (Bisen *et al.*, 2018).

The development of a social network of small vegetables producers in Acatzingo is an example of a production chain. This strategy allowed them to diversify the commercialization of their products and enabled them to generate capital and innovation (Lugo-Morin, 2013). Another example is the development of local strategies of public procurement from family agriculture (FAO, 2016). A further example is the supply development strategy. However, this alternative requires information about the clients, their needs, their characteristics, and the products they require. Understanding that producers supply products and provide services to a wide clientele (Yacuzzi, 2012), the objective of this study is to identify the supply requirements of the sector, analyzing the characteristics and demand of jalapeño pepper among the restaurant sector in Quintana Roo. The hypothesis is that specific quality attributes determine which type of jalapeño pepper is sought.

#### MATERIALS AND METHODS

#### Study area location

The study was carried out in Quintana Roo. In 2020, this state had 1,857,985 inhabitants, which accounts for 1.5% of the total population of the country. Ninety percent of the population of Quintana Roo lives is urban areas, while the remaining 10% lives in rural areas. This state has 44,705.2 km², which accounts for 2.3% of the total area of the country. Regarding weather, a sub-humid warm climate with summer rains prevails in 98.91% of the state, while only 1.09% of the surface has a warm climate with abundant summer rains (21° 36' 20" N, 17° 53' 38" S, 86° 42' 37" E, and 89° 17' 48" W) (INEGI, 2018; 2020).

#### Sample selection

From September to November 2020, the Restaurants demand for Jalapeño pepper in the tourist sector of Quintana Roo survey was design and applied. As a consequence of the SARS-CoV-2 (COVID19) pandemic the survey was randomly applied using Google documents. Seventy-three restaurants answered the survey. The restaurants were located as follows: Bacalar (13), Chetumal (21), Playa del Carmen (17), and Tulum (22). According to the Directorio Estadístico Nacional de Unidades Económicas (DENUE) of the INEGI, 129 establishments engage in food processing, food production, and food sale (INEGI, 2021). Based on the data from April 2020, the sample accounts for 56.58% of the total of regional restaurants in business included in the DENUE (INEGI, 2021b).

#### Data source

The following data was obtained: characteristics of the restaurants (time they have been in business, number of employees, location, their menu, etc.); characteristics of the current jalapeño pepper demand (suppliers, purchase frequency, volume of the purchase); and the conditions required to establish a purchase arrangement with the producers (willingness to take part in the buying-and-selling process with jalapeño pepper producers of the state, supply and demand requirements).

#### Data analysis

Based on the applied surveys (n=73) and the variables already mentioned, a data base was developed. The descriptive statistic method was used to analyze these data, in order to characterize the restaurants' current demand and the feasible supply arrangements.

The minimum, maximum, and mean monthly demands were determined for each municipality and for all the municipalities. In order to estimate the monthly demand (kg), the minimum and maximum volume that the owners of each restaurant are willing to purchase per week were multiplied. Using this method, the potential consumption (minimum and maximum) per week was determined. Subsequently, the monthly consumption of each restaurant was also determined. The year has 52.143 weeks; this figure was divided by 12. The resulting number (4.34525) was multiplied by the weekly consumption. As a result, the potential, minimum, and maximum monthly and yearly consumption of each restaurant was determined.

#### RESULTS AND DISCUSSION

#### Characteristics of the restaurants

The restaurants are mainly located in Chetumal and Tulum; the remaining are located in Playa del Carmen and Bacalar (Table 1). In Playa del Carmen and Bacalar, the restaurants are brand new and 7.7% (Bacalar) have been in business from 3 to 6 years. The rest of the restaurants in these municipalities have not been in business for more than three years. Meanwhile, the restaurants in Tulum have been in business from 1 to 3 years and from 3 to 6 years (≈50% for both ranges). The restaurants in Chetumal have been in business for longer periods: up to 85.7% of them have been in business from 3 to 6 years. The restaurants in Bacalar and Chetumal are located in the main avenues of both

Table 1. Tears that	a restaurant nas	been in b	asincss and	i location i	or the restr	tararits (70)	•	
Place	Restaurants	<1	1-3	3-6	>6	Beach	Hotel	Avenue
Tace	Restaurants		yea	ars		Deach	110161	major
Bacalar	13	38.5	53.8	7.7	0.0	100	0	0
Chetumal	21	0.0	0.0	85.7	14.3	100	0	0
Playa del Carmen	17	29.4	70.6	0.0	0.0	17.6	23.5	58.8
Tulum	22	0.0	54.5	45.5	0.0	40.9	18.2	40.9

**Table 1**. Years that a restaurant has been in business and location of the restaurants (%).

Source: Own elaboration based on survey data.

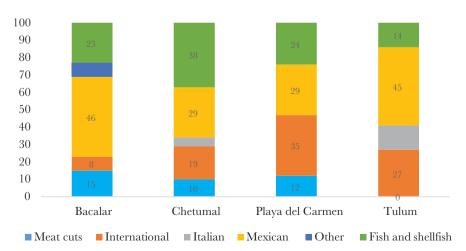
municipalities, while in Playa del Carmen and Tulum, the restaurants are located both in the main avenues and in beach areas.

Another characteristic of the restaurants in Bacalar and Chetumal is that they are not partners with hotels or hotel chains. In fact, this type of partnerships was only recorded in Playa del Carmen and Tulum; however, less than 25% of the restaurants work in partnerships with hotels. In this study, the most popular food in Quintana Roo was Mexican cuisine, followed by international cuisine, and fish and seafood. In Bacalar, Chetumal, and Playa del Carmen, a small percentage of restaurants offers beef and, in Chetumal and Tulum, there are Italian cuisine restaurants (Figure 1).

#### Characteristics of the current demand of jalapeño pepper in restaurants

Out of the n=73 restaurants surveyed, only 44% included jalapeño pepper in their dishes (Table 2). The demand was characterized based only on the information provided by the 32 restaurants that used jalapeño pepper in their dishes.

Most restaurants in Bacalar and Chetumal prefer to buy jalapeño pepper in supermarkets, while restaurants in Playa del Carmen and Tulum prefer to buy in public markets. While some restaurants in other municipalities purchase peppers each week, restaurants in Playa del Carmen and Tulum purchase peppers on a fortnightly basis.



**Figure 1**. Type of cuisine that restaurants offer per municipality (%). Source: Figure developed by the authors, based on data from the surveys.

**Table 2**. Restaurants that use jalapeño pepper in their dishes.

Municipality	Yes	Not
Bacalar	5	8
Chetumal	9	12
Playa del Carmen	9	8
Tulum	9	13
Total	32	41

Source: Table developed by the authors, based on data from the surveys.

Most restaurants consume 1 to 10 kg of jalapeño pepper per week. A restaurant from the municipality of Bacalar uses 11 to 25 kg of jalapeño pepper per week (Table 3). The volumes required per each municipality and all municipalities were estimated based on the volume purchased and the number of restaurants.

For example, to calculate the monthly demand (kg) for Bacalar, the number of restaurants (4) that fall within the weekly purchase range (1-10) was multiplied by the minimum value (1) of the weekly purchase range (1-10), obtaining a weekly demand of 4 kg. Subsequently, the number of restaurants in Bacalar (1) that fall within the weekly purchase range (11-25) was multiplied by the minimum value (11) of the weekly purchase range (11-25), obtaining a weekly demand of 11 kg. Subsequently, Bacalar's minimum values were added up, obtaining a weekly consumption of 15 kg. Multiplying that result by the number of weeks per month (4.34525), the result was 65.12 kg—the approximate monthly demand in Bacalar. Taking into consideration the minimum values, the maximum value was 282.44 kg (65 kg per week times 4.34525). The same calculation was performed for the maximum weekly purchase values for each municipality.

Therefore, the minimum, maximum, and average potential monthly demand was estimated in 182.45, 1,455.65, and 819.05 kg per month. The value of the sales of the restaurant was estimated based on the quantities demanded and the average rural price of jalapeño pepper in 2020. Consequently, the minimum and maximum demands can generate a net income of \$1,733.27 and \$13,828.67 pesos per month, respectively, based on an average rural price of \$9.5 per kg. These amounts can reach a minimum and maximum annual income of \$20,799.24 and \$165,944.10, respectively. However, these amounts may be higher, given the price escalation of 2022.

**Table 3**. Calculation of the demand of jalapeño pepper per restaurant.

M:	Weekly pu	rchase (kg)	Estima	ted monthly dema	and (kg)
Municipality	1 a 10	11 a 25	Minimum	Maximum	Average
Bacalar	4	1	65.12	282.44	173.78
Chetumal	9	0	39.11	391.07	215.09
Playa del Carmen	9	0	39.11	391.07	215.09
Tulum	9	0	39.11	391.07	215.09
Total	-	-	182.45	1,455.65	819.05

Source: table developed by the authors, based on data from the survey.

Therefore, restaurants' demand for jalapeño pepper reached a potential maximum demand of 1.5 tons per month and 18 tons per year. Consequently, at least 1.5 ha would be required each year for the staggered production of pepper. This situation provides an opportunity for jalapeño pepper producers, which are currently located in the municipalities of Bacalar and Othón P. Blanco.

#### Supply development

Determining the willingness of restaurants (consumers) to establish alliances with jalapeño pepper producers (producers) is important for the evaluation of the feasibility of establishing a supply arrangement among them.

Only two restaurants are unwilling to establish an alliance to buy directly from farmers. In contrast, 30 restaurants (93.75%) would agree to establish a supply agreement with local jalapeño pepper producers (Table 4).

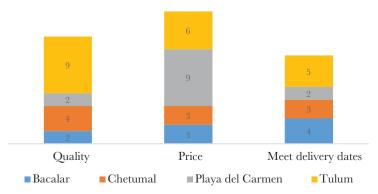
Additionally, restaurants that wish to establish supply arrangements with jalapeño pepper producers set forth the requirements of this supply agreement. Restaurants demand the following items: price (21 restaurants), quality (19), and fulfilment of delivery dates (14). Figure 2 shows that Tulum is the municipality with more restaurants that demand quality (9), while most restaurants in Playa del Carmen demand price (9).

Although some requirements are more important for restaurants, the long-term survival of these linkages requires, at the very least, the complete fulfilment of the three major

**Table 4**. Willingness of restaurants to establish supply arrangements with producers.

Municipality	Yes	Nope
Bacalar	5	0
Chetumal	7	2
Playa del Carmen	9	0
Tulum	9	0
Totals	30	2

Source: table developed by the authors, based on data from the survey.

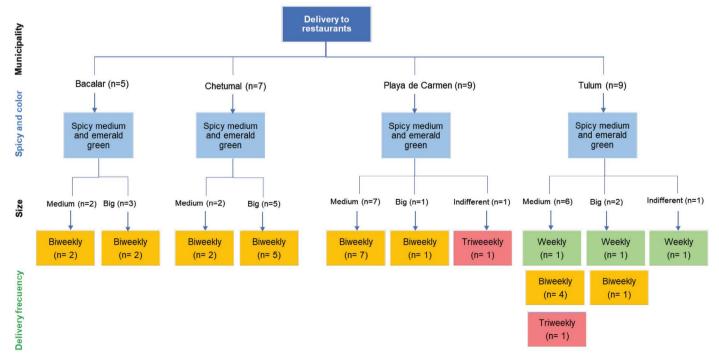


**Figure 2**. Number of restaurants and their requirements to establish arrangements with producers. Source: table developed by the authors, based on data from the survey.

requirements: price, quality, and delivery dates. Figure 3 shows the supply arrangements for restaurants that use jalapeño pepper. The results are shown per municipality and according to the four relevant headings required by corporate consumers (restaurants): medium-sized and big peppers, medium-level pungency, and emerald green skin. In this respect, the local supply level implies a differentiated and standardized product, as well as a constant supply.

The low association and branding level problems —the latter characterized by production and commercialization individuality— reduce the negotiating power of small producers in the market, turning them into agents that accept the lowest prices (Caicedo, 2013). Achieving a supplier development between producers and an industry or company (e.g., restaurants) could improve the commercialization, price, and profits of small rural producers. The income for the sale of jalapeño peppers recorded in this study takes into consideration the average rural price for 2020. However, negotiations could lead to better purchase prices and the satisfaction of the requirements made by the consumers: quality and delivery frequency.

Supplier development studies usually focus on the offer aimed at established industries —which demand a product with constant quantity and quality. For example, there are supplier development studies for the following established industries: vanilla (Barrera et al., 2014), rubber (González-Ramírez et al., 2019), and avocado (Espejel et al., 2022). However, this study seems to be one the first of its kind that seeks to identify if direct consumers (in this case, restaurants) would be interested in a supplier development arrangement with local producers. This process could be described as a demand-based



**Figure 3**: Supply arrangement for Quintana Roo's restaurants. Source: table developed by the authors, based on data from the survey.

supplier development, whose priority would be the establishment of production- and consumption-focused economic relationships, based on solidarity and the common good (López, 2012).

Both cases (offer-based or demand-based supply) would fulfill the concept proposed by Castro *et al.* (2016): supplier development are all those activities that benefit both the purchasing company and the suppliers. Those activities strengthen their capacities, through trust relationships and the promotion of a joint development, making joint contributions, adding value to the supply chain, and improving performance. Likewise, the concept of short commercialization channels should be worked upon. According to López (2012), this concept is understood as an agri-food circulation system in which only one or no intermediary stands between production and consumption, and which provides an advantage in the search for fair prices between the offeror and the consumer.

The main requirements of the restaurants that pointed out that they intended to establish a supply development with local producers of jalapeño pepper are quality and fulfilment of delivery dates. Quality is one of the main attributes that companies require. Torres (2012) obtained similar results regarding the supply of macadamia (a gourmet product) to supermarkets and retailers: quality is guaranteed for this kind of products.

This study identified restaurants as a market niche for local jalapeño pepper producers. This is a first step towards a supply arrangement. Achieving ongoing contracts and arrangements requires social organization, institutional state support, technical assistance to obtain quality products, and the fulfilment of sale commitments. Reina-Usuga *et al.* (2018) add that changes in governance mechanisms in the territorial food system are also required.

According to Fernández and Natividad (2018), this situation would create a direct linkage between producers and consumers, invigorating local economy, increasing the income of small producers (men and women), and becoming a point of entry to other institutional local markets. Additionally, according to Mauleón (2012), this direct sale relationship between producers and consumers —which increases the direct contact between both groups— supports family farming, preserves rural employment, creates new entrepreneurial opportunities, and breaks the isolation of farmers. According to Connell *et al.* (2008), purchasing fresh product grown by local and known farmers would benefit consumers (*i.e.*, restaurant owners).

#### **CONCLUSIONS**

Only 44% of the surveyed restaurants use jalapeño pepper in their recipes. The restaurants that do use them are willing to establish supply links with producers. There is a potential demand among local restaurants for jalapeño peppers. However, achieving this kind of agreements requires a differentiated supply level in the local sphere —i.e., satisfying the demand of local restaurants. This process would imply the development of a differentiated and standardized product and the overall fulfilment of three major points: price, quality, and delivery dates. Potential consumers require products with the following characteristics: medium-sized and big peppers, medium-level pungency, and an emerald green skin.

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# Study of the allelopathic effect of some weeds on the germination of durum wheat (*Triticum durum* desf.).

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#### **ABSTRACT**

**Objetive**: the presence of weeds in a cereal field is detrimental for several reasons. In this study, we chose four adventitious species to test their allelopathic effect on the germination of seeds and the development of seedlings of the durum wheat variety "Simeto".

**Design/Methodology/Approach**: we tested the allelopathic effect at the laboratory level, by mixing weed seeds and durum wheat. In these trials, the allelopathic effect was measured.

**Results**: the inhibitory effect of these adventitious species manifests itself much more on the development of the seedlings, especially on the aerial parts.

Findings/Conclusions: the bindweed (Convolvulus arvensis L) is the most inhibiting.

Keywords: Allelopathy, Weed, Inhibition, Germination, Durum wheat (Triticum durum Desf.).

Citation: Hannachi, A. (2023). Study of the allelopathic effect of some weeds on the germination of durum wheat (*Triticum durum* desf.). Agro Productividad. https://doi.org/10.32854/agrop. v16i1.2356

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: August 03, 2022.
Accepted: January 05, 2023.
Published on-line: February 22, 2023.

*Agro Productividad*, *16*(1). January. 2023. pp: 23-31.

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#### INTRODUCTION

Cereals have long been produced in Algeria in all agro-ecological zones. This culture is very old in Algeria, durum wheat holds a first-rate place among cultivated plants. It is cultivated under rainfed regime in the majority of production areas and therefore is dependent on climatic conditions, more precisely on the amount of rain received during the plant's development cycle (Zaghouane and Bouziri, 2013).

The raison d'être of agriculture means that, in a cultivated field, any plant that is not sown or planted voluntarily is considered undesirable and the farmer has not stopped destroying these weeds, which are easy to show the harmfulness as they sometimes penalize yields. Weeds have always been a problem for agricultural producers. Heavy losses in yields and crop quality result from weed competition (Hannachi, 2010).



Among the many enemies of crops, weeds occupy an important place. The presence of weeds or weeds in a grain field can be detrimental for several reasons. Competition for water, minerals and light, as well as toxic chemical interactions between weeds and cultivated plants (allelopathy), directly affects crop growth and yield. The massive infestation of these weeds hinders the plowing and harvesting tools and makes the success of these operations problematic. The mixture of weed seeds with cereal seeds degrades the commercial quality of the harvested product. Cereal weeds should therefore be effectively combated. Competition between weeds and crops also plays a role in yield losses (Hossain *et al.*, 2016).

The study of weeds therefore aims to control the weed flora and set up the appropriate process to eliminate it. The objective of this study is to test the allelopathic effect of some weeds on the germination of seeds and the development of seedlings of durum wheat (*Triticum durum* Desf.) Variety "Simeto".

#### MATERIALS AND METHODS

#### Plant material

Our work consists in studying the allelopathic effect of some weeds on the germination of durum wheat (*Triticum durum*) variety "Simeto", which represents the plant material used provided by the CCLS Sahki Mouhamed (Matéra) El harouche (Algeria) seed used for the test is a crop from the period 2013-2014.

#### **Simeto**

Is an Italian variety, obtained following a cross between two varieties which are capeiti and valvona, registered in 2001 and requested by ITGC. The characteristics of the variety Simeto according to the national center for control and certification of seeds and plants (2009) are:

#### Morphological characteristics

Medium-sized plant, white ear at maturity and short with black beard, with a semi elongated grain, habit with semi-upright tillering, and early cycle. Moderately susceptible to disease but resistant to powdery mildew.

#### Technological characteristics

Weight of a thousand grains is high, with very good semolina quality and protein content of 15.80%, and resistant to mitination (CNCC, 2009).

#### Weeds

In order to choose weed species to study their allelopathic effect on durum wheat and barley, we asked CCLS Sahki Mouhamed El harouche (Algeria) some weed species, we chose four (04) species belonging to 04 botanical families:

- Wild oats (*Avena sterilis* L.), Family: Poaceae (grasses).
- Wild bindweed (Convolvulus arvensis L.), Family: Convolvulaceae.

- Field mustard (Sinapia arvensis L.), Family: Brassicaceae (Crucifers).
- Centaury (Centaurea nicaeensis All.), Family: Asteraceae (Compositae).

The majority of the species chosen are problematic cereal species in the Mediterranean region.

#### Laboratory experimentation Germination tests

All germination tests are carried out in Petri dishes. We used sterile plastic dishes 85 mm in diameter and 15 mm high. Tissue disks with a diameter equal to that of the dishes are placed in the Petri dishes. The same type of boxes are used for durum wheat and barley and also for each weed species. Each box is numbered with labeling.

#### Preliminary germination tests

In order to obtain maximum germination rates and to choose an average duration for germination tests, we carried out preliminary germination tests. All seeds used (durum wheat and weeds) are subjected to these tests.

We used two petri dishes for each species. We initially introduced 20 ml of distilled water with a graduated pipette (10 ml). Then 10 seeds of each species are placed on tissue paper in each box. Follow the germination of the seeds every day at the same time, for 10 days the speed and duration of germination of the seeds do not change significantly at ambient temperatures (from 15 to 25 °C). However, at 10 °C the seeds have a slow germination (Buhler and Leroux, 1997). On the 8<sup>th</sup> day of incubation we observed that all the seeds which germinate develop a radicle (or coleorrhize) and a tigella (or coleoptile). After this incubation period we have noticed that there is no more germination and that some roots are starting to dry out. The preliminary germination tests allowed us to finalize the list of weeds. The selected species are those which have presented a germination rate greater than or equal to 50%. These preliminary tests also allowed us to determine the duration of the final germination tests (8 days). The species used for the allelopathic tests were: field mustard (*Sinapia arvensis* L.), centaury (*Centaurea nicaeensis* All.), wild oats (*Avena sterilis* L.) and bindweed (*Convolvulus arvensis* L.).

#### Final germination tests

We used Five Petri dishes to test the allelopathic effect of each weed species on the germination of durum wheat and barley.

Five boxes are used for durum wheat and others are used for barley. These represent the witness. Using a graduated pipette we initially introduced 20 ml of distilled water for all the petri dishes. In each Petri dish we placed 10 seeds of durum wheat or barley and 10 seeds of each adventitious species except the witnesses. The boxes are covered immediately. We chose healthy seeds (without anomalies) and which are almost the same size. After installing the prepared petri dishes in the laboratory, we followed the germination and took notes.

#### Determination of germination percentages

After 8 days of incubation, the experiment is stopped and the percentage of germination of durum wheat (photos 1, 2, 3,4) in each box is determined. We considered as germinated seed that which developed a coleorhize.

#### Measurement of the lengths of the roots and aerial parts

After determining the number of seeds that germinated in each box, we measured the lengths of the root part (LPR) and the aerial part (LPA). The LPR represents in durum wheat and barley the length of the coleorhize or the longest of the primary roots. LPA for durum wheat and barley is the length of the coleoptile or first leaf.

We followed a measurement method, by direct measurement of LPR and LPA on millimeter paper.

#### Data analysis

The percentage of seed germination for each Petri dish is determined according to the following formula:

$$PG\%$$
 = number of seeds that germinated  $\times$  100/10

To compare the effects of the four weed species on durum wheat and barley, we converted the germination percentages and the LPR and LPA measurements to inhibition percentages. The conversions are carried out according to the following formula [5]:

$$%I = \lceil (Witness - CAd) / Witness \rceil \times 100$$

%I: the percentage of inhibition compared to the control; *Witness*: the average of the 5 repetitions of the witness; CAd: the percentage of germination or the length of the LPR or LPA of each box containing durum wheat (C) with an adventitious species (Ad) (Figure 1-4).

The %I of each species (germination, length of root and length of aerial part) is calculated separately, such as: %GI: The percentage of inhibition of germination (G).%ILR: Percentage inhibition of root length (LR), %ILPA: The percentage of inhibition of the length of the aerial part (LPA). The data obtained relate to the percentage of germination (%PG), the length of the root part (LPR), the length of the aerial part (LPA) of durum wheat. We presented for each species, the differences between mixing durum wheat with a weed and the control, the effect on the three variables (LR, LPA and PG).

#### RESULTS AND DISCUSSION

## Effect of different weed species on durum wheat: effect on durum wheat: "Simeto" variety

The analysis of variance indicates that the percentage of germination (PG), the length of the root part (LPR) and the length of the aerial part (LPA) of durum wheat are significantly



Figure 1. Durum wheat germination test with Avena sterilis L.

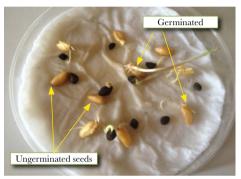


Figure 2. Durum wheat germination test with Convolvulus arvensis L.

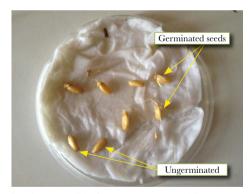


Figure 3. Durum wheat germination test with Centaurea nicaeensis All.



Figure 4. Durum wheat germination test with Sinapia arvensis L.

affected at P=0.95 and P=0.99 by the four weed species. The comparison of the means indicates that all the weed species significantly affect the length of the aerial part at P=0.95. For the length of the root, the means of the durum wheat and adventitious mixtures are significantly different in comparison with the control. For germination, wild mustard and wild oats did not have a significant effect in comparison with the control, on the other hand, wild bindweed (*Convolvulus arvensis* L.) and centaury (*Centaurea nicaeensis* All.) Are significant.

#### Effect on germination

We note that the highest inhibition is obtained by the bindweed (*Convolvulus arvensis* L) represents a percentage rate of 40%, and then the centaury (*Centaurea nicaeensis* All.) At 24.44%. On the contrary field mustard (*Sinapia arvensis* L.) and wild oats (*Avena sterilis* L.) can stimulate the germination of durum wheat variety "Simeto" (Figure 5).

#### Effect on the length of the root portion

The results obtained (Figure 6) show that the species most inhibiting the development of the root part is the bindweed (*Convolvulus arvensis* L.) per percentage of inhibition exceeds 35%. The inhibitory effect of the other three species is moderately weak and does not exceed 20%.

#### Effect on the length of the aerial part

The three species field mustard (Sinapia arvensis L.), centaury (Centaurea nicaeensis All.), And wild oats (Avena sterilis L.), show a moderately weak inhibitory effect on the length of the aerial part. They have values that do not reach 23% (Figure 7). In contrast, the inhibition percentage is relatively higher for the bindweed (Convolvulus arvensis L) at 48.05%. The results show that the species most inhibiting seed germination and seedling development is the bindweed (Convolvulus arvensis L). As well as wild oats has a positive effect which stimulates the germination of grains of the durum wheat variety "Simeto". All species except wild oats (Avena sterilis L.) inhibit the length of the aerial part more than the length of the root.

The germination of a seed can only take place if certain favorable conditions are met, namely: oxygen, temperature, water. Furthermore, it is well known that natural substances produced by plants are capable of delaying or even inhibiting the germination of seeds and the growth of seedlings, this is the phenomenon of allelopathy.

This work determines the existence of an allelopathic phenomenon in experimental conditions, it provides proof that weeds contain toxic compounds whose action can potentially be exerted in natural conditions. In what follows, we will discuss the results presented in the 1<sup>st</sup> part of this chapter.

The results we obtained show that the four weed species: bindweed (*Convolvulus arvensis* L.), Centaury (*Centaurea nicaeensis* All.), Field mustard (*Sinapia arvensis* L.), and wild oats (*Avena sterilis* L.), affect durum wheat variety "Simeto" and barley variety "Saida" in different ways.

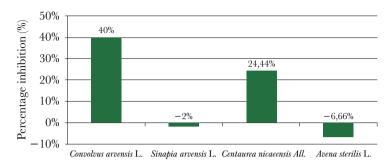


Figure 5. Percentage inhibition of weeds on germination (PG) of durum wheat (Simeto).

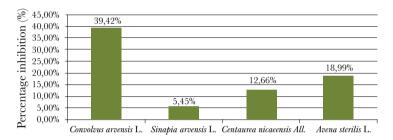


Figure 6. Percentage inhibition of weeds over the length of the root portion (LPR) of durum wheat (Simeto).

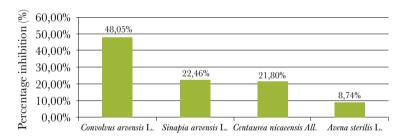


Figure 7. Percentage inhibition of weeds over the length of the aerial part (LPA) of durum wheat (Simeto).

The effects of these weeds are observed on seed germination and seedling development. We have noticed that the germination of the seeds is delayed or it stops in an advanced stage or it does not occur.

When seed germination is not inhibited, we have observed other effects on seedling development (inhibition or stimulation). In the case of inhibition, we noted effects on the radicle (coleorrhize), on the tigella (coleoptile) or on both showed that when sensitive plants are exposed to allelopathy, the germination of seeds is delayed (Kheddam, 1998). With some seeds, germination stops in the swelling stage of the seed. Others believe that germination stops at the onset of the radicle.

According to Lamamra, 2007, the percentage of germination increases and the length of the root increases. Germination is accelerated and the development of the aerial part is important.

Some have an inhibitory effect on germination like bindweed (*Convolvulus arvensis* L.) on durum wheat variety "Simeto". Others cause an inhibiting effect on their growth such

as field mustard (*Sinapia arvensis* L.), according to Rouimel, 2012, cruciferous plants often tend to be "toxic"; especially mustard, which should not be combined with cereals such as wheat and barley.

In the reality of the cultivated field, it seems that the growth inhibitions observed in the laboratory are much more difficult to explain (Sayoud, 1978). Summarize the reasons for these differences between controlled conditions and agricultural field. The first difficulty is linked to the multiple interactions between two species that coexist or succeed one another: how to distinguish allelopathy and competition for example. The second difficulty stems from the fact that, even if there is a production of compounds for which the depressive action on the biological functions of target species has been shown under controlled conditions, this does not mean that this effect is expressed field. For this, it would be necessary that at the appropriate time the quantities available of these molecules in the medium are sufficient, and that the target plant is in an adequate state of sensitivity. Finally, the third difficulty is linked to the intervention of other living organisms in the system: phytotoxic compounds can also have microbial origins, independent of the presence of a culture. Allelopathy is therefore, the chemical interference of one or more substances of a plant species with the germination, growth or development of other plant species, which covers both inhibition and stimulation effects. When susceptible plants are exposed to allelopathy, germination, growth and development can be affected.

#### **CONCLUSION**

The allelopathic effect of weeds, bindweed (Convolvulus arvensis L.), Centaury (Centaurea nicaeensis All.), Field mustard (Sinapia arvensis L.), and wild oats (Avena sterilis L.) vary according to the species. Some have an inhibiting effect on germination such as bindweed. The results obtained give that the majority of species significantly inhibit the germination of durum wheat. The highest inhibition is noted for the weed bindweed species. Agronomic solutions almost all have assumed and recognized consequences on the organization of work (shifting the sowing date, establishment of inter-crop or allelopathic precedent) or the energy used (tillage before sowing and mechanical weeding). These technical routes reducing the use of herbicides must therefore be evaluated on several criteria: economic performance of course, but also energy (efficiency, cost), working time (duration, site conflict), agronomic value (objective achieved, effects unwanted induced effects, long term effects on flora).

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# Morphological characterization of Elephant grass (*Pennisetum purpureum* Schumach) ecotypes collected in Chiapas, Mexico

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#### ABSTRACT

**Objective**: To characterize the morphology of 18 ecotypes of Elephant grass (*Pennisetum purpureum* Schumach) in Chiapas.

**Design/Methodology/Approach**: The morphological characterization was carried out with 34 quantitative and qualitative descriptors. A principal component analysis and a hierarchical cluster analysis were performed based on the average data.

**Results**: In the principal component analysis, five of the principal components accounted for 70.7% of the total variability in the 18 ecotypes of Elephant grass. The variables that made the most significant contributions in each CP were: in CP1, internode diameter (p<0.01), internode length (p<0.05), color of internode without wax (p<0.01), number of innovations (p<0.01), prophylls (p<0.01), number of prophylls (p<0.01), external length of the sheath (p<0.01), internal length of the sheath at its opening point (p<0.01), opening of the auricle (p<0.01); for CP2, the number of visible internodes (p<0.05), channel width (p<0.05), size of innovations (p<0.05), adventitious root, number of internodes (p<0.01), wax under the sheath (p<0.05); and for CP3, number of visible internodes (p<0.05), color of the internode with wax (p<0.001), channel depth (p<0.001), ligule shape (p<0.05) and leaflet tip (p<0.05). As a result of the hierarchical cluster analysis and the semipartial correlation coefficient, five morphologically distinct groups were determined.

**Study Limitations**: A more accurate description of the morphological diversity of the grasses requires the characterization of the inflorescence and the spikelet.

**Findings/Conclusions**: The 18 ecotypes of the Elephant grass (*Pennisetum purpureum* Schumach) collected and characterized were dissimilar with each other; consequently, they are considered a genetic resource with potential importance as forage on the Chiapas coast.

Keywords: tropical grasses, livestock, feeding.

Citation: Maldonado-Méndez, J. de J., Guerra-Medina, C. E., Avendaño-Arrazate, C. H., Gálvez-Marroquín, L. A., & Ariza-Flores, R. (2023). Morphological characterization of Elephant grass (*Pennisetum purpureum* Schumach) ecotypes collected in Chiapas, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop. v16i1.2366

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: August 23, 2022.

Accepted: December 28, 2022.

Published on-line: February 22, 2023.

*Agro Productividad*, *16*(1). January. 2023. pp: 33-43.

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#### INTRODUCTION

The Elephant grass [Pennisetum purpureum Schumach or Cenchrus purpureus Morrone (Schumach)] is native to eastern Africa and has been introduced to the tropics and subtropics of both hemispheres. It is a C<sub>4</sub> grass adapted to environments with high temperatures, high solar radiation, drought, and N or CO<sub>2</sub> limitations (SiB Colombia, 2020). It has a wide dispersion and high genetic diversity (Cavalcante and Lira, 2010). Its morphological characteristics have been researched during the vegetative and reproductive stages (Tcacenco and Lance, 1992). Elephant grass accessions have high genetic diversity, which is distributed in different groups. They have various uses, including the production of biofuels (Silva et al., 2017; Rocha et al., 2017; López et al., 2014) and the production of ethanol (Scholl et al., 2015). Likewise, their lignin, cellulose, and ash contents are suitable for direct combustion (Morais et al., 2009) and they can be used to make paper, due to their amount of lignocellulosic biomass (Madakadze et al., 2010). We also studied the roots and stems of Elephant grass genotypes that benefited from the biological nitrogen fixation in the soil, carried out by a variety of nitrogen-fixing bacteria from the Herbaspirilum, Azospirilum, Gluconacetobacter, Burkholderia, Bradyrhizobium, Klebsiella, Leptotrix, Enterobacter, and Diazotrophic genera (Videira et al., 2012 and 2013).

Taking into consideration the forage potential of the Napier grass (Rusdy, 2016; Wangchuk et al., 2015), the ILRI conducted a study of 345 genotypes, in order to analyze and compare genetic diversity between and within world collections. The results provide useful information for the management, conservation, improvement strategy, and improvement of the genetic diversity within the said collections (Muktar et al., 2021). In addition, evaluations of agronomic traits have been carried out (Kebede et al., 2016a) selecting cultivars with high biomass production (Cunha, 2011), as a result of their chemical composition and in vitro digestibility (Kebede et al., 2016b). Finally, Nyambati (2010) reported cultivars that produce 6.2 kg per day in grazing systems aimed at milk production.

Given the high potential of Elephant grass, the germplasm bank of the Rosario Izapa experimental field has a genetic improvement program. The first step of the said program was the collection of naturalized ecotypes. The most productive ecotypes, as well as those with the best quality, were selected as forage, both in humid and dry tropical areas. These ecotypes can be used for grazing, haymaking, and silage (mainly during the season when forage is less available), as well as green manure and to control the erosion of the hillsides. Additionally, they improve the feeding of bovines, resulting in a greater production of milk and meat.

The objective of this study was to collect and characterize naturalized clones of the Elephant grass (*Pennisetum purpureum* Schumach) in Chiapas. The collected specimens would be used to develop a genetic base for the selection of outstanding ecotypes with greater production potential and nutritional quality that can be used in grazing.

#### MATERIALS AND METHODS

The Elephant grass (*Pennisetum purpureum* Schumach) specimens were collected along the Pacific Coastal Plain of the state of Chiapas, from the municipality of Ciudad Hidalgo

to the municipality of Tonalá, in a 360-km long and 15-40 km wide area. Table 1 shows the 18 collection sites, where 18 germplasm ecotypes were collected, as well as their respective passport data (Villanueva-Avalos *et al.*, 2012; Morales, 2008). A 1 m² sample was obtained and 34 quantitative and qualitative descriptors of 10 randomly selected stems were measured *in situ* (Table 2) (Maldonado-Méndez *et al.*, 2019; Quero-Carrillo *et al.*, 2012; Kretschmer *et al.*, 1979). The quantitative characteristics were measured with a measuring tape, a ruler, and a RoHS<sup>™</sup> digital vernier; the qualitative characteristics were evaluated by observation and the color was compared with the Royal Horticultural Society color chart.

#### Statistical analysis

The mean, the minimum value, the maximum value, the coefficient of variation of the quantitative variables, and the mode of the qualitative data of the 18 ecotypes were determined. A multivariate analysis was used to analyze the data. The principal component analysis (PCA) was carried out using the SAS (2020) PRINCOMP procedure, where the eigenvalues and eigenvectors were considered. The principal components 1, 2, and 3 were plotted on a Cartesian plane to observe the distribution of the characterized ecotypes. In addition, the hierarchical cluster analysis was used, and the groups of the different ecotypes were divided using the squared Euclidean distance and the hierarchical clustering algorithm.

**Table 1.** Passport data of 18 ecotypes of Elephant grass (*Pennisetum purpureum* Schumach) collected on the Chiapas Coast.

	Ecotype	Location	Latitude (N)	Longitude (O)	Altitude (m)
1	INIFAP Pp-01	Cacahoatán	15° 01' 53.779"	92° 9' 44.009"	643
2	INIFAP Pp-02	Tuxtla Chico	14° 56' 36.956"	92° 10′ 12.730"	318
3	INIFAP Pp-03	Frontera Hidalgo	14° 48' 15.721"	92° 15' 21.338"	63
4	INIFAP Pp-04	Ciudad Hidalgo	14° 41' 33.390"	92° 16' 55.102"	14
5	INIFAP Pp-05	Metapa	14° 51' 17.994"	92° 11' 31866"	77
6	INIFAP Pp-06	Tapachula	14° 54' 33.405"	92° 16' 40.399"	173
7	INIFAP Pp-07	Tapachula	14° 54' 38.875"	92° 16' 39.372"	161
8	INIFAP Pp-08	Mazatán	14° 51' 57.620"	92° 25' 23.917"	27
9	INIFAP Pp-09	Mazatán	14° 49' 31.841"	92° 28' 17.311"	7
10	INIFAP Pp-10	Huehuetán	15° 00' 51.364"	92° 23' 22.231"	41
11	INIFAP Pp-11	Huehuetán	14° 59' 27.286"	92° 27' 06.065"	10
12	INIFAP Pp-12	Huehuetan	14° 59' 26.833"	92° 27' 09.548"	17
13	INIFAP Pp-13	Huixtla	15° 05' 42.492"	92° 28' 30.359"	34
14	INIFAP Pp-14	Villa Comaltitlán	15° 19' 54.752"	92° 33' 48.779"	348
15	INIFAP Pp-15	Mapastepec	15° 25' 32.689"	92° 00' 20.872"	29
16	INIFAP Pp-16	Pijijiapan	15° 38' 23.970"	93° 00' 20.872"	22
17	INIFAP Pp-17	Tonalá	15° 58' 27.652"	93° 38' 11.210"	63
18	INIFAP Pp-18	Pijijiapan	15° 44' 33.534"	93° 17' 36.484"	44

**Table 2**. Morphological characteristics used for the characterization study of the ecotypes of the *Pennisetum purpureum* grass collected on the Chiapas coast.

	Character	Acronym	Character state (scale)
	Stem	_	
1	Number of visible internodes	SNVI	Number
2	Internode diameter (measure the middle internode)	SID	mm
3	Internode length	SIL	mm
4	Presence of wax	SPW	1. Absence, 2. weak, 3. strong
5	Color of the internode with wax	SCIWW1	1. Yellowish, 3. Yellowish green, 5. Black and white, 7. Black
6	Color of the internode without wax	SCIWW2	1. Yellowish, 2. Green, 3. Yellowish green
7	Channel width	SCW	Mm
8	Channel depth	SCD	1. Little deep, 2. intermedium, 3. Deep
9	Innovations	SI	1. Absence, 2. Presence
10	Number of innovations	SIN	Number
11	Size of innovations	SSI	Mm
12	Prophylls	SP	1. Absence, 2. Presence
13	Number of prophylls	SNP	Number
14	Size of prophylls	SSP	Cm
15	Adventitious root	SAR	1. Absence, 2. Presence
16	Number of internodes	SIN	Number
	Leaf		
17	Wax under the leaf sheath	LWULS	1. Absence or very little, 3. Litte, 5. Very little
18	Leaf sheath color	LLSC	1. Green, 2. Yellowish, 3. lime green
19	Leaf sheath hairiness	LSH	1. Absence o very low, 3. low, 5. medium, 7. high, 9. Very high
20	External length of the leaf sheath	LELLS	Cm
21	Internal length of the leaf sheath at the opening point	LILLSOP	Cm
22	Ligule shape	LLS	1. Flat, 2. convex, 3. biconvex
23	Ligule pubescence	LLP	3. low, 5. medium, 7. high
24	Development of the auricle	LDP	1. Rudimentary, 2. developed
25	Opening of the auricle	LOA	Cm
26	Lamina position	LLP	1. Erected, 3. semi-erect, 5. open
27	Lamina lenght	LLL	Cm
28	Lamina width	LLW	Cm
29	Lamina color	LLC	1. Green, 2. Yellowish green, 3. Lime green, 4. Dark green
30	Central veins width	LCVW	Mm
31	Central veins color	LCVC	1. Whitish, 3 Green
32	Hairness in the upper side of the lamina	LHUSL	1. Absence o very low, 3. low, 5. medium, 7. high, 9. Very high
33	Leaflet tip	LLT	1. Absence, 2. Presence
34	Leaf-blade apex length	LLBAL	Cm

#### RESULTS AND DISCUSSION

#### **Principal Component Analysis (PCA)**

Five main components accounted for 70.7 % of the total variability in the 18 ecotypes of the Elephant grass (*Pennisetum purpureum* Schumach): PC1 accounted for 25.4%, PC2 14.2%, PC3 12.3%, PC4 10%, and PC5 8.6% (Table 3).

The variables that made the most significant contributions in each CP were: in CP1, internode diameter (SID), internode length (SIL), color of the internode without wax (SCIWW2), number of innovations (SIN), prophylls (SP), number of prophylls (SNP), external length of the sheath (LELLS), internal length of the leaf sheath at the opening point (LILLSOP), opening of the auricle (LOA); in CP2, number of visible internodes (SNVI), channel width (SCW), size of innovations (SIN), adventitious root (SAR), number of internodes (SIN), wax under the sheath (LWULS); and for CP3, number of visible internodes (SNVI), color of the internode with wax (SCIWW1), channel depth (SCD), ligule shape (LLS), and leaflet tip (LLT) (Table 4).

CP1 is related to stem and leaf variables, while CP2 is related to the stem. Consequently, the distribution on the cartesian coordinates, according to CP1 and CP2 shows the variation found in the ecotypes of the Elephant grass (*P. purpureum* Schumach). In addition, both CPs account for about 40 % of the variation (Figure 1a). The variation in CP1 and CP3 is the result of their relationship with stem and leaf variables (Figure 1b).

#### Hierarchical Cluster Analysis (HCA)

According to the hierarchical cluster analysis (Kaufman and Rousseuw, 1990), based on 34 stem and leaf morphological variables of the 18 Elephant grass ecotypes, five groups (GI, GII, GIV, and GV) were determined, using the 0.07 semipartial correlation coefficient (Figure 2).

Table 5 shows the quantitative and qualitative morphological descriptors of stems and leaves, grouped according to the results of the dendrogram.

Group I include the following ecotypes: INIFAP Pp-01, INIFAP Pp-03, INIFAP Pp-10, INIFAP Pp-14, and INIFAP Pp-15. According to its characteristics, the stem grows rapidly by producing more internodes (26) than the other groups; however, its diameter is smaller (20.51 mm) and it is 14.9 cm long. The internode with wax is black and white, while the internode without wax is yellowish green. The highest channel is 4.78 mm wide, which

**Table 3**. Eigenvalues and ratio of the total variance per principal component, based on the morphological characteristics of 18 ecotypes of the Elephant grass (*Pennisetum purpureum* Schumach).

PC	Eigenvalues	Difference	Proportion	Acumulate
1	6.627	2.934	0.254	0.254
2	3.692	0.479	0.142	0.396
3	3.213	0.609	0.123	0.520
4	2.604	0.351	0.100	0.620
5	2.252	0.514	0.086	0.707

PC=Main component.

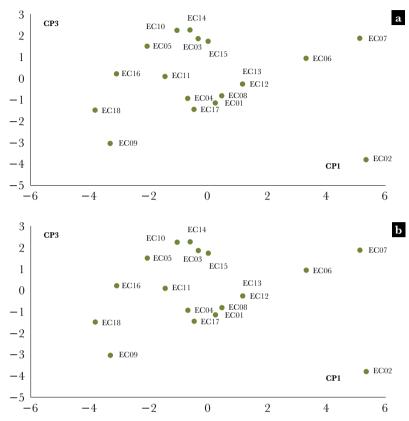
**Table 4**. Values of the principal components and the correlation coefficient of each morphological variable of 18 ecotypes of Elephant grass (*Pennisetum purpureum* Schumach).

***		Eigenvectors		Pearson's	s coefficient co	rrelation
Variable	PC1	PC2	PC3	PC1	PC2	PC3
SNVI	0.110	0.346	0.264	0.283ns	0.664*	0.472*
SID	0.339	0.178	-0.055	0.873**	0.342ns	-0.097ns
SIL	-0.200	0.163	-0.093	-0.515*	0.312ns	-0.167ns
SPW	-0.058	0.176	-0.013	-0.150ns	0.337ns	-0.022ns
SCIWW1	0.000	-0.274	0.381	0.000ns	-0.525*	0.683**
SCIWW2	-0.248	-0.092	0.143	-0.639**	-0.176ns	0.255ns
SCW	0.118	0.255	-0.076	0.302ns	0.490*	-0.135ns
SCD	-0.184	0.009	-0.351	-0.472*	0.017ns	-0.629**
SI	0.130	0.177	0.164	0.335ns	0.340ns	0.293ns
SIN	0.235	0.190	0.075	0.604**	0.364ns	0.135ns
SSI	0.110	0.230	-0.017	0.284ns	0.442*	-0.030ns
SP	-0.320	0.170	0.044	-0.822**	0.326ns	0.078ns
SNP	-0.261	0.263	0.152	-0.671**	0.504*	0.271ns
SSP	-0.254	0.190	0.212	-0.654*	0.366ns	0.379ns
SAR	-0.125	0.223	-0.076	-0.322ns	0.428ns	-0.135ns
SIN	0.000	0.330	0.316	-0.001ns	0.634**	0.565*
LWULS	-0.077	-0.279	0.287	-0.198ns	-0.536*	0.515*
LLSC	-0.051	0.195	-0.004	-0.130ns	0.375ns	-0.007ns
LSH	0.173	-0.144	-0.226	0.445ns	-0.276ns	-0.405ns
LELLS	0.312	0.027	-0.037	0.802**	0.052ns	-0.065ns
LILLSOP	0.323	-0.001	0.015	0.832**	-0.002ns	0.026ns
LLS	-0.079	0.007	-0.385	-0.202ns	0.013ns	-0.689*
HPLI	0.055	-0.209	0.097	0.142ns	-0.401ns	0.174ns
LLP	-0.106	0.096	-0.232	-0.273ns	0.184ns	-0.416ns
LOA	0.351	0.059	0.075	0.903**	0.113ns	0.133ns
LLP	0.032	0.191	-0.253	0.081ns	0.366ns	-0.452*

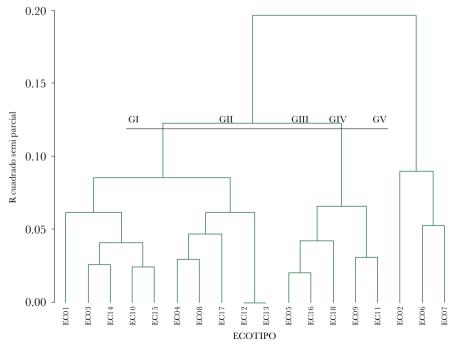
SNVI, SID, SIL, SPW, SCIWW2, SCW, SCD, SI, SIN, SSI, SP, SNP, SSP, SAR, SIN, LWULS, LLSC, LSH, LILLSOP, LLP, LDP, LOA, LLP; \*\* Statistically highly significant with alpha=0.001, \*=significant with alpha=0.05, and ns=Not statistically significant.

means it is not deep. It tends to form a larger number of innovations (10), bigger prophylls (10.6), and more internodes with adventitious roots (10) and consequently it quickly enters the reproductive phase. Under the sheath, it has scarce wax. It has an external length of 17.7 cm; at the opening point, the internal length measures 8.6 cm. Its ligule is convex, its auricle measures 1.7 cm, and the tip on the leaflet measures 2.7 cm.

Group II was made up of five ecotypes: INIFAP Pp-04, INIFAP Pp-08, INIFAP Pp-12, INIFAP Pp-17, and INIFAP Pp-13. Its stems are 20.78 mm wide and 14.7 cm long, with less internodes (22) than the previous group. The internodes with wax and without wax are both yellowish green. The channel is 4.37 mm wide, and it is deep. It presents



**Figure 1**. Distribution of 18 Elephant grass (*Pennisetum purpureum* Schumach) ecotypes collected in the state of Chiapas, based on: a) main components 1 and 2 (CP1 and CP2), and b) 1 and 3 (CP1 and CP3).



**Figure 2**. Dendrogram of 34 stem and leaf morphological characteristics of 18 ecotypes of Elephant grass (*Pennisetum purpureum* Schumach) collected in Chiapas, Mexico. GI=Group one, GII=Group two, GIII=Group three, GIV=Group four, and GV=Group five.

Table 5. Quantitative and qualitative morphological descriptors of the stem of 18 ecotypes of the Elephant grass (Pennisetum purpment Schumach) collected in the Chiapas

Group																		
Group		1	2	3	5	9	7	8	10	12	13	16	17	20	21	22	25	33
	Ecotype	IANS	ais	TIS	SCIMMI	SCIMM5	RCM	гсD	NIS	dS	dNS	NIS	FMNFS	refer	TILLSOP	STI	гоч	LLT
GI	INIFAP Pp-01	56	21.63	13.2	_	_	5.45	2	7	2	13.1	5	3	16.7	9.1	2	1.9	0.1
GI ID	INIFAP Pp-03	23	20.42	18.3	5	33	4.01	-	4	2	13.4	10	3	21.0	12.0	2	1.8	4.0
GI I	INIFAP Pp-14	28	19.76	18.8	5	3	4.80	2	15	2	7.3	15	5	18.2	7.2	2	1.8	3.6
GI I	INIFAP Pp-10	29	18.53	12.6	5	3	4.69	1	12	2	12.6	11	3	15.2	8.3	2	1.7	2.7
	INIFAP Pp-15	26	22.23	11.6	5	3	4.97	1	10	2	6.7	10	5	17.5	6.3	2	1.6	2.9
MEAN		26	20.51	14.9	5	3	4.78	1	10	2	9.01	10	3	17.7	9.8	2	1.7	2.7
GII II	INIFAP Pp-04	21	19.10	18.6	3	33	4.32	3	7	2	12.4	7	3	17.2	9.5	2	1.7	0.1
GII II	INIFAP Pp-08	22	20.02	13.7	3	33	4.29	3	10	2	3.5	3	3	16.7	11.3	2	1.7	1.9
GII II	INIFAP Pp-17	27	19.45	15.5	3	33	4.79	3	12	2	4.7	2	5	17.9	7.0	3	1.6	3.2
GII II	INIFAP Pp-12	20	22.66	13.0	5	3	4.23	1	15	2	5.4	4	3	18.3	8.0	3	2.0	2.7
GII	INIFAP Pp-13	20	22.66	13.0	5	3	4.23	1	15	2	5.4	4	3	18.3	8.0	3	2.0	2.7
MEAN		22	20.78	14.7	3	3	4.4	3	12	2	6.3	4	3	17.7	8.8	3	1.8	3.2
GIII	INIFAP Pp-05	23	17.25	13.9	3	3	3.24	2	10	2	13.8	8	5	13.1	8.1	2	1.4	5.1
GIII	INIFAP Pp-16	20	15.28	16.0	5	3	3.98	2	5	2	12.4	2	3	14.7	5.5	2	1.3	2.7
GIII	INIFAP Pp-18	14	12.59	16.3	5	3	2.97	3	3	2	7.4	3	5	14.0	5.0	3	6.0	2.3
GIII	INIFAP Pp-09	14	17.11	15.8	3	3	5.16	3	-	2	9.9		3	15.2	5.3	3	1.4	1.9
GIII	INIFAP Pp-11	18	17.38	15.1	5	3	3.98	2	1	2	0.9	2	5	18.5	9.8	2	1.8	3.0
MEAN		18	15.92	15.4	5	3	3.87	2	4	2	9.5	2	5	15.1	7	2	1.4	3.0
GIV	INIFAP Pp-02	23	27.78	14.5	1	-	4.67	2	91	-	0.1	9	_	22.9	14.5	3	2.2	3.3
GV	INIFAP Pp-06	21	20.48	10.4	5	3	4.11		12	1	9.0	0	5	18.8	12.3	2	2.0	2.7
GV I	INIFAP Pp-07	22	22.94	11.0	7	-	4.63	_	10	-	0.1	2	5	20.5	13.8	_	2.5	3.2
MEAN		21	21.71	10.7	7	3	4.37	П	11	_	0.3	5	5	19.7	13.0	2	2.2	2.9

SNVI=Number of visible internodes; SID=Internode diameter (measure the middle internode); SIL=Internode length; SCIWW1=Color of the internode with wax; SCIWW2=Color of the internode without wax; SCW=Channel width; SCD=Channel depth; SIN=Number of innovations; SP=Prophylls; SNP=Number of prophylls; SIN=Number of internodes; LWULS=Wax under the leaf sheath; LELLS=External length of the leaf sheath; LILLSOP=Internal length of the leaf sheath at the opening point; LLS=Ligule shape; LOA=Opening of the auricle; LLT=Leaflet tip. 10 innovations, 5 prophylls, and 4 internodes with adventitious root; therefore, like the previous group, it quickly enters the reproductive phase. The external length of the sheath is 17.7 cm: at the opening point, the internal length measures 8.8 cm. The opening of the auricle measures 1.8 cm and the tip of the leaflet measures 3.2 cm.

Group III was made up of five ecotypes: INIFAP Pp-05, INIFAP Pp-16, INIFAP Pp-18, INIFAP Pp-09, and INIFAP Pp-11. It has thinner and longer stems (with a 19.92 mm diameter and a 15.4 cm length) and fewer internodes (18). The internodes with a lot of wax are black and white, while the internodes without wax are yellowish green. The channel is 3.87 mm wide and has an intermediate depth. It has a lower number of innovations (4), a high number of prophylls (9), and few internodes with adventitious roots (2). Like the previous groups, it quickly enters the reproductive phase. The sheath measures 15.1 cm (external length) and 6.7cm at the opening point (internal length). A scarce amount of wax can be found underneath. The ligule is biconvex. The auricle measures 2.2 cm (opening) and the tip of the leaflet measures 3.0 cm.

Group IV consisted of a single ecotype: INIFAP Pp-02. It stands out for having the maximum averages in descriptors of high forage value (Wouw *et al.*, 1999). Its stem has the following characteristics: the number of internodes is one of the highest (23), their thickness is greater (27.78 mm), and the internodes are of medium length (14.5 cm). The internodes with and without wax are both yellowish. The channel is less wide than the highest specimen (4.67 mm) and it is of intermediate depth. It has the highest number of innovations (16). The absence of prophylls sets this group apart from the others, because it does not enter the reproductive stage. It has 6 internodes with adventitious roots. It has scarce wax under the sheath. The external length of the sheath measures 22.9 cm and, at the opening point, the internal length of the sheath measures 14.6 cm. The ligule is convex, the auricle measures 1.7 cm, and the tip of the leaflet measures 3.3 cm.

Finally, group V is made up of two ecotypes, which are distinguished by a low number of internodes (21), with a medium diameter (21.75) and of short length (10.7). The internodes with wax are deep black, while the internodes without wax are yellowish green. The channel is 4.37mm wide and it is not deep. It has an average number of innovations (11) and adventitious root in five internodes. It does not present prophylls. Its sheath shows scarce wax underneath and has an external length of 19.7 cm. The ligule is convex, the auricle measures 1.7 cm, and the tip of the leaflet measures 2.9 cm. Xavier et al. (1995) observed that Napier grass has 15.60 internodes, with an 18.0 mm diameter and a 9.80 cm length. It has prophylls and adventitious roots. Its sheath is 21.30 cm long and the ligule measures 3.80 mm. These data match the range of the characterized ecotypes. For their part, Wouw et al. (1999) characterized 53 accessions of Pennisetum purpureum and P.  $purpureum \times P$ . glaucum, based on their agronomic and morphological characteristics, and obtained five groups. The separation was the result of the following characteristics: the length of the internodes, the thickness of the stem, the hairiness, the length and width of the leaf, how they grow, and the rhizomes or roots in the internodes. In the present work, these characteristics were used. Five groups were also obtained, which have been described in the previous paragraph.

#### **CONCLUSIONS**

The 18 ecotypes of the Elephant grass (*Pennisetum purpureum* Schumach) collected and characterized show dissimilarities between them. Therefore, they are considered a genetic resource with potential forage importance on the Chiapas coast.

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## The innovation networks of blackberries

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#### **ABSTRACT**

**Objective**: Map the innovation networks of blackberry producers in the Los Reyes Michoacán region, as well as to identify the degree of centrality, density and intermediation of the network and some options for improvement.

**Design/methodology/approach**: Visits and interviews were made to the main collection centers Sunbell, El Cerrito, SPR and Driscoll, to the personnel of Trusts Established in Relation to Agriculture of the Western Region, the Zamora Agency. For the baseline survey, a universe of 50 producers was considered, including partners and main clients; then, all the red was relieved with the "snowball" technique.

**Results**: 39% of the producers are also dedicated to the production and marketing of avocado. Most of the production (92%) is destined for export. The main collection centers for export are Sunbelle, Splendor and Driscoll. El Molinito and Moradely account for 70% of the total national market.

Agrofertilizadores de Michoacán Agromich, Fertimich, Bucosa and Agrupe concentrate 70% of the inputs sold to producers. The company Procal stands out in the supply and service management network.

**Limitations on study/implications**: It is a study focused on the Los Reyes region, which could be extended to other regions, crops, and expand the number of actors involved in the study.

**Findings/conclusions**: The relationships between different actors centralization, inputs-outputs, and intermediation indicators take higher values, which shows the role of producers and key companies (Ere03 and OP01) as source, collector, and articulator actors. It is important to promote high productivity and profitable technologies for innovation.

Keywords: Blackberries, Networks, Innovation, region, snowball.

Citation: Infante-Jiménez, Z. T., Ortega-Jiménez, P., Coutiño-Puchuli, A. E. (2023). The innovation networks of blackberries. *Agro Productividad*. https://doi.org/10.32854/agrop. v16i1.2389

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: September 29, 2022. Accepted: January 08, 2023. Published on-line: February 22, 2023.

*Agro Productividad*, *16*(1). January. 2023. pp: 45-57.

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### INTRODUCTION

World consumption of berries has been growing over the last decade. Several studies anticipate a strong demand for strawberries, blueberries, raspberries, and blackberries, following the trend of recent years. This is expected to remain if these fruits continue to be considered a delicacy by certain consumers who are willing to pay higher prices than those paid for traditional fruits.



International markets require quality and safety certificate; the fruit must be produced through appropriate agricultural practices, free of diseases and quarantine pests as defined by receiving countries; the containers must be clean with open perforations and complete labeling; producers must adhere to the instructions for use of pesticides observed in the Quality Certification Standards determined by the imports-exports agency. In addition, all varieties must prove good flavor, color, and size.

International trends point to the development of new commercial strategies aimed at consolidating external markets, exploiting the increased demand and consumption of berries in developed countries. The world's main producing countries are the United States, Russia, Poland, Turkey, Holland, Germany, former Yugoslavia, and Canada. The largest exporters are Spain, United States, Belgium, and Canada, which account for 60% of the total value. They are followed by Mexico, France, Chile, and Poland, with an aggregated share of 17%. Mexico contributes about 344,000 tons of berries, only 7.3%, so there is a great opportunity to grow considering the comparative advantages of some regions.

It is estimated that the world production of blackberries and hybrids is around 60,000 tons, and most of is frozen (75%). In Mexico, most blackberries used in the agro-industry are frozen, using the *Individual Quick Frozen* (IQF) system. This allows to use the product by piece or in blocks.

The state of Michoacán contributed 96% of blackberries and 27% of raspberries, most of which was produced in the valley of Los Reyes. Mexico is the leading supplier of berries to the United States (USA), contributing 40% of the volume and value of total imports in average, followed by Chile with 16%, Canada with 13%, Netherlands with 12%, and France with 5%.

The USA remains the main export market for Mexico's production. Exports grow in an annual average of 12%. The purpose is to take advantage of the scarcity of berries in the U.S. Market considering the following:

- There is a variety with a longer shelf life (Tupi variety).
- · An existing unsatisfied demand
- There is a shortage of production in the USA during the winter season.
- Mexico holds a favorable geographical position compared to other producing countries.
- Mexico has climate advantages as a berry producer. In other words, the production
  and export in the region of Los Reyes happens precisely when there is no production
  in the United States; it is harvested from October to April and exported from
  November to March.

Mainly one crop of one variety predominates in the valley of Los Reyes; it is the blackberry *Tupi*, which replaced the *Brazos* variety approximately 5 years ago. Other varieties that are better adapted to regional conditions have also been developed. This is carried out with a wide entrepreneurial vision by all producers and actors in the network. For this reason, there is a significant input network and collection centers within the municipality.

The main change driver originates from the broker's end, which puts pressure on the Collection Centers to obtain fruit with longer shelf life and acceptable to consumer tastes. Collection Centers establishes the fruit's specifications for arrival in their warehouses (cold rooms). These specifications can only be achieved through proper crop management and technical screening. The supervising technician is accredited by the *Comité Estatal de Sanidad Vegetal* (State Committee on Plant Health).

Producers are aware of the need to innovate and adopt new technologies to achieve greater competitiveness. Some of the most recently implemented and widely used production technologies are macro tunnels, irrigation systems, and safety practices. They put increasing pressure to managing resources in a sustainable way. The blackberry is highly profitable as it is a perennial crop. It has the greatest investment cost, as observed in its cultivation process and healthy growth measures. A reasonable harvest is obtained from the first cultivation year and during the following years. Proper management implies pruning, fertilizing, and ensuring the crop's healthy development, especially during the flowering and harvesting seasons.

The sanitary factor of crop management is the most widely observed parameter. This is because producers must meet special requirements to grow fruits for export.

A good harvest depends to a large extent on the strength and nutrition of the plant, so fertilization is the second most important activity (the first is harvesting) in terms cost of cultivation. Harvesting is the most critical and rigorous stage. Women harvesters must comply with sanitation and safety measures, and the containers they use must be thoroughly clean.

The area of study is similar to that covered by PROCAL, including their partners and customers. It covers six municipalities in Michoacán, mainly involved in the production of blackberries. The municipalities are Los Reyes, Tocumbo, Periban, Tancítaro, Cotija, and Uruapan.

This study will help the Blackberry Networks in Los Reyes become more efficient through organizational and technical innovation related to production and commercialization. The study will carry out the following actions: identify the relationship between actors and their links within the network; observe the behavior of the network and individual nodes; evaluate the performance of actors to obtain a group perspective from the network; acknowledge the characteristics of the network: characterization, density, and centrality; design scenarios to improve the network's performance and competitiveness; develop schemes for a harmonious participation of stakeholders; develop all stakeholders' actions under innovation and mutual benefit schemes that lead to improved competitiveness and congruent use of financial, technological, commercial, administrative, and organizational instruments for producers.

#### **INNOVATION**

Innovation is originated from ideas and proposals conceived and expressed by people. It takes the form of plans and projects, and it is people who put innovative projects into practice. Innovation represents any change based on knowledge that generates wealth; the goal of any innovative process is the generation of wealth (Muñoz *et al.*, 2007). Any type of

innovation-oriented change must be based on knowledge and supported by information and data. The goal of any innovative process is economic and social welfare. Innovation Capacity is the ability of a company to integrate tangible and intangible resources to achieve a specific result.

Joseph A. Schumpeter (1883-1950), an Austrian economist of the twentieth century, introduced the process of business innovation as a central element in economic analysis. He conceptualized innovation as the production of different things, or the same things through different methods (Schumpeter, 1968).

For Schumpeter, quoted by Montoya (2004), change emerges from the system (endogenous process). His proposal for economic development introduced the concept of innovation as a cause of development and the innovative entrepreneur as an enabler of the innovation process. Schumpeter (1978) considers the production process as a combination of productive forces, which in turn are composed of material forces. These are the so-called factors of production such as labor, land, and capital, which add to other intangible forces such as technical facts and social organization.

For Schumpeter (1978), technological innovation is the fundamental force that moves capitalist production and the source of its constant transformation. Innovation is understood as an invention that is introduced into the market. For Schumpeter, radical innovations account for social transformation as they are capable of provoking revolutionary changes and decisive turns for the society and the economy. By radical innovation he means the introduction of new consumer goods into markets; the emergence of new production and transportation methods; the opening of new markets; the generation of new sources of raw materials; organizational and managerial changes in companies.

Schumpeter (1996) sees entrepreneurs as people who have the capacity and initiative to propose and implement new production means; they are people, with or without a business, who can generate and manage radical innovations within or outside organizations. The 21<sup>st</sup> century is characterized by a rapid innovation of technologies, processes, and products. Emerging innovations change the way goods and services are produced, traded, exchanged, and consumed.

Innovations are disseminated through individuals within a social system; the communication pattern across these individuals makes up a social network (Valente, 1999). Context is an essential reference, interaction is a key strategy, and ethics guarantee the sustainability and evolution of any innovative venture.

Drucker (1985) defines innovation as a systematic analysis that transforms changes into business opportunities. He lists the possible sources of innovation as follows: Surprises, inconsistencies, process needs, industry and market changes, demographic or population changes, trends or changes in consumer perception, and new knowledge.

The actors who generate innovations are found in both the public and private sectors. The public sector in Latin America is mainly represented by universities and innovation research centers. The actors involved in innovation are not only those who innovate but also those who acquire innovations. The private business sector is a fundamental recipient of innovations as well as labor unions. However, labor unions are passive recipients while the private sector has decision power (Sutz, 2002).

Innovation is recognized as the key process for the economic growth of companies, regions, and countries (Grossman and Helpman, 1994). Innovation can occur while developing new products, processes, services, or business models in the agroindustrial sector. Innovation processes in rural areas have received little attention compared to urbanindustrial contexts.

Through the theory of diffusion of innovations, Rogers (1962) explains how, why, and at what speed new ideas and technologies move across cultures. For Rogers, ideas, practices, or objects perceived by individuals as new constitute the main innovation elements.

According to the most relevant researchers on the subject, some of the most important concepts of innovation are as follows: Schumpeter (1978) sees innovation from an evolutionary perspective; he refers to product innovation, which includes raw material, process evolution, and new market structures. Freeman (1995) describes innovation as the process of integrating existing technology and inventions to create or improve a product, process, or system; it represents the consolidation of a new and economically improved product, process, or system.

There is a difference between invention and innovation, but they are frequently used as synonyms. An invention is any human creation that allows the transformation of matter or energy for its use, whereas innovation is aimed at a market under a business approach detecting opportunities and organizational capabilities to generate new products, processes, or services. On the other hand, technological innovation is a process that combines a market opportunity with a need and/or a technological invention.

Innovation is vital to sustain and move businesses forward. It is a very complex process, and it needs effective innovation management to create radical change and generate adaptations to lengthen the life of a business. The most accepted definition of innovation today is the one offered by the OECD (2018). It describes innovation as a new or improved product or process (or a combination thereof) that differs significantly from the unit's previous products or processes and has been made available to potential users (product) or put into use by the unit (process).

According to the OECD (2005), four types of innovation can be distinguished:

- 1. Product innovation: it is a good or service that is new or significantly improved with respect to its features or uses. This includes improvements in components, materials and technical specifications, software, ease of use, and other functional characteristics.
- 2. Process innovation: it is the implementation of a new or significantly improved method of production or distribution. This includes changes in technique, equipment, or software.
- 3. Marketing innovation: it is the implementation of a new marketing method that involves significant changes in product design or packaging, placement, promotion, or price.
- 4. Organizational innovation: a new organizational method in business practice, site organization, or in the company's external relationships.

Desouza (2009) describes the innovation process in the following 5 stages:

- a) Generation and mobilization of ideas. The generation of new ideas must be spurred by creativity and by the pressure coming from competitors. The mobilization of ideas among different agents is essential in this process and facilitates their development.
- b) Review and selection of ideas. This stage consists of considering only the ideas with the greatest potential for generating value as well as economic and social growth.
- c) Experimentation. Experimentation tests the feasibility of ideas in a given environment and helps confirm that an invention or idea effectively solves a problem.
- d) Commercialization. It allows innovation to be taken to a larger scale and to expand and deploy new products, services, or organizational methods in a particular market.
- e) Diffusion and implementation. This stage is the innovation's final approval and the implementation of the necessary structures and resources for its production, maintenance, and diffusion.

These stages do not necessarily appear in this order. Each stage involves different actors who contribute to the generation of new products, services, marketing methods, and organizational processes based on their knowledge, resources, and experience.

#### **Innovation Life Cycle**

According to OECD (2018), the main activities of innovation are research and development, knowledge transfer within the company, and innovative marketing and commercialization. The linear approach to the research and development process starts with research, then it proceeds to development, where assimilation, transfer, and protection can be integrated to finally reach innovation.

Innovation is a systemic, interactive, multidisciplinary, and multi-authored process. Among innovation authors are public institutions, such as universities and research and development centers, the State, society, social media, and companies.

A smart innovator first validates whether the market will accept his/her product, understands how to make it reach future customers, and develops it at low cost, seeking market-oriented improvements over time.

The study of innovation networks allows for understanding information flows between producers, companies, and institutions as well as identifying factors related to the existence of such relationships in favor of decision making.

#### MATERIALS AND METHODS

A few visits and interviews were made to the main collection centers to learn about the situation of the most important stakeholders (Sunbell, El Cerrito, SPR, and Driscoll). We also interviewed Banco de México - FIRA personnel from the Western Regional Office and the Zamora Agency. Producers United for Quality were also interviewed. A universe of 50 producers was considered for the baseline survey, including partners and main clients; then, the entire network was surveyed using the "snowball" technique.

#### RESULTS AND DISCUSSION

The population analyzed was mostly adults of 46.4 years of age in average: a minimum of 23 years, and a maximum of 68 years of age. They had high-school education level in average (12.6 years) and work experience in the sector between 1 and 18 years, averaging 7.4 years. The gender composition was mostly men (89%) and only 11% women.

All producers grow the Tupi, but only one has an innovative variety. On the other hand, 39% of producers have another crop or activity (avocado), so the average area for blackberry per farmer is 6.7 ha, with an average total area of 12.6 ha and a high coefficient of variation. All producers have a small land tenure. They all consider their production a good market opportunity because:

- They adopt better technologies to increase production.
- The lease of land to grow blackberries represents a constant.
- Cultivation in new areas (Jalisco and Colima) is a trend.

Most of the production (92%) is destined for export, and only 8% is for the domestic market. The latter represents the "rejection", *i.e.*, produce that did not meet the quality standards established by collectors-traders and is mainly destined for regional markets. As for the main collection centers for export, only three of them —Sunbelle, Splendor, and Driscoll— concentrate all produce in approximately 80% of the population of study. Two of the main collection centers that sell to the domestic market —El Molinito and Moradely—account for 70% of it all.

As for the perception of production problems, farmers mention five main issues in order of importance: the cost of inputs, the cost of labor, low prices, road quality, pests and diseases.

They also perceive five problems regarding access to formal credit through bank and non-bank financial institutions in order of importance: high-value collateral required, complex procedures, low profitability of the activity, high interest rates, and lack of information on the procedures involved.

Because the production network is highly developed in the area, we found all kinds of local providers of inputs, equipment, and machinery for the development of the crop and harvest, financial services, as well as technical, fiscal, economic-financial, and marketing advisory.

Five companies concentrate 70% of the inputs sold to producers: Agrofertilizantes de Michoacán holds 29.4%, followed by Agromich with 11.8% and Fertimich, Bucosa and Agrupe with 8.8% each. According to data provided by the surveyed producers, the average profit per hectare is \$78,000.00. However, the most efficient producers achieve a profit of approximately \$250,000.00 per hectare.

#### The dynamics of innovation

The highest rate of innovation adoption is seen in Tocumbo with 81%, followed closely by Los Reyes with 73%. Producers in the city of Peribán have a lower level of adoption. An average innovation adoption rate (INAI) of 71% is estimated according to the information

provided by the producers interviewed. This situation can be explained by the following facts: In Peribán, blackberries are produced together with avocado and peach. The adoption of this crop and the categories or activities included in the calculation of the INAI has been slower. So, 50% of the producers interviewed were above the general INAI average of 76.5%, and six producers remained within that average. We also identified two producers with a high percentage of technological innovation and the group with the most lagging producers.

Three categories present the greatest lag and therefore need appropriate attention. These categories serve as the basis for establishing the Innovation Strategy to improve the INAI.

Organization: There is a perceived need for producers to rearrange their purchase systems and access new, safer inputs to generate scale economies and lower costs for goods and services.

Seedling reproduction and genetic management: This was the second most problematic category as perceived by producers. Currently ALL growers have the Tupi variety established. Only one of them has also a new variety: the Sliping Bioner.

Plantation establishment and management: Blackberry growers in Michoacán, especially in the valley of Los Reyes, are aware of their international competitors. They know that establishing a healthy, vigorous crop and a properly managed orchard is essential to remain in the market.

For producers to obtain a code, they must accept the supervision of technicians authorized by the Collection Center, who are also authorized and certified by the State Committee on Plant Health (CSV).

A good harvest depends to a large extent on the plant's strength and nutrition. Fertilization is then second place (the first is harvesting) in terms of cultivation cost with 17.7%.

Harvest is the most critical and rigorous stage. For this reason, women harvesters must show up with their nails trimmed, groomed, their hair tied back and secured with a

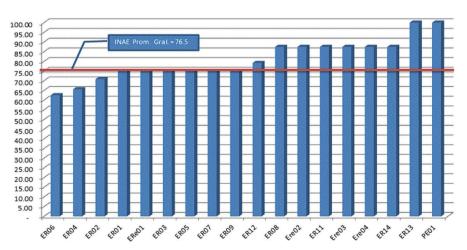


Figure 1. Categories of producers and levels of innovation.

cap. The containers must be completely clean. All boxes (clamshells and container boxes) together with the code are supplied by the company that holds the sales commitment.

Blackberry growers are aware of the need to manage resources in a sustainable way as Good Practices are supervised by CSV-authorized technicians.

There is a defined area in each farm for workers to prepare and ingest their food in isolation from the crop; the toilet must be clean and always have clean water for personal hygiene. The preparation of the products to be applied is also isolated from the crop, and empty containers are immediately collected; they are first confined and then removed from the farm.

The sanitary aspect of crop management is the parameter with the highest level of adoption. This makes sense considering the requirements or specifications that the producer must meet to supply fruit for export.

A letter of intent to sell the fruit to the packing house or Collection Center is signed. This grants a CODE or identification number (ID) for each land property. This data label is attached to each of the plastic boxes (220-gram clamshells) and to the cardboard box containing ten of them.

This code allows traceability in case of problems at any stage of the collection process, during transportation to the destination, or even at the distribution point.

The Task Force (EGI) establishes goals for producers participating in the exhibition to adopt technological innovations that will increase their competitiveness.

Some activities within the categories need to be developed for producers to adopt.

Training, demonstrations, and result evaluation events will be carried out to promote such activities. Adopting new varieties, hiring technical assistance services, and generating the use of macro-tunnels are practices that need the most promotion.

#### Innovation network

We obtained elements through producer surveys for mapping 16 types of networks, which are shown in the Table 1. The combination of three additional networks was mapped out to better understand the network.

We obtained measures of density, centrality (inputs and outputs), centralization, and intermediation in addition to each network's map (Table 2).

As displayed in the maps and indicators, all networks show the following:

- The number of relationships between actors (density) is low.
- With a diffuse structure,
- no network dominance or predominance (centralization) is shown.
- for input and output indicators or for the intermediation level of each low actor, but it shows a trend for some actors according to the network.

These values were due to the number of producers surveyed as well as the type of response provided. However, the types of producers and the roles they play in each network are identified in each of the mappings.

Code Actor **ERLT** Leading technology producer ER Typical producer ERe Referred producer ы Supplier of inputs PE Machinery and equipment supplier PG Genetics supplier PSP Professional service provider ΙE Teaching and research institution PF Financial services provider IG Government institution CIIntermediary customer CAStaff at collection center, commercial, or agroindustrial OR Trade organization OP Producer organization FAM Family EP Own experiment AoCFriend or Godfather RoB Journals or newsletters CAP Training courses INET Internet

Table 1. Classification of Actors for Network Analysis.

Density is very low in the communication network. There are actors with low inputs and outputs, and there is a small group was with a collector producer (ERELT04), who is known as a technological leader.

Other (specify)

The relationships in the communication learning network are low, but a group is clearly identified where a referred producer (Ere05) is seen as a source and articulating actor.

Three small groups stand out in the financing network. It shows collector actors where only one is a financial intermediary, and the other is a marketer-collector.

There are two main collectors in the marketing networks for both the domestic and export markets: El Molinito for domestic and Sunbelle for export, the latter being also an important source of financing for producers.

We identified a significant participation of a main input provider in the input supply network (PI01). It is a fertilizer company called Agro-fertilizers of Michoacán. The company Agromich appears with less importance for other types of inputs.

The producer referred to as Ere03 appears in the innovation network with a significant number of entries. It acts as a source and articulating actor and is referred to by a significant number of producers as the innovator.

The company *Procal* stands out in the inputs and services management network, acting as a conglomerating part in the organization.

Table 2. Types of Networks.

						C	entralizati	on
		Types of networks	Density	Cent	rality	(In	termediati	on)
		Types of networks	Density			Rate	Main	Value
				Input	Output		Actor	
1		Who you talk to	4.50%	9.19	1.5	0.67	Ere03	5
2	Social	Who you learn from	3.23%	1.66	8.32	0	-	0
3		Conflict						
4		Working capital credit	6.32%	1.66	8.79	0	-	0
5	Funding	Investment credit						
6	1 amanig	Joint investments in equipment, facilities, and infrastructure	2.77%	3.09	7.85	0	-	0
7		With Quality	5.23%	1.44	34.72	0	-	0
8	Marketing	No Quality	3.62%	2.64	20.79	0	-	0
9		Consolidated sale						
10		Who do you buy main inputs from? (Fertilizers)	7.62%	1.22	38	0	-	0
11	Provision	Who do you buy secondary inputs from?	4.92%	1.66	9.92	0	-	0
12		Who do you buy tertiary inputs from?						1
13		With whom do you buy on a consolidated basis?						
14		Innovative	7.14%	1.55	33	2.08	Ere03	8
15	Various	Joint management	8.19%	1.23	30.55	0.65	Ere03	2
16		Belongs to an organization	8.82%	1.95	48.43	0	-	0
17		Communication + learning	3.66%	1.53	2.83	0.62	Ere03	10
18	Combination	Communication + learning + innov.	3.94%	2.24	10.38	1.4	Ere03	27
19	Combination	Communication + learning + lnnov. + management	4.53%	1.94	7.74	1	Ere03	22

The communication, learning, innovation, and management networks were mapped together for a better understanding of the network and actors. This resulted in 3 mappings:

- The first one grouped the communication network together with the learning network.
- The second mapping covered the communication, the learning, and the innovation networks.
- The third mapping included the communication, the learning, the innovation, and the management networks.

The relationships between different actors become more evident and clearer in these 3 mappings. The centralization, inputs-outputs, and intermediation indicators take higher values, which shows the role of producers and key companies (Ere03 and OP01) as source, collector, and articulator actors.

It is also observed that these 4 aspects of the network (communication, learning, innovation, and management) have several communities; one of them hosts the largest number of people and is intercommunicated.

The first part described the network, its members, and the importance of the activity at the regional, state, national and global levels. Then we learned about the relevant characteristics of actors in the network through different tools. These characteristics were considered as important factors in intervention strategies.

Additionally, the activity and innovation dynamics were known, and their status or baseline was established. Also, the roles of different actors were made visible through network mappings and indicators. We therefore suggest strengthening the capacities of PROCAL's staff so they can plan their businesses, increase their membership, their organization, and their market development. We also propose the development of adequate credit schemes, input procurement, risk management, marketing, manufacturing, and technology transfer services. It is important to promote high productivity and profitability technologies for innovation categories such as: Plantation establishment and management, breeding and genetics of new varieties.

#### **CONCLUSION**

Our multi-criteria selection indicates the feasibility of supply schemes in their technical, economic, and social aspects as they prompt the producers' interests. Then, the development of the producers' organizational and integration capacities through companies is recommended.

Third, but no less important, is the alternative of developing, transferring, and adopting high productivity and profitability technologies. The network's current situation (baseline) in its different aspects was established; then the interactions of those involved were recorded, and their problems and solutions were defined. This enables a timely follow-up and a clear glimpse to document the degree of development. It also allows us to take preventive and corrective measures and to redirect our strategies if necessary.

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# Potential use of water saved with technification of gravity irrigation in non-agricultural sectors

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#### ABSTRACT

**Objective**: To analyze conceptually the potential use of water that the technification of gravity irrigation allows in saving and generating alternatives of use according to the extrapolation of volumes, both in the agricultural sector or outside of it.

**Design/methodology/approach**: The Modernized Gravity Irrigation Program (RIGRAT) will be evaluated, by measuring the volumes of irrigation used in the Irrigation Districts (ID) 075 Río Fuerte, 076 Valle del Carrizo, and 063 Guasave, Sinaloa, during the 2015-2018 agricultural cycles. The measured and statistical data are integrated for the analysis of volumes saved by the program and its national statistical projection.

**Results**: The volume saved in the ID 075 was 2,401.02 thousand m<sup>3</sup> (2.4 hm<sup>3</sup>) in 6,114.5 ha under technification of the RIGRAT program. The volume of water saved on that surface represents 10% of the water used by the industrial sector of Sinaloa. It is inferred that the modernization of the ID 075 Río Fuerte in the planted area of 289,780 ha, would imply a saving of 40% of the water that can be used in urban areas of Sinaloa. In the same agricultural sector, it would be possible to save a volume of 187 thousand m<sup>3</sup>, which represents 6.4% of the water used by the irrigation district at plot level.

**Limitations on study/implications**: Current regulations do not allow the transfer of water volume in its different uses, with the aim of optimizing the value of water.

**Findings/conclusions**: With actions implemented in the RIGRAT program, water saving is achieved at the farm level and there would be a great impact, since agriculture is the main consumer of water and there could be volumes saved to be used in other sectors.

Keywords: Efficiency, irrigation sheet, water use, modernization.

Citation: Flores-Velázquez, J., Roblero-Hidalgo, R., Rojano-Aguilar, A., & Aguilar-Rodríguez, C. E. (2023). Potential use of water saved with technification of gravity irrigation in non-agricultural sectors. *Agro Productividad*. https://doi.org/10.32854/ agrop.v16i1.2230

**Academic Editors**: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: February 21, 2022. Accepted: January 15, 2023. Published on-line: February 22, 2023.

Agro Productividad, 16(1). January. 2023. pp: 59-67.

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#### INTRODUCTION

Irrigated agriculture consumes globally 69% of the useful water available (Martínez *et al.*, 2021). The use of technology to elevate the efficiency of water in the agricultural sector acquires increasingly more relevance, with actions in the various stages of their cycle. The proposals contemplate implementation of techniques and policies directed at increasing their efficiency and obtaining resource savings and the possibilities regarding the task with this new volume available.



Water distribution in the different sectors has diverse implications; there are ways of saving water before its use, that is, reducing the application of water by increasing the efficiency of the processes involved, in the conduction, storage, distribution and consumptive use (Chávez and Fuentes, 2019; Chávez *et al.*, 2020). Another way is after its use, through recovery, with actions such as capture, drainage or treatment for reuse (de Anda and Shear, 2021). China is the country with the highest percentage of water reuse. The United States occupies the first place in treated residual water, with 7.6 million m³/day (Jiménez and Takashi, 2008).

Administratively, in Mexico, the use and management of agricultural water is distributed through the so-called basin agencies, local management offices and other steering organizations; however, it is in practice where the greatest challenge happens. Specifically, the agricultural sector has a historical backwardness with efficient water management, because it uses 77% of the water for crop production (CONAGUA, 2013), quite above public (12.2%), industrial (4.2%) and energy-generation (4.9%) uses. Most of the water demand is for consumptive uses, with the sources of water being superficial (61.3%) and the remainder (38.7%) that originates from underground sources. From these sources, 263,551 million cubic meters (hm<sup>3</sup>) have been licensed, with 65,155 hm<sup>3</sup> corresponding to the agricultural sector, understood to be basically for irrigation (SEMARNAT, 2018). The water sources for irrigation in Mexico are basically storage dams; one of the main inconveniences for irrigation planning in Mexico has to do with the variability in the volume of storage; thus, for one year it can have only 200 hm<sup>3</sup>, and the next reach 2,000 hm<sup>3</sup>. Facing this variability, the challenges are diverse: 1) how to operate an irrigation zone with this uncertainty, 2) how to project the crop mosaic in this irrigation zone, 3) what to do with the water when an average of 1,165 hm<sup>3</sup> was projected and in the dam there are 2,000 hm<sup>3</sup>.

Concerning the non-consumptive use, or alternative uses for water, for example for the generation of electricity, 133,000 hm<sup>3</sup> have been used in a specific period. Regardless of whether this value is high or low, the substantial fact is that it is an alternative form of water use, and that it can be counted again in other uses. The relative scarcity of water in Mexico for urban (domestic) use is reaching critical dimensions, because of the continuous and accelerated population growth, and consequently the need for food in urban zones (Cummings and Nercissiantz, 1992).

In the presence of constant climate variations, and the need to sustain crop production, the possibility to improve the stability in storage volumes is suggested, through the constant improvement in the use of water directly by the crops (Unver *et al.*, 2017; Nikolaou *et al.*, 2020). That is, reducing the use of agricultural water at the farm level, through the technification of irrigation, which at the government level was the national program, Modernized Gravity Irrigation Program (*Riego por Gravedad Tecnificado*, RIGRAT).

Mexico has nearly 6.5 million hectares of irrigation, with more than 90% being gravity irrigation in its different modalities, versus about 600,000 ha with pressurized irrigation. To carry out this process there are administratively 13 basin agencies and 23 local management offices, supported by 85 irrigation districts, 23 districts of technified rainfed conditions and irrigation units. As a whole, it is a licensed surface which represents the agricultural surface in Mexico, responsible for supplying harvests to the country.

Other sources such as the National Water Plan (*Plan Nacional Hídrico*, PNH, 2008) and CONAGUA, define that irrigated agriculture in Mexico is developed within a great diversity of conditions of climate, soil, technological levels, and sociocultural factors. Mexico has nearly 6.5 million hectares with irrigation infrastructure that represent 25% of the cultivated surface and from which 50% of the production value is obtained. Of the total irrigation surface, 91% is superficial irrigation and the remainder, 560,000 ha, represents pressurized irrigation systems. However, presently they have reached the limit of the agricultural frontier in the irrigation surface due to lack of water, reason why it is necessary to increase the productivity of irrigation water and to optimize the way of cultivating the largest surface with the minimum amount of water.

With the creation of the National Water Commission (Comisión Nacional del Agua, CONAGUA) in 1989, and the enactment of the new National Waters Law in 1992 and its regulations, the transference of the administration of the irrigation districts to users began, supported by a partial rehabilitation program of the infrastructure that is licensed to the Irrigation Users' Civil Associations (Associaciones Civiles de Usuarios de Riego, ACUR) constituted into Irrigation Modules. By 2011 more than 99% of the total surface of the irrigation districts had been transferred to the ACURs. The RIGRAT involves these agencies directly with the primordial purpose of saving water. Each module has a specific amount of water licensed, which is given by the Irrigation District. The volume of water is managed by the module, and it is its main source of income, since it is an input in the production process.

The approach consisted in implementing technification of gravity irrigation in three main aspects: training, design and measurement. Activities were carried out for training and technical assistance with technicians and users of irrigation in design, trace and application of irrigation; the leveling of lands that implies a greater uniformity of irrigation and, therefore, contributes to reducing the volume of water applied; the agronomic and hydraulic design of irrigation, its trace and controlled application to the farm; the forecast of irrigation in real time, which means adjusting the irrigation programs according to rainfall, evaporation, temperature, and crop development; the monitoring and evaluation of irrigation, to guarantee that irrigation is applied according to program, valuing its efficiencies and impacts. The objective of this study was to analyze conceptually the potential use of water that technification of gravity irrigation can achieve and the alternatives of use according to the extrapolation of volumes, both in the agricultural sector or outside of it.

#### MATERIALS AND METHODS

The field work was carried out in the state of Sinaloa during the agricultural years 2016-2018. Sinaloa is the state with largest surface devoted to agricultural production; form the point of view of water use, it is organized into 8 Irrigation Districts (IDs) as shown in Table 1. The main crops are corn, bean and vegetables, using an average sheet of 113 cm, considering the surface requires a distributed volume approximately of 8,743 million m<sup>3</sup>, to attain sowing annually a surface in the order of 800,000 ha.

Irrigatio	on district	Number	Irrigated	Vol. Dist.		Sown	Harvested
Numbre	Name	Users	area (ha)	Thousand m <sup>3</sup>	Lb (cm)	area (ha)	area (ha)
10	Culiacán	18,797	195,549	2,160,688	110	237,481	204,213
63	Guasave	14,607	107,448	1,367,025	127	135,545	115,140
74	Mocorito	5,518	41,578	463,065	111	49,300	40,022
75	Rio Fuerte	21,075	215,536	2,930,397	136	289,780	264,251
76	El Carrizo	8,830	66,191	726,685	109	79,711	65,434
108	Elota	2,531	20,381	225,976	110	20,381	19,086
109	El Dorado	8,769	61,808	849,505	136	67,020	62,621
111	Baluarte	627	2,595	20,134	79	2,933	2,840
Т	otal	80,753	711,087	8,743,475	113	882,150	773,607

Table 1. Characteristics of the Irrigation Districts that operate in Sinaloa, Mexico (CONAGUA, 2007-2012).

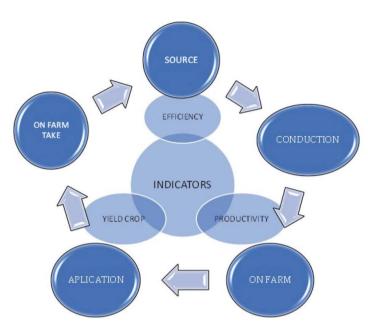
Addressing the surface sown, the analysis of water saving is detailed in the Irrigation Districts 075 Río Fuerte, 062 Guasave, and 076 Valle del Carrizo, and projections are made for the rest of the state. The analysis of irrigation district 075 Río Fuerte was conducted in 6000 ha, in which the technification activities were implemented. During the period when the project was developed, activities were carried out in five main axes:

- i. Training and technical assistance to users on basic aspects of design, trace and application of irrigation;
- ii. Leveling of lands to favor a greater uniformity of irrigation and reduction of the volume of water applied;
- iii. Controlled application of water in the farm; through plot capacity systems, from the control points or hydrants to the plot, with the aim of estimating application and inter-plot efficiencies;
- iv. Forecast of irrigation, to adjust the irrigation programs initially elaborated; this is where the tensiometer is used, through continuous sampling, the irrigation calendar is adjusted in function of the moisture content of the soil;
- v. The evaluation of plot irrigation to guarantee that water is applied according the crop's demand in interval irrigation.

The estimation of irrigation sheets, efficiencies and calendar follows standardized procedures (Allen *et al.*, 1998; Ojeda and Flores, 2015). From the information gathered in the field and agricultural statistics, for the purpose of this study, results were organized to estimate indicators with which to establish the volumes used and with them to estimate the volume saved by stage (plot, district and dam) and in function of the amount, to conduct the similarity of use in other sectors.

One way of conceiving the implementation of the project is presented in Figure 1.

The process of technification is relatively clear; at the plot level, there are waterdistribution systems where plot irrigation efficiencies are officially reported that fluctuate at 38%. This value implies a volumetric investment with a high percentage of diverse losses,



**Figure 1**. Indicators of efficiency, productivity and yield to measure water savings derived from technification of gravity irrigation.

which is why the increase of this efficiency increased with technification. An increase in the efficiency of plot irrigation implies a reduction in the water volume that has to come out of the source, that is, from the dam in most cases.

#### RESULTS AND DISCUSSION

The measurements in the control points and the general monitoring of activities for irrigation technification can be summarized in Table 2. The surface established is where the monitoring of the crops was carried out to estimate the volumes saved during agricultural cycles. For the surface established, the water volumes used are shown; at the farm level it was considered global plot efficiency, and from this there is an estimation upstream based on the efficiencies considered by CONAGUA in the different points from the source to the point of delivery to the module.

The implementation of activities destined to technification of irrigation allows decreasing the irrigation sheet applied in the participating irrigation plots. With this modernization an increase in efficiency is achieved, in function of the surface. The goal would be to

Table 2. Average yield of crops per irrigation district (CONAGUA, 2013).

T	Sown area		Wate	rsheet irrig	ation		Saved Volume	Saved
Irrigation district Name	(ha)	Req. (cm)	Farm (cm)	Module (cm)	DR (cm)	Source (cm)	(source) (Thousand of m <sup>3</sup> )	watersheet (cm)
063 Guasave	10,102.58	48.2	86.7	114.6	127.3	148.2	5,051.07	5.0
075 Río Fuerte	6,114.50	43.0	103.9	139.2	162.7	171.3	3,960.05	6.5
076 Valle del Carrizo	6,037.87	38.2	65.0	80.2	93.0	102.8	2,808.09	4.7
Three districts	22,254.95	44.0	85.6	111.3	126.8	141.5	11,819.21	5.3

attain a recovered volume of approximately 3,470 Mm<sup>3</sup>, which implies an increase in the efficiency of application of 13%. In this sense, in a cycle is observed the three districts of northern Sinaloa, savings at the farm level in the order of 7000 cubic meters, Table 3.

Water saving is possible through technification of irrigation; the volume saved is stored in the works of the dams, which leaves open the possibility of their use, in the agricultural sector and outside of it. Among the possibilities suggested there was a water bank, the reconversion of crops, and the expansion of the agricultural frontier; however, water savings and its use outside the agricultural sector is of the greatest relevance without there actually being a framework for its distribution; virtual water, ecological water, alternative energies, and non-consumptive used, are the challenges in the study of water.

In the irrigation district 076 Valle del Carrizo, there were 5 modules where monitoring of irrigation was carried out in the same number of ha (5000). At the beginning of the cycle, this module had a water productivity of 0.68 kilograms per cubic meter, and it was using average irrigation sheets of 105.18 cm, which gives a water yield of 2.58 pesos for each cubic meter of water. With the implementation of the program, water productivity was estimated in 1.03 kilograms per each cubic meter and the sheets in the case of the grains of 64 cm and 70 cm combined with vegetables, with which substantial water saving is inferred.

In the fall-winter cycle, 922 plots were attended to, with 799 beneficiaries distributed in the 7,017.27 ha that are dealt with in 6 irrigation modules, from irrigation districts 075 Río Fuerte and 076 Valle del Carrizo. In these plots, the irrigation sheets that originally exceeded 100 cm, and which currently fluctuate around 80 cm on average, have been achieved for all the crops with plot efficiencies above 42 % (Figure 2).

The indicators that allow evaluating the effectivity of irrigation or measuring the impact of technification of irrigation, which include the five main activities mentioned, are exposed in Figure 2. The estimation of these indices contribute accurate and timely information about the volumes used in the farm, measured and contributed, which together with the yields allows estimating the productivity of water. With the volumes measured and estimated in each control point, the efficiencies are estimated and as consequence the water volumes saved from the fact of adopting an irrigation design, an irrigation trace, and a technique in its application. The water volumes are available, and addressing the water quality conditions (Asit *et al.*, 2006 and Asit *et al.*, 2009), there will be the possibility of direct use in the public sector, which has an effect at the domestic level and is contingent on the distance from cities, among other variables.

Table 3. Results of the technification of gravity irrigation in three irrigation districts (RIGRAT, 2016, 2017).

Irrigation district	Watershee	t irrigation	Used V	Volume	Saved '	Volume
Name	Farm (cm)	Source (cm)	Source Thousand m <sup>3</sup>	Farm Thousand m <sup>3</sup>	Source Thousand m <sup>3</sup>	Farm Thousand m <sup>3</sup>
063 Guasave	86.7	148.2	149,695.8	87,605.8	5,051.1	2,956.0
075 Río Fuerte	103.9	171.3	104,738.6	63,558.8	3,960.1	2,403.1
076 El Carrizo	65.0	102.8	62,046.9	39,269.6	2,808.1	1,777.2
Three districts	85.6	141.5	314,858.9	190,434.2	11,819.2	7,148.5

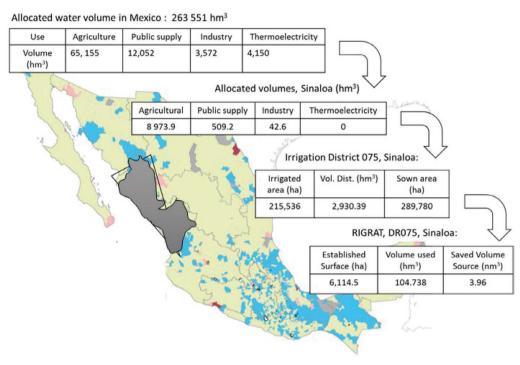


Figure 2. Volume of water saved and uses in the ID 075, Río Fuerte.

The use and management of water in general has different contexts, although the basic aspect is that there is water, which under the technification scheme is achievable; the next step will be to involve the authorities and the citizens to consider the nature of the national good whether from the environmental, social or economic point of view (Carabias and Landa, 2005). In Mexico, with the advances in matters of water and specifically related to the free trade agreement, NAFTA, which liberates the transit of virtual water, problems persist of criteria standardization, among which the price difference stands out (Cummings & Nercissiantz, 1992) which can derive into a water crisis similar to the cross-border crisis, affecting the production of crops.

The agricultural sector is one of the most affected by climate change (Ringler *et al.*, 2010) which is why the implementation of actions directed at saving water must go through improving water productivity (Kijne *et al.*, 2003); however, the management and availability of the volume saved is still pending, which is why the regulating institution is the one in charge of quantification and management of the resource (OECD, 2013).

The technological implications for this activity are clear: a) having gauging structures available at the farm to determine the volumes delivered, b) land levelling with the purpose of irrigation, c) irrigation programming based on soil and climate variables, d) calculation of the irrigation requirement per crop and for the crop mosaic; these actions were implemented in each of the participating farms of the program.

#### CONCLUSIONS

The volumes saved at the dam level are presented, estimated according to the methodology established. The dam is presently maintained from this volume saved.

Among the alternatives initially suggested, there is increase in the surface sown and as consequence increase in the harvest. There is the possibility of double planting or double cultivation. However, the use of this volume in other sectors, such as the energetic and a new ecological concept, directed at the recharge of aquifers and the stratification of the environment, is unclear. The strategies for development and conservation of the water resource in the agricultural sector should go through aspects of water optimization, addressing the productive uses. Selecting technologies that can be appropriated by the producer, based on the understanding of the process by the user. Managing the irrigation system and sensitizing the producer by addressing the problem of water use beyond the farm, giving an added value to the use of water saved, which is not used due to the increase in efficiency, and of reuse, recovered after irrigation.

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# Associativity in the case of vegetable producers in the Central Valleys of Oaxaca, Mexico

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#### ABSTRACT

**Objective**: To evaluate the associativity in groups of vegetable producers from six municipalities of the Central Valleys of Oaxaca, through an analysis of the attributes that are established to measure whether or not there is associativity, under the assumption that associativity is high in the analyzed communities. Economic globalization transforms the behavior of people and their values, devaluing the national identity of associative work. **Design/methodology/approach**: The study was carried out in the region of the Central Valleys of Oaxaca, with a qualitative and quantitative approach, in two phases: the first, documentary research on the theoretical approaches of associativity; the second, with field questionnaires, for which six municipalities were selected to represent vegetable producers.

**Results**: The results show the absence of associativity in the production of vegetables.

**Limitations on study/implications**: The number of the sample is within the limitations, so by expanding the number of municipalities the probability of association would be higher.

**Findings/conclusions**: The conclusion is that the increase in productivity and income and the reduction of production costs would favor the continuity of vegetable production and associativity would be promoted with this.

Keywords: associativity, development, collective work.

Citation: Pérez-Esteban, F., Santiago-Martínez, G., Miguel-Velasco, A., Castañeda-Hidalgo, E., Lozano-Trejo, S., Hernández-Hernández, B. (2023). Associativity in the case of vegetable producers in the Central Valleys of Oaxaca, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop. v16i1.2273

**Academic Editors**: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: May 11, 2022. Accepted: December 29, 2022. Published on-line: February 22, 2023.

*Agro Productividad*, *16*(1). January. 2023. pp: 69-77.

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#### INTRODUCTION

Globalization has had a drastic impact on the regularization of markets and the opening of new borders, which has transformed the way of producing, consuming, and the daily behavior of people and their values (Romero and Vera, 2012); likewise, it devalues the traditional references of national identity (Ospina, 2001). It has also led to the destruction of the rural community organization and to the loss of customs and collective work, causing the abandonment of the Mexican farmland and food shortage (Ordóñez and Rodríguez, 2008). The Mexican farmland has suffered changes that have altered the traditional rural



reality; social representations of the inhabitants are more heterogeneous, diverse and unequal compared to prior times (Fawaz, 2007). These changes have brought challenges in small-scale farmers and involve the import of foods (Reardon and Berdegué, 2002). Therefore, there is a need to seek strategies to strengthen the Mexican farmland, and in this sense, associativity is a strategy that contributes key elements to potentiate the productive activity, through internal and external factors, among them trust (Vasilica and Coba, 2017). When the decision is made to opt for associativity, the social fabric of a territory can be strengthened and consolidated, since it favors the capacities for production; however, it is necessary for associative processes to be conducted with a high level of commitment, willpower, without selfishness and with plenty of trust, in order to allow farmers to improve their quality of life (Arguello et al., 2017). It is necessary for both institutions and organizations to strengthen the capacities of collective action of farmers (Lázaro and Aranda, 2019). Oaxaca is a Mexican state where associative work has been implemented and it is reflected in agricultural work, since there is biological and cultural diversity and a complex environmental heterogeneity (Ordóñez and Rodríguez, 2008). It is considered that associativity in the state of Oaxaca can function as a strategy for the successful production of vegetables, since part of the history of the state is based on collective forms of work such as the Tequio and the Guelaguetza (Maldonado, 2015). Gallicchio (2004) maintains that the production of vegetables in places with environmental diversity is beneficial, since thanks to their heterogeneity a diversity of these can be produced throughout the year, which promotes and contributes to rural development. In addition to being a starting point where territorial development is attained through associativity. Therefore, the objective of this study is to evaluate the degree of associativity in groups of vegetable producers in the Central Valleys of Oaxaca, in the municipalities of El Carrizal in Cuilapam de Guerrero, Santa María Roaló in Villa de Zaachila, San Antonino Castillo Velasco and San Pedro Mártir in Ocotlán de Morelos, Santa María Atzompa and La Ciénega, Zimatlán de Álvarez in the Central Valleys of Oaxaca, through an analysis of the attributes that are established to measure whether or not there is associativity, under the assumption that associativity is high in the communities analyzed.

#### MATERIALS AND METHODS

The research that sustains this study has a mixed approach, supported by documentary research on theoretical approaches and concepts of associativity. According to Guerrero-Castañeda et al. (2016), it serves to support the explanation of a phenomenon, combining its perspectives for a broad vision and both are not complemented as methods, but rather as knowledge production. For the documentary research, indexed articles were reviewed from Web of Science, Google Academic and Scopus, Redalyc, RefSeek, Scielo, related to the theme of research giving priority to the concept of associativity, collective work and leadership. To search for information, a temporality from 1995 to 2020 was used. Likewise, a questionnaire was applied for the generation of information to evaluate the degree of associativity in agricultural vegetable producers of the six communities mentioned before. The variables considered were participation (PN), cohesion (CN), organizational climate (CO), teamwork (TE), leadership (LD), and communication (CM); in the economic, family

and social impact. The questionnaire was applied to a sample of 90 producers selected randomly by strata. In San Antonino Castillo Velasco 23 questionnaires were applied, in San Pedro Mártir 14, in El Carrizal 15, in Santa María Roaló 11, in La Ciénega 15, and in Santa María Atzompa 12. To calculate the sample size the technique of stratified random sampling was used, with a distribution proportional to the size of each municipality and the formula proposed by Badii *et al.* (2008) was applied.

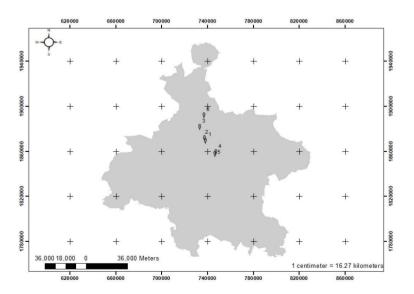
$$n = \left(z^2 pq\right) / d^2$$

Where: n: Population size; z: Value of tabulated z; p and q: Probabilities of success and failure; d: Coefficient of reliability.

The statistical analysis of the information was carried out with Duncan's multiple range technique with reliability of 90%. The percentage values obtained from a binomial distribution were transformed by the arcsine function to fulfill with the assumptions of homoscedasticity and independence of errors (Vázquez *et al.*, 2017).

# RESULTS AND DISCUSSION

The research that sustains the study was carried out in the region of Central Valleys of the state of Oaxaca, in five municipalities that produce vegetables: El Carrizal, Cuilapam de Guerrero, Santa María Roaló, Villa de Zaachila, San Antonino Castillo Velasco, San Pedro Mártir, Ocotlán de Morelos, Santa María Atzompa and La Ciénega, Zimatlán de Álvarez. The selection criteria were the representativeness that they have on vegetable production and the proximity with the capital of Oaxaca, where they generally trade their products (Figure 1).



### Simbology

- 1. Agencia del Carrizal, Cuilapam de Guerrero.
- 2. Agencia de Santa María Roaló, Trinidad Zaachila
- 3. Municipio de San Antonino Castillo Velasco, Ocotlán.
- 4. La Ciénega, Zimatlán
- 5. Municipio de Santa María Atzompa.
- 6. Municipio de San pedro Martír, Ocotlán.

## Vegetables

- 1. Mexican husk tomato (Physalis ixocarpa)
- 2. Chile de agua (Capsicum annuum L.).
- 3. Green bean (Phaseolus vulgaris L.)
- 4. Pumpkin (Curcubita pepo).
- 5. Coriander (Coriandrum sativum)

Source: Own elaboration.

Figure 1. Localities of study in the Central Valleys of Oaxaca and the main vegetables they produce. Source: Prepared by the authors.

# Context of analysis

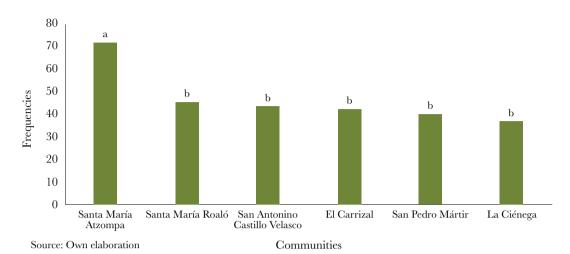
Associativity in the vegetable sphere of the Central Valleys of Oaxaca can be explained by the contrast of the agricultural sector in other regions of the state of Oaxaca, which are devoted to perennial species such as coffee, mango, avocado, maguey, papaya, banana, among others. These are in regions with absence of frequent climate phenomena such as frosts, hailstorms, and droughts, or else, they are recovered in case of facing them; in addition, the behavior of the offer and the demand does not fluctuate so drastically in these regions, because they are traded with added value by companies devoted to agroindustry, which ensures their permanence in the market. On the contrary, in the Central Valleys of Oaxaca the presence of adverse physical (hailstorms, reduction of water tables and frosts) and biological (pests) factors is frequent in the production of vegetables, in addition to socioeconomic aspects related to the agricultural policies of the agriculture and livestock sector, among other factors (low educational level, high degree of marginalization). Specifically, vegetable producers highlight that the horticultural activity is limited fundamentally by physical, biological, technological, and socioeconomic factors. Among the physical factors, they mentioned the reduction of water tables, since vegetable production requires irrigation; they also mention problems of alkalinity and salinity of water for irrigation, and occasional hailstorms and frosts. Regarding the biological problems, they expressed the constant presence of pest insects, diseases in crops, and competition with other plant species over water, light and nutrients. To face this situation, they mention that they depend on the acquisition of agrichemicals, since their production model is based on technology inherited from the green revolution.

It is in the socioeconomic aspect where producers make decisions about how, when, where, why and what for, a particular species will be planted. The farmers recognize the need for technical assistance, frequently expressing the neglect from government agencies for many years, and that they have even presented problems of intoxication from the ill use and management of agrichemicals. This, in addition to the fact that vegetable species are of short cycle, which makes them more vulnerable to suffer the effects from physical and biological factors, combined with the absence of government support with regards to the technical assistance for agriculture.

## Analysis of the current situation of associativity

Regarding associativity, the surveys showed that there are 15 projects and active initiatives in the rural sphere of the region of the Central Valleys of Oaxaca to produce vegetables, with activities developed in the rural sphere that range from transformation of raw materials, management of certified forests, exploitation and conservation of wildlife, ecotourism, among others, called rural social enterprises (Hernández *et al.*, 2018). Regarding the variables of associativity analyzed, Figure 2 and Figure 3 show that for the number and volume of vegetables produced, San Antonino and San Pedro Mártir presented the highest values.

The educational level in the community of Atzompa presented higher schooling because it was found in the metropolitan zone of the city of Oaxaca, compared to the five other municipalities located in the rural zone and with greater educational



**Figure 2**. Educational level in the communities. Source: Prepared by the authors.

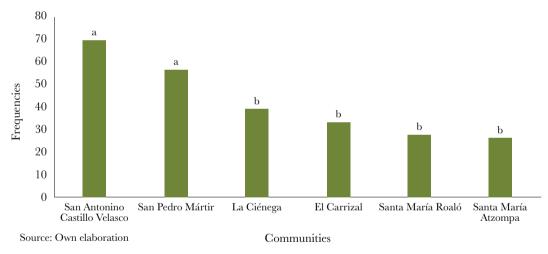


Figure 3. Vegetables cultivated in each community. Source: Prepared by the authors.

backwardness. With regards to family participation, it was found that the communities of Santa María Roaló and El Carrizal show more family integration for work (Figure 4), while San Antonino Castillo Velasco, San Pedro Martír, La Ciénega and Santa María Atzompa resort more to hiring workforce since the species cultivated and the continuous production require it.

Table 1 shows the behavior of the variables related with the associativity of the communities studied, with a greater significance found in the socioeconomic variables related to vegetable production. The variables that were significant were: other crops (OTROCULT), years in agriculture (AÑOAGRI), place of sale (LUVENT), profitability of the vegetables (RENHOR), requirement for workforce (REQOBRA), activity in which hiring workforce is required (ACTCONTRA), how many laborers they hire (JORNALES), and volume of production in tons (VOLPRO).

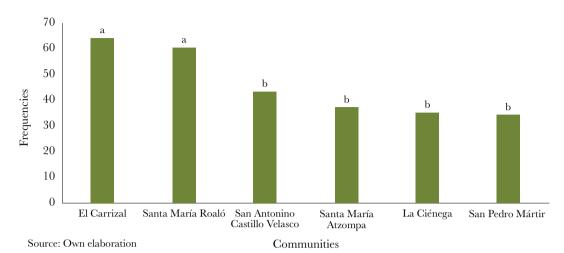


Figure 3. Family participation in the farmland. Source: Prepared by the authors.

**Table 1**. Analysis of the variables studied. Source: Prepared by the authors.

Source of variation	Model	*Error	Total Adjusted	Coeff. of Var.
DF	5	84	89	
OTROCULT	4796.14397**	383.87238		43.06079
AÑOAGRI	1498.82384*	610.29025		54.29462
LUVENT	5428.49084**	349.95293		41.11435
RENHOR	4339.83840***	339.58105		40.50049
REQOBRA	715.53557**	225.53360		33.00610
ACTCONTRA	3662.80324**	313.28552		38.90082
JORNALES	729.11349 <sup>*</sup>	226.49920		33.07668
INTEASOCI	215.48644 <sup>ns</sup>	623.60200		54.88357
CATTIERRA	836.76584 <sup>ns</sup>	615.28180		54.51621
VOLPRO	3487.94729**	416.38409		44.84723
OTRACTI	712.82906 <sup>ns</sup>	531.65303		50.67605
VENINDI	678.56366 <sup>ns</sup>	306.75216		38.49306

OTROCULT=Other AÑOAGRI=Years in crops, agriculture, LUVENT=Place RENHOR=Profitability of the vegetables, REQOBRA=Requirement ACTCONTRA=Activity in which hiring workforce is required, JORNALES=How many laborers they hire, INTEASOCI=Interest in participating in an associative way, CATTIERRA=Amount of land available, VOLPRO=Production volume in tons, OTRACTI=Other activities carried out besides agriculture, VENINDI=Advantages of individual work. \*significant, \*\*highly significant, ns=non-significant. Regarding the most representative variables of associativity, such as interest in participating in an associative way (INTEASOCI), it does not present significance, which reflects the lack of interest over the associative work to produce vegetables, since this activity is developed individually, which does not happen with the variables of the socioeconomic context that showed greater significance in the production.

Concerning the assumption of the article that a high associativity would be expected in the communities analyzed, it is rejected because the statistical results show an absence of statistical significance in the variables that promote associativity among vegetable producers. The agricultural sector is considered of great importance in the country; it provides basic foods to Mexican families and generates many jobs. However, many small-scale farmers present problems to become associated, for teamwork and in their ability to relate with others. State that farmers need to put aside their individualism, the null participation, and the lack of trust to become associated. And with this, to attain opportunities that strengthen their competitiveness to become integrated to trade their products, since this way they could cut out the intermediary and appropriate their own value chain (Rivas et al., 2018). There is a need for commitment, participation, and an attitude of leadership to organize the producers to improve their processes and their capacity for production, teamwork, way of thinking and values, which are attitudes that the members must adopt to fulfill their objectives. In the communities analyzed, despite their tradition to cooperate traditionally as it happens with the Tequio and the Guelaguetza for public festivities, in agricultural production associativity is still not understood as something that tends to contribute to the creation of strategies to strengthen development and growth by improving processes that give added value to the products, which also provides attributes that contribute to their development. Therefore, it can be said that it is necessary to change paradigms and consider the contribution of the attributes that give organizations soundness, and consider that productivity, reliability, stability, and equity between all the members are of utmost importance for the integration. They maintain that the level of trust between members, the transparency and their participation contribute to associativity, the same as they mention that democracy, honesty, equality, equity, and participation of the members are a basic complement to achievement the objectives (Barrera et al., 2016). This considers that commitment, leadership, effort, discipline, and teamwork are also judged as attributes that complement associativity and agree that attributes such as leadership, respect, transparency, and self-sufficiency do (Callejas, 2017; Melo 2017; Del Carmen and Malueños 2018). The research that sustains this study also analyzed other members who participate in associativity, since it is not just small-scale producers who participate, but also intermediaries, government, and some organizations, which somehow favor this relationship of rural associativity. It was observed that the problems of association between small-scale producers are generated due to the lack of attributes between them, the lack of communication, and the support both from government and non-government organizations; such is the case of this study where from a total of 90 producers from six different localities, none declared or showed that they were associated (Graph 1). This is because although the sector and the commercialization of their products demand it to improve their quality of life, they have not had the willingness to become associated.

### CONCLUSIONS

The absence of associative schemes in vegetable production is also due to the socioeconomic problems linked to marginalization and social backwardness that prevail in the six communities studied from the region of Central Valleys of Oaxaca, since the overriding urgency of covering basic human needs makes them seek other alternatives for subsistence. Other factors are physical and biological, since given the characteristics of traditional agriculture in the state, these do not allow their development from the constant exposure to natural phenomena, which is why small-scale producers do not have the capacity

to withstand productive risks, due to the marginal yields they obtain, which at the same time is reflected in the high costs of inputs used in agricultural production in Oaxaca. This also contributes to the lack of support and policies for agriculture and livestock production from the federal and state governments, according to the conditions of the smallholding, represented in basic backing to produce species of annual cycle. In conclusion, there is no associativity in the communities of the vegetable producers analyzed, which, if it were to exist, would favor the reduction of their production costs, as well as the increase of their productivity and income, and would likewise counteract the lack of public backing for their activity.

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# Evaluation of the application of fertilizers and biostimulants in *Zephyranthes lindleyana* Herb (Amarylidaceae) under greenhouse conditions

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**Objective**: To evaluate the effect of different doses of N, P, K fertilizers and two biostimulants, on growth parameters and vegetative development in plants of the species *Z. lindleyana* Herb.

**Design/methodology/approach**: An experiment was established under greenhouse conditions under a CRD; ten treatments with five repetitions were tested, with different doses of N, P and K and two biostimulants. The experimental unit was one plant per pot. Data were analyzed with descriptive statistics, non-parametric Kruskal-Wallis tests ( $\alpha$ =0.05). Budding, flowering, leaf height, number of leaves and seed production were measured.

**Results**: Vegetative development was distinguished in all plants. Only six treatments showed flowering. The percentage of floral and vegetative sprouting did not show delay or advance in the physiology of the plant. In leaf height and number of leaves, different behaviors were detected in the treatments, not detected in the Kruskal-Wallis tests. The plants treated with high doses of NPK fertilization and combined with biostimulants showed inflorescences and seed production.

**Limitations on study/implications**: Being a native plant with ornamental potential, the collection of this species is a challenge due to the lack of studies on the potential distribution of the species.

**Findings/conclusions**: This species can be cultivated under a greenhouse and pot planting system. Floral budding, height and number of leaves is stimulated by high doses of nitrogen. The combination of NPK and biostimulants favors development/growth and seed production.

**Keywords**: biodiversity, biomass, endemic, nutrient, ornamental.



Citation: Rodríguez-Flores, Z. E., Fernández-Pavía, Y. L., García-Cué, J. L., De la Cruz-Torres, E., Jaen-Contreras, D. (2023). Evaluation of the application of fertilizers and biostimulants in *Zephyranthes lindleyana* Herb (Amarylidaceae) under greenhouse conditions. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i1.2441

**Academic Editors**: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: November 29, 2022.
Accepted: January 18, 2022.
Published on-line: February 22, 2023.

*Agro Productividad*, *16*(1). January. 2023. pp: 79-87.

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### INTRODUCTION

2016).

Mexico occupies the fifth place in wealth of flora and houses in its territory between 25,000 and 30,000 plants, among which around 11,000 species are endemic vascular plants, and which offer a broad ornamental potential, as is the case of some genera such as *Zephyranthes*, *Hymenocallis* and *Sprekelia* whose species are not used for commercial production (Mapes and Basurto, 2016; Villaseñor,

The genus *Zephyranthes* contains approximately 90 species of which 37 are native to Mexico, which are distributed in subtropical and



tropical regions (Fischuck, 2021), primarily in the north and center-south of the country (Afroz et al., 2018; Spurrier et al., 2015). On the other hand, Zephyranthes lindleyana Herb. is a native species from the Mexican territory with high ornamental potential (Leszczyńska et al., 2000), and the same as other species such as Z. citrina, Z. carinata and Z. minuta lack reliable reports in different areas, from taxonomic to agronomic management, since there is scarce information published about the optimal doses of fertilization. Karavidas et al. (2022) point out that, as the biological basis for primary production, practices such as fertilization in endemic plants must be established, since these practices allow preserving and potentiating natural resources.

The study of endemic plants promotes a scenario so that those that are native are sources of biological and genetic resources that promote the diversity of crops and at the same time allow their conservation. The potential of these native species, such as *Zephyranthes lindleyana* Herb., as a result of their value as ornamental and phytochemical product, creates the opportunity to contribute to the increase of genetic diversity and allows new opportunities for species with ornamental potential to be traded. Cardoso and Vendrame (2022) consider that in order to achieve this, it is necessary to identify the ideal nutrition that allows conserving genetic resources since there is the danger of losing these species due to climate change or the disturbance of their habitats.

Because of the aforementioned, the following question emerged: What effect does the application of different doses of N, P and K and biostimulants have on Z. lindleyana Herb (Amarylidaceae)? To answer it, a study was conducted that had the objective of evaluating the effect of different doses of N, P, K fertilizers and two biostimulants, on growth parameters and vegetative development in plants of the species Z. lindleyana Herb (Amarylidaceae). The hypothesis proposed was that different doses of fertilization and two biostimulants have an influence on the growth and development of the plant. There is the intention to induce the ideal development and growth of Z. lindleyana Herb., with the addition of different doses of N, P and K and two commercial biostimulants based on brown algae (Ascophyllum nodosum), under homogeneity of the substrate, planting methods, irrigation, and greenhouse conditions to make known the management of this endemic species.

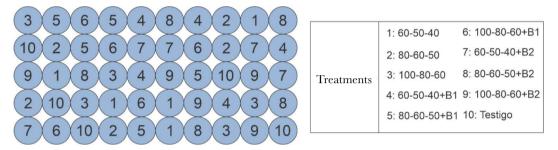
# MATERIALS AND METHODS

# Location and experimental conditions

The experiment was conducted within the facilities of the Research Unit "Ramón Fernández González" in the urban locality of San Miguel Coatlinchán, Texcoco, Estado de México (19° 27' 47.19" N and 98° 52' 54.71" W and 2272 masl) with coordinates. The experimental site has a semi-dry temperate climate with maximum annual temperature of 25 °C and minimum of 7.7 °C, as well as a maximum seasonal rainfall of 3.8 mm in the summer and a minimum of 0.3 mm in the winter. The plant material was donated by the Centro de Investigación en Plantas Nativas of the Universidad Popular Autónoma del Estado de Puebla, Atlixco, Puebla, Mexico, collected in the limits of the municipality of Tepeojuma e Izúcar de Matamoros, Puebla, Mexico.

# Treatments and experimental design

An experiment was established with plants from the species *Z. lindleyana* Herb. under greenhouse conditions established with a completely randomized design, where ten treatments were tested with five repetitions (Figure 1). The treatments were designed according to the Exploratory Batch Technique (FAO, 2022). The experimental unit was one plant per pot.



**Figure 1**. Distribution plan of the treatments of the fertilization experiment. Doses of N, P, K expressed in percentage. b1: Biostimulant MC Cream $^{\text{(E)}}$ ; b2: Biostimulant MC Extra $^{\text{(E)}}$ .

# Management of the experiment

Planting was carried out in the month of March 2021 in black polyethylene bags with capacity of 3 L. The physicochemical properties of the substrate were determined before sowing (Table 1), and the texture was reported as Sandy Loam (SL). The irrigation management was manual every third day using tap water. One week after sowing, when vegetative sprouting happened in most of the plants, application of the treatments was conducted.

Table 1. Physicochemical characterization of the soil.

T.T	E.C	O.M.	N	P	CEC	Fe	Cu	Zn	Mn
pН	dS m <sup>-1</sup>	%	%	mg kg <sup>-1</sup>	meq/100 g	${ m mgkg}^{-1}$			
5.4	2.2	12.4	0.6	23	32	65	2	39	80

E.C.: Electric Conductivity. O.M.: Organic Matter, CEC: Cation Exchange Capacity.

## Data analysis

Data analysis was done in different ways. For the sprouting and flowering, the percentage of floral and vegetative sprouting was calculated per treatment. These measurements were conducted in a range of three days since the first sprouting and flowering. To quantify the vegetative development, measurements were carried out in botanical terms of plant height or leaf length (LH) and number of leaves (NL). Taking these measurements was done periodically with a millimetric ruler of 30 cm. The height was taken from the neck of the bulb and start of the leaf to the apex of the largest leaf. The growth response of the plants was provided by taking data in the flowering stage, which is when it expresses its greatest growth habit and expression of the plant, according to the crop's physiology. Then, the LH and NL data were analyzed with univariate descriptive statistics. Furthermore, the

normality of the variables was tested through the Shapiro Wilk test ( $\alpha$ =0.05). Next, the nature of the variables and the results were analyzed. Since the assumption of normality was not fulfilled, the decision was made to use non-parametric Kruskal-Wallis tests ( $\alpha$ =0.05) to identify similarities or differences between their medians. Seed production was calculated through the total number per treatment and then the treatments were compared by their median with the Kruskal-Wallis test ( $\alpha$ =0.05).

## RESULTS AND DISCUSSION

During the month of April, vegetative sprouting began in most of the treatments in a uniform manner, except for the treatments T05 (80-60-50 kg ha<sup>-1</sup>+MC Cream® 1mL  $L^{-1}$ ) and T09 (100-80-60 kg ha<sup>-1</sup>+MC Extra® 0.5g/L) which only obtained 60% of sprouting. The floral emergence began during the second week of May 2021.

The dose 80-60-50 kg ha<sup>-1</sup> was also where these buds were most present in T02, T05 and T08 (Figure 2). The dates agree with what was reported by Tapia *et al.* (2017) which coincide with the genus Z. The presence of floral buds was found in treatments T01, T02 and T03 which do not have biostimulants. The plants presented different behavior in the different treatments that were noted visually in a qualitative manner.

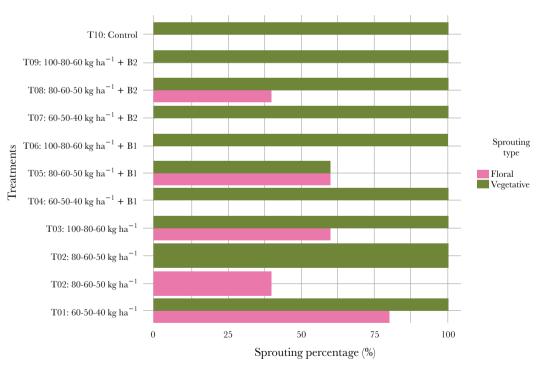


Figure 2. Percentage of floral and vegetative sprouting per treatment.

The univariate descriptive analyses on LH (Table 2) evidence the behavior by treatment. In the minimum values, it can be observed that the treatments T04 and T09 obtained values of 1 cm height, while the treatments T01, T07 and T08 exceeded 10 cm. In maximum values, this difference is evidenced in the same way since all the plants

			Leaf heig	ght (cm)			
Trat	Min	Max	Mean	Median	Std.dev	Coef. var	Pl
T01	10.4	19.5	16.36	17.5	3.8	23.22	5
T02	5	24.6	18.78	21.4	7.8	42.01	5
T03	4.9	27	16.6	14	9.61	57.73	5
T04	1	24.7	15.78	17.9	9.09	57.61	5
T05	6.7	24.6	17.22	19.2	7.51	43.64	5
T06	2	25	17.7	22.5	9.73	55.01	5
T07	14.8	25	21.66	22.9	3.97	18.33	5
T08	14.2	21	17.72	17.9	2.42	13.65	5
T09	1	24.3	12.78	10.4	9.95	77.86	5
T10	4.3	20	14.24	13.5	6.45	45.29	5

**Table 2**. Descriptive statistics of the variable leaf height (LH) of *Zephyranthes lindleyana* Herb. plants under different nutrition treatments.

Where: Trat: Treatment, Min: Minimum, Max: Maximum, Mean Mean, Median: Median, Std.var, Standard Deviation, Coef.var: Coefficient of variation in %, Pl: Number of sprouted plants. Plant: Number of sprouted plants, T01: 60-50-40 kg ha $^{-1}$ , T02: 80-60-50 kg ha $^{-1}$ , T03: 100-80-60 kg ha $^{-1}$ , T04: 60-50-40 kg ha $^{-1}$ + MC Cream, T05: 80-60-50 kg ha $^{-1}$ + MC Cream, T06: 100-80-60 kg ha $^{-1}$ + MC Cream, T07: 60-50-40 kg ha $^{-1}$ + MC Extra, T08: 80-60-50 kg ha $^{-1}$ + MC Extra, T09: 100-80-60 kg ha $^{-1}$ + MC Extra, T10: Control.

surpassed 20 cm of height, the effect of eight of nine treatments exceeded the control T10, of which the tallest heights corresponded to treatments with high doses of nitrogen with and without biostimulants (T02:80-60-50 kg.ha<sup>-1</sup> and T06:100-80-60 kg.ha<sup>-1</sup>+MC Cream® 1mL/L). Treatment T07 (60-50-40 kg.ha<sup>-1</sup>+MC Extra®) exceeded the control by nearly 10 cm. The coefficients of variation in T01, T07 and T08 are low, and in the others there is a heterogeneous behavior within the treatment. Then, a non-parametric Kruskal-Wallis test was carried out, where no differences were distinguished between the medians of the treatments ( $\chi^2$ =6.6324, P value=0.6753).

For NL the same procedure was followed and after performing the descriptive statistics (Table 3), the results were also contrasting, since the vegetative development was not homogenous in the treatments. The minimum values show that leaf formation for some treatments was affected importantly, since some treatments only obtained one leaf in a plant; however, since it was a discreet variable, the best value to represent a difference, descriptively, is the median. The value in which the best treatments were found quantitatively were treatments T02 (80-60-50 kg ha<sup>-1</sup>) and T06 (100-80-60 kg.ha<sup>-1</sup>+MC Cream<sup>®</sup> 1mL/L) with a median of six leaves. The lowest value was the control T10. The coefficients of variation in T01, T07 and T08 are low, and in the others, there is a heterogeneous behavior within the treatment. Likewise, a non-parametric Kruskal-Wallis test was carried out, where no differences were found between the treatment medians ( $\chi^2$ =8.6305, P value=0.4721).

When contrasting the results obtained with others reported in species of the same family (such as *Iseme amancaes*), subjecting the plants to different doses of NPK did not show a significant effect in growth variables (Jaulis & Pacheco, 2018); however, there are

unaci amei	ciit iiutiitioii	ti catificitis.					
			Num	ber of leaves	5		
Trat	Min	Max	Mean	Median	Std.dev	Coef.var	Pl
T01	3	5	3.8	4	0.83666	22.01	5
T02	1	8	5.2	6	2.5884358	49.77	5
T03	2	5	3.8	4	1.3038405	34.31	5
T04	1	6	4	4	1.8708287	46.77	5
T05	1	6	3.8	4	1.9235384	50.61	5
T06	2	8	5.2	6	2.280351	43.85	5
T07	3	6	4.8	5	1.0954451	22.82	5
T08	4	6	5	5	0.7071068	14.14	5
T09	1	6	3.6	4	2.0736441	57.60	5
T10	1	5	6.4	3	1.6733201	49.21	5

**Table 3**. Descriptive statistics of the variable number of leaves (NL) of *Zephyranthes lindleyana* Herb. plants under different nutrition treatments.

Where: Trat: Treatment, Min: Minimum, Max: Maximum, Mean Mean, Median: Median, Std.var, Standard Deviation, Coef.var: Coefficient of variation in %, Pl: Number of sprouted plants. Plant: Number of sprouted plants, T01: 60-50-40 kg ha $^{-1}$ , T02: 80-60-50 kg ha $^{-1}$ , T03: 100-80-60 kg ha $^{-1}$ , T04: 60-50-40 kg ha $^{-1}$  + MC Cream, T05: 80-60-50 kg ha $^{-1}$  + MC Cream, T06: 100-80-60 kg ha $^{-1}$  + MC Cream, T07: 60-50-40 kg ha $^{-1}$  + MC Extra, T08: 80-60-50 kg ha $^{-1}$  + MC Extra, T09: 100-80-60 kg ha $^{-1}$  + MC Extra, T10: Control.

other studies that report that the combination of high doses of nitrogen and biostimulants show improvements in other parts of the plant such as the root, thickness, length, weight and number of bulbs (Anbarasi & Haripriya, 2020). During the first week of the month of May, T01 (60-50-40 kg ha<sup>-1</sup>), T03 (100-80-60 kg ha<sup>-1</sup>), T05 (80-60-50 kg ha<sup>-1</sup> + MC Cream<sup>®</sup> 1mL L<sup>-1</sup>) and T08 (80-60-50 kg ha<sup>-1</sup> + MC Extra<sup>®</sup> 0.5g/L) were the only ones that presented inflorescences. The inflorescences behaved in the same way in all the treatments; in each of the repetitions, only a single magenta flower was produced (Figure 3).



**Figure 3**. Floral expression and morphology of the *Zephyranthes lindleyana* Herb. plant. A: Solitary flower with magenta color, B: Complete plant of *Z. lindleyana* Herb.

One of the flowering variables was the stem height (SH), measurement indicating the flower stem height. The number of flowers in all the treatments was from a single solitary flower with the same characteristics. Figure 3 shows the treatments that did obtain flower. It can be seen that there is a wide dispersion in the treatments because not all plants presented inflorescences. Likewise, it can be seen that treatment T08 (80-60-50 kg ha<sup>-1</sup>+MC Extra®) was the one that behaved in the least dispersed way and with a mean of around 7 cm of height, which contrasts with treatment T05 (80-60-50 kg ha<sup>-1</sup>+MC Extra®). However, in some treatments, as in T01 where all the repetitions presented inflorescences, not all the flowers produced seeds because the fruit did not reach maturity or simply was detached before producing them (Figure 4).

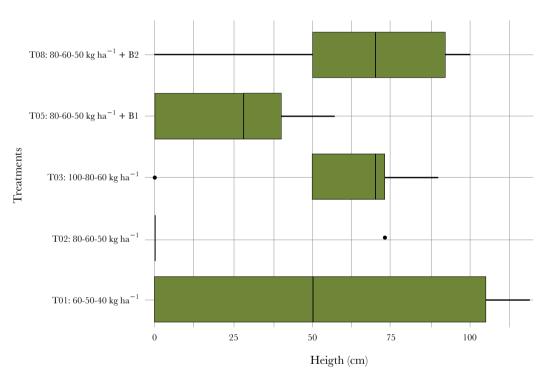


Figure 4. Stem height of Zephyranthes lindleyana Herb. from the treatments that presented inflorescence.

It should be highlighted that in the treatments where a biostimulant was not included, there was seed production, although the dose that did produce seeds with or without biostimulants was the 80-60-50 kg ha<sup>-1</sup> dose, even if this production was affected in its application without biostimulant (T02). A Kruskal-Wallis test was conducted between treatments T01, T02, T03, T05 and T08 and no statistically significant differences were identified for the values of the average number of seeds. Table 4 shows that not all the plants of the corresponding treatments produced seeds, and in fact only two plants could produce seeds in treatments T02 and T08. Likewise, when comparing the maximum number of each treatment (except T02) the seed production exceeded 30 units. Taking into account the number of plants and the mean, it can be defined that qualitatively the treatment with highest number of seeds corresponded to the lowest doses of N, P, K without biostimulant T01 (60-50-40 kg ha<sup>-1</sup>).

Table 4 shows the descriptive statistics of the number of plants sprouted that gave seeds. It was detected that in T02 and T08 only two plants could produce them from a total of five. Treatment T01 was the one with highest production with a total of 101 seeds that corresponded to the lowest dose of N, P, K without biostimulant (T01: 60-50-40 kg ha<sup>-1</sup>). The treatments (T01, T02 and T03), where no biostimulant were included, had some plants with seed production. The dose 80-60-50 kg ha<sup>-1</sup>s produced seeds with and without biostimulants. This dose produced less seeds in treatment T02 where no biostimulant was applied. When applying an unbalanced Kruskal-Wallis non-parametric test (by the number of plants where seeds emerged), the following was obtained:  $\chi^2$ =4.41, P value=0.3531; that is, no statistical differences were found in the medians of the seeds per treatment (P≤0.05).

**Table 4**. Descriptive statistics of the number of plants emerged that gave seeds of *Zephyranthes lindleyana* Herb.

	Seeds (number)									
Trat	Min	Max	Mean	Median	Std.dev	Coef.var	SE. mean	Suma	Pl	
T01	20	34	25.25	23.50	6.7019	26.54	3.350	101	4	
T02	15	16	15.50	15.50	0.7071	4.56	0.500	31	2	
T03	11	42	27.00	28.00	15.524	57.49	8.962	81	3	
T05	15	42	26.66	23.00	13.868	52.00	8.006	80	3	
T08	30	53	41.50	41.50	16.263	39.18	11.50	83	2	

Where: Trat: Treatment, Min: Minimum, Max: Maximum, Mean Mean, Median: Median, Std.var, Standard Deviation, Coef.var: Coefficient of variation in %, Pl: Number of sprouted plants. Plant: Number of sprouted plants, T01: 60-50-40 kg ha $^{-1}$ , T02: 80-60-50 kg ha $^{-1}$ , T03: 100-80-60 kg ha $^{-1}$ , T04: 60-50-40 kg ha $^{-1}$  + MC Cream, T05: 80-60-50 kg ha $^{-1}$  + MC Cream, T06: 100-80-60 kg ha $^{-1}$  + MC Cream, T07: 60-50-40 kg ha $^{-1}$  + MC Extra, T08: 80-60-50 kg ha $^{-1}$  + MC Extra, T09: 100-80-60 kg ha $^{-1}$  + MC Extra, T10: Control.

# **CONCLUSIONS**

The hypothesis proposed is not rejected. The native species *Zephyranthes lindleyana* Herb (Amarylidaceae) responded to the application of different doses of NPK and biostimulants in a sandy loam soil. *Z. lindleyana* Herb. can be cultivated in the central zone of the country under a greenhouse planting system and plot without showing an advance or delay in vegetative and floral sprouting. Floral emergence will be stimulated by high doses of nitrogen and show different responses in variables of height and number of leaves. The effect in treatments with different doses of NPK and biostimulants favored the development or growth of plants and the production of seeds. This was noted with regards to the control treatment.

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# Fruit characterization and plant quality of Spanish cedar (*Cedrela odorata* L.) during the early nursery stage

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#### ABSTRACT

**Objective:** The aim of this study was to characterize Spanish cedar (*Cedrela odorata* L.) fruits and seeds, and to evaluate the effects of provenance, substrata, and fertilizer on germination and plant quality in nursery.

**Design/Methodology/Approach**: The fruits and seeds from Spanish cedars were collected in three different towns: C-32 (Francisco Trujillo Gurria), C-40 (Ernesto Aguirre Colorado), and C-41 (Carlos A. Madrazo). Those towns are in Plan Chontalpa, Tabasco, Mexico. After they were harvested, the samples were morphometrically characterized. Seed production efficiency (SPE), germination (%), and Dickson Quality Index (DQI) were estimated. Two completely randomized experimental designs with factorial arrangement of treatments were used. The factors were the provenance, four or three substrata, and two levels of Greenfool <sup>®</sup> 600 fertilizer.

**Results**: Fruits of 3.46 cm in length and 1.81 cm in width were collected; the mean number of seeds per fruit was 49.36. Seed production efficiency ranged from 48.1% to 52.72%, with 32.86% germination. The seeds from Town C-41 obtained the highest germination percentage, with the use of black soil:sand as substrate. The plants fertilized and developed in black soil:sand substrate

obtained a higher DQI.

Study Limitations/Implications: The activity restriction caused by the Sars-Cov-2 pandemic was the main limitation. The lower number of producers and plantations from Town C-32 was the implication.

Findings/Conclusions: Fruit and seed characteristics were different

among provenances. The provenance impacted on seed germination, but not on the plant quality index (DQI). Instead, the substrate and fertilizer impacted on the DQI.

**Keywords**: *Cedrela odorata*, fruit and seeds, morphometric characteristics, Dickson quality index, Meliaceae.

Citation: Hernández-Méndez, N. Y., Sánchez-Gutiérrez, F., Sol-Sánchez, Á., Suárez-Islas, A., & Pérez-Flores, J. (2023). Fruit characterization and plant quality of Spanish cedar (Cedrela odorata L.) during the early nursery stage. Agro Productividad. https://doi. org/10.32854/agrop.v16i1.2325

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: July 05, 2022. Accepted: January 13, 2023. Published on-line: February 22, 2023.

*Agro Productividad*, *16*(1). January. 2023. pp: 89-96.

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# INTRODUCTION

The timber of Spanish cedar (*Cedrela odorata* L., Meliaceae) has a significative value.

They can be commonly found as scattered trees in anthropogenic landscapes (such as milpas, pastures, and hedgerows) and provide shade in coffee (*Coffea arabica* L.) (Soto *et al.*, 2008) and cacao (*Theobroma cacao* L.) plantations, from where they are also collected for commercial purposes.

Timber commercialization and harvesting of Spanish cedar have been limited in order to guarantee its survival. However, seeds are gathered from the trees without prior selection, a practice that does not guarantee the quality of the plants. Therefore, determining the use and characteristics of the tree, fruits, and seeds is fundamental to establish the quality of future plants.

The evaluation of the quality of the plant will determine its capacity to adapt and develop under the different environmental conditions of the sowing site (Rueda *et al.*, 2012). Morphological and physiological parameters define plant quality in nurseries. Appropriate cultural labors with the seeds, the substrata, the plant containers, and the nutrition are required to fulfill the said parameters (Rodríguez-Ortiz *et al.* 2020). Ramos-Huapaya and Lombardi-Indacochea (2020) attribute plant quality to the genetic characteristics of the seeds and the nursery management. For example, even seeds that come from the same site can have different quality levels (Cano and Cetina, 2004).

Various indexes are used to measure plant quality. For instance, the slenderness rate relates the height and the diameter of a plant (León-Sánchez et al., 2019); the lignification index evaluates the weight parameters (Sáenz et al., 2014); the above ground/below ground part of the plant ratio (Sáenz et al., 2014), and the Dickson Quality Index (DQI) which integrates all the parameters of these indexes (Villalón-Mendoza et al., 2016). High DQI values show a better plant quality (Ramos-Huapaya and Lombardi-Indacochea, 2020). In order to produce good quality plants, nurseries use commercial substrata, such as peat moss, agrolite, and vermiculite. However, the use of these materials reduces profit margins. Consequently, communities use the resources that are available to them as growth substrata: sand, compost, peat, or mountain floor.

Therefore, the objective of this study was to characterize the Spanish cedar fruits harvested in cacao plantations in Plan Chontalpa, Tabasco and to evaluate the impact of provenance, substrata, and fertilization on plant germination and quality during the early nursery stage.

## MATERIALS AND METHODS

# Study site and selection of the trees from which the fruits were harvested

The identification and selection of the Spanish cedar trees from which the fruits were harvested was carried out in cacao plantations in Town C-32 (Francisco Trujillo Gurría), Town C-40 (Ernesto Aguirre Colorado), and Town C-41 (Carlos A. Madrazo), located in the municipality of Huimanguillo, Tabasco, Mexico. The area has a subhumid warm climate, with abundant rainfall in summer (Am). It has a 26.2 °C mean annual temperature and a 2,290.3 mm mean annual precipitation (IMTA, 2009).

In order to enter the plantations, we approached the community authorities, the technicians from the Sembrando Vida program, and the producers. We asked the producers how many Spanish cedar trees grew in their plantations and if they were willing to harvest the fruits. All the Spanish cedar trees in the plantations were counted and measured (height and diameter; DBH 1.30). A Suunto clinometer (Sáenz *et al.*, 2014) was used to measure total height and a diametric tape was used to measure the diameter at breast height (DBH).

Based on the CONAFOR (2016) classification, fruits and seeds were harvested from Category 1 trees.

# Harvest and characterization of fruits and seeds

The largest number of fruits possible was harvested from each tree. Subsequently, the fruits were weighted, and 100 fruits were randomly selected. Then, their diameter and length were measured. Afterwards, each fruit was placed in a paper bag to ease their identification and the counting of seeds at the dehiscence stage. The seeds were separated in viable (winged seeds with embryo) and embryoless seeds. Subsequently, the seeds of each tree were mixed, and 100 seeds were randomly selected per tree, in order to measure their length.

# Plant germination and development in the nursery

The DQI was used to evaluate plant quality, after a plant germination and development analysis was carried out. The experimental design for the germination was completely random, with a factorial arrangement of treatments. The first factor was the provenance (Towns C-32, C-40, and C-41) and the second factor was the substrata: 1, black soil + sand substrate (1:1); 2, compost; 3 black soil + sugar cane waste (1:1); 4, peat moss + agrolite (1:1). A total of 12 treatments with four repetitions of 100 seeds each were established.

The same experimental design and factorial arrangement with treatments were used for plant development. The first factor was provenance (three towns), the second factor was the substrata (1, 2, and 3) and the third factor was the Greenfool 600 fertilizer, with a 15-31-15 formulation (2: with and without). A total of 18 treatments with four repetitions and four plants per repetition were established.

The following variables were measured: germination (%), root collar diameter (RCD), and plant height (H). Germination was recorded at 65 days after the sowing; the plants that came out of the soil were considered as germinated seeds. Germination percentage was obtained dividing the number of germinated seeds by the number of sown seeds and multiplying the result by 100. The RCD was measured using a Surtek<sup>®</sup> digital vernier and H was measured from the base of the stem to the apical bud using a 50-cm rule. Both variables were recorded according to the Mexican Standard NMX-AA-170-SCFI-2016 (DOF, 2016) every 8 days for 3 months.

At the end of the development experiment, a destructive sampling was carried out to quantify above ground and root biomasses: one randomly selected plant was extracted per repetition, the root was washed to remove the substrate, and the root was separated from the above ground part of the plant in order to record fresh weight. The samples were placed in paper bags and were dried until they reached a constant weight. A VE-5000 VELAB electronic scale with milligram accuracy was used to measure fresh and dry weight and the result was used to estimate DQI. The following formula was used:

$$DQI = \frac{Total\ dry\ mass(g)}{\frac{Height(cm)}{Diameter(mm)}} + \frac{Above\ ground\ dry\ mass(g)}{Root\ dry\ weight(g)}$$

# Data analysis

The data were subjected to an analysis of variance and a mean comparison test (Tukey,  $p \le 0.05$ ), using the InfoStat software. Previously, a normality and homogeneity of variance tests were carried out, as well as a data transformation (if required).

## RESULTS AND DISCUSSION

## Tree selection and fruit harvest

Twenty out of 239 Category 1 trees were selected as source of fruits and seeds. According to CONAFOR (2016), Category 1 includes high-quality, straight, and vigorous trees, without forks and with few branches in the upper two thirds. These trees have a circular crown, are dominant or codominant, are pest and disease free, and they must be good seed producers. Therefore, Town C-41 had the greatest number of trees and the tallest and widest trees, as well as the highest fruit production rate (Table 1).

# Characterization of fruits and seeds

The ANOVA found differences ( $F_{1,2}$ =23.72 and 100.70; p<0.001) in fruit width and length and in the number of viable, empty, and total seeds per provenance. The Town C-32 fruits had the same statistical size and the same statistical number of viable seeds than Town C-40 fruits; however, their viable seeds were bigger and more numerous than Town C-41 fruits. The same trend was observed in the length of the winged seeds and of seeds without wings (Table 2).

Overall, the fruits from the three towns were longer and wider than the fruits recorded by Ureta *et al.* (2019) which were 2.51 cm long and 0.76 cm wide. In contrast, Márquez *et al.* (2020) reported similar or longer (3.25 cm) and narrower (1.87 cm) fruits. Specifically, the fruits collected at Town C-32 were longer and had a similar diameter than those reported by Rodríguez *et al.* (2001), who recoded 3.34-cm long and 1.82-cm wide fruits.

**Table 1**. Descriptive measurements of the Spanish cedar trees and quantity of fruits harvested from three provenances in Plan Chontalpa, Tabasco, Mexico.

Variable	Provenance	Mean	SD	Minimum	Maximum
	C-32	37.1	7.5	28.0	48.0
DBH (cm)	C-40	39.0	1.0	38.0	40.0
	C-41	42.2	14.0	26.0	75.0
	C-32	14.1	0.9	13.0	16.0
Total height (m)	C-40	18.0	4.0	14.0	22.0
	C-41	18.4	4.2	10.0	25.0
	C-32	24.5	3.7	20.0	32.0
Fruits (kg)	C-40	24.5	1.5	23.0	26.0
	C-41	26.6	7.7	18.0	45.0
	C-32	15.5	3.6	10.8	22.0
Crown diameter (m)	C-40	15.2	3.6	11.6	18.8
	C-41	14.2	3.3	11.3	22.0

DBH, Diameter at breast height (1.30 m). SD, Standard deviation.

**Table 2**. Descriptive measurements of the Spanish cedar trees and quantity of fruits harvested from three provenances in Plan Chontalpa, Tabasco, Mexico.

Variable	Provenance	Mean	SD	Minimum	Maximum
	C-32	1.81 b	0.17	1.10	3.20
Fruit diameter (cm)	C-40	1.78 b	0.10	1.50	2.10
	C-41	1.74 a	0.22	1.20	3.70
	C-32	3.46 b	0.33	2.30	4.20
Fruit length (cm)	C-40	3.42 b	0.27	2.70	3.90
	C-41	3.21 a	0.45	1.90	4.30
	C-32	24.37 b	5.51	8.00	41.00
Viable seeds per fruit	C-40	25.12 b	3.29	14.00	36.00
	C-41	22.76 a	5.72	0.00	37.00
	C-32	24.99 b	5.61	10.00	49.00
Vain seeds per fruit	C-40	23.19 a	3.43	9.00	32.00
	C-41	25.71 b	5.37	0.00	55.00
	C-32	49.36 b	6.42	22.00	69.00
Total seeds per fruit	C-40	48.30 a	3.31	31.00	59.00
	C-41	48.46 a	4.05	21.00	64.00
	C-32	24.53 b	2.61	16.95	30.28
Seed length with wing (mm)	C-40	24.77 b	2.60	18.75	31.23
	C-41	22.53 a	3.29	8.61	30.35
	C-32	8.75 b	1.14	5.49	12.28
Seed length without wing (mm)	C-40	8.62 b	0.86	6.37	10.67
	C-41	8.17 a	1.14	5.02	11.41

SD, Standard deviation. Different letters for each variable indicate significant differences between provenances (Tukey, P≤0.05). n=100.

Regarding the production of total seeds, Town C-32 had the highest production: 49.36 seeds in average, 24.99 of which were empty. The production of seeds from this origin is greater than the one reported by Rodríguez *et al.* (2001), who recorded an average of 43 total seeds (including 21 embryoless seeds). According to the coefficient of variation, the fruits showed a greater variation in seed weight and its SPE and greater homogeneity regarding their width and seed production potential. The seed production recorded by Márquez *et al.* (2020) was also different (39.9 seeds per fruit). Likewise, there were differences regarding the production reported by Alderete and Márquez (2004), who recorded 42.35 seeds per fruit and a 56.35% SPE. Meanwhile, Márquez *et al.* (2020) reported a 58.86% SPE. Both reported higher SPEs than those reported in this work: 52.72% (C-41), 50.62% (C-32), and 48.01% (C-40).

The morphometric variables of the fruits collected in Town C-32 had a highly significative correlation (p<0.05), which proves that there is a close relationship between the size and seed number variables. The variables with higher correlation were fruit width vs fruit length (59%) and empty seeds vs. total seeds (59%). No correlation was higher than 50% in Town C-40, while fruit width vs. fruit length had a 77% correlation in Town C-41 (non-tabulated data).

# **Seed germination**

The ANOVA showed that provenance has an impact on germination. This effect was corroborated by the comparison of means: the germination percentage of the seeds from Town C-41 was statistically greater than the seeds from Town C-32 (Table 3). Germination in the substrata was statistically the same; nevertheless, the germination percentage of seeds in black soil+sand was 7.04% higher than in black soil+sugarcane waste (Table 3).

The germination percentages obtained in this study were lower than the results reported by Ureta *et al.* (2019) and Torres-Torres *et al.* (2018): 85 and 47.2%, respectively. Nevertheless, the seed used in this study did not receive any pre-germination treatment, unlike the seeds germinated by Torres-Torres *et al.* (2018), who soaked them for 24 h, before sowing them in a substrate made of chicken manure and sand (2:1).

# **Plant Development**

Different RCD and H statistics were recorded in the various levels of the evaluated factors at 15, 45, and 90 days after the experiment was established. The plants with highest RCD and H were grown in Town C-40, using fertilizer and a black soil:sand substrate (Table 4).

For this experiment, an average height of 18.66 cm was recorded in Town C-40; the height of the plants was statistically higher than plants from other provenances. Nevertheless, the said height is lower than the 38.12 cm reported by Orozco *et al.* (2020) for three-month old

Provenance	Germination (%)	Substrate	Germination (%)
Town C-32	14.96 b	Black soil + sugarcane cachaza	29.02 a
Town C-40	24.03 ab	Compost	22.41 a
Town C-41	32.86 a	Black soil + sand	21.98 a
		Peat Moos + Agrolite	22.39 a

**Table 3**. Effect of provenance and substrate on the germination of cedar seeds.

Different letters for each column indicate significant differences between provenances. (Tukey, p<0.05).

**Table 4**. Effect of provenance, fertilizer, and substrate on root collar diameter and plants height of Spanish cedar, at 15, 45, and 90 days after transplanting.

Variable	Day 15			Day 45			Day 90		
	Provenance	Fertilizer	Substrate	Provenance	Fertilizer	Substrate	Provenance	Fertilizer	Substrate
D.C.D.	1) 1.38 b	1) 1.51 a	1) 1.52 a	1) 2.74 b	1) 3.03 a	1) 3.50 a	1) 4.63 b	1) 5.27 a	1) 5.79 a
RCD (mm)	2) 1.70 a	2) 1.25 b	2) 1.35 b	2) 3.29 a	2) 2.47 b	2) 2.18 с	2) 5.33 a	2) 3.99 b	2) 3.81 с
(11111)	3) 1.07 с		3) 1.27 b	3) 2.27 с		3) 2.57 b	3) 3.92 c		3) 4.29 b
	1) 7.91 b	1) 7.98 a	1) 8.62 a	1) 10.78 b	1) 10.47 a	1) 12.27 a	1) 16.95 b	1) 16.75 a	1) 17.63 a
PH (cm)	2) 10.34 a	2) 7.52 b	2) 7.81 b	2) 12.45 a	2) 9.62 b	2) 9.02 b	2) 18.66 a	2) 14.89 b	2) 16.65 a
	3) 5.00 c		3) 6.83 c	3) 7.27 с		3) 9.01 b	3) 11.84 с		3) 13.18 b

RCD, Root collar diameter. PH, plant height. Provenances: 1, Town C-32; 2, Town C-40; 3, Town C-41. Fertilizer: 1, With; 2, Without. Sustrates: 1, Black soil + Sugarcane cachaza; 2, Compost; 3, Black soil + sand. Different letters for each column and variable indicate significant differences (Tukey, p<0.05). n=12.

Spanish cedars. Meanwhile, Torres-Torres *et al.* (2018) describe 15-cm tall plants produced with chicken manure, sand, and anthill soil (1:1:1), without pre-germination treatment.

# **Dickson Quality Index**

According to the DQI, the best quality was recorded in Spanish cedars grown in 60:40 black soil:sand substrate with fertilizer (Table 5). Based on the comparison of means, there were no significant statistical differences among provenances. Therefore, proper nursery management is essential to obtain quality plants that guarantee the success of reforestation efforts or the establishment of a commercial plantation.

**Table 5**. Effect of provenance, fertilizer, and substrate in the Dickson Quality Index of three-month old Spanish cedars.

Provenance	DQI	Sustrate	DQI	Fertilizer	DQI
Town C-32	0.86 a	Black soil + sugarcane cachaza	1.02 b	With	1.04 b
Town C-40	0.87 a	Compost	0.94 b	Without	0.71 a
Town C-41	0.88 a	Black soil + sand	0.65 a		

DQI, Dickson quality index. Different letters for each column indicate significant differences (Tukey, p < 0.05). n = 12.

Mateo-Sánchez *et al.* (2011) used a substrate made of 70% raw sawdust and 30% peat moss-agrolite-vermiculite (60:20:20) and applied 12 kg/m<sup>3</sup> of Osmocote plus<sup>®</sup> (15-9-12); 100 days later, they obtained a lower DQI (0.19) than the one obtained for plants grown with seeds collected from the towns of Plan Chontalpa, Tabasco. Díaz *et al.* (2013) also recorded lower results (0.39 DQI), 90 days after the sowing, with plants produced with cacao compost. Finally, Basave *et al.* (2016) grew plants in 500-ml black polyethylene bags and recorded a 0.3 DQI.

### CONCLUSIONS

Although they come from the same region and study area, the fruit and seed characteristics varied between the three towns, which points out a provenance effect. The said characteristics influenced seed germination. Regardless of the substrate, the biggest seeds from the biggest fruits had a lower germination percentage than the smallest seeds from the smallest fruits. This provenance effect is lost with a proper nursery management.

Under nursery conditions, irrespective of their origin, Spanish cedar plants fertilized with Greenfol<sup>®</sup> 600 and grown in a 60:40 black soil:sand substrate had better quality than those produced without fertilizer or grown in other evaluated substrata, according to the DQI.

### ACKNOWLEDGMENTS

The authors are grateful to the Consejo Nacional de Ciencia y Tecnología for the postgraduate scholarship granted to the first author. They would also like to thank the cacao producers who allowed them access to plantations and who supported them during the selection of Spanish cedars and fruit collection.

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# Forage diversity and selection in white-tailed deer (*Odocoileus virginianus Texanus* MEARNS) in Coahuila, Mexico

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#### ABSTRACT

**Objective**: To identify diet diversity and selection among white-tailed deer (*Odocoileus virginianus texanus* Mearns) at UMA Rancho San Juan, Monclova, Coahuila, Mexico, from October 2018 to August 2019.

**Design/methodology/approach**: The composition of the white-tailed deer's diet was identified by applying the microhistological technique. The line interception method was used to estimate the seasonal availability of forage. Diet and forage diversity were established based on the Shannon index, while their relation was identified using a simple linear regression. Diet selection was determined using the chi-squared test and Ivlev's electivity index.

**Results**: We identified 49 species and 20 families in the diet, which comprised 49.84% shrubs, 18.38% succulents, 16.02% herbaceous plants, and 15.72% grasses. Deer selected *Opuntia engelmannii*, consumed *Acacia rigidula* and *Cenchrus ciliaris* in proportion to their availability, and consumed *Acacia berlandieri*, *Jatropha dioica*, and *Karwinskia humboldtiana* below their availability.

**Study Limitations/Implications**: This line of research should be further pursued, including nutritional quality aspects of the forage and diet variations between sampling years. We also recommend fostering the presence of herbaceous plants through habitat improvement techniques.

**Conclusions**: No relation was found between diet and forage diversity. When forage diversity decreased, grass intake increased.

Key words: Line interception method, availability, Ivlev, desert scrub, microhistological technique.

Citation: Gastelum-Mendoza, F. I., González-Saldívar, F. N., Cantú-Ayala, C. M., Uvalle-Sauceda, J. I., Guerrero-Cárdenas, I., & Lozano-Cavazos, E. A. (2023). Forage diversity and selection in white-tailed deer (Odocoileus virginianus Texanus MEARNS) in Coahuila, Mexico. Agro Productividad. https://doi. org/10.32854/agrop.v16i1.2363

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: August 18, 2022.

Accepted: December 19, 2022.

Published on-line: February 22, 2023.

*Agro Productividad*, *16*(1). January. 2023. pp: 97-107.

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# INTRODUCTION

The white-tailed deer (*Odocoileus virginianus texanus* Mearns) is the most important game species in Mexico. Although they inhabit widely diverse ecosystems (Villarreal *et al.*, 2014), white-tailed deer are mainly associated with the desert scrubs of northeastern



Mexico (Mandujano et al., 2010; Gastelum-Mendoza et al., 2020). Unlike other herbivores, they are selective ruminants that feed on forage depending on food type and availability, physiological state, and density-dependent factors (Ramírez, 2004). White-tailed deer's tendency to prefer shrubs and herbaceous plants to other types of forage has been well documented, as has the fact that habitat conditions have a direct influence on their selection of certain plant groups (Gallina, 1993; Ramírez et al., 1997; Ramírez, 2004). Likewise, foraging intensity on certain plant species directly affects the habitat's carrying capacity (Ramírez et al., 1997; Fulbright and Ortega-Santos, 2007).

The white-tailed deer's foraging habits have been studied in Mexico and the United States. In northern Mexico, for instance, 51 species are considered important for their diet (Villarreal, 1999). Ramírez (2004) reports that 79 plant species make up the deer's diet in northeastern Mexico. Although white-tailed deer feed on a large number of plants, most of them belong to just a few species (Quinton and Horejsi, 1977; Murden and Risenhoover, 1993; Pietz, 2000). In habitats with a high diversity of foraging species, deer can consume over 160 different species. However, 50% of their diet can consist of less than 10% of the said species (Chamrad and Box, 1968). In the State of Durango, only 18 out of the 135 species consumed by deer were found in more than 1% of the diet (Gallina, 1984). In the South Texas Plains region (USA), 10 out of 83 consumed species comprised 53% of the diet (Everitt and Drawe, 1974). Although the Texan white-tailed deer is the most important game species in northeastern Mexico, its foraging habits in the State of Coahuila have not been studied. In order to provide the white-tailed deer's handlers with the information they need to improve this species' habitat —focusing on its in situ conservation—, this study sought to determine the deer's diet composition and diversity, as well as the way in which it selects its food depending on availability.

# MATERIALS AND METHODS

## Study area description

The study was conducted at the Unidad de Manejo para la Conservación de la Vida Silvestre (UMA) [Wildlife Conservation Management Unit] Rancho San Juan (26° 49′ 31.11" N, 101° 01′ 57.77" W), located in the municipality of Monclova, Coahuila, Mexico (Figure 1), 38 km in a straight line east of the municipality's capital and 43 km west of the municipality of Candela. The predominant vegetation type is the microphyllous desert scrub (Rzedowski, 1978). The climate is dry (BSohw), with an annual average temperature of 21 °C. Annual rainfall varies between 200 and 900 mm. Elevation fluctuates between 600 and 1,000 m.a.s.1 (García, 2004). An area of 1,532.14 ha within the UMA is allocated for the handling of Texan white-tailed deer (26° 48′ 09.96" N, 101° 00′ 15.77" W).

## Forage availability

An herbivore's food selection is expressed as the relation between each species' contribution to its diet and their availability in the habitat (Strauss, 1979). The said relation was assessed using Canfield's line interception method (1941). To this end, 18 lines of 25 m of length each were randomly placed for each season: autumn (October 2018), winter (February 2019), spring (May 2019), and summer (August 2019). In all cases,

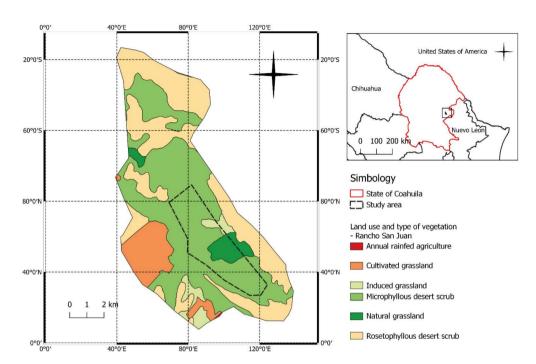


Figure 1. Location of the study area and the vegetation types at UMA Rancho San Juan, Monclova, Coahuila, Mexico.

the plants intercepting the lines were quantified. The species were classified according to their biological form as bushy, herbaceous, grasses, and succulents. Their availability was expressed as each species' relative frequency per season, according to the following formula (Curtis and McIntosh, 1951):

Relative frequency = 
$$\left(\frac{Number\ of\ lines\ containing\ species\ i}{Total\ number\ of\ lines\ in\ the\ season}\right) * 100$$

# Diet diversity and selection

In order to establish the composition of the white-tailed deer's diet, we used the microhistological technique, which identifies fragments of plant epidermis in fecal samples (Sparks and Malechek, 1968; Peña and Habib, 1980). With this aim, 50 white-tailed deer fecal groups were collected per season: autumn (October 2018), winter (February 2019), spring (May 2019), and summer (August 2019). Likewise, we compiled a photographic reference catalog of 150 plant species and their characteristic cell structures. The fecal samples were placed in paper bags, labeled, and dried in an INOX oven (120VAC, 60HZ) at 75 °C during 48 h. Subsequently, they were grinded in a Wiley mill and grouped in four composite samples (one per season). These were diluted with sodium hypochlorite, according to the procedure described by Sparks and Malechek (1968). Finally, they were placed on 20 microscope slides (five per each composite sample), using a metal plate with 7-mm-diameter holes, in order to homogenize the sample size in each slide. Twenty fields of view per slide were observed under the microscope at 100x magnification, in order to identify and count the plant cell structures. The identified plant species were classified

according to their biological form as: shrubs, herbaceous, grasses, and succulents. The results were expressed as the relative frequency of each plant species in the diet, following Peña and Habib (1980).

To establish forage selection, we compared the availability of the species in each season and their contribution to the deer's diet. This was quantified according to two analyses. The first one was based on the chi-squared test ( $\alpha \le 0.05$ ), used to estimate the differences between the percentage of the plant in the diet and its availability in the habitat; and the second one was based on Ivlev's electivity index (Strauss, 1979) according to the following formula:

$$Ei = \frac{\left[r(i) - p(i)\right]}{\left[r(i) + p(i)\right]}$$

Where: Ei = Forage selectivity index; r(i) = Relative frequency of species i in the diet; p(i) = Relative frequency of species i in the habitat.

The values for this index were classified according to Stuth (1991): >0.35, preferred plants (S); -0.35 to 0.35, plants consumed in proportion to their availability (P); <-1.0, avoided plants (E). The diversity of diet and vegetative cover was estimated using the Shannon diversity index (1948); both were then compared by means of a simple linear regression model.

# **RESULTS AND DISCUSSION**

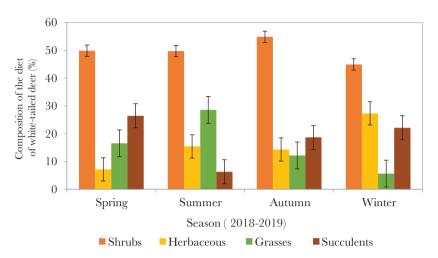
We identified 49 species and 20 families in the white-tailed deer's diet (Table 1). However, 50.23% of the diet included only seven species (Acacia rigidula, Erioneuron pulchellum, Eysenhardtia texana, Leucophyllum frutescens, Opuntia engelmannii, Opuntia leptocaulis, and Prosopis glandulosa). The most common families in the diet were Poaceae, Fabaceae, and Asteraceae. The annual diet comprised 49.84% brush species, 18.38% succulents, 16.02% herbaceous plants, and 15.72% grasses. Brush species were predominant in the diet during the four seasons. Herbaceous plants were more common in winter, grasses in summer, and succulents in spring (Figure 2). Herbaceous plants were the only group selected by the white-tailed deer for its diet ( $\chi^2$ =44.43, P<0.05), which proves the deer's preference for this plant group.

No values over 20% were identified for grasses in the diet; therefore, no preliminary indicators of habitat overuse and poor nutrition were observed (Kie *et al.*, 1980). *Opuntia engelmannii* was the most consumed among the succulent species (Table 1 and Figure 3). We were also able to infer that white-tailed deer avoided consuming succulents in summer ( $\chi^2$ =68, P<0.05). During the rest of the year, succulents were proportionally consumed. Although water requirements usually increase in summer (Ramírez, 2004), UMA has ten artificial water sources that cover these requirements, therefore reducing the intake of prickly pears. Moreover, we identified filler plant species that were consumed depending on their availability; this category included *Acacia rigidula*, *Cenchrus ciliaris*, and *Eysenhardtia texana*. Meanwhile, the most avoided species were *Acacia berlandieri*, *Euphorbia antisyphilitica*,

**Table 1**. Seasonal composition of the white-tailed deer's diet per biological form, family, and species at UMA

Rancho San Juan, municipality of Monclova, Coahuila, Mexico.

Family	unicipality of Monclova, Coahuila, Mo	.,	Season (2018-2019)			
		Spring (%)	Summer (%)	Autumn (%)	Winter (%)	
Shrubs						
Fabaceae	Acacia berlandieri	0.28	0.29			
Fabaceae	Acacia farnesiana	0.28	0.57	0.65		
Fabaceae	Acacia rigidula	11.29	12.57	6.94	4.51	
Verbenaceae	Aloysia macrostachya	1.1	1.71	0.65	0.23	
Asteraceae	Baccharis texana	1.65	0.57	1.3		
Bignoniaceae	Chilopsis linearis	0.83				
Euphorbiaceae	Croton punctatus			4.12	5.19	
Euphorbiaceae	Croton torreyanus	0.83	1.14	9.33	5.87	
Ebenaceae	Diospyros texana		0.57			
Ephedraceae	Ephedra pedunculata	1.1			0.23	
Euphorbiaceae	Euphorbia antisyphilitica	3.86	0.57	1.74	7.67	
Fabaceae	Eysenhardtia texana	8.54	7.43	11.28	1.81	
Oleaceae	Forestiera angustifolia	1.65		4.12	0.45	
Zygophyllaceae	Guaiacum angustifolium	7.16	3.14	0.43	0.23	
Asteraceae	Gymnosperma glutinosum	7.10	0.11	0.22	0.20	
Asteraceae	Hymenoxys odorata		0.29	0.43		
Euphorbiaceae	Jatropha dioica		0.29	0.43		
Rhamnaceae	Karwinskia humboldtiana		0.29		0.23	
Krameriaceae			2	0.22	0.23	
	Krameria erecta				1 10	
Zygophyllaceae	Larrea tridentata	1.00	0.86	0.22	1.13	
Scrophulariaceae	Leucophyllum frutescens	1.93	0.57	5.42	1.61	
Verbenaceae	Lippia graveolens	2.24	0.57	2.0		
Fabaceae	Mimosa zygophylla	3.31	2	3.9	1.81	
Fabaceae	Prosopis glandulosa	5.23	7.43	1.52	4.29	
Solanaceae	Solanum elaeagnifolium	0.83		0.22	0.68	
Rhamnaceae	Ziziphus obtusifolia		6.86	2.17		
Herbaceous					1	
Malvaceae	Abutilon wrightii	2.2	1.71	2.82	0.45	
Asteraceae	Acourtia runcinata	0.28				
Nyctaginaceae	Allionia incarnata	0.55	1.43	1.8	7.9	
Asteraceae	Ambrosia dumosa	0.28		3.47	4.74	
Fabaceae	Dalea bicolor	0.55	4.29	1.74	1.13	
Convolvulaceae	Evolvulus alsinoides		1.14	0.65	0.23	
Fabaceae	Medicago sativa	2.75	4.29	1.74	4.29	
Asteraceae	Parthenium argentatum		0.29			
Asteraceae	Parthenium sp.	0.55			2.3	
Brassicaceae	Physaria fendleri				3.16	
Boraginaceae	Tiquilia canescens		2.29	2.82	3.39	
Grasses	<b>1</b>					
Poaceae	Aristida adscensionis		1.14	1.3	0.23	
Poaceae	Aristida purpurea	1.65	1.1.1	3.9	0.23	
Poaceae	Bouteloua curtipendula	1.00		1.95	3.40	
Poaceae	Bouteloua hirsuta	0.55		1.95	1.58	
Poaceae	Cenchrus ciliaris	3.3	4.29	2.39	1.81	
Poaceae	Cynodon dactylon	0.83	7.43	0.22	0.45	
		1.1	6.57	0.22	0.45	
Poaceae	Heteropogon contortus	1.1	6.57	0.22	0.40	
Poaceae	Hilaria mutica	0.07	0.57	0.00	0.0	
Poaceae	Erioneuron pulchellum	9.37	16	0.22	0.9	
Succulents	0	10 =0	4.00	15.4	14.0	
Cactaceae	Opuntia engelmannii	18.73	4.29	15.4	14.9	
Cactaceae	Opuntia leptocaulis	7.71	2	3.4	7	
Cactaceae	Opuntia microdasys			0.22	0.23	



**Figure 2**. Seasonal composition of the white-tailed deer's diet according to the biological form of plants at UMA Rancho San Juan, Monclova, Coahuila, Mexico (the vertical lines over the bars indicate the typical error).



Figure 3. Evidence of the white-tailed deer grazing on *Opuntia engelmannii* at UMA Rancho San Juan, Monclova, Coahuila, Mexico.

Jatropha dioica, and Karwinskia humboldtiana. The only preferred species was Opuntia engelmannii (Table 2).

Shrub grazing is the base of the white-tailed deer's diet (Gallina, 1993; Ramírez et al., 1996). Particularly in the southeastern United States and northeastern Mexico, where a seasonal shortage of herbaceous vegetation occurs, this kind of deer survives by feeding on a shrub diet (Campbell and Hewitt, 2014). The chi-squared test indicates that there is no difference between contribution of shrubs to the diet and their availability in the habitat. This means that deer consumed these species either randomly or in proportion to their availability. In this regard, Fulbright and Ortega-Santos (2007) mention that the deer's shrub intake serves as a nutritional bridge between periods of herbage availability. White-tailed deer have a strong preference for herbs as compared with grazing, since herbs are generally more digestible and have a higher nutritional value than shrubs (Ramírez et al., 1997; Ramírez, 2004; Fulbright and Ortega-Santos, 2007). However, herbage represented only 16.31% of the annual diet in this study; it was more important for the winter diet and

**Table 2**. Chi-squared test values ( $\alpha \le 0.05$ ) in diet composition and forage availability, and Ivlev's electivity index for the white-tailed deer's diet at UMA Rancho San Juan, municipality of Monclova, Coahuila, Mexico.

	Expected use + (%)	Observed use <sup>++</sup> (%)	Ivlev	Type of use <sup>+++</sup>
Acacia berlandieri				
Spring	6.18	0.28	-0.91	E
Summer	3.28	0.29	-0.84	Е
Autumn	2.66	0.00	-1.00	Е
Winter	4.52	0.00	-1.00	E
$\chi^2 = 155.15$ , g. l.=3, P	< 0.05			
Acacia rigidula				
Spring	6.74	11.29	0.25	P
Summer	4.92	12.57	0.44	S
Autumn	4.79	6.94	0.18	P
Winter	5.03	4.51	-0.05	P
$\chi^2$ =7.22, g. l.=3, NS <sup>+</sup>	++			
Cenchrus ciliaris				
Spring	3.93	3.30	-0.09	P
Summer	4.92	4.29	-0.07	P
Autumn	2.13	2.39	0.06	P
Winter	4.02	1.81	-0.38	E
$\chi^2$ =2.94, g. l.=3, NS	1			
Euphorbia antisyphilitica				
Spring	8.99	3.86	-0.40	E
Summer	7.10	0.57	-0.85	E
Autumn	9.04	1.74	-0.68	E
Winter	8.04	7.67	-0.02	P
$\chi^2 = 112.27$ , g. l. = 3, P·	< 0.05			
Eysenhardtia texana				
Spring	3.93	8.54	0.37	S
Summer	4.37	7.43	0.26	P
Autumn	4.26	11.28	0.45	S
Winter	2.01	1.81	-0.05	P
$\chi^2 = 8.14$ , g. l. = 3, P<0	.05			
Forestiera angustifolia				
Spring	2.81	1.65	-0.26	P
Summer	1.09	0.00	-1.00	E
Autumn	0.53	4.12	0.77	S
Winter	1.01	0.45	-0.38	E
$\chi^2 = 4.64$ , g. l.=3, NS	1			

Table 2. Continues...

	Expected use + (%)	Observed use <sup>++</sup> (%)	Ivlev	Type of use ++-
Guaiacum angustifolium				
Spring	1.69	7.16	0.62	S
Summer	1.09	3.14	0.48	S
Autumn	2.66	0.43	-0.72	E
Winter	2.01	0.23	-0.79	Е
$\chi^2 = 30.86$ , g. l. = 3, P<	0.05			
Jatropha dioica				
Spring	4.49	0.00	-1.00	Е
Summer	4.92	0.29	-0.89	E
Autumn	2.66	0.00	-1.00	E
Winter	3.52	0.00	-1.00	Е
$\chi^2 = 73.92$ , g. l. = 3, P<	0.05	l .		
Karwinskia humboldtian				
Spring	1.69	0.00	-1.00	Е
Summer	2.19	0.29	-0.77	Е
Autumn	1.60	0.00	-1.00	Е
Winter	4.52	0.23	-0.90	Е
$\chi^2 = 92.47$ , g. l. = 3, P<	0.05			
Opuntia engelmannii				
Spring	4.49	18.73	0.61	S
Summer	6.01	4.29	-0.17	P
Autumn	3.19	15.40	0.66	S
Winter	4.02	14.90	0.58	S
$\chi^2 = 29.14$ , g. l.=3, P<	0.05			
Opuntia leptocaulis				
Spring	3.37	7.71	0.39	S
Summer	2.19	2.00	-0.04	P
Autumn	2.13	3.40	0.23	P
Winter	0.50	7.00	0.87	S

<sup>&</sup>lt;sup>+</sup> Expected use: forage availability expressed in relative frequency; <sup>++</sup> observed use: percentage in diet; <sup>+++</sup> type of use: selected plants (S), plants consumed in proportion to their availability (P), avoided plants (E), NS=not significant.

less important in spring and summer (Figure 2). Winter herbaceous plants that reproduce from seeds and complete their life cycle in a year are generally absent during the summer in Texas and northern Mexico (Ramírez, 2004; Fulbright and Ortega-Santos, 2007). Moreover, herbage represents only a small fraction of the diet in arid environments such as northeastern Mexico (Arnold and Drawe, 1979). However, in areas with greater rainfall, herbage can be predominant in the white-tailed deer's diet. Navarro *et al.* (2018) found that

herbage comprised 70.80% of the deer's diet in Tlachichila, Zacatecas. Likewise, Olguín *et al.* (2017) report that white-tailed deer consumed 36.5% herbage in a thorn scrub in Tamaulipas.

Deer do not usually eat grass, since they cannot efficiently digest mature grasses due to their digestive anatomy (Hanley, 1982). Nevertheless, the deer can efficiently consume regrowths after a rainy period (Fulbright and Ortega-Santos, 2007). Meyer *et al.* (1984) mention that grasses can constitute over 40% of the deer's diet during humid periods. This is a potential explanation for the higher percentage of grasses in spring and summer (Figure 2). Besides, deer did not avoid grass intake during these seasons, as they did in autumn and winter ( $\chi^2$ =68, P<0.05), when grass contribution to their diet was lower (Figure 2).

Succulents, particularly prickly pear (*Opuntia* spp.), are considered buffering species in arid areas, since deer consume them to meet their water requirements (Espino-Barro and Fuentes, 2005). Brush species, such as guajillo (*Acacia berlandieri*) and blackbrush acacia (*Acacia rigidula*), can constitute over 70% of the deer's diet in northeastern Mexico (Ramírez *et al.*, 1996). Still, they only amounted to 8.97% of the deer's annual diet in this study. Nevertheless, succulents as a whole were the most important species in their diet. Deer avoided guajillo throughout the year, while they consumed blackbrush acacia in proportion to its availability (Table 2). Since these plants were not among the deer's preferred species, their nutritional contribution to this animal's diet might be deficient. In this regard, Campbell and Hewitt (2014) found that the concentration of calcium, phosphorus, and sodium in the deer's diet diminished as the guajillo intake increased.

Deer change their plant selection depending on the season: Eysenhardtia texana, for instance, was a filler species in the summer and winter diet, while in spring and autumn it was a preferred species; Forestiera angustifolia was avoided in summer and winter, regularly consumed in spring, and preferred or selected in autumn; Guaiacum angustifolium was preferred in spring and summer, and avoided in autumn and winter. Unlike Opuntia engelmannii, tasajillo (Opuntia leptocaulis) was only preferred in spring and winter.

Out of the 43 available plant species, 14 are not reported as part of the white-tailed deer's diet (Ramírez, 1989; Ramírez et al., 1997). These can be considered avoided species among white-tailed deer and their function might be related to thermic and escape covers. Although food diversity in the habitat is important to maintain an adequate nutritional diet, no relation between diet and forage diversity was observed (R<sup>2</sup>=0.13; P<0.05). While diet diversity remained relatively constant throughout the year, vegetative cover diversity decreased from spring to winter. According to Ramírez (2004), no plant meets the deer's nutritional needs throughout the year; consequently, deer try to maintain a diverse diet, despite the decrease in forage diversity. However, when forage diversity decreases, deer increase their intake of less palatable plants with a low nutritional quality (e.g., grasses) and decrease their intake of plants with a high protein and nutrient content (e.g., herbaceous species) (Ramírez et al., 1997; Fulbright and Ortega-Santos, 2007).

### **CONCLUSIONS**

Shrub intake turned out to be the foundation of the white-tailed deer's diet. Although not highly available, herbaceous plants were preferred. Besides, a high grass intake was observed. Grasses were randomly selected and might be evidence of a poor forage diversity. *Acacia berlandieri* was avoided and *Acacia rigidula* was proportionally consumed. Diet diversity did not depend on vegetative cover diversity. The study results are relevant to conduct habitat improvements that favor the presence of species preferred by white-tailed deer in the desert scrubs of northeastern Mexico.

### **ACKNOWLEDGEMENTS**

The authors would like to express their gratitude to Mr. Gerardo Benavides Pape (engineer), owner of UMA Rancho San Juan, as well as to his operational staff. We are also in debt with the technical staff of the Laboratorio de Fauna Silvestre of the Facultad de Ciencias Forestales [Wildlife Laboratory of the School of Forest Sciences], Universidad Autónoma de Nuevo León.

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### Sowing of stevia (*Stevia rebaudiana* Bertoni) in the humid tropical climate of Tabasco, México

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### **ABSTRACT**

**Objective**: To evaluate *Stevia rebaudiana* under the climatic conditions of the state of Tabasco, Mexico.

**Design/Methodology/Approach**: Three consecutive sowings of stevia plants were carried out for one year, progressively adapting the management and crop establishment according to the results obtained in the previous sowing. The survival percentage at 15 days after transplantation (dat) was calculated. Plant height was measured at 60 days and limitations observed in each crop cycle were recorded, as well as climatic variables.

**Results**: bending problems, soil splashing on the leaves, and foliar fungal diseases were recorded during the December 2019 sowing; however, 71% of the plants survived the transplant. In the April 2020 sowing, 40% of the plants survived the transplant; nevertheless, the plants showed generalized chlorosis and lack of growth, as a consequence of excessive solar radiation. Finally, in August 2020, the survival rate reached 89% and an average plant height of 20.55 cm was recorded.

**Study Limitations/Implications**: the rainfall that caused flooding in June and October 2020 limited the development of this research. Likewise, to determine the steviosides content and establish the quality of the harvest, the cultivation cycle must conclude.

**Findings/Conclusions**: Stevia can be grown in the climatic conditions of Tabasco, as long as the rainy seasons and high temperatures are avoided during the first stage of cultivation. Water is a limiting factor that causes phytopathological problems and the death of the plant.

Keywords: non-caloric sweetener, alternative crop, survival.

Citation: Arcos-López, J., Gayosso-Rodríguez, S., Estrada-Botello M. A., & Sánchez-Hernández R. (2023). Sowing of stevia (*Stevia rebaudiana* Bertoni) in the humid tropical climate of Tabasco, México. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i1.2378

**Academic Editors**: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: September 12, 2022. Accepted: January 23, 2023. Published on-line: February 22, 2023.

*Agro Productividad*, *16*(1). January. 2023. pp: 109-115.

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### INTRODUCTION

Stevia rebaudiana Bertoni or "candy leaf" is native to Brazil and Paraguay (Ferrazzano et al., 2016). It is a perennial plant of the Asteraceae family. Stevia is a major sugar substitute because it is 250 to 300 times sweeter than sucrose (Latarissa et al., 2020). Its leaves contain steviol glycosides, making it suitable for consumption by people with blood sugar concentration and overweight problems (Hossain et al., 2017). It provides other health benefits, such as anticancer, antidepressant, antiviral, and antimicrobial properties and it also helps to control hypertension (Oviedo-Pereira et al., 2015). In

addition, it can be used in cosmetics, as a soil improver, and as a supplement in animal diets, among other uses (Martínez, 2015). In Mexico, stevia was introduced approximately a decade ago (Ramírez et al., 2011; Herrera et al., 2012). In 2017, the Servicio de Información Agroalimentaria y Pesquera reported that a total of 55.85 hectares of stevia were cultivated in the country, in the states of Nayarit, Chiapas, Michoacán, Oaxaca, Campeche, and Ouintana Roo. However, there is no record of the production of this crop in Tabasco. Its establishment in new production areas requires diverse information about its adaptation and management, including density, fertilization, irrigation, etc., which vary according to the following and other factors; climate, relative humidity, luminosity, and soil fertility (Espitia, 2007; Jarma, 2010; Jarma, 2012; Daza, 2015). For México, stevia is an innovative and profitable crop with promising national and international market conditions. It is a labor-intensive crop that generates many rural jobs, making it an option for small producers (Espitia et al., 2009; Ramírez et al., 2011). Additionally, as a result of the accelerated trend expansion towards natural and organic eating habits, the interest in growing backyard plants for food or for their medicinal properties has increased. In view of the health benefits of cultivating stevia and the possible benefits to the economy of family gardens, the response of Stevia rebaudiana to the environmental conditions in the state of Tabasco, México, was evaluated.

### MATERIALS AND METHODS

The experiment consisted of a series of three consecutive sowings, progressively adapting the crop management and establishment, based on the results obtained in the previous sowing. Therefore, S. rebaudiana was sown in December 2019 and harvested in March 2020. The crop was established in the nursery and greenhouse area of the División Académica de Ciencias Agropecuarias of the Universidad Juárez Autónoma de Tabasco (DACA UJAT), located in the community of La Huasteca (17° 47' 15" N, 92° 57' 15" W), municipality of Centro, Tabasco, México, at an altitude of 19.7 m.a.s.l. During the cultivation period, temperature and relative humidity were recorded with a HOBO Data Logger. Meanwhile, according to the Comisión Nacional del Agua (CONAGUA, 2019), the monthly rainfall registered was 144.4 mm, 75.3 mm, 137.8 mm, and 18.8 mm, in December 2019 and January, February, and March 2020, respectively. The plants were established in a sandy clay loam texture soil, with an OM of 4.3%, pH of 4.5, and EC of 0.096 dS/cm. In the area, cleaning with a machete, leveling, cleaning, and preparing drains were carried out by hand. Twelve 1 × 3 meters long and 30 cm high beds were made. Rooted cuttings of the Morita II variety from Akil Yucatán, México were established. The one-month-old plants were grown in vermicompost trays. They were established directly on the previously designed beds with a 20×20 cm density in a square system. Crop management was carried out by manual weeding and watering according to the crop needs (Figure 1).

The second sowing was established in April 2020 at the Instituto Tecnológico de la Zona Olmeca, located in Ocuiltzapotlán in the municipality of Centro, Tabasco (18° 09' 39" N and 92° 50' 55" W). The area has a warm humid climate with abundant rains in summer. The mean monthly temperature was recorded in April, May, and June with a



**Figure 1**. a) Manual preparation of planting beds, irrigation, and manual weeding; b) Soil and mechanized preparation of planting beds, drip irrigation system; and c) manual preparation of planting beds, drip irrigation, silver plastic mulch.

HOBO Data Logger. The monthly precipitation was 36.3 mm, 281.7 mm, and 385.7 mm in the said months, according to the Comisión Nacional del Agua (CONAGUA, 2020). The cultivation area has a soil with a clayey texture, with an OM of 2.3%, pH of 7.9, and an EC of 0.47 dS/cm.

The cultivation area was cleaned by hand with a machete, harrowed twice, and the land was weevilled using mechanized means. We prepared 1.50-m wide, 25-m long, and 30-cm high planting beds, with 50-cm furrows between beds for crop management. Planting was carried out with rooted cuttings of stevia (Morita II variety) from the company "Stevia Huntulchaac" from Yucatán, México. The plants were established with a  $20 \times 20$  cm density between plants in a square system, while a drip irrigation system with tape was installed with 30 cm between rows and outlets every 30 cm (Figure 1).

In August 2020, the third sowing was carried out at the Instituto de la Zona Olmeca de Ocuiltzapotlán Centro, Tabasco. Average and maximum temperatures were recorded with a HOBO Data Logger. According to the Comisión Nacional del Agua (CONAGUA, 2020), the monthly precipitation was 303.1 mm, 461.3 mm, and 550.4 mm in August, September, and October 2020, respectively. The land was cleaned by hand with a machete, it was left to dry in the sun for four days, and the stubble was subsequently burned. We made 25-m long beds with a useful surface of 70 cm wide; the soil was turned over to form a 30-cm tall elevation. A 50-cm furrow was left between beds. An irrigation system with a strip was installed with 40 cm between rows and with outlets every 30 cm in the row. Rooted cuttings of S. rebaudiana (Morita II variety) from Tekax, Yucatán, were established. A  $20 \times 20$  cm density was used in the square system (Figure 1).

For plant management, a formative pruning was carried out one week after the transplant and a second formative pruning at 30 dat in the three sowings. For nutritional purposes, the dose reported by Casaccia and Álvarez (2006) was used: 162 N, 19 P, 140 K, 40 Ca, 9 Mg kg ha<sup>-1</sup>. Fifty percent of the fertilizer was applied the first week after the transplant and the rest at 45 dat. The variables were: survival percentage at 15 dat

and plant height at 60 d, taking 15 plants at random per sowing. The data obtained were subjected to basic statistics.

### RESULTS AND DISCUSSION

Regarding the *S. rebaudiana* established in the humid tropical conditions of Tabasco, the results show that 71% of the plants sown in December 2019 survived the transplant (Table 1). The norte wind season —a period characterized by heavy rains and winds resulting from the formation of tropical storms and cyclones— caused lodging and the deposit of soil on the leaves, leading to foliar fungal diseases (Arturo *et al.*, 2009; Salazar *et al.*, 2015). The rainfall, the size of the plants at the time of transplantation (10 cm), and the uncovered soil (without vegetation cover) contributed to this problem. Under these conditions, it was necessary to design drains to evacuate excess water and avoid flooding that would affect the crop. In addition, a sanitation cutting of the diseased plants was carried out and a 2.5 ml L<sup>-1</sup> dose of Metalaxil<sup>®</sup> was applied to control the fungus. During this period, lepidopteran larvae consumed tender shoots, without causing severe damage to the crop. Individuals were captured by hand to control the larval population.

The lowest survival percentage among the plants sown in April 2020 was the result of the extreme environmental factors recorded in May of the same year, such as the high solar intensity (9,974.56 lux), as well as the highest temperature (52 °C) and the lowest relative humidity (34.52%) of the year (Table 1 and 2). Consequently, the plants stopped growing and showed general chlorosis and leaf thickening. This physiological response

**Table 1.** Survival of rooted cuttings of *Stevia rebaudiana* (Morita II variety), established in Tabasco on three different dates.

Sowing	Survival (%)	Temperature* 30 ddt (°C)	Precipitation* 30 ddt (mm)
December 2019	71	24.4	144.4
April 2020	40	30.8	36.3
August 2020	89	28.8	303.1

<sup>\*</sup>Average for Tabasco (CONAGUA, 2021 and 2022); dat (ddt): days after transplant.

**Table 2.** Temperature (T), relative humidity (RH), and light intensity, recorded in the cultivation areas of *Stevia rebaudiana* (Morita II variety), in Tabasco, Mexico.

Date	Average		Maximum		Minimum		
Date	T (°C)	HR (%)	Intensity (lux)	T (°C)	HR (%)	T (°C)	HR (%)
December 2019	27.06	79.57	-	45.42	87.44	20.71	58.18
January 2020	25.40	87.10	-	40.99	96.03	19.87	65.54
February 2020	25.83	85.75	-	41.34	95.50	19.31	63.42
April 2020	29.50	73.97	698.41	38.38	86.17	23.05	56.87
May 2020	32.66	70.44	9974.56	52.07	92.60	23.93	34.52
June 2020	29.95	78.96	4393.89	45.09	93.19	25.14	50.24
August 2020	31.14	76.00	6459.02	47.93	97.82	23.24	34.93
September 2020	30.36	79.55	4677.49	45.8	98.3	23.5	40.9

was caused by the stress resulting from light radiation which increases water and nutrient consumption (Canovas, 1995). High solar radiation can lead to photo-oxidation, causing the decomposition of photosynthetic pigments, which results in chlorosis (Tadeo and Gómez, 2008). For his part, Gómez (2012) mentions that high radiation, high temperatures, and water stress increase photorespiration, affecting productivity, because of the consumption of the carbohydrates produced in the photosynthetic process.

Finally, all the plants of this crop cycle were impacted by the tropical storm "Cristobal", which recorded 611 mm to 635 mm rainfalls (CONAGUA, 2020), flooding of the land and causing the death of the plants in three days. In relation to excess water in the crop, Bentancour-Ancona and Segura-Campos (2015) mention that stevia requires 1,000 to 1,400 mm rainfall per year and the state of Tabasco exceeds the rainfall required by the crop with an annual average of 2,550 mm; the area of the mountains has recorded the most copious rainfall in the state (INEGI, 2017). Therefore, the local precipitation must be taken into consideration and the rainy season should be avoided in the early stages of the crop, in order to prevent soil saturation, fungal diseases, and root anoxia (Salisbury and Ross, 1992).

The highest survival percentage was recorded among the plants sown in August 2020 (Table 1). Turgid plants without sanitary problems or transplant stress were observed in this cycle. The climatic conditions during this season were favorable (Figure 2): the average temperature was 31 °C, slightly higher than the optimal range (24-28 °C) reported for stevia by Cruz (2016). There were no significant rains and the use of plastic mulch prevented soil splashing on the leaves and the presence of fungal diseases; it also helped with weed control and soil moisture conservation (Bériot, 2022).

The growth of the stevia plants varied in each growing cycle. Average plant heights ranged from 9.8 cm to 20.55 cm at 60 dat. The April sowing was the most affected in terms of growth, due to extreme weather factors coupled with a silt loam soil that limited the movement of water. Table 3 shows that the plants sown in August 2020 had

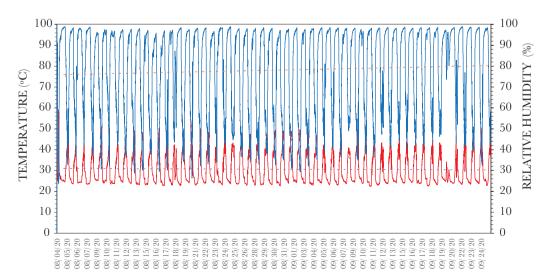


Figure 2. Temperature and relative humidity in August and September 2020, in the *Stevia rebaudiana* cultivation area in Tabasco.

Sowing date Height 60 ddt (cm)		Planting conditions	Observations				
December 2019	12.5	Bedding, weeding and manual irrigation	Bending by splash and presence of leaf spots				
April 2020	9.86	Mechanized soil and drip irrigation system	leaf thickening, general chlorosis, no growth				
August 2020	20.55	Manual bedding, drip irrigation, plastic mulching	Turgid and healthy plants				

**Table 3.** Average height of *Stevia rebaudiana* (Morita II variety) plants, established under different climatic conditions and infrastructures in Tabasco.

a higher growth (20.55 cm, in average) than those reported by Herrera *et al.* (2012) in Nayarit (14 cm).

Several authors mention that the development of the stevia plant is affected by such factors as sowing density (Espitia, 2007), solar radiation (Jarma, 2012), irrigation (Daza, 2015), nutrition (Jarma, 2010), and crop management (Ramírez et al., 2011). However, the limiting factor observed during this study was the water-soil ratio. Soil texture defines the movement of water and determines drainage and water retention and consequently root development (Gayosso et al., 2021). Stevia plants did not tolerate soil saturation for more than three days, resulting in the total loss of the crop; therefore, the water factor (rainfall or irrigation) and the type of soil are important in the selection of the cultivation area. Regarding the soil, Ramírez et al. (2011) mention deep soils (20-50 cm), loamy and sandy soils, luvisols, regosols, and fluvisols, with a 5-7 pH.

### **CONCLUSIONS**

A determining factor for the cultivation of stevia in Tabasco is excess water; therefore, areas with less rainfall and sandy loam soils should be selected. High temperatures and low relative humidity are critical during the first stage of cultivation. The transplant of stevia to soil in the month of August in Tabasco has a similar survival and growth percentage than the average of other states. However, the cultivation of stevia can be an option in Tabasco, if the quality of the harvest is valued by determining the steviosides content and the cost-benefit ratio.

### **ACKNOWLEDGEMENTS**

The authors would like to thank the PRODEP fund for financing the research project "Producción y manejo agronómico de Stevia rebaudiana en Tabasco, México" (code: UJAT-EXB-241).

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### Agricultural production and market prices in Mexico

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### **ABSTRACT**

**Objective**: To establish if the price of the main products of the Mexican agricultural sector is cointegrated with the production of the main producing states in five agricultural regions of Mexico.

**Design/Methodology/Approach**: Thirty cointegration tests were carried out to determine if the prices of the main agricultural products in Mexico (corn kernel, sorghum kernel, and beans) influence the production of the main producing states in the five agricultural regions of Mexico.

**Results**: In the northwestern region, the price does not influence the production of the main producing states of corn kernel, sorghum kernel, and beans. In the northeastern, central-western, and central regions, the price does influence the production of the main producing states of the said produce; meanwhile, in most of the states in the southern-southeastern region, the price does not affect the production of the main producing states.

Study Limitations/Implications: The analysis did not include all states and their main products.

**Findings/Conclusions**: The price influences production, but its influence is not even in the five regions of Mexico or in the main producing states of corn kernel, sorghum kernel, and beans.

Keywords: agricultural production; market prices; cointegration; states.

Citation: Cruz-Lázaro, L. M., Banda-Ortiz, H., & Vivanco-Palacios, P. I. (2023). Agricultural production and market prices in Mexico. *Agro Productividad*. https://doi.org/ 10.32854/ agrop.v16i1.2437

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: November 23, 2022. Accepted: January 19, 2023. Published on-line: February 22, 2023.

*Agro Productividad*, *16*(1). January. 2023. pp: 117-126.

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### INTRODUCTION

The agricultural sector produces the food that society requires for its survival and forms value chains; therefore, it is considered the sector that creates the economy. Likewise, its production is influenced by several factors, including the prices of its products. For example, higher product prices encourage producers to increase their production, because high prices mean higher income. On the contrary, lower products prices would decrease production, which would cause the income of the producers to diminish (Bambilla *et al.*, 2014; Méndez, 2011; OECD-FAO, 2011; Terrones and Sánchez, 2010; García *et al.*, 2018). This phenomenon is based on the neoclassical economics theory, which has been used to analyze the agricultural sector of various countries and sets forth that producers seek to maximize their profit, minimize their costs, and increase the efficient use of their factors of production. However, this relationship is not the same for all producers, because it depends on several factors, such as the level of their technological and economic resources.



Therefore, producers with greater access to economic and technological resources will be in a better position to take advantage of the increase in product prices than those producers with fewer economic and technological resources (de Grammont, 2010; Rosenzweig and Hillel, 2015; Roldán, 2012; Cardona *et al.*, 2007; Roitbarg, 2021). The agricultural sector has a twofold relevance: on the one hand, it provides the food that society demands for its survival; on the other hand, it creates value chains, since its processes require various inputs, such as tractors, credits from commercial and development banks, fertilizers, etc. Therefore, it is considered the creative sector of the economy. Likewise, its evolution has led to the creation of two groups: a highly-productive group with access to technological and economic resources; and a not very productive group with little access to technological and economic resources (de Grammont, 2010; Moreno *et al.*, 2011; Tonconi, 2015; Sosa and Ruíz, 2017; García *et al.*, 2018; Garcia, 2020; Roitbarg, 2021).

The agricultural sector is also influenced by the prices of its products. Therefore, when the population demands more products, prices increase, which causes producers to increase their production, in order to increase their income. Likewise, farmers compete among themselves and demand more production inputs; consequently, not all producers are able to take advantage of the price increase (Bambilla et al., 2014; Tonconi, 2015; Flores, 2014; Guzmán et al., 2012; García, 2020; Benítez, 2022; Cardona et al., 2007; OECD-FAO, 2011). The neoclassical approach to economics indicates that economic agents in the agricultural sector seek to maximize profit, to minimize costs, and to maximize the efficient use of factors and that prices affect the production (Cardona et al., 2007; Roitbarg, 2021). The latter phenomenon has been pointed out by various authors, including Bambilla et al. (2014), Tonconi (2015), Márquez et al. (2006), and OECD-FAO (2011), who have highlighted that product prices affect agricultural production. However, not all producers are affected the same way: only those with economic and technological resources can take advantage of a price increase (and are consequently able to considerably increase their production), unlike producers with fewer economic and technological resources (de Grammont, 2010; Moreno et al., 2011; Rosenzweig and Hillel, 2015; Guajardo, 2012; Roldán, 2012). In Mexico, agricultural production is also affected by the prices of the products, which impact the income of the producers: when prices increase, the production of the Mexican agricultural sector also increases, in order to obtain a higher income (Márquez et al., 2006; Flores, 2014; Acosta, 2005; Appendini, 2008; Cardona et al., 2007; Roitbarg, 2021). Researches like those carried out by Guzmán et al. (2012) and Bambilla et al. (2014) indicate that, in Mexico, price and production have a positive relationship. Therefore, in order to determine the existence of this relationship and establish the conditions in which it occurs, it is necessary to determine whether or not the price of the main products of the agricultural sector in Mexico are cointegrated with the production of the main producing states of the five regions of the Mexican agricultural sector.

### MATERIALS AND METHODS

Thirty cointegration tests were performed. The 1980-2021 databases of the states were obtained from the website of the Secretaría de Agricultura y Desarrollo Rural (SADER,

2022a). The five producing regions are the same as those used by SADER (2022b) to supervise the agricultural sector of Mexico. Likewise, according to SADER (2022a), the states with the highest production of the three main agricultural produces in Mexico in 2021 were chosen. Table 1 shows the main products and the states that had the highest production of each one in 2021.

Table 1 shows that the main products of the Mexican agricultural sector in 2021 were corn kernel, beans, and sorghum kernel. The table also shows the main producing states for each of the three products in the five regions analyzed. These states were examined by means of cointegration tests, to determine if their production is influenced by the prices of the products. Likewise, in order to carry out an analysis of actual data, the price database was deflated using the Índice Nacional de Precios al Consumidor (INPC) of the Instituto Nacional de Estadística y Geografía (INEGI, 2022).

### **Cointegration tests**

Thirty cointegration tests (fifteen with a trend and fifteen without a trend) were carried out to determine if prices impact the agricultural production of the main producing states, regarding the main products of five Mexican regions. Cointegration tests determine whether or not the relationship has a long-term and non-spurious nature. Data for the 1980-2021 period was analyzed and a total of 42 observations were made for each cointegration test (Table 2).

Table 2 shows the influence of prices in the states with the highest production of the main agricultural products in the regions analyzed. For each state, cointegration tests (with and without trend) were used to examine the relationship of its production with the product prices from 1980 to 2021. According to Gujarati and Porter (2010), to perform the cointegration tests, it is first necessary to determine that the variables analyzed are non-stationary and of the same integration order (order one), by means of two Augmented Dickey Fuller (DFA) tests for unit root (with and without trend, one of order zero and the other of order one). The EViews software was used for this purpose. The DFA tests will have the form of the following equation (1).

$$\Delta Y_t = \beta_1 + \beta_2 t + \delta Y_{t-1} + \sum_{i=1}^m \alpha_i \Delta Y_{t-1} + \varepsilon_t \tag{1}$$

Where:  $\varepsilon_t$  is a white noise pure error term;  $\Delta Y_{t-1}$  the number of lagged difference terms that are frequently included.

Table 1. Products, regions, and states analyzed.

Main products of the Mexican agricultural	Northwest	Northeast	Center-West	Center	South- southeast
Corn kernel	Sinaloa	Chihuahua	Jalisco	Edo. de México	Veracruz
Beans	Sinaloa	Zacatecas	Guanajuato	Puebla	Chiapas
Sorghum kernel	Sinaloa	Tamaulipas	Guanajuato	Morelos	Campeche

Source: Table developed by the authors based on SADER (2022ab).

TO 11 A	$\alpha$		
Table 2.	Cointegration	tests per	region.

Region	States	State analyzed product	Cointegration tests (with and without trend)
Northwest	Sinaloa Sinaloa Sinaloa	Corn kernel Beans Sorghum kernel	
Northeast	Chihuahua Zacatecas Tamaulipas	Corn kernel Beans Sorghum kernel	It will be analyzed by means of cointegration tests (with and without trend) if the production of the main producing states of
Center-West	Jalisco Guanajuato Guanajuato	Corn kernel Beans Sorghum kernel	the main products produced by the Mexica agricultural sector of the five regions of the Mexican agricultural sector is influenced
Center	Edo. de México Puebla Morelos	Corn kernel Beans Sorghum kernel	by prices. Where the independent variable is prices and the dependent variable is production.
South-southeast	Veracruz Chiapas Campeche	Corn kernel Beans Sorghum kernel	

Source: Developed by the authors.

In all DFA tests, the Durbin-Watson statistic will be examined to verify the absence of autocorrelation problems. Therefore, the value of the Durbin-Watson statistic must be higher than the significance point ( $\alpha$ =5%) and their corresponding k and n values. Next, in the order zero DFA tests, the p-value will be analyzed. If it is greater than 0.05, the series has a unit root and is non-stationary and, if it is lower than 0.05, the series has no unit root and is stationary. If the variables do not have a unit root and are non-stationary in order zero, it must be determined whether or not they belong to the integration order one (i.e., they must be stationary in the first difference). Therefore, a second DFA test is carried out on the variables (with and without trend), but with differences (order one), analyzing again the Durbin-Watson statistic and the p-value of the test. To verify that the variable is of integration order one, the series must not have a unit root and must be stationary in the second difference. Therefore, the tests must have a <0.05 p-value. If the variables meet the two conditions, the thirty cointegration tests are carried out using the EViews software, based on Gujarati and Porter (2010). In this way, the augmented Engle-Granger test will be used to estimate the cointegrating regression —that will have the form of Equation (2)—, obtaining the residuals of the cointegrating regressions for each of the thirty tests.

$$Y_i = \beta_1 + \beta_2 X_{1i} + u_i \tag{2}$$

Where:  $Y_i$ =state production for a given month;  $\beta_1$ =intercept;  $\beta_2$ =cointegrating parameter;  $X_{1i}$ =real price of the product for a given month i;  $u_i$ =estimated residuals of the cointegrating regression; i=month within the study period.

The augmented Engle-Granger unit root tests will be applied to the residuals of the cointegrating regressions obtained with Equation (2), in order to obtain the Engle-Granger

tau statistic. The residuals are determined to be stationary or not based on that statistic, consequently establishing if the variables are cointegrated. The *p*-value of the Engle-Granger tau statistic was evaluated for that purpose. If the said statistic is lower than 0.05, the cointegrating residuals lack a unit root, are stationary, and are cointegrated in the long run; otherwise, the cointegrating residuals have a unit root, are non-stationary, and are not cointegrated.

### RESULTS AND DISCUSSION

Table 3 shows the results of the application of the DFA tests with and without trend, of order zero and one, from 1980 to 2021, to the actual price and produced quantity variables in the states that produce most corn kernel, sorghum kernel, and beans in the five regions of the Mexican agricultural sector.

Table 3 shows that, according to the results of the DFA tests of order zero and one, there is no evidence of a positive serial correlation in the variables examined, since the value of the Durbin-Whatson statistic is above the point of significance in all cases. Likewise, the third column includes the p-values of the DFA tests without trend and with a trend of order zero, for which, in most cases, the p-values are greater than 0.05, indicating that the series have a unit root and are non-stationary ( $\alpha = 5\%$ ). The only exceptions are the actual price of sorghum kernel in Campeche (without trend), the amount of corn kernel produced in the State of Mexico and the amount of beans produced in Puebla (both without and with trend), and the amount of corn kernel produced in Veracruz (with trend). In Table 3, column five, all the p-values of the DFA tests of order one are lower than 0.05, indicating that the series do not have a unit root and are stationary, with and without trend ( $\alpha = 5\%$ ); therefore, all the variables belong to integration order one. In conclusion, the variables in the tests with and without a trend meet the two conditions: to be non-stationary and to belong to integration order one (with the abovementioned exceptions). Based on this, Table 4 shows the results of the augmented Engle-Granger unit root tests, applied to the residuals of the cointegrating regressions (with and without trend), corresponding to the prices of the production of corn kernel, sorghum kernel and beans in each analyzed state (with the exception of the states that did not meet the conditions for the application of the cointegration test, which are Puebla and the State of Mexico).

Table 4 shows the *p*-values of the Engle-Granger tau statistic for the augmented Engle-Granger test applied to the residuals of the cointegrating regressions. The *p*-values are greater than 0.05 in the northwestern region, which indicates that the cointegrating residuals have a unit root and are non-stationary; therefore, the series are not cointegrated —*i.e.*, the price does not affect production. In the northeastern, central-western, and central regions, the *p*-values are lower than 0.05; consequently, the cointegrating residuals do not have a unit root and are stationary, indicating that the series are cointegrated in the long run —*i.e.*, price affects the production. In the southern-southeastern region, the *p*-values were greater than 0.05, in most of the producing states, indicating that the cointegrating residuals have unit roots and are therefore non-stationary. Consequently, the series are not cointegrated —*i.e.*, the price does not affect production. However, the corn kernel produced in the state of Veracruz with a trend is an exception regarding price: the *p*-value is lower than 0.05,

**Table 3**. Results of the DFA tests of order zero and one (with and without trend).

Región	Variable Real price and quantity produced by the State.	P-value (zero- order DFA test).	Durbin-Watson and Significance point of the Durbin-Watson statistic for an alpha of 5% and a n=42. There is positive serial correlation (zero order).	P value (first order DFA test).	Durbin-Watson and Significance point of the Durbin-Watson statistic for an alpha of 5% and a n=42. There is positive serial correlation (order one).
	Real price of corn kernel Sinaloa	0.2486	No, because 2.30>1.60	0	No, because 2.07>1.600
sst	Real price of corn kernel Sinaloa (with trend)	0.3544	No, because 2.16>1.659	0	No, because 2.06>1.721
Northwest	Real price of beans Sinaloa	0.131	No, because 2.083>1.854	0	No, because 2.28>1.659
Nor	Real price of beans Sinaloa (with trend)	0.1292	No, because 2.0298>1.854	0	No, because 2.29>1.721
	Real price of sorghum kernel Sinaloa	0.092	No, because 2.026>1.60	0	No, because 2.083>1.659
	Real price of sorghum kernel Sinaloa (with trend)	0.243	No, because 1.959>1.60	0	No, because 2.09>1.721
	Real price of corn kernel Chihuahua	0.5847	No, because 2.15>1.60	0	No, because 2.04>1.600
st	Real price of corn kernel Chihuahua (with trend)	0.6041	No, because 2.029>1.60	0.0001	No, because 2.02>1.721
Northeast	Real price of beans Zacatecas	0.4729	No, because 2.155>1.854	0	No, because 2.164>1.659
Nor	Real price of beans Zacatecas (with trend)	0.5479	No, because 2.087>1.924	0	No, because 2.171>1.721
	Real price of sorghum kernel Tamaulipas	0.1309	No, because 2.0613>1.60	0	No, because 2.71>1.659
	Real price of sorghum kernel Tamaulipas (with trend)	0.2396	No, because 2.956>1.60	0.0001	No, because 2.077>1.721
	Real price of corn kernel Jalisco	0.3618	No, because 1.914>1.854	0	No, because 2.13>1.600
est	Real price of corn kernel Jalisco (with trend)	0.6212	No, because 2.0732>1.60	0	No, because 2.15>1.659
Center-West	Real price of beans Guanajuato	0.7183	No, because 2.324>1.854	0	No, because 2.12>1.600
ente	Real price of beans Guanajuato (with trend)	0.2686	No, because 2.16>1.854	0.0003	No, because 2.06>1.924
0	Real price of sorghum kernel Guanajuato	0.1269	No, because 2.4134>1.60	0	No, because 1.916>1.600
	Real price of sorghum kernel Guanajuato (with trend)	0.2456	No, because 2.30>1.659	0	No, because 1.914>1.659
	Real price of corn kernel Edo. de México	0.9953	No, because 1.855>1.600	0	No, because 1.854>1.600
	Real price of corn kernel Edo. de México (with trend)	0.6669	No, because 1.865>1.659	0.0009	No, because 1.891>1.721
Center	Real price of beans Puebla	0.9995	No, because 1.721>1.600	0.0002	No, because 1.942>1.600
$C_{e}$	Real price of beans Puebla (with trend)	0.7208	No, because 1.739>1.659	0.0003	No, because 1.936>1.659
	Real price of sorghum kernel Morelos	0.9994	No, because 1.919>1.600	0.0008	No, because 1.845>1.659
	Real price of sorghum kernel Morelos (with trend)	0.9043	No, because 1.900>1.721	0.0001	No, because 1.822>1.721
	Real price of corn kernel Veracruz	0.9997	No, because 1.706>1.600	0.0005	No, because 1.833>1.600
east	Real price of corn kernel Veracruz (with trend)	0.8061	No, because 1.797>1.659	0.0004	No, because 1.7781>1.659
outh	Real price of beans Chipas	0.9939	No, because 1.749>1.600	0.0001	No, because 1.983>1.600
South-southeast	Real price of beans Chiapas (with trend)	0.6333	No, because 1.745>1.659	0.0003	No, because 1.943>1.659
$\mathbf{Sou}$	Real price of sorghum kernel Campeche	0.0195	No, because 2.042>1.600	0	No, because 2.063>1.600
	Real price of sorghum kernel Campeche (with trend)	0.0775	No, because 2.021>1.659	0	No, because 2.064>1.659
	Produced amount of corn kernel Sinaloa	0.6475	No, because 2.11>1.600	0	No, because 2.07>1.600
ب	Produced amount of corn kernel Sinaloa (with trend)	0.1689	No, because 1.85>1.600		No, because 2.078>1.659
Northwest	Produced amount of beans Sinaloa	0.0503	No, because 2.20>1.721	0	No, because 1.8260>1.721
Jort	Produced amount of beans Sinaloa (with trend)	0.1357	No, because 2.18>1.786	0	No, because 1.8286>1.786
4	Produced amount of sorghum kernel Sinaloa	0.258	No, because 1.946>1.786	0	No, because 2.03>1.659
	Produced amount of sorghum kernel Sinaloa (with trend)	0.0777	No, because 1.8578>1.659	0	No, because 2.04>1.721

Table 3. Continues...

	ie 3. Continues				
Región	Variable Real price and quantity produced by the State.	P-value (zero- order DFA test).	Durbin-Watson and Significance point of the Durbin-Watson statistic for an alpha of 5% and a n=42. There is positive serial correlation (zero order).	P value (first order DFA test).	Durbin-Watson and Significance point of the Durbin-Watson statistic for an alpha of 5% and a n=42. There is positive serial correlation (order one).
	Produced amount of corn kernel Chihuahua	0.274	No, because 2.081>1.786	0.0001	No, because 2.15>1.786
t.	Produced amount of corn kernel Chihuahua (with trend)	0.3667	No, because 2.002>1.854	0.001	No, because 2.16>1.854
heas	Produced amount of beans Zacatecas	0.211	No, because 1.97>1.786	0	No, because 2.1184>1.659
Northeast	Produced amount of beans Zacatecas (with trend)	0.3562	No, because 1.95>1.91	0	No, because 2.127>1.721
~	Produced amount of sorghum kernel Tamaulipas	0.2246	No, because 2.03>1.659	0	No, because 2.16>1.600
	Produced amount of sorghum kernel Tamaulipas (with trend)	0.5232	No, because 2.014>1.721	0	No, because 2.175>1.659
	Produced amount of corn kernel Jalisco	0.4072	No, because 1.822>1.721	0	No, because 1.8581>1.659
٠	Produced amount of corn kernel Jalisco (with trend)	0.6454	No, because 2.0802>1.91	0	No, because 1.8698>1.721
West	Produced amount of beans Guanajuato	0.2331	No, because 2.060>1.91	0	No, because 2.07>1.600
Center-West	Produced amount of beans Guanajuato (with trend)	0.1395	No, because 2.060>1.91	0	No, because 2.074>1.659
Çe	Produced amount of sorghum kernel Guanajuato	0.2608	No, because 2.0843>1.786	0	No, because 1.90>1.600
	Produced amount of sorghum kernel Guanajuato (with trend)	0.2246	No, because 2.027>1.91	0	No, because 1.9010>1.659
	Produced amount of corn kernel Edo. de México	0.0005	No, because 1.917>1.600	0	No, because 2.076>1.600
	Produced amount of corn kernel Edo. de México (with trend)	0.0035	No, because 1.918>1.659	0	No, because 1.821>1.659
Center	Produced amount of beans Puebla	0.0027	No, because 2.080>1.600	0	No, because 2.027>1.786
Ce	Produced amount of beans Puebla (with trend)	0.0005	No, because 2.051>1.659	0.0003	No, because 2.026>1.854
	Produced amount of sorghum kernel Morelos	0.3651	No, because 2.148>1.659	0	No, because 1.960>1.659
	Produced amount of sorghum kernel Morelos (with trend)	0	No, because 1.9606>1.659	0	No, because 1.992>1.721
	Produced amount of corn kernel Veracruz	0.2378	No, because 2.336>1.600	0	No, because 2.059>1.600
ast	Produced amount of corn kernel Veracruz (with trend)	0.0187	No, because 1.959>1.659	0.0001	No, because 2.048>1.786
South-southeast	Produced amount of beans Chipas	0.257	No, because 2.114>1.600	0	No, because 2.030>1.600
h-soı	Produced amount of beans Chiapas (with trend)	0.5721	No, because 2.036>1.659	0	No, because 2.045>1.659
outl	Produced amount of sorghum kernel Campeche	0.9018	No, because 2.238>1.600	0	No, because 2.071>1.600
	Produced amount of sorghum kernel Campeche (with trend)	0.5437	No, because 2.153>1.659	0	No, because 1.925>1.721

Source: Table developed by the authors.

**Table 4**. Augmented Engle-Granger unit roots applied to the residuals of the cointegrating regressions (with and without trend).

Region	Cointegration test	P-values of the Engle-Granger tau statistic	They are cointegrated
Northwest	Price of corn kernel with corn kernel production in the state of Sinaloa without trend.	0.1945	No.
	Corn kernel price with corn kernel production in the state of Sinaloa with trend.	0.1296	No.
	Bean price with bean production in the state of Sinaloa without trend.	0.371	No.
	Bean price with bean production in the state of Sinaloa with trend.	0.6082	No.
Nor	Sorghum kernel price with sorghum kernel production in the state of Sinaloa without trend.	0.0545	No.
	Sorghum kernel price with sorghum kernel production in the state of Sinaloa with trend.	0.1726	No.
	Price of corn kernel with corn kernel production in the state of Chihuahua without trend.	0.0056	Yeah.
	Corn kernel price with corn kernel production in the state of Chihuahua with trend.	0.0195	Yeah.
east	Bean price with bean production in the state of Zacatecas without trend.	0.001	Yeah.
Northeast	Bean price with bean production in the state of Zacatecas with trend.	0.0003	Yeah.
Ž	Sorghum kernel price with sorghum kernel production in the state of Tamaulipas without trend.	0.0009	Yeah.
	Sorghum kernel price with sorghum kernel production in the state of Tamaulipas with trend.	0.0042	Yeah.
	Price of corn kernel with corn kernel production in the state of Jalisco without trend.	0.0368	Yeah.
	Corn kernel price with corn kernel production in the state of Jalisco with trend.	0.0149	Yeah.
'est	Bean price with bean production in the state of Guanajuato without trend.	0	Yeah.
er-W	Bean price with bean production in the state of Guanajuato with trend.	0.0001	Yeah.
Center-West	Sorghum kernel price with sorghum kernel production in the state of Guanajuato without trend.	0.0258	Yeah.
	Sorghum kernel price with sorghum kernel production in the state of Guanajuato with trend.	0.049	Yeah.
	Price of corn kernel with corn kernel production in the Edo. De México without trend.	0.0024	Yeah.
	Corn kernel price with corn kernel production in the Edo. De México with trend.	0.0101	Yeah.
•	Bean price with bean production in the state of Puebla without trend.	0.0012	Yeah.
Center	Bean price with bean production in the state of Puebla with trend.	0.0007	Yeah.
ŭ	Sorghum kernel price with sorghum kernel production in the state of Morelos without trend.	0.0339	Yeah.
	Sorghum kernel price with sorghum kernel production in the state of Morelos with trend.	0.0023	Yeah.
	Price of corn kernel with corn kernel production in the state of Veracruz without trend.	0.1028	No.
#	Corn kernel price with corn kernel production in the state of Veracruz with trend.	0.0262	Yeah.
heas	Bean price with bean production in the state of Chiapas without trend.	0.5034	No.
sout	Bean price with bean production in the state of Chiapas with trend.	0.2553	No.
South-southeast	Sorghum kernel price with sorghum kernel production in the state of Campeche without trend.	0.9706	No.
- 4	Sorghum kernel price with sorghum kernel production in the state of Campeche with trend.	0.5611	No.

Source: Table developed by the authors using EViews.

which indicates that the cointegrated residuals do not have a unit root and are stationary; consequently, the series are cointegrated in the long run—*i.e.*, price affects the production.

### CONCLUSIONS

The cointegration tests indicate that, in the northwestern region, the price is not cointegrated with the production of the main producing states of corn kernel, sorghum kernel, and beans -i.e., the price does not affect the production of these states. In the northeastern, central-western, and central regions, the price is cointegrated with the production of the main producing states of corn kernel, sorghum kernel, and bean —i.e., the price affects the production of the states. In most of the cases of the southernsoutheastern region, the price is not cointegrated with the quantities produced in any of the main producing states of corn kernel, sorghum kernel, and beans —i.e., the price does not affect production in these states. The only exception is the price of corn kernel produced in the state of Veracruz with a trend, where the price is cointegrated with the quantities produced -i.e., the price affects production. This indicates that price influences the production of the agricultural sector, but its influence is not the same, neither in the five regions of Mexico, nor in the main producing states of corn kernel, sorghum kernel, and beans. The production of some states and regions is influenced by other elements beside prices, although this relationship does take place in other states and regions. This phenomenon can be attributed to the conditions of each state and region of the Mexican agricultural sector.

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## Plant growth and early *in vitro* floral differentiation of vanilla (*Vanilla planifolia* Jacks. ex Andrews)

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### ABSTRACT

**Objective**: To induce *in vitro* flowering of vanilla (*Vanilla planifolia*) with different plant growth regulators (PGRs) using the double-layer technique.

**Design/methodology/approach**: A layer of semi-solid Knudson C (KC) medium, added with 40 g L<sup>-1</sup> sucrose, 15% coconut water (CW; v/v) and 7 g L<sup>-1</sup> agar, was placed in 100 mL flasks. A liquid layer of the same composition without agar was placed on top. It was supplemented with different doses (mg L<sup>-1</sup>) of PGRs: 6-benzyladenine (BA) (7), thidiazuron (TDZ) (6), paclobutrazol (PBZ) (0.5) and gibberellic acid (GA<sub>3</sub>) (2). Plus two controls, G1: no PGRs + no CW; G2: no PGRs + CW. Vanilla shoots of 2 cm in length and with at least one axillary shoot were placed. They were incubated at a temperature of  $26\pm2$  °C day and 18 °C darkness, with light intensity of  $55\,\mu$ mol m<sup>-2</sup> s<sup>-1</sup> during 13 weeks. The number of shoots, leaves and roots was evaluated, as well as the length of shoots and fresh weight. Floral differentiation was evaluated at the tenth week by conventional microtechnique.

**Results**: The number shoots and leaves and shoot length were significantly higher in C2. The number of roots increased with PBZ 0.5 mg  $L^{-1}$ . C1 and C2 promoted higher fresh weight. Floral differentiation was observed with GA<sub>3</sub> 2 and PBZ 0.5 mg  $L^{-1}$  treatments.

**Limitations on study/implications**: Further evaluation of other PGR doses and environmental conditions is required to achieve full floral differentiation of vanilla.

**Findings/conclusions**: CW increased vegetative growth. GA<sub>3</sub> and PBZ showed early floral differentiation in *Vanilla planifolia*, which is the first report of this phenomenon for the species.

**Keywords**: Coconut water, orchid flowering, *in vitro* double-layer culture medium, floral meristems, plant growth regulators (PGRs).

### Citation: Ríos-Barreto, Y., Arellano-Ostoa, G., Fernández-Pavía, Y. L., García-Villanueva, E., & Tejeda-Sartorius, O. (2023). Plant growth and early in vitro floral differentiation of vanilla (Vanilla planifolia Jacks. ex Andrews). Agro Productividad. https://

**Academic Editors**: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

doi.org/10.32854/agrop.v16i1.2439

Received: November 29, 2022. Accepted: January 14, 2023. Published on-line: February 22, 2023.

*Agro Productividad, 16*(1). January. 2023. pp: 127-135.

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### INTRODUCTION

Vanilla planifolia is an orchid species of economic importance due to the aromatic extract that is obtained from its fruits, vanillin (Soto Arenas, 2006; Xochipa-Morante et al., 2016; Bautista-Aguilar et al., 2021). Vanilla plantations in Mexico begin to produce at three years after their establishment and they have an average life of 5 years; therefore, in



many cases, there are harvests only for two years (Kelso-Bucio et al., 2012, Garza-Morales, et al., 2021). This long period of plant development and the short productive cycle of the crop, in addition to its susceptibility to diseases (Lee-Espinoza et al., 2008; Bautista-Aguilar et al., 2021), climate change, and lack of technology transference (Vázquez-Trujillo, 2020; Garza-Morales et al., 2021), have caused the reduction of the cultivated surface of vanilla in Mexico. V. planifolia has been widely studied for its in vitro propagation and conservation, and until today there are several protocols about it (Gonzáles-Luna, 2003; Lee-Espinoza et al., 2008; Bello-Bello et al., 2015; Ramírez-Mosqueda and Iglesias-Andreu, 2015; Halim et al., 2017). Although there are many protocols for plant growth of this species, no studies of *in vitro* flowering have been reported. Among all the biological phenomena, flowering is without a doubt one of the most fascinating and difficult to understand, as in the Orchidaceae family, which is why the induction of *in vitro* flowering is a valuable tool to understand it more deeply in physiological, genetic and molecular terms (Teixeira et al., 2014). Flowering is influenced by environmental and endogenous factors that are integrated in perfect correspondence to trigger flowering at the adequate time (Amasino and Michaels, 2010; Tuan-Ha, 2014). In vitro floral induction has been reported in orchids and other ornamental species, through the use of plant growth regulators (PGRs). Among the most widely used, there are cytokinins such as BA in Cymbidium niveo-marginatum Mak (Kostenyuk et al., 1999), in Dendrobium Chao Praya Smile (Hee et al., 2007), and in D. huoshanense (Lee and Chen, 2014). Likewise, some compounds with similar action to cytokinins, such as TDZ, have shown effects that induce flowering in orchids, as in D. Second Love (de Melo Ferreira et al., 2006), and D. wangliangii (Lawrie et al., 2021). In addition, PBZ has also been evaluated in the genus Dendrobium (Te-Chato et al., 2009; Zhao et al., 2013), as well as GA<sub>3</sub> in combination with BA in *Phalaenopsis amabilis* (Semiarti et al., 2015). In addition, the use of GA<sub>3</sub> has been reported to induce in vitro flowering in other species such as *Phlox paniculata* L (Anuar et al., 2017). The induction of in vitro flowering of vanilla would open a new field for a better understanding of flowering in the species; for example, for genetic improvement referring to the quality of the aromatic compounds of the pods. Because of the aforementioned, the objective of this study was to induce in vitro flowering of Vanilla planifolia using different doses of plant growth regulators (PGRs) in a double-layer culture medium.

### MATERIALS AND METHODS

### Establishment of induction to flowering in a double-layer culture medium

To establish the experiment in double-layer mediums for flowering induction, the methodology proposed by Sim *et al.* (2007) was adapted.

### Plant material

Shoots with length of approximately 2 cm were used, with at least one axillar shoot of *Vanilla planifolia*, from material from Papantla, Veracruz, Mexico, and they were multiplied through several *in vitro* sub-cultures, in a RITA<sup>®</sup> type temporary immersion system (TIS), supplemented with 2 mg  $L^{-1}$  of BA, plus 0.5 mg  $L^{-1}$  of naphthaleneacetic acid (NAA), plus 30 g  $L^{-1}$  of sucrose.

### Culture medium

The culture medium that induced flowering consisted of a double-layer medium, which included: Knudson's semi-solid medium (C KC; Phytotechlab<sup>®</sup>), supplemented with 40 g  $\rm L^{-1}$  of sucrose (Phytotechlab<sup>®</sup>), 150 ml  $\rm L^{-1}$  of sterilized coconut water (v/v), from fresh immature coconuts from the local market, and 7 g of agar (Sigma<sup>®</sup>). The liquid phase consisted of the same composition as the semi-solid phase, except for the agar. The semi-solid phase was placed in containers in the amount of 20 mL, and once gellified, 10 mL of liquid medium were added.

### **Treatments**

The treatments consisted in doses of PGRs: BA, TDZ, PBZ and GA<sub>3</sub> (Sigma<sup>®</sup>), with two controls, C1: without PGRs and without coconut water; C2 without PGRs supplemented with coconut water (Table 1). Both phases of the medium were supplemented with those doses. Glass containers of 240 ml capacity were used, where four explants were placed per container, considered as the experimental unit with four repetitions per treatment.

The pH was adjusted to 5.7 before sterilization at 121 °C for 20 minutes in an autoclave (STIK<sup>®</sup> model Mj-504-A) at 1.5 atmospheres of pressure. They were incubated at a temperature of  $26\pm2$  °C daylight and 18 °C darkness, with a luminous intensity of 55  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, during 13 weeks.

### Morphological analysis

Ten weeks after culture in a double-layer medium for flowering induction, part of the growing tissue was extracted from each explant by treatment, which contained mainly structures different from the commonly observed growth were observed *in vitro*, with the aim of performing anatomical cuts and observing a possible morphological change with floral structures.

The samples were placed in a fixing solution for herbaceous plant tissues, because they are very soft tissues (FAA) at 50%. Then, the samples were dehydrated (Sandoval, 2005) and processed through the conventional technique of inclusion in paraffin, dyed with safranine 0 at 0.1%, complemented with fast green at 0.1%, mounted with synthetic resin and observed with the photonic microscope (Johansen, 1940).

**Table 1.** Flowering inductive treatments in double-layer medium supplemented with different doses of plant growth regulators (mg  $L^{-1}$ ).

	1 0	0	( )		
Treatments	CW	BA	TDZ	PBZ	$GA_3$
C1	0%	0	0	0	0
C2	15%	0	0	0	0
BA	15%	7	0	0	0
TDZ	15%	0	6	0	0
PBZ	15%	0	0	0.5	0
GA <sub>3</sub>	15%	0	0	0	2

CW: coconut water; BA: 6-benzyladenine, TDZ: thidiazuron, PBZ: paclobutrazol,  $GA_3$ : gibberellic acid.

### Statistical analysis

The treatments were distributed in a completely random design. The normality of the data was determined through the Shapiro-Wilk test, and the homoscedasticity test through the Levene test. The range of a transformation was carried out, and the data were analyzed through ANOVA and means comparison with Tukey's test (0.05%). The data were analyzed through R software, version 4.1.0 (R Core Team, 2021).

### RESULTS AND DISCUSSION

The treatments affected the plant growth of *V. planifolia*. It was observed that the number of shoots presented significant differences between treatments (Figure 1). The S/PGRs+CW15% treatment was statistically higher since it presented the highest number of shoots (6.5) (Figure 1A) and the highest number of leaves (17.2) (Figure 1B). Likewise, it was statistically higher in the length of the shoots, with them being 35% higher than the shoots in BA, TDZ and GA<sub>3</sub> (Figure 1D).

The results indicate that coconut water can have a positive effect on plant growth. According to some authors this effect can be due to the cytokinins present in this compound (Gupta, 2016). Coconut water has been used for the germination and the proliferation of different orchid species. In *Dendrobium* 'Gradita 31', adding coconut water at 15% to the culture medium increased both the number of protocorms generated (37%) and the fresh weight of the protocorms from 0.28g to 0.46 g (Winarto and Teixeira, 2015).

In this experiment, the CW showed a higher number of vanilla shoots, with an average of 6.5 shoots per explant, compared to BA, which yielded 4 shoots per explant, even when BA is one of the cytokinins most frequently used in *in vitro* culture for the proliferation

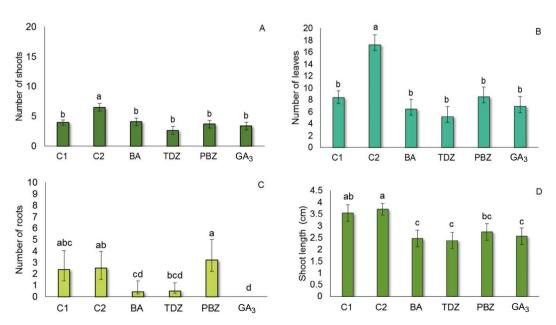


Figure 1. Number of shoots (A), leaves (B), roots (C), length of shoots (D) derived from nodal segments of *Vanilla planifolia*, in flowering inductive treatments based on different doses of plant growth regulators (mg  $L^{-1}$ ): BA, 7; TDZ, 6; PBZ, 0.5; GA<sub>3</sub>, 2. All the doses supplemented with coconut water. Bars with different letters + SD indicate significant differences (Tukey,  $p \le 0.05$ ).

(Halim et al., 2017; Inderiati et al., 2019). Better results were obtained in this study by adding coconut water, compared to other studies. Carranza-Álvarez et al. (2021) indicate that it generated 4.6 shoots per explant with the addition of 3% (v/v) of coconut water, although they do not report significant differences in comparison to their control. It is important to consider that in this study the BA was used in higher doses to attempt to induce flowering in vanilla, compared to the doses normally reported for vegetative growth, which could decrease the number of shoots. Some authors report thresholds of BA doses for plant growth. Lee-Espinosa et al. (2008) indicate that dose of 2.1 mg L<sup>-1</sup> generated an average of 18 shoots per vanilla explant, although the use of the dose under or over this dose generated a lower number of shoots per explant. Similarly to the data in this study, Abebe et al. (2009) reported that the dose of 3 mg  $L^{-1}$  of BA generated up to 4.2 shoots per explant in vanilla, after 45 days of growth. Meanwhile, Inderiati et al. (2019) showed that dose of 1.5 mg L<sup>-1</sup> of BA in vanilla promoted the highest number of shoots per explant (3.27). Thus, the doses most frequently reported for vanilla proliferation are from 1 to 3 mg L<sup>-1</sup>. In previous studies (data not shown), different doses of BA were tested to try to understand its potential effect on the induction of flowering in vanilla, and in addition to the reports for other orchid species, a high dose was used in this study, although a decrease was found in the plant growth (Figure 1A, B and C).

The treatment with 0.5 mgL<sup>-1</sup> of PBZ significantly increased the average number of roots per explant (3.2) (Figure 1C), compared to the doses of BA (0.43), TDZ (0.5), and GA<sub>3</sub> which inhibited it completely. These results are similar to what was reported by Bello-Bello et al. (2015) who indicate that the dose of 1 mg  $L^{-1}$  generated on average 3.8 roots per explant. And this result is similar to those reported in other species. In *Dendrobium* Friederick's, the dose of 0.5 mg L<sup>-1</sup> of PBZ promoted the highest average number of roots per explant (6; Te-Chato et al., 2009). Although PBZ increased the number of roots in vanilla, these roots were, in addition, quite thickened, inhibiting the aerial growth, which is why exploring it for rooting during its phase of ex vitro acclimation is proposed. These results are similar to what Gimenes et al. (2018) report, who mentioned that the PBZ in Zygopetalum crinitum (Orchidaceae) seedlings decreased the length of the aerial part and roots, and in addition, promoted their thickening, although the authors mention that in the acclimation phase, the application of PBZ did not imply a higher survival of the orchid Z. crinitum. Meanwhile, Wen et al. (2013) report that in Dendrobium nobile, the application of 0.8 mg L<sup>-1</sup> of PBZ increased by 41.6% the survival rate in the acclimation of this species, in comparison to a control. Therefore, it would be pertinent to explore this effect in vanilla. In addition to this, the treatment with  $GA_3$  inhibited root emission, which contrasts with what was reported by Coello *et al.* (2010), who indicate that  $GA_3$  is an important factor for the promotion of roots in Guarianthe skinneri. Other authors report similar results from those in this study, such as Rodrigues et al. (2015), who indicate that the dose of 5 mg  $L^{-1}$ GA<sub>3</sub> decreased to 1.87 the number of roots in Cyrtopodium saintlegerianum, compared to the control without GA<sub>3</sub> (4.53 roots per explant). This shows that each species can have differential responses in the presence of the same growth regulator.

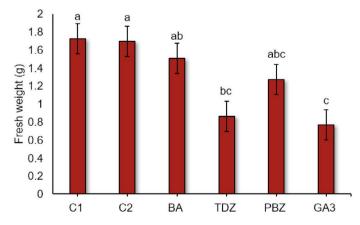
The length of shoots was statistically higher in C2 (Figure 1D), compared to GA<sub>3</sub>, BA and TDZ. Similar results were reported by Carranza-Álvarez *et al.* (2021), since the

addition of coconut water at 3% (v/v) increased the lengths of vanilla shoots, from 2.0 cm to 3.5 cm, compared to the control. On the other hand, Roy *et al.* (2012) mention that higher doses of TDZ in *Cymbidium giganteum* decrease the length of explants. For example, 0.2 mg  $L^{-1}$  of TDZ generated shoots of 1.85 cm, while the dose of 2.0 mg  $L^{-1}$  reduced their length to 0.67 cm. In contrast to our results, it has been reported that GA<sub>3</sub> increases the elongation of shoots in other species, for example, doses of 5 and 10 mg  $L^{-1}$  promoted the highest average length, from 7.74 and 8.06 cm, respectively, in comparison to the dose without GA<sub>3</sub> (4.53 cm), in the *in vitro* culture of *Cyrtopodium saintlegerianum* (Rodrigues *et al.*, 2015). However, these reports differ from our results, since this study found that dose of 2 mg  $L^{-1}$  of GA<sub>3</sub> generated shoots of 2.5 cm, while the control promoted shoots of 3.5 cm of length.

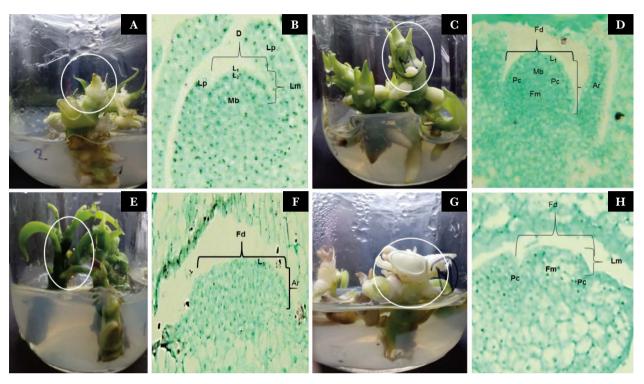
The treatment with BA and TDZ promoted oxidation of the explants 6.25 and 25%, respectively (data not shown). This is similar to other growth variables (number of leaves, length of explant), the treatments with PGRs also affected negatively the fresh weight of the shoots generated in *V. planifolia* (Figure 2) since the control treatments were statistically higher.

### Floral differentiation of Vanilla planifolia

The treatments with GA<sub>3</sub> and PBZ (Figure 3) showed formation of tissues different from the vegetative ones reported in vanilla. In the cuts carried out, it was observed that treatments of GA<sub>3</sub> and PBZ presented flattening of the meristem dome (Figure 3 B-D), sign of the floral initiation. Meanwhile, the rest of the treatments presented a convex meristem dome, characteristic of vegetative growth (Figure 3A). The use of various PGRs has been reported to attain *in vitro* flowering. Te-chato *et al.* (2009) achieved 29% of *in vitro* floral induction in *Dendrobium* Friederick's, with the addition of 0.05 mg L<sup>-1</sup> of PBZ. The addition of PBZ and TDZ, in concentrations of 1 mg L<sup>-1</sup> in each PGR have promoted up to 62.2% of *in vitro* flowering in *Dendrobium nobile* (Wang *et al.*, 2009). Likewise, GA<sub>3</sub> has been used



**Figure 2**. Fresh weight (g), of shoots derived from nodal segments of *Vanilla planifolia*, in flowering inductive treatments based on different doses of plant growth regulators (mg  $L^{-1}$ ): BA, 7; TDZ, 6; PBZ, 0.5; GA<sub>3</sub>, 2. All the doses were supplemented with coconut water. Bars with different letters + SD indicate significant differences (Tukey,  $p \le 0.05$ ).



**Figure 3**. *In vitro* growth of shoots (A, C, E, G) with fragments for anatomical cuts (white circles) and longitudinal sections of apical shoots (B, D, F, H) of *Vanilla planifolia* subjected to treatments that induce flowering with PGRs (mg L<sup>-1</sup>): A-B: BA, 7; C-D: PBZ, 0.5; E, F, G, H: AG<sub>3</sub>, 2. B) Vegetative apical meristem. D and F) Apical meristem at the beginning of floral differentiation, which consists in the lengthening of the meristem body (Ra) and beginning of dome flattening (Da). H) Apical meristem at the beginning of floral differentiation where dome flattening is seen. Cm: meristem body; D: curve dome; Da: flat dome; L: meristem length; Mf: fundamental meristem; Pc: procambium Pf: leaf primordium; Ra: region of apical meristem lengthening; T1: tunic 1 or protodermis; T2: tunic 2.

to attain flowering induction. Semiarti *et al.* (2013) indicate that the dose of 15 mg L<sup>-1</sup> of GA<sub>3</sub> plus 9 mg L<sup>-1</sup> of BA can induce flowering initiation in *Phalaenopsis amabilis* (L.) Blume. Britto *et al.* (2003) indicate that *in vitro* flowering was observed in *Ceropegia bulbosa* Roxb. var. *Bulbosa* cultivated in Gamborg B5 medium with addition of 1 mg L<sup>-1</sup> GA<sub>3</sub> + 0.5 mg L<sup>-1</sup> BA. Chaari-Rkhis *et al.* (2006) indicate that they obtained floral induction in some olive cultivars such as "Marsaline" and "Picholine" with dose of 10 mg L<sup>-1</sup> of GA<sub>3</sub>. One of the most frequently reported PGRs for *in vitro* floral induction in orchids, mainly *Dendrobium*, is BA (Kostenyuk *et al.*, 1999; Hee *et al.*, 2007; Sim *et al.*, 2007, Zhao *et al.*, 2013), although in this study the treatment with BA did not show characteristics of floral initiation in vanilla (Figure 3A).

### **CONCLUSIONS**

A high dose of BA affects negatively the vegetative growth of vanilla. The use of PBZ increases the number and thickening of roots. Coconut water in the culture medium promotes a greater growth of vanilla. The GA<sub>3</sub> and PBZ generate changes in the meristems, which indicate early floral differentiation. This study is a first report that shows the inductive potential of *in vitro* flowering of *Vanilla planifolia*, although it is necessary to

conduct more research to attain complete meristem differentiation and floral development of the species.

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# Effect of different land uses on soil quality indicators in Lixisols from La Sabana, Huimanguillo, Tabasco, Mexico

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### ABSTRACT

**Objective**: To evaluate the physical indicators of the quality of soils subjected to four different land uses in Lixisols from La Sabana in Huimanguillo, Tabasco, Mexico.

**Design/methodology/approach**: Nine variables were evaluated in soil samples from Lixisols located in La Sabana, Huimanguillo: infiltration rate, resistance to penetration, bulk density, total porosity, aggregate stability, thinning, depth of the horizon, volume and weight of soil loss. We used a completely randomized sample design, with a factor with four levels (each land use: pasture, rubber tree, rubber-cacao and rubber-mahogany), and five treatment repetitions; each sampling point of the plot with the five-of-golds method, with the exception of the use with pasture, which was linear.

**Results**: The reference soil group (RSG) corresponds to a Ferric Lixisol (Cutanic, Endoloamic, Epiarenic, Humic, Profondic), whose RSG has not been reported for the study area. The quality indicators are within acceptable limits. In soil loss, the pasture has conserved more over time, storing greater volume and weight of soil per hectare; the use with rubber (monoculture) has lost a greater amount of soil from the A horizon, evidenced by the decrease in its depth, volume and weight per hectare.

**Limitations on study/implications**: Until a few years ago, in the study area within La Sabana in Huimanguillo, Acrisol had been described as the dominant RSG.

**Findings/conclusions**: The presence of Acrisol in the study area is ruled out. Soil quality for all uses is acceptable.

**Keywords**: soil quality, erosion, land-use change, bulk density, tropical agriculture.

Palma-López, D. J., Zavala-Cruz, J., Sánchez-Hernández, R., & Palma-Cancino, D. J. (2023). Effects of different land uses on soil quality indicators in Lixisols from La Sabana, Huimanguillo, Tabasco, Mexico. *Agro Productividad*. https://doi.org/10.32854/ agrop.y16i1.2285

Citation: Domínguez-Angulo, S.,

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: June 01, 2022.

Accepted: December 13, 2022.

Published on-line: February 22, 2023.

*Agro Productividad*, *16*(1). January. 2023. pp: 137-145.

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### INTRODUCTION

Soils are an essential resource for the conservation of ecosystems and the welfare of society, since they are the main productive base of economic activities in the primary sector (Palma-López *et al.*, 2007; 2017). The lack of a correct management in their use, primarily due to anthropic causes, for example an intensive use that is not in accordance to their capacity, deforestation and the expansion of the agricultural frontier, change in land use, among others, negatively affects the physical, chemical and biological properties

of the soils, inducing them to erosion and degradation (Drobnik *et al.*, 2018). This is why evaluating the soil quality, understood based on Karlen *et al.* (1997), as the capacity of a soil type in particular to function within the limits of a natural or managed ecosystem through indicators, physical, chemical and biological, is at the same time a sustainability measure of the soil use and management practices. The objective of this study was to evaluate physical indicators of the soil quality in Lixisols with different land uses in La Sabana, Huimanguillo, Tabasco, Mexico.

### MATERIALS AND METHODS

### Study area and description

The study was conducted from 2020 to 2021 in the *Ejido* Tecominoacán, in Huimanguillo, Tabasco, inside the area of La Sabana, between coordinates 17° 55' 20.43" N and 93° 36' 18.787" W. The climate is warm humid with rains during the entire year Af(m) and temperatures between 26 and 28 °C annual mean, with records of total annual precipitation from 2000 to 3000 mm (Salgado-García *et al.*, 2010; Tinal-Ortiz *et al.*, 2020). Until a few years ago, the dominant soils in the region have been Acrisols (Palma-López *et al.*, 2017).

### Establishment of study plots

Four plots of 20×20 were established, each with a particular use and management of the soil: pasture (*Bracharia humidícola*) with 36 years of semi-stabling and alternate rotational grazing and 3 heads of livestock per hectare; rubber tree (*Hevea brasiliensis*) as

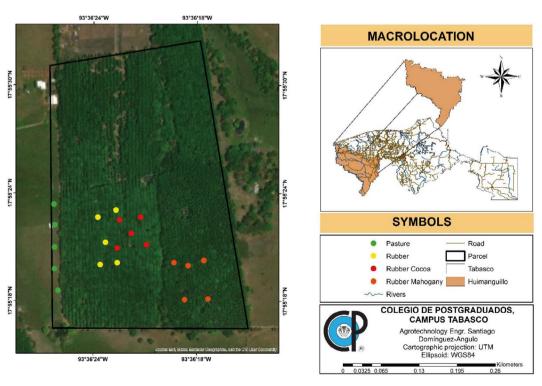


Figure 1. Localization of the study area.

monocrop with 6 years of conventional management with application of the following agrichemicals: Glyphosate, Paraquat, Diuron, Cypermethrin, Yaramila, Urea, DAP, KCl, Maxigrow and Bayfolan; the use with rubber-cacao (*Theobroma cacao*) with 6 and 3 years; and rubber-mahogany (*Swietenia macrophylla*) with 6 and 12 years of establishment, respectively. The soil preparation prior to the establishment of the plantations was executed with primary and secondary mechanized farming (four rake passes). The land uses were identified through field visits and semi-structured interviews with the cooperating producers. The plots with rubber-cacao and rubber-mahogany present the same management as the ones with rubber tree (monocrop), showing similar soil conditions to those of the monocrop.

### Extraction of the samples and characterization of the reference soil group

Five simple samples were extracted of the A horizon for each land use, through the five-of-golds method and linear in the use with pasture. From the profile, four simple samples were taken by drilling, one for each horizon in different depths: 0-43; 43-84; 84-101 and 101-150 cm. Characterization in the field of a soil profile was made using the description manual by Cuanalo, 1990. The samples from the profile horizons were moved to the laboratory, previously identified and dried in the shade for their later analysis with the methods established in the NOM-021-RECNAT-2000 on the occasion of the RSG classification, according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2015). Likewise, the samples from each land use were taken to the file identified to evaluate the physical indicators of the A horizon soil quality.

### Variables evaluated and methods used

The methodologies from the "Guidelines for soil quality assessment in conservation planning" from the Soil Quality Institute (USDA, 2001) were used, to evaluate the following variables: bulk density (known cylinder method), total porosity (%), through the formula  $\varphi = 1 - \frac{\rho_b}{\rho_b}$  (where:  $\varphi$ =total porosity,  $\rho_b$ =bulk density, and  $\rho_p$ =real density 2.65 t m<sup>-3</sup>), infiltration speed, average evaluation of thinning, and stability of aggregates. The resistance to penetration was measured in the field with an AMS pocket piston penetrometer, brand Addag, model 77114, carried out on the soil surface at 30 cm from the excavation done to measure the depth of the A horizon, and the area selected was cleared of plant residues, roots, or other solid materials that could alter the reading of resistance to penetration. For each plot, the sheets of soil loss (reduction in depth), the volume (m<sup>3</sup> ha<sup>-1</sup>) and the weight (t ha<sup>-1</sup>) were estimated with the figure of the soil's bulk density and the depth of the horizon through the following formulas: Vs = (ph)(s), where Vs = volume of soil in  $m^3$  ha<sup>-1</sup>, ph=horizon depth in meters, and s=surface (generally considered to be 10,000 m<sup>2</sup> or 1 hectare); and  $Ps = (Vs)(\rho_h)$ , where Ps = soil weight in t ha<sup>-1</sup>, Vs = soilvolume, and  $\rho_h$ =bulk density. To estimate the reduction in depth, the following was used: pph = ushmsp - ushmp, pph = depth lost from the horizon, ushmsp = soil use with the deepest horizon, *ushmp*=soil use with the least deep horizon.

### Experimental design and statistical analyses

The experimental design was completely random, where each land use was a factor with four levels and five repetitions for each treatment, each sampling point within the plot was a repetition. The data were subjected to a one-way analysis of variance (ANOVA) and Tukey's multiple means comparison test was applied with significance of p<0.05 when differences were found between the treatments, through the free software Past 4.09 version 2022 and the Real Statistics complement for Excel 2013.

### RESULTS AND DISCUSSION

The RSG of the study area resulted in a Ferric Lixisol (Cutanic, Endoloamic, Epiarenic, Humic, Profondic), differentiating from an Acrisol where the Lixisols have clay of low activity in the argic horizon and a high saturation of bases between 50-100 cm of depth, while the Acrisols, even though they also have clays of low activity in the argic horizon the base saturation is low in the depth of 50-100 cm. In this regard, no information was found reporting the presence of Luxisols in the hillside zone of La Sanana in Huimanguillo (La Chontalpa), although Zavala-Cruz et al. (2016) refers to their presence in Balancán and Tenosique in the river meadow zone (Región de los Ríos), and on the other hand, Palma-López et al. (2017) mention that until that year this type of soil had not been identified in Tabasco, although it was found since 2016 in regions between the municipalities of Tenosique and Balancán. The soil profile description in ejido Tecomatán, Huimanguillo, Tabasco, is shown in Table 1.

### Depth of the A horizons

There are no significant differences (p>0.05) between the means of the depths of the A horizons of each land use (Table 2). The use with pasture has conserved more soil through time, since it obtained the greatest depth (30.4), while the rubber-cacao use has the second place, since it reduced 1.20 cm null mpas, followed by rubber-mahogany (1.80 cm), and the soil with rubber tree is the one that has been reduced most, 2.80 cm. Similarly, Alejandro-Martínez *et al.* (2019) did not observe significant differences between the depths of the A horizons of the land uses with: second growth forest, pineapple, yucca, sugarcane and pasture, in another *ejido* in La Sabana, Huimanguillo, Tabasco, where the A horizon of the use with second growth forest the deepest (42.50 cm) thanks to the plant cover of the soil that minimizes erosion; on the other hand, the pasture use was the lowest (17.50 cm), differing from this study where it was the most conserved.

### Volume and weight of the A horizon

There are no significant differences between the volume of the A horizons from the different land uses (Table 2). On the contrary, Alejandro-Martínez *et al.* (2019) found highly significant differences in different land uses where the second growth forest (reference use) presents the highest volume (4250 m<sup>3</sup> ha<sup>-1</sup>), probably due to a greater depth.

There are no significant differences between the weight of the A horizon and the land uses. In this regard, Alejandro-Martínez *et al.* (2019) indicate that they did not find significant differences when evaluating the weight of the A horizon of the soil with second

Table 1. Description of the profile in Ejido Tecominoacán, Huimanguillo, Tabasco.

Profile	Horizon (cm)	Soil description
	A1 (0-43)	Thin and horizontal transition; wet; black color (10 YR 2/2), with presence of roots; sandy loam texture; no stoniness; strongly developed structure, with subangular blocks and crumbs, thin and very thin in size; very friable wet consistency; sticky and slightly plastic very wet consistency; numerous pores, with small and very small diameter, continuous, chaotic orientation, inside and outside of aggregates, with tubular morphology; very fast permeability; extremely abundant roots, thin, small and medium size; with the presence of earthworm droppings, ants and termites; with a field pH of 5.5
	E (43-84)	Thin and horizontal transition; wet; black color (10 YR 2/2), with dark brown mottled (10 YR 3/3), marked contrast, common abundance, thin, medium and large size; sandy loam texture; no rockiness; moderately developed structure, subangular blocks, thin and medium size; friable wet consistency; slightly sticky and slightly plastic very wet consistency; cutans by eluviation, on faces of aggregates and pores, with clay minerals and iron oxides; numerous pores, with very small diameter, continuous, chaotic orientation, inside and outside of aggregates, with tubular morphology; very fast permeability; common roots, thin and very small in size; with the presence of earthworm droppings, ants and termites; with a field pH of 5
	Bt1 (84-101)	Medium and irregular transition; saturated moisture; brownish yellow color (10 YR 6/1), with red mottled (2.5 YR 6/8), thin, few specks, thin and very thin in size; sandy loam texture; with very few rockiness, grit, rounded quartz, heavily weathered; strongly developed structure, with subangular blocks, very thin, thin and medium in size; friable wet consistency; sticky and slightly plastic very wet consistency; cutans by eluviation, continuous, with clay minerals and iron oxides; frequent small and medium sized nodules, dark reddish brown color, with subangular shape, soft hardness, composition of iron oxides; frequent pores, thin to thick diameter, continuous, chaotic orientation, inside and outside of aggregates, with tubular morphology; fast permeability; few and very small roots; with a field pH of 5
	Bt2 (101-1-150)	Medium and irregular transition; saturated moisture; brownish yellow color (10 YR 6/1), with red (2.5 YR 6/8) and pale brown (10 YR 7/3) mottled, contrasting, many, thin and medium size; sandy clay loam texture; few rounded gravels of weathered quartz; strongly developed structure, with subangular blocks, thin and medium in size; friable wet consistency; sticky and slightly plastic very wet consistency; cutans by eluviation, continuous, with clay minerals and iron oxides, on faces of aggregates and pores; frequent small sized nodules, red color, with subangular shape, soft hardness, composition of iron oxides; frequent pores, thin to thick diameter, continuous, chaotic orientation, inside and outside of aggregates, with tubular morphology; moderate permeability; rare and thin roots; with a field pH of 4.2

growth forest, pineapple, yucca, sugarcane and pasture use, from among which the greatest weight was 4579.50 t ha<sup>-1</sup> for second growth forest, exceeding by 1,320.61 t ha<sup>-1</sup> what the A horizon weighs from pasture use.

### Bulk density and total porosity

There were no significant differences between treatments of bulk density (Dap), although on average the Dap was accentuated in the pasture use (Table 3). Noguera & Vélez (2011) evaluated five land uses in three silvopastoril systems, concluding that the pasture use with Kikuyo (*P. clandestinum*) grass did not only obtain the highest Dap

Table 2. Soil quantity by	land-use and	horizon A red	uction comparison.

Treatments	Horizon depth (cm)	Horizon volume (m³ ha <sup>-1</sup> )	Horizon weight (t ha <sup>-1</sup> )	
Pasture	30.4a	3040a	3258.39a	
Rubber	27.6aa	2760a	2644.53a	
Rubber-cocoa	29.2a	2920a	2943.06a	
Rubber-mahogany	28.6a	2860a	2946.20a	
VC (%)	19.01	19.01	19.81	
F	0.88	0.88	0.45	
Statistical differences	ND	ND	ND	

The deepest land-use was pasture, soil sheets loss volume and weight was calculated, subtracting from the highest value the value of each land-use. Equal letters indicate the absence of statistical differences (p>0.05).

Soil depth reduction						
Treatments	Soil depth loss (cm)*	Volume of soil lost (m <sup>3</sup> ha <sup>-1</sup> )*	Weight of soil lost (t ha <sup>-1</sup> )*	Soil depth loss (cm)**	Volume of soil lost (m <sup>3</sup> ha <sup>-1</sup> )**	Weight of soil lost (t ha <sup>-1</sup> )**
Pasture				12.10	1210	1311.6
Rubber	2.80	280	267.764	14.90	1490	1424.9
Rubber-cocoa	1.20	120	121.137	13.30	1330	1342.6
Rubber- mahogany	1.80	180	186.069	13.90	1390	1436.9

<sup>\*</sup> Values estimated taking as reference the deepest horizon, in this case it was the use with pasture (30.4 cm).

 $(1.17 \text{ t m}^{-3})$ , but rather was at par with the lowest values in the variables associated such as porosity and water conductivity, caused by indiscriminate and constant stomping of animals within the plot.

There were no significant differences between the means of porosity of the treatments (Table 3). If we consider the average results obtained, the lowest porosity corresponds to treatment one —the soil use with pasture, which at the same time represents the intensive use— with 59.09 %. It is necessary to stress that the percentage of porosity of the pasture

**Table 3.** Averages of the soil quality indicators. Different letters indicate statistical differences (p<0.05).

Treatments	Infiltration speed $(cm water h^{-1})$	Penetration resistance (MPa)	Bulk density (t m <sup>-3</sup> )	Porosity (%)	Aggregate stability (%)	Average soil meltdown (%)
Pasture	162.39b	0.40a	1.08a	59.09a	32.38a	5.94a
Rubber	657.35c	0.29a	0.96a	63.91a	24.76a	5.75a
Rubber-cocoa	1472.73a	0.29a	1.01a	61.91a	20.83a	5.82a
Rubber-mahogany	1317.89a	0.27a	1.03a	60.99a	22.5a	5.77a
VC (%)	63.18	29.56	10.10	6.33	65.27	3.48
F	0.008	0.13	0.30	0.30	0.70	0.49
Statistical differences	**	ND	ND	ND	ND	ND

<sup>\*\*</sup> Values estimated considering the depth of the acahual (secondary vegetation) from Alejandro-Martínez et al. (2019).

use was considered high (50-60%), and on the other hand, the other uses fall into the category of Very high porosity (>60%) according to the interpretation table by Flores & Alcalá (2010). The low porosity is associated to the Dap evaluated and scarce organic matter, which implies problems such as water infiltration and storage with the restriction of growth and development of the roots (Drobnik *et al.*, 2018).

# Thinning and stability of aggregates

No significant differences were observed (p>0.05; CV=3.480%) between the averages of thinning in the land uses. The average of thinning ranges between 5.31 and 6%. According to Francis *et al.* (2018), soil thinning is a physical process that is characterized by the rupture of the air-dried soil macroaggregates into microaggregates and much finer primary particles when they are drastically dampened. It is believed that the thinning results obtained could be related to a good organic matter content (OM), since it benefits the structure of the soil reducing the permeability, while it reduces the forces that destroy the aggregates from bursting (Gabioud *et al.*, 2011).

The stability of aggregates was not statistically different between the land uses (p>0.05) (Table 3). The stability of aggregates is low, since they fluctuate from 20.83 to 32.8% (USDA, 2001). Bernal & Hernández (2017), when evaluating the stability of aggregates in a red Ferralitic soil lixiviated under three uses, obtained the best stability values (44.5%) for the first 20 cm of depth under a forest system, considered satisfactory; this result is higher than those obtained for the use with rubber-cacao (20.83%), rubber-mahogany (22.5%), and rubber tree 24.76 (%).

#### Resistance to penetration and infiltration speed

There were no significant differences between treatments (p>0.05, CV=29.56) (Table 3). The resistance to penetration was greater in the land use with pasture (0.40 MPa), while the lowest value was observed in rubber-mahogany (0.27 MPa). Similarly, Noguera & Vélez (2011) did not find significant differences in the resistance to penetration at different depths (0-15 cm, 15-30 cm and 30-45 cm), although obtaining higher resistance values in grasses with livestock with 3 MPa (30.60 kgf cm²) in the first five centimeters, which is very high compared to that found for the pasture evaluated (0.40 MPa). However, the low levels of resistance to penetration of all the soils suggest low physical degradation, since starting from 2 MPa (20.40 kgf cm²), the soil can face significant restriction for the development of roots (López, 2002). White (2006) suggests that in forests, a resistance to penetration lower than 1 MPa (10.20 Kgf cm²) represents low restriction for the roots and soils of better quality, which allows suggesting that the uses with rubber tree, rubber-cacao and rubber-mahogany present a good state.

Highly significant differences were observed (p<0.001, CV=63.181%) in the infiltration speed (Table 3). Although the results categorized all the treatments as with *very fast infiltration*, in the pasture land use the infiltration speed is affected since on average it is the lowest with 162.386 cm  $\rm H_2O\,h^{-1}$  (centimeters of water sheet per hour), which agrees with the highest values in the variable resistance to penetration, lowest porosity, and high Dap (López, 2002). The values obtained were 657.536 and 1327.886 cm  $\rm H_2O\,h^{-1}$  for

rubber tree and rubber-mahogay, respectively, and the highest value was obtained with rubber-cacao with  $1472.734 \text{ cm H}_2\text{O h}^{-1}$ .

#### CONCLUSIONS

The classified RSG corresponds to a Ferric Lixisol (Cutanic, Endoloamic, Epiarenic, Humic, Profondic). The quality of the physical properties evaluated from the A horizon for all the land uses were found in acceptable levels. The results suggest that the pasture has conserved more soil through time and stores more volume and weight of soil per hectare. The physical variables for the use with pasture have the lowest average values in the case of infiltration speed, and highest for resistance to penetration, bulk density, total porosity, percentage of stability of aggregates, and thinning.

## **AKNOWLEDGEMENTS**

The authors wish to thank CONACyT for the financial support for the study; Colegio de Postgraduados; producers Wilber Pulido Izquierdo, Aníbal Pulido Valencia for access to their productive areas; likewise, PhD. Isidro Pérez Hernández and M.Sc. Alain Lois D'Artola Barceló for the support given in the Basic Sciences Laboratory 1 of Universidad Politécnica del Golfo de México.

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# Pregerminative treatments in *Tillandsia ionantha* seeds to obtain seedlings under *in vitro* culture

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#### **ABSTRACT**

**Objective**: To search for an *in vitro* strategy to favor both germination and a greater number of seedlings in *Tillandsia ionantha*; also, to promote the development of future research on this species.

**Design/methodology/approach**: Factor one: lighting conditions (light-dark), factor two: 13 preconditioning treatments, which included storage at room temperature and in refrigeration at 10 °C, soaking (12 and 24 hours), with hydrogen peroxide (10 and 20%), potassium nitrate (0.2 and 0.4%), gibberellins (50 and 150 ml.l<sup>-1</sup>), three alternate incubation temperatures (28, 32 and 36 °C). They were sown in MS medium (Murashige and Skoog, 1962) at 25%, adding 20 g.l<sup>-1</sup> of sugar, 2 g.l<sup>-1</sup> of activated carbon, and 5.5 g.l<sup>-1</sup> of agar. A flask with three seeds which coma removed was established as an experimental unit; 15 repetitions were established and placed in the incubation room at 24 °C with a photoperiod of 16:8. The germination process was recorded, and the seedlings were extracted two months after their establishment.

**Results**: The treatment that resulted in the highest number of seeds that initiated the germination process and the highest number of plants was when the seeds were kept at room temperature. The highest contamination was observed in the treatment exposed to 32 °C. It was observed that 80% of the experimental units showed signs of imbibition within a few days, although the vast majority did not complete the process and the maximum yield was on average 1.4 seedlings.

**Conclusions**: The best treatment is to use seeds stored at room temperature and if storage is necessary, to soak them for 12 h.

**Keywords**: native, conservation, bromeliad.

Citation: García-Granados, B. I.,
Valdez-Hernández, E. F., RodríguezDe la O, J. L., Juárez-Hernández,
M. de J., & Flores-Cruz, M. (2023).
Pregerminative treatments in *Tillandsia*ionantha seeds to obtain seedlings under in vitro culture. Agro Productividad.
https://doi.org/10.32854/agrop.
v16i1.2442

**Academic Editors**: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: November 29, 2022.
Accepted: January 15, 2023.
Published on-line: February 22, 2023.

*Agro Productividad*, *16*(1). January. 2023. pp: 147-153.

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# INTRODUCTION

Illegal trade of exotic plants and other lifeforms is a common practice in the world. This article seeks to promote alternative forms of production and propagation of certain genera of Bromeliaceae, which occupy the eleventh place in specific wealth among angiosperms and the third place among monocotyledons in Mexico. The genus *Tillandsia* at the global level has more than 649 species and includes members with distinctive morphological and physiological



characteristics (Pickens et al., 2006; Gouda et al., 2016; Granados et al., 2016). Most of the species of the genus are epiphytes or live on rocky hillsides, preferring to become established on tree branches of Quercus spp. due to its coarse bark. They have leaves covered with pelted trichomes and are arranged in rosettes, while the inflorescences are bracts with bright colors (Diego-Escobar et al., 2013); in Mexico, Tillandsia occupies the first place with 230 species. T. ionantha is an example of these and is generally traded illegally; it is commonly known as an air plant with compact rosette growth that has red pigment in certain seasons. Therefore, an in vitro strategy is sought to favor both the germination and a larger number of seedlings in Tillandsia ionantha, in order to promote the development of future research on this species.

## MATERIALS AND METHODS

# Location of the study

The study was developed in the Laboratory of Plant Tissue Cultivation and seeds, of the Plant Production Department at Universidad Autónoma Chapingo; T. ionantha seeds were used from wild plants from the state of Morelos. The growth medium was Murashige and Skoog (1962) at 25%, with addition of 20 g L<sup>-1</sup> sucrose and 2 g L<sup>-1</sup> activated carbon, pH adjusted to  $5.7\pm1$ ; 5.5 g L<sup>-1</sup> Deiman® brand agar as gelling agent was added to 10 mL of the medium in a Gerber® brand jar, which was then introduced into an autoclave to be sterilized for 25 min at a temperature of 121 °C and a pressure of 1.1 to 1.2 kg cm<sup>2-1</sup>.

# **Preconditioning of seeds**

The coma (crest of hairs that ease the dispersion through wind) was eliminated from the seeds to avoid possible sources of contamination. Groups of 100 seeds were quantified and placed in filter paper envelopes to facilitate their management in each treatment. Once the pre-treatment was done, the seeds were washed inside the leaf flow chamber with the following procedure: a solution of 10% of commercial chlorine was added for 10 minutes, and after this time they were washed three times; a solution of 10% alcohol at 96% was added for 10 min, and after this time they were washed three times with sterile water; then a solution of 1.5 mL  $\rm L^{-1}$  colloidal silver was added for 10 min, and after this time they were washed five times with sterile water.

Once the seeds were washed, the envelopes were opened individually and three seeds were sown per jar, then they were sealed with film tape and labeled; then they were incubated in the predetermined conditions. Concerning the light factor, it was supplied with a fluorescent lamp with a designation of cold white color (CW) with color code 33 with a relative color temperature of 4100 K and a mean design lumen of 9000 lm with nominal chromatic reproduction index of 62 and nominal potency of 185 Watts and exposure to a photoperiod of 16 light hours and 8 dark hours.

#### **Treatments**

Twenty-six treatments were established, which consisted in ten preconditioning and three incubation conditions. The control consisted in seeds that were stored since the day of harvest at room temperature in the laboratory, the rest of the seeds were stored in refrigeration at 10 °C for 15 days and a group was established with this characteristic; seeds with previous soaking of 12 to 24 h were established, the treatments were exposed to a solution described in Table 1, immersed during 24 h in constant agitation. Other groups of seeds were incubated at different temperatures. All of these treatments (Table 1) were incubated in two conditions, light and dark.

# Experimental design and establishment

The experimental design was completely random factorial with 15 repetitions, where each experimental unit was a jar with three seeds.

## Response variables

The experiment was monitored three times per week, recording the following variables. Germination: a seed was considered germinated when the imbibition process was visible. Percentage of germination: it was evaluated in function of the number of germinated seeds from the total deposited in each jar and according to the date of data collection. Percentage of necrotization: it was evaluated in function of the number of seeds that visibly presented this condition according to the total deposited in each jar. Percentage of contamination: the jar that presented signs of contamination, whether fungus or bacteria, was eliminated and the seeds in them were counted as contaminated; in addition, in some jars the proliferation only affected one or two specimens, and they were the only ones quantified. Percentage of normal plants: at the end of the experiment the plants that did not present any type of physical abnormal condition, or any damage from contamination or necrotization, were extracted and quantified according to the treatment that they belonged to.

**Table 1.** Pregerminative treatments evaluated in *Tillandsia ionantha* Planch seeds.

	Treatments			
Pregerminative	Incubation condition			
	Light	Darkness		
Room temperature	T1	T1		
Cold stored	T2	T2		
Soaking 12 hrs	Т3	Т3		
Soaking 24 hrs	T4	T4		
Hydrogen peroxide 10 %	Т5	Т5		
Hydrogen peroxide 20 %	Т6	Т6		
Potassium nitrate 0.2 %	Т7	Т7		
Potassium nitrate 0.4 %	Т8	Т8		
Gibberellins 50 mg.l	Т9	Т9		
Gibberellins 150 mg.l	T10	T10		
Incubated 28 °C	T11	T11		
Incubated 32 °C	T12	T12		
Incubated 36 °C	T13	T13		

# Statistical analysis

To analyze the variables, analysis of variance was used and since there were differences, Tukey's means test (p=0.05) was applied using the statistical software SAS version 9.0.

#### RESULTS AND DISCUSSION

## Light factor

Regarding factor 1, no significant differences were found except at two days after sowing when the germination was higher with the light condition with 39.974%, the dark condition with 31.559% which was provided by keeping them in totally black boxes on both sides and an exposure of 24 h darkness; this could be contradicted by what was observed by Vadillo *et al.* (2004) when they evaluated *Puya raimondii* Harms seeds when considering the positive photoblast seeds, since *Tillandsia ionantha* germinated similarly in both conditions. However, since differences were found in terms of contamination, these could be attributed to the microclimate that was generated in the germination chamber, since in order to establish darkness cardboard boxes were designed within these pieces of equipment and with the moisture fungal formation colonies were observed around the box, which could inoculate the culture mediums within that box.

# Beginning of germination

According to Tukey's test (p=0.05) at two days after sowing (das), the treatments T1, T3, T6 and T7 are statistically equal; however, it should be mentioned that treatment T1 (room temperature) was the one that presented a higher mean (64.567%) with specimens in phase 1 (Figure 1), which is why we could assume that germination of *Tillandsia ionantha* seeds is promoted under storage conditions at room temperature, as indicated by Klekailo *et al.* (2012), where the authors point out that temperature was a key factor, because germination was only found (a reduced fraction of the seeds and slowly) in the treatment at 20/30 °C; on the other hand, treatments T4, T5, T7, T9, T10, T11 and T13 are statistically equal, with treatment T9 (gibberellins 50 mg.l<sup>-1</sup>) being the one that presented the lowest mean (13.2%) with specimens in phase 1, which is why we can speculate that the use of this plant hormone at this dose does not promote germination in this species. In this regard, Saldívar Iglesias *et al.* (2010) indicate that gibberellic acid in *Jaltomata procumbens* at a dose of 250 mg.l<sup>-1</sup>, increased the speed compared to that reported in treatments of 200 and 150 mg.l<sup>-1</sup> and that the slower germination speeds are found with treatments of 100, 50 and 0 mg.l<sup>-1</sup> gibberellic acid; in general it was observed that as the concentration of gibberellic

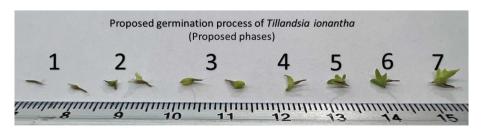


Figure 1. Germination process of Tillandsia ionantha Planch.

acid increases, the germination speed increases. According to the means test, at 4 (das) the treatments T1, T3, T6 and T8 are statistically equal; however, it should be mentioned that again the treatment T1 (room temperature) was the one that presented a higher mean (83.433%) with specimens in phase 1 (Figure 1). On the other hand, treatments T2, T4, T5, T7, T9, T10, T11 and T13 are statistically equal; however, it should be mentioned that the treatment T10 (gibberellins 150 mg.l<sup>-1</sup>) presented the lowest mean (30.967) with specimens in phase 1 (Figure 1), which is why we can speculate that it contradicts what was observed by Saldívar Iglesias *et al.* (2010), since it indicates that with a higher concentration of gibberellins a higher germination speed was not observed in comparison to other treatments.

According to Tukey's test (p=0.05) at 7 (das) the treatments T1, T3, T6 and T8 are statistically equal; however, it should be mentioned again that the treatment T1 (room temperature) was the one that presented a higher mean (83.433%) with specimens in phase 1 (Figure 1). On the other hand, treatments T2, T4, T5, T7, T9, T10, T11 and T13 are statistically equal, although it should be mentioned that treatment T11 (incubated at 28 °C) presented the lowest mean (48.833%) with specimens in phase 1 (Figure 1).

#### Germination

According to Tukey's test (p=0.05), the treatments T1, T3, T5,T6, T7, T8, T11, T12 and T13 are statistically equal, although it should be mentioned that treatment T1 (room temperature) was the one that presented a higher mean (95%) of germinated specimens, and until this moment it could be seen that in order to guarantee a higher percentage of germination, it is not necessary to conduct pre-conditioning; regrettably, due to logistics the seed lots must be stored, generally under refrigeration to maintain the quality and health, according to what is described by Márquez (2019) who point out that seeds can remain viable for 38 months under refrigeration conditions at 10 °C with average germination of 78.9%, while, this study shows 73.467% under this condition; however, some pregerminative treatments promoted an increase in germination, almost equaling the seed without refrigeration.

Table 2 shows that the lowest percentage of germination is with seeds stored in cold (treatment T2) as well as continuous soaking for 24 hours (treatment T4). The latter is possibly linked to the seed's biology, and no information was found regarding the maximum imbibition of seeds of this species or similar, which is why it could be speculated that they suffered drowning.

#### Necrotization

Necrotization was present in all the treatments, with treatment T1 being the lowest with 48.867% which could agree with what was exposed by Calderón *et al.* (2011) when they pointed out that due to the biology of the wild Tillandsias, they can be very sensitive to the concentration of salts since a higher percentage of necrotization was actually observed in the seeds subjected to treatments such a potassium nitrate, hydrogen peroxide, and gibberellins, although 25% of the DM was used. Necrotization was possibly promoted

Treatments	2 Days after sowing	4 Days after sowing	7 Days after sowing	% Germination	% Necrosed	% Contamination	% Normal plants
Light	39.97 <sup>a</sup>	49.35 <sup>a</sup>	60.87 <sup>a</sup>	87.80 <sup>a</sup>	75.27 <sup>a</sup>	5.9	19.61 <sup>a</sup>
Darkness	31.55 <sup>b</sup>	50.42 <sup>a</sup>	64.56 <sup>a</sup>	85.06 <sup>a</sup>	70.50 <sup>a</sup>	10.9	16.37 <sup>a</sup>
Tl	64.56 <sup>a</sup>	83.43 <sup>a</sup>	91.17 <sup>a</sup>	95.6 <sup>a</sup>	48.87 <sup>c</sup>	6.67 <sup>b</sup>	46.7 <sup>a</sup>
T2	41.06 <sup>bcd</sup>	48.9 <sup>bcd</sup>	54.53 <sup>ef</sup>	73.47 <sup>d</sup>	73.4 <sup>abc</sup>	7.77 <sup>b</sup>	13.27 <sup>bc</sup>
Т3	55.5 <sup>ab</sup>	65.6 <sup>ab</sup>	74.53 <sup>abcd</sup>	92.3 <sup>abc</sup>	62.37 <sup>bc</sup>	4.43 <sup>b</sup>	31.0 <sup>ab</sup>
T4	32.1 <sup>cdef</sup>	42.23 <sup>cd</sup>	54.53 <sup>ef</sup>	73.47 <sup>d</sup>	65.77 <sup>abc</sup>	6.67	13.2 <sup>bc</sup>
T5	25.36 <sup>def</sup>	36.5 <sup>cd</sup>	48.9 <sup>f</sup>	81.3 <sup>abcd</sup>	84.467 <sup>ab</sup>	$0_{\rm p}$	10 <sup>bc</sup>
Т6	54.53 <sup>ab</sup>	75.66 <sup>a</sup>	82.3 <sup>ab</sup>	94.5 <sup>ab</sup>	81.2 <sup>ab</sup>	11.1 <sup>b</sup>	14.3 <sup>bc</sup>
Т7	27.67 <sup>cdef</sup>	41.03 <sup>cd</sup>	60.17 <sup>cdef</sup>	91.2 <sup>abc</sup>	89 <sup>a</sup>	$0_{\rm p}$	9.9 <sup>bc</sup>
Т8	46.73 <sup>abc</sup>	64.53 <sup>ab</sup>	77.93 <sup>abc</sup>	95.6 <sup>a</sup>	68.9 <sup>abc</sup>	16.6 <sup>ab</sup>	24.4 <sup>bc</sup>
Т9	13.2 <sup>f</sup>	33.17 <sup>cd</sup>	48.87 <sup>f</sup>	79.03 <sup>bcd</sup>	88.93 <sup>a</sup>	5.53 <sup>b</sup>	5.533 <sup>c</sup>
T10	19.87 <sup>ef</sup>	30.97 <sup>d</sup>	48.9 <sup>f</sup>	76.83 <sup>cd</sup>	87.83 <sup>a</sup>	7.73 <sup>b</sup>	3.333 <sup>c</sup>
T11	17.7 <sup>f</sup>	32.07 <sup>d</sup>	48.83 <sup>f</sup>	90.1 <sup>abc</sup>	73.4 <sup>abc</sup>	12.17 <sup>ab</sup>	18.83 <sup>bc</sup>
T12	38.93 <sup>bcde</sup>	52.27 <sup>bc</sup>	69.07 <sup>bcde</sup>	90.1 <sup>abc</sup>	48.93 <sup>c</sup>	30 <sup>a</sup>	22.167 <sup>bc</sup>
T13	27.73 <sup>cdef</sup>	42.2 <sup>cd</sup>	55.6 <sup>def</sup>	90.1 <sup>abc</sup>	74.43 <sup>ab</sup>	1.1 <sup>b</sup>	21.167 <sup>bc</sup>

**Table 2.** Responses observed in the 26 pregerminative treatments in *Tillandsia ionantha* Planch.

Averages per column with different letters indicate a significant difference according to Tukey's test (p=0.05).

when maintaining the seedlings during 60 days in this medium.

## Contamination

According to Tukey's test (p=0.05), the treatments T11 and T12 are statistically equal, although it should be mentioned that treatment T12 (incubated at 32 °C), was the one that presented a higher mean (30%) with specimens contaminated as described previously; the highest contamination was seen in the germination chambers in which these treatments were found, as consequence of the microclimate generated inside them. The treatments T5 (hydrogen peroxide 10%) and T7 (potassium nitrate 0.2%) presented the lowest percentages of contamination with 0% both.

# Normal plants

Although in percentages higher than 70% of germination were found all the treatments (Table 2), at 60 days when the seeds were extracted and a loss of more than 50% of specimens was found, due to contamination, oxidation or a case that was not reported where the seeds presented some of the germination phases, primarily phase 1 or 2 (Figure 1), and remained there without generating complete or normal seedlings.

## **CONCLUSION**

The disinfection technique used was adequate, as well as the culture medium, although

it is recommended to conduct trials with low concentration of salts or monthly or biweekly replacements of the explants to decrease the oxidation percentages. Likewise, using seeds stored at room temperature eliminating the coma. The best treatment is to use seeds stored at room temperature and if their storage is necessary, to use soaking for 12 hours and tentatively potassium nitrate at a concentration of 0.4%, addressing the replacements or the decrease in salts to decrease possible causes of oxidation.

Differences were not seen between the treatments of light and dark.

#### **ACKNOWLEDGEMENTS**

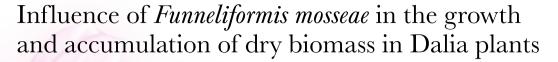
The corresponding author wishes to thank CONACyT for the scholarship to conduct a postdoctoral stay in Mexico and Professor Flavio Valdés for the suggestions.

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#### ABSTRACT

**Objetive:** in the production of *Dahlia* spp., only chemical fertilization has been used, and an option that has not yet been explored is the implementation of a microbial inoculant. Therefore, the objective of this study was to evaluate the effect of the mycorrhizal fungus *Funneliformis mosseae* on the growth and development of the dahlia (*Dahlia variabilis* var. Variegated dwarf).

**Design/methodology/approach**: the seeds were sown in polyethylene bags containing a mixture of black soil, peat moss, and agrolite. A completely randomized design was used, and the treatment structure was  $2 \times 3$  factorial. The study factors were *F. mosseae*, chemical fertilization, and substrate sterilization.

**Results**: an analysis of variance was performed, and the mean values of the treatments were compared with Tukey's test ( $\alpha$ =0.05).

**Conclusions**: with the inoculation of *F. mosseae*, a significant increase was obtained in the study variables: plant height, stem diameter, number of buds and flowers per plant; leaf + stem, flower, root, and total biomass, compared to non-inoculated plants. A colonization of 89% in the roots was recorded. A limitation of the study is that the effect of the inoculum on plant growth can vary according to the mycorrhiza species used. In conclusion, inoculation with *Funneliformis mosseae* increased growth and biomass accumulation in Dahlia plants.

Mexico is the center of biological diversity of the genus *Dahlia*, and its cultivation began

Keywords: Dahlia variabilis var. Variegated dwarf; biomass; plant quality; mycorrhizal colonization.

Citation: García-Sánchez, Claudia; Becerril-Román, A. Enrique; Jaén-Contreras, David; Hernández-Melchor, Dulce J.; López-Morales, Fernando; Gaytán-Acuña, E. Araceli. Influence of Funneliformis mosseae in the growth and accumulation of dry biomass in Dalia plants. Agro Productividad. https://doi. org/10.32854/agrop.v16i1.2443

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: November 29, 2022.
Accepted: January 15, 2022.
Published on-line: February 22, 2023.

 $\label{eq:Agro-Productividad} \textit{Agro-Productividad}, \textit{16} (1). \ January. \\ 2023. \ pp: 155-161.$ 

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# INTRODUCTION

in the times of the Aztec empire. Due to its importance, in the year 1963 it was declared the symbol of National Floriculture (Jiménez-Mariña, 2015). Despite this, its production does not occupy a predominant place in national floriculture, while in countries such as the Netherlands, Japan, England, and the United States its production is higher (Vidalie, 2001). Dahlias belong to the Asteraceae family and can be herbaceous or shrub plants, their leaves are simple or tripinned-compound, with hollow stems between the internodes. Their inflorescences are arranged in chapters made up by ligulated and tubular flowers. Their fruit is an achene and their roots are tuberous (Bye and Linares, 2008).

In the cultivation of dahlia, it is recognized that applying chemical fertilizers has increased their development (Arenas-Julio *et al.*, 2011), although their excessive use has caused problems of environmental contamination, such as the acidification of soils which affects fertility and the eutrophication of bodies of water (Bertani and Lu, 2021); because of this, the use of fertilizers that are not damaging to the environment, such as biofertilizers and microbial inoculants, has been promoted.

Arbuscular Mycorrhizal Fungi (AMFs) are members of the *Glomeromycota phylum* and they form symbiosis with 80 to 95% of plant species. The fungus colonizes biotrophically the root crust, without damaging the plant and becoming part of it, physiologically and morphologically (Koltai, 2010). In ornamental species of the Asteraceae family, inoculation with AMF has been found to increase the height, number of flowers per plant, and biomass (Vaingankar and Rodrigues, 2012; Uc-Ku *et al.*, 2019).

It is necessary to continue with the implementation of biofertilizers through the use of AMFs, since they promote growth and flowering in various species, in addition to representing a sustainable alternative in flower production (De Pascale *et al.*, 2020). Dahlia is appreciated internationally because of the large variety of shapes and colors that it presents, which is why it is necessary to rescue its cultivation in Mexico, in addition to the application of AMFs as an organic fertilization alternative, which has not been explored in its growth. Therefore, the research objective was to evaluate the effect of *Funneliformis mosseae* on the growth and development of dahlia (*Dahlia variabilis* var. Variegated dwarf) in pots under greenhouse conditions.

# MATERIALS AND METHODS

The experiment was established in a greenhouse with milky plastic cover, in Colegio de Postgraduados (CP), Campus Montecillo, Texcoco, Estado de México. Commercial Hortaflor® dahlia seeds were used, which were sown in germination trays that contained peat moss and vermiculite (1:1); the substrate was sterilized in an autoclave at 120 °C during three hours. Germination began in February and seed emergence was observed 10 days after sowing. The transplant was carried out 15 days after emergence, for which black polyethylene bags of 35×35 cm were used, which contained a mixture of black soil, peat moss and agrolite (1:1:1), which were sterilized in autoclave at 120 °C during three hours. At the time of transplant, each of the seedlings was inoculted with 10 g of inoculant that contained 350 spores of the mychorrhizal fungus Funneliformis moseae. An orifice with the size of the ball of the seedling was made in the center of the pot where the inoculum and the seedling were placed. Chemical fertilization was carried out 30 days after transplant, 1 g L<sup>-1</sup> of chemical fertilizer of continuous liberation 11-7-7 (N-P-K) was placed in each of the seedlings, which was distributed to the maximum possible, since dahlia is sensitive to excess salts. The variables of study were plant height (cm) which was considered from the basal area to the apex of the main stem, the stem diameter (mm) was measured at the base, the number of buds and of flowers per plant were counted, and the dry matter weight (g) of each of the plant structures was recorded: leaf + stem, flower buds, flower, root and total.

The mychorrizal colonization in the dahlia roots was determined, for which the clearing and dyeing method by Phillips and Hayman (1970) was used. And the observation of roots was carried out in an American optical <sup>®</sup> optical microscope.

The experimental design was completely random and the treatment structure was a complete 2×3 factorial arrangement; the variation factors, and the levels of application were: 1) Inoculation with the arbuscular mychorrizal fungus *F. mosseae* (M): 0 and 10 g of inoculant; 2) Chemical fertilization (F) with 11-7-7: 0 and 1 g L<sup>-1</sup>; and 3) The sterilized and non-sterilized substrate (S). With that, the combination of eight treatments was obtained: M0F0S0, M1F0S0, M0F1S0, M1F1S0, M0F0S1, M1F0S1, M0F1S1 and M1F1S1.

Fifteen plants were established per treatment; one plant was considered as experimental unit, and three repetitions per treatment were selected, for each of the variables evaluated. The sampling was done in the flowering period, in the month of June.

The results obtained from each of the factors and their interactions were examined in the statistical software Statistical Analisis System (SAS®), version 9.0, with which an analysis of variance (ANOVA) was conducted, and the mean values of the factors and their interactions were compared with Tukey's test ( $\alpha$ =0.05).

## RESULTS AND DISCUSSION

In the analysis of factors in study, it was observed that the inoculation with *F. mosseae* (M1) promoted and increased height, stem diameter, number of buds, and number of flowers per plant, compared to the non-inoculated plants (M0) (Table 1). When analyzing the interaction of the three factors in study, mychorrizal fungus, chemical fertilization and substrate (M\*F\*S), the greatest height was obtained with the treatment (M1F0S0), without

**Table 1.** Mean values of height, stem diameter, number of buds and flowers in dahlia plants (*Dahlia variabilis* var. Variegated dwarf).

TD	Plant height	Stem diameter	Buds	Flowers
Treatments	(cm)	(mm)	Number per plant	
M				
M0	46.7 b	7.7 b	15.5 b	9.2 b
M1	55.8 a	11.2 a	41.3 a	20.2 a
DMS	7.8	1.5	11.8	6.1
M*F*S				
M0F0S0	50.6 a	6.8 b	17.3 bc	10.0 с
M1F0S0	68.5 a	10.7 ab	25.3 abc	32.7 ab
M0F1S0	45.0 a	7.6 ab	13.7 с	12.0 с
M1F1S0	46.6 a	12.6 a	56.3 a	10.0 с
M0F0S1	43.8 a	7.7 ab	17.7 abc	1.7 с
M1F0S1	61.2 a	11.6 ab	28.3 abc	33.7 a
M0F1S1	47.2 a	8.7 ab	13.3 с	13.3 bc
M1F1S1	47.2 a	9.9 ab	55.3 ab	4.7 с
DMS	25.5	5.0	38.8	19.9

M=Inoculation factor with Funneliformis mosseae; M\*F\*S=Interaction of the inoculation with F. mosseae, with chemical fertilization and the substrate; M0=Without F. mosseae; M1=With F. mosseae; F0=Without chemical fertilization; F1=With chemical fertilization; S0=Substrate without sterilization; S1=Sterilized substrate; DMS=Minimum Significant Difference. Means with the same letter in each column are equal according to Tukey's test ( $\alpha \le 0.05$ ).

statistically differing from the other treatments (Table 1). Regarding the stem diameter, the best results were found with the treatment (M1F1S0) and the smallest diameter with the control (M0F0S0). With the treatment (M1F1S0), the highest number of buds was obtained with 56.3 and the lowest number of buds was 13.3 with the interaction (M0F1S1). The highest number of flowers per plant was 33.7 with the treatment (M1F0S1) and the lowest number was 1.7 with the interaction (M0F0S1) (Table 1).

Concerning the dry biomass, the greatest accumulation of biomass was observed in leaf + stem, flower, root and total with inoculation of the mycorrhizal fungus (M1), compared to the control (M0) (Table 2). And in the interaction of the three factors (M\*F\*S) a similar behavior was observed, since with the M1\*F0\*S0 interaction the greatest accumulation of dry biomass was found in leaf + stem (37.4 g), flower (13.5 g), root (20.5 g) and total (71.4 g), compared to the other treatments (Table 2).

The increase in plant height, stem diameter, number of buds and flowers, as well as the biomass in each of the structures and total, can be attributed to the fact that the mycorrhized roots can explore a greater volume of soil, due to the presence of external hyphae that mycorrhizal fungi develop, and these results are associated to a higher absorption of nutrients from the less soluble sources (Sohn *et al.*, 2003); it is considered that the fungi hyphae are the ones responsible for transporting more photosynthates to the plant, which contributes to the increase in growth, in the development and in the production of biomass of the plant species (Ibarra-Puón *et al.*, 2014).

**Table 2**. Mean values of dry biomass in each one of the dahlia plant structures (*Dahlia variabilis* var. Variegated dwarf).

T	Dry weight (g)					
Treatments	Leaf + Stem	Flower	Root	Total		
M						
<b>M</b> 0	9.2 b	3.7 b	6.5 b	19.4 b		
M1	30.3 a	9.4 a	11.8 a	51.6 a		
DMS	4.5	1.5	4.6	8.5		
M*F*S						
M0F0S0	10.1 bc	3.9 cd	9.1 abc	23.1 cd		
M1F0S0	37.4 a	13.5 a	20.5 a	71.4 a		
M0F1S0	10.2 bc	4.7 cd	7.7 abc	22.6 cd		
M1F1S0	27.7 a	7.4 bc	6.9 abc	41.9 bc		
M0F0S1	4.8 с	1.4 d	1.2 с	7.4 d		
M1F0S1	32.8 a	11.7 ab	16.4 ab	60.8 ab		
M0F1S1	11.8 bc	4.7 cd	7.9 abc	24.5 cd		
M1F1S1	23.4 ab	5.0 cd	3.6 bc	32.1 cd		
DMS	14.8	5.0	14.9	27.8		

M=Inoculation factor with Funneliformis mosseae; M\*F\*S=Interaction of the inoculation with F. mosseae, with chemical fertilization and the substrate; M0=Without F. mosseae; M1= With F. mosseae; F0=Without chemical fertilization; F1=With chemical fertilization; S0=Substrate without sterilization; S1=Sterilized substrate; DMS=Minimum Significant Difference. Means with the same letter in each column are equal according to Tukey's test ( $\alpha \le 0.05$ ).

With the inoculation of AMFs, ornamental species can acquire the nutrients efficiently, which is reflected in a higher growth and development of the plant (Jiménez-Moreno *et al.*, 2018).

However, in this case no significant differences were found in the plant height between treatments, and this can be influenced by the plant's genetics, the soil and the climate conditions, as well as the species of inoculant used (Linderman and Davis, 2004).

In the roots of dahlia plants, a colonization of 89.36% was found with the M1F0S0 treatment. The highest mycorrhizal colonization was found when the dahlia plants were only inoculated with *F. mosseae*, while the addition of chemical fertilizer caused a decrease in the percentage of colonization in the roots observed (Table 3, Figure 1C), which is because the addition of chemical fertilizers affects the establishment of AMFs (Rouphael *et al.*, 2015). Meanwhile, the substrate sterilization did not influence this result.

The M0F0S0, M0F1S0, M0F0S1 and M0F1S1 interactions, where the mycorrhizal inoculant was not applied are not shown, since the presence of fungal structures was not found.

**Table 3**. Percentage of mycorrhizal colonization in the dahlia roots (*Dahlia variabilis* var. Variegate dwarf).

Treatments	Mycorrhizal colonization (%)		
M1F0S0	$89.36 \pm 2.30 \mathrm{a}$		
M1F1S0	$70.31 \pm 8.50 \mathrm{bc}$		
M1F0S1	87.61 ± 2.62 ab		
M1F1S1	69.20 ± 4.13 c		
DMS	17.44		

M1=With *F. mosseae*; F0=Without chemical fertilization; F1=With chemical fertilization; S0=Substrate without sterilization; S1=Sterilized substrate. The values are means  $\pm$  standard error. Means with the same letter in each column are equal according to Tukey's test ( $\alpha \le 0.05$ ). DMS=Minimum Significant Difference.



Figure 1. A-B: Dahlia flower. C: Arbuscule, fungal structure of the arbuscular mycorrhizal fungus Funneliformis mosseae, on the roots of the dahlia.

The level of mycorrhizal colonization among ornamental species is variable, it depends on the species, the inoculum used, and the conditions in which the experiment is developed (Vaingankar and Rodrigues, 2012). To consider that the mycorrhizal symbiosis with the plant roots is helpful, the plants inoculated with the AMF must be capable of producing more dry matter in comparison to non-inoculated plants (Asrar et al., 2012). In this case, it can be stated that inoculation of the dahlia plants with F. mosseae was beneficial since it fostered the highest accumulation of dry biomass in each of the structures and in total (Table 2). The implementation of microbial inoculants such as mycorrhizal fungi represents an option that is environmentally friendly, where a representative increase in the production of ornamental crops is achieved, and in addition, they can provide savings in the application of chemical inputs (Zulueta-Rodríguez et al., 2013). In this case, the inoculation with F. mosseae promoted the growth and the accumulation of biomass in dahlia plants.

## **CONCLUSION**

The inoculation with *F. mosseae* promoted a significant increase in plant height, stem diameter, number of buds and flowers, and dry biomass accumulation; because the AMF helped the absorption of nutrients, it represents a viable option for the production of dahlia in pots under greenhouse conditions.

# **AKNOWLEDGEMENTS**

The authors wish to thank Mexico's National Science and Technology Council (Consejo Nacional de Ciencia y Tecnología, CONACyT) and Colegio de Postgraduados for the financial and logistic support granted to perform PhD studies.

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