Agrociencia

eISSN: 2521-9766

VOLUME 58, NUMBER 8 | NOVEMBER 16 - DECEMBER 31, 2024 | MEXICO

AGRICULTURA
SECRETARÍA DE AGRICULTURA Y DESARROLLO RURAL

Editorial Team

Editor in Chief, Agrociencia
Fernando Carlos Gómez Merino

Deputy editor, Agrociencia
Libia Iris Trejo Téllez

International Editorial Council
Roger Austin (**UK**)
José Sarukhán Kermez (**Mexico**)
Barry C. Arnold (USA)

Internal Editorial Advisory Committee
Jorge Alvarado López
Jorge D. Etchevers Barra
Víctor A. González Hernández
Said Infante Gil
Leopoldo E. Mendoza Onofre
José A. Villaseñor Alva

Responsibles of the Edition
Fernando Carlos Gómez Merino

Design and Composition
L. Brenda Espejel Lagunas

Translators
Inés Enríquez
Joel Castillo González
Nicolas Crossa

Metadata harvester
Moises Quintana Arévalo

Platform support
L. Brenda Espejel Lagunas
Valeria Abigail Martínez Sias
Ana Luisa Mejía Sandoval

Secretarial assistance
Yolanda Fermoso Meraz

COPYRIGHT AND RELATED RIGHTS, **Volume 58, Number 8, November 16 - December 31, 2024**, Agrociencia is a semi-monthly publication edited by Colegio de Postgraduados. Carretera Mexico-Texcoco, Km 36.5, Montecillo, Texcoco, State of Mexico. C. P. 56264. Phone: 5959284427. www.colpos.mx. Editor in chief: Dr. Fernando Carlos Gómez Merino. Reservations of Rights to Exclusive Use 04-2021-031913431800-203. eISSN: 2521-9766, granted by the National Copyright Institute. Last modification date, **December 31, 2024**.

The opinions expressed by the authors do not necessarily reflect the position of the editor of the publication.

All correspondence (subscription information, sales, advertising, author contributions, etc.) should be addressed to:

Central Office:

Agrociencia
Guerrero No. 9, Esquina con Avenida Hidalgo,
San Luis Huexotla, Texcoco 56220,
State of Mexico. MEXICO
Tel.: +52-595 92 84427

https://agrociencia-colpos.org/index.php/agrociencia

Disclaimer: Trade marks or any commercial representations cited on scientific articles, essays or notes do not imply nor should be inferred as Agrociencia endorsement. No criticism, disclosure or rejection should be assumed either. Likewise, statements or recommendations expressed by authors are solely their responsibility and may not totally agree with those of the Editor.

Cover: *Saccharum* spp.
Photography and credits: Colegio de Postgraduados

AGRICULTURA
SECRETARÍA DE AGRICULTURA Y DESARROLLO RURAL

CONTENTS

Volume 58, Number 8 | November 16 - December 31, 2024

AGRICULTURAL MACHINERY

ANIMAL SCIENCE

APPLIED MATHEMATICS-STATISTICS-COMPUTER SCIENCE

Biotechnology

Crop Science

Socioeconomics

Wildlife

Agrociencia

DEVELOPMENT OF MECHANICAL EQUIPMENT FOR TRANSPLANTING SUGARCANE (*Saccharum* spp. HYBRIDS) SPROUTS

Hipólito **Ortiz-Laurel**[1*], David **Rosas-Calleja**[1], Héctor **Debernardi-de la Vequia**[1],
Arturo Abraham **Gómez-Jiménez**[1], Israel Antonio **Gómez-Juárez**[2]

[1]Colegio de Postgraduados Campus Córdoba. Carretera Córdoba-Veracruz km 348, Manuel León, Amatlán de los Reyes, Veracruz, Mexico. C. P. 94953.
[2]Ingenio Central Motzorongo S. A. de C. V. Vía Central Motzorongo Sur S/N, Motzorongo, Tezonapa, Veracruz, Mexico. C. P. 95090.
* **Author for correspondence**: hlaurel@colpos.mx

Citation: Ortiz-Laurel H, Rosas-Calleja D, Debernardi-de la Vequia H, Gómez-Jiménez AA, Gómez-Juárez IA. 2024. Development of mechanical equipment for transplanting sugarcane (*Saccharum* spp. hybrids) sprouts. **Agrociencia** 58(8): 934-942. https://doi.org/ 10.47163/ agrociencia.v58i8.2971

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: February 18, 2023.
Approved: October 28, 2024.
Published in Agrociencia:
November 11, 2024.

ABSTRACT

Manual planting of sugarcane (*Saccharum* spp. hybrids) in Mexico is slow, and eye bud germination is deficient. Alternative planting methods must be adopted in order to improve sugarcane field productivity. This study evaluated the field performance of a tractor-mounted planter prototype when transplanting two-month-old nursery-grown sugarcane sprouts in relation to the following parameters: sound sprout treatments, row separation between seedlings, upright placement into the soil, appropriate soil covering, and field uniformity at planting. This mechanical equipment was tested on a readily tilled soil suitable for sugarcane planting, although slightly stoney on the surface. The tractor and implement combination were set to travel at a field speed of 0.3 m s^{-1} when laying seedlings into the row at 900 mm separation. Transplanting separation between seedlings onto the row had a variation of 3.4 %. When assisted by two laborers for planting, the tractor and implement combination achieved a field capacity of 2.5 ha d^{-1}. Thus, for a field capacity of 2 ha d^{-1} and 120 workdays per planting season, the investment in the machine can be returned in two years. Consequently, planting sugarcane sprouts using the planter prototype is up to eight times more cost-effective than manual transplanting.

Keywords: mechanization, agricultural machinery, mechanical transplanting, planting, seedlings.

INTRODUCTION

Mexico is the seventh global producer of sugar. The Mexican sugar industry is one of the most important due to its economic and social relevance for farmhouse inhabitants. This industry generates more than 2.5 million jobs. It takes place in 15 out of 32 Mexican states and benefits 227 municipalities. Raw produce has a value of approximately $1714 million USD. During the 2016–2017 harvest season, 51 sugar factories in Mexico milled 54.329 million Mg of sugarcane (*Saccharum spp*. hybrids) stalks from 0.777 million ha, with an average yield of 68.6 Mg ha^{-1} (CONADESUCA, 2017b). Yield values are far from excellent, so the sugar sector has prioritized increasing crop productive efficiency. Two factors have been identified as critical to achieving this goal: farming practices and environmental concerns.

Planting is the first step in ensuring the consistent growth and profitability of sugarcane (Yadav *et al*., 2003). Each sugar factory in Mexico schedules a series of field operations to prepare the soil for seed placement. However, none of them fully complies according to soil types and available field conditions. The entire planting process is considered semi-mechanized because soil processing for the seedbed is carried out using equipment attached to the farm tractor. This soil processing consists of old stool destruction and field clearing, subsoiling, plowing, disking, leveling, and furrowing (Ortiz-Laurel *et al*., 2016). Farmers are unaware of the required soil tilt for planting. Soil crumbling is poor, and they do not explore more rational and less costly methods, arguing the unavailability of suitable machines and farm equipment. In addition, seed planting is carried out by hand. Sugarcane stalks or billets are placed at the furrows' bottom and covered with a 30 to 50 mm soil layer by using manual tools. A thicker layer or excessive compaction can jeopardize bud eye germination and delay emergence.

Mechanized sugarcane planting is a relatively recent and innovative practice worldwide (Ripoli and Ripoli, 2010). Nonetheless, Mexico has no experience. Sugacane fields in central Veracruz usually have clay soil and are slightly covered with medium-sized stones. Fully mechanized planting has some advantages when compared to a semi-mechanized system. Soil moisture is maintained, as soil is opened and closed straightforwardly. Furrows are more parallel, and germination is improved. Protection chemicals and fertilizers can be applied at once. The number of mechanized operations once the crop develops is reduced. Soil compaction is decreased, and adequate soil consolidation promotes better soil-seed contact (Compagnon *et al*., 2017).

Few sugarcane producer countries have promoted mechanized planting. They use planting machines that perform all the tasks in an integral form (Ripoli and Ripoli, 2010). Generally, those machines handle pieces of sugarcane stalks that are provided by a mechanical harvester (Nova, 2009). However, despite more than a decade of experiences, the main problem is that the amount of seed has not been reduced (Robotham, 2004), and commonly, buds show damages due to deficient handling from harvesters, affecting germination. Nonetheless, whatever planting method is used in Mexico, the quality of the job is less than optimum, expensive, germination is poor (around 70 %), and plant emergence is unreliable. Usually, replanting has to be undertaken on defective spots. The growth of seedlings other than bud chips for seed material is expanding (CONADESUCA, 2017a; Radha *et al*., 2010). Although sugarcane can be planted using seedlings (Patnaik *et al*., 2016), it requires more intensive soil processing compared to using billets, as a suitable tilth and loose soil has to make good contact with roots from seedlings in order to achieve a rapid adaptation to this new environment.

Mechanized planting of sugarcane using seedlings can guarantee an improved crop establishment and could reduce planting costs when compared with the traditional procedure (Srathongtiw and Choedkiatphon, 2022). Similarly, planting operations can be quickly carried out. A uniform planting depth can be achieved, helping develop a healthy root system. However, the need for nurturing seedling facilities, the supply of growing inputs, and the labor involved in germination of buds and management of seedlings until they are ready for field transplanting makes this system expensive (Palma-López, 2018). Similarly, as enough care is put on growing seedlings, it is also important that field transplanting is carried out efficiently, adding that manual transplanting is costly and inefficient (Castillo *et al*., 2003).

Personnel of the Postgraduate College were involved in the design, development, and manufacture of a transplanting device for sugarcane seedlings. A protocol was scheduled to carry out a set of field trials to evaluate the performance of this equipment. Factors to be analyzed were reliability, field capacity, and precision when planting into the soil. The above will lead to the promotion of technological development to increase planting efficiency and reduce production costs of sugarcane cropping.

MATERIALS AND METHODS

The Motzorongo Regional Experimental Center (CERMOTZ) in Veracruz, Mexico, has gained experience for growing, caring for, and handling sugarcane seedlings. They also provide advice on manual transplanting; thus, their expertise was valuable for the purpose of this study. A randomized sample of 100 sugarcane (*Saccharum* spp. hybrids) sprouts from several varieties, of the same age and ready for transplanting, were selected for physical identification (Figure 1). Useful dimensions were selected for manufacturing materials and designing mechanical equipment that ensures the precise distribution of seedlings in the field, such as the dosing mechanism. The main parameters were those for the root system capsule, including shape, large and small diameter, height, resistance (moisture content, root size), weight, and the size of final foliage before transplanting.

Mechanical seedling transplantation can be a substitute for conventional sugarcane manual planting, where the operation uses seedlings sprouted from several weeks. This is achieved by designing some mechanical mechanisms according to the specific features exhibited by seedlings, likely dimensions of root portion, weight, and total length of seedling for planting, as well as caring processes. Calculating those mechanisms is required to adjust planting density. Furthermore, a successful system of this kind can increase its versatility by including novel attachments for spraying sanitary and growth facilitators as seedlings reach the soil.

CERMOTZ makes business by selling sugarcane seedlings at a cost of $0.15 USD each, regardless of variety, with a maximum age of two months. Seedlings have uniform physical dimensions; their root capsule has a cone shape, with large and small diameters of 65 and 40 mm, respectively, and a height of 60 mm. When seedlings are

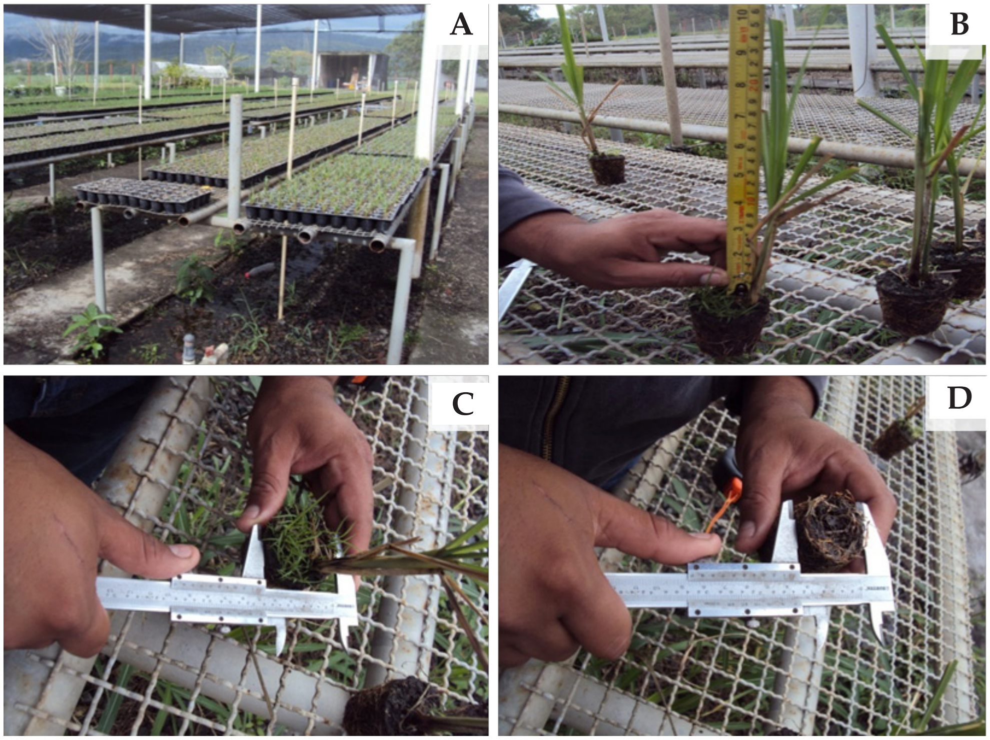


Figure 1. Evaluation of the main dimensions of sugarcane (*Saccharum* spp. hybrids) seedlings raised at the Motzorongo Regional Experimental Center in Veracruz, Mexico. A: nursery for sugarcane seedlings; B: measurement of sugarcane seedling height; C: measurement of the large diameter of the root system capsule; D: measurement of the small diameter of the root system capsule.

delivered to buyers, CERMOTZ personnel cut the top end of foliage so the seedling final length is 200 mm from the top side of the root system capsule (Figure 2).

The information was useful for designing and setting the specifications for the transplanting mechanism, as well as the entire transplanting module. This planter prototype weighs 350 kg and has the following dimensions: 1.4 m high, 1.25 m wide, and 2 m long. Simultaneously, it was necessary to record the manual method for field transplanting of the seedlings. The labor required, time consumption, activities needed, and keeping track of each task were all recorded. The latter was required to improve and reduce operations, as well as to assign value to those tasks according to a level of difficulty to estimate the entire cost of the operation.

CERMOTZ follows a sequence of detailed operations for manual transplanting: where furrows have already been constructed, the separation distance between seedlings is marked on the bottom of the furrows; a hole is dug on each marked spot and a sprout is placed inside it; and finally, the seedling is covered with soil and water. As a reference, transplanting into 1.2 m-separated rows with a 700 mm spacing between

Figure 2. Two-month-old sugarcane (*Saccharum* spp. hybrids) seedlings ready for planting.

seedlings requires 11 904 seedlings per hectare and 18 laborers who charge $8.5 USD each. Thus, labor costs for transplanting are $153 USD per hectare.

The main component from the planter prototype is a carrousel-type distributor with five free-ends vertical tubing. Seedlings with a diameter less than 60 mm can go into those compartments, and when in operation, the distributor turns because of the transmission through the drive wheel when the tractor moves (Figure 3). The discharge tube is located at the bottom section of the distributor, so when turned, the bottom end of one compartment lines up exactly with it, and the seedling slips through the tube into the open furrow, followed by a soil pressing around the seedling to keep it upright, and loose soil is added around it by cover discs. The largest length

Figure 3. Carrousel-type distributor for sugarcane (*Saccharum* spp. hybrids) seedlings dropping into the soil.

allowed for a seedling to slip freely through the discharge tube without being pulled by the tube itself because of forward movement once the seedling is into the soil is 300 mm. When the drive wheel turns, it connects to a calibration mechanism that matches the level of turning of the seedling distributor, so by exchanging some spare parts, the distance each seedling travels in the field can be changed.

Evaluation of the mechanical planting of sugarcane seedlings was carried out on a field prepared for planting at the Postgraduate College Campus Cordoba (18° 51′ 20″ N, 96° 51′ 37″ W, at an altitude of 660 m). The soil on this site has a sandy clay texture and a 500 mm arable layer, and the plot is lightly covered by 150 mm-diameter stones on average. This soil is representative of the sugarcane production region (Figure 4). Performance was evaluated during field work on the transplanting mechanical planter prototype. The main parameters measured were: a) planting distance between seedlings; b) positioning condition; c) soil covering; and d) planting depth. Similarly, the machine's power demand for field transplanting, fuel consumption from the mobile power device, field efficiency for transplanting operations, and time savings and operation costs were compared to manual transplanting.

Figure 4. Evaluation of the mechanical transplanting device for sugarcane (*Saccharum* spp. hybrids) seedlings in fields at the Postgraduate College Campus Cordoba, Veracruz, Mexico.

Furthermore, the transplanting capacity was measured by varying the travel velocity from 0.3 to 2.5 m s^{-1}. The objective was to compare efficiency, field behavior, and transplanting capacity to manual planting with seedlings and varying travel speed up to a maximum slippage of 12 % from the tractor's drive wheels. Once transplanted, the distance between seedlings was measured to determine how much they differed from the initial set of 900 mm. Seedlings placed at various inclination angles were recorded.

RESULTS AND DISCUSSION

The device's performance was evaluated, and its behavior was examined in the field under various operating conditions. When operating in a straight line, an average travel speed of 0.3 m s^{-1} was selected and adjusted to ensure that a seedling was properly placed every 900 mm. Three sections of the planting field, each 50 m long, were chosen at random for accounting measurements. The tractor and planter prototypes spent an average of 140 s planting each 50-m section. After data analysis, it was discovered that seedling separation placement had an average efficiency of 3.4 % higher than established.

For the field transplanting process, power demand was calculated as 20.52 kW per planting unit, average fuel consumption was determined as 13.28 L ha^{-1}, and by using data from operation timing and costs, a comparison was made against manual transplanting and also conventional planting using stalk billets. In all cases, trials showed that a mechanized system for transplanting sugarcane seedlings has the largest advantages. Field cleaning from previous crops and soil preparation for the seedbed are not included in the calculations.

The planter prototype had a field capacity of 2.5 ha d^{-1} on a plot with previously worked soil and an average travel speed of 1.2 m s^{-1}, with seedling spacing of 900 mm and rows separated by 1.2 m. Therefore, with similar field and soil conditions and a single transplanting unit operated by two laborers, it is possible to plant 12 000 seedlings in an eight-hour shift. Field capacity and efficiency can be increased by attaching two units on the toolbar, based on the tractor's capacity. Therefore, it was determined that the operation cost for the transplanting device is $18.3 USD per hectare; thus, the capital investment for the machine can be recovered in two years, as long as it operates at least on 120 d per season and maintains a field capacity of 2 ha d^{-1}. The effectiveness of the transplanter, like any mechanized task in sugarcane, is subject to human factors in terms of their skills and abilities, as well as other factors such as soil and weather conditions.

One of the best features of this planter prototype was soil moisture conservation; by opening a slot into the soil, placing the seedling, and closing the furrow after seedling transplantation, there is no wet soil exposed to the environment or sun radiation. This process is achieved with 99 % efficiency. Finally, it was observed that 9 out of 10 seedlings remained upright after reaching the bottom of the furrow, indicating a slight inclination towards the direction of movement due to inertia.

Mechanized seedling transplantation can be carried out as a planting task. Its main advantage is that the seedlings have already germinated; the best ones with an average length of 250 mm are selected, while those with less vigor are discarded. As a result, it is possible to guarantee a sound crop establishment of more than 90 %, with uniform field distribution and parallel rows. The mainframe of the planter prototype will allow in the near future to add apparatus for applying sanitary and agrochemical products for securing a better crop establishment and plant nutrition in order to reduce crop production costs, although the cost of the machine will increase (Naik *et al*., 2013, 2015).

CONCLUSIONS

The transplantation process of sugarcane (*Saccharum* spp. hybrids) seedlings by using the mechanical planter prototype was 99 % efficient when carried out at an average forward velocity of 0.3 m s^{-1}. In addition, adequate uniformity was achieved for seedling depth placement in the soil, enough soil was added around the seedling after planting, and 2 % of lay-down seedlings were observed after machine discharge onto the furrow. Efficiency for the separation distance between seedlings into the soil was 3.4 % higher than established. Thus, operating the planter prototype along 120 days during the planting season in order to achieve a field capacity of 2 ha day^{-1} had a reduced operation cost by up to eight times than manual transplanting. Capital investment on the machine can be recovered in two years' time.

ACKNOWLEDGEMENTS

To the manufacturing workshop Talleres y Servicios Agroindustriales Gómez S.A. de C.V. (TYSAGSA), who provided facilities, raw materials, machine tools, and technicians for manufacturing the planter prototype. To enterprise Dampf Ingeniería S.A. de C.V. (D.I.) for financing the purchase of the assembly components, drives, and transmission elements. To Centro Experimental Regional Motzorongo (CERMOTZ), who provided the expertise from technicians and donated seedlings for the trials.

REFERENCES

Castillo RO, Gómez A, Garcés F. 2003. Multiplicación masiva de semilla sana de variedades de caña de azúcar mediante cultivo de tejidos vegetales. Centro de Investigación de la Caña de Azúcar del Ecuador. El Triunfo, Ecuador. 9 p.

Compagnon AM, da Silva RP, Furlani CEA, Cavichioli FA, Arriel FH. 2017. Operational uniformity for a sugarcane planter. African Journal of Agricultural Research 12 (12): 953–962. https://doi.org/10.5897/ajar2016.11300

CONADESUCA (Comité Nacional para el Desarrollo Sustentable de la Caña de Azúcar). 2017a. Producción de plantas de caña de azúcar *in vitro*. Gobierno de México. Secretaría de Agricultura y Desarrollo Rural. Comité Nacional para el Desarrollo Sustentable de la Caña de Azúcar. Ciudad de México, México. 10 p.

CONADESUCA (Comité Nacional para el Desarrollo Sustentable de la Caña de Azúcar). 2017b. Estadísticas de la Zafra 2015–2016. Gobierno de México. Secretaría de Agricultura y Desarrollo Rural. Comité Nacional para el Desarrollo Sustentable de la Caña de Azúcar. Ciudad de México, México. https://www.gob.mx/conadesuca/documentos/estadisticas-conadesuca (Retrieved: November 2023).

Naik R, Annamalai SJK, Nair NV, Prasad NR. 2013. Studies on mechanisation of planting of sugarcane bud chip settling raised in protrays. Sugar Tech 15 (1): 27–35. https://doi.org/10.1007/s12355-012-0187-4

Naik R, Annamalai SJK, Nair NV, Prasad NR. 2015. Mechanization package for chipping and planting of sugarcane bud chips grown in protrays for sustainable sugarcane initiative in India. Agricultural Mechanization in Asia, Africa and Latin America 46 (4): 14–21.

Nova VJ. 2009. Siembra mecanizada de la caña de azúcar en Brasil. Revista Tecnicaña 13 (22): 31–36.

Ortiz-Laurel H, Rosas-Calleja D, Rössel-Kipping D, Salgado-García S, Debernardi-de la Vequia H. 2016. Efectividad y rentabilidad de técnicas de siembra de caña de azúcar (*Saccharum* spp.). Agroproductividad 9 (3): 40–47.

Palma-López D. 2018. Evaluación de sustratos orgánicos para la producción de plántulas de caña de azúcar. Agroproductividad 11 (12). https://doi.org/10.32854/agrop.v11i12.1318

Patnaik JR, Singh SN, Sarangi D, Nayak PK. 2016. Assessing potentiality of bud chip technology on sugarcane productivity, profitability and sustainability in real farming situations under south east coastal plain zone of Odisha, India. Sugar Tech 19 (4): 373–377. https://doi.org/10.1007/s12355-016-0481-7

Radha J, Solomon S, Shrivastava AK, Chandra A. 2010. Sugarcane bud chips: A promising seed material. Sugar Tech 12 (1): 67–69. https://doi.org/10.1007/s12355-010-0013-9

Ripoli MLC, Ripoli TCC. 2010. Evaluation of five sugar cane planters. Engenharia Agrícola 30 (6): 1110–1122. https://doi.org/10.1590/s0100-69162010000600012

Robotham BG. 2004. Sugarcane planters: Characteristics of different types, soil disturbance and crop establishment. Proceedings of the Australian Society of Sugar Cane Technology 26.

Srathongtiw T, Choedkiatphon S. 2022. Development of sugarcane seedling transplanter. Rajamangala University of Technology Srivijaya Research Journal 14 (1): 62–77.

Yadav RNS, Yadav S, Tejra RY. 2003. Labour saving and cost reduction machinery for sugarcane cultivation. Sugar Tech 5 (1–2): 7–10. https://doi.org/10.1007/bf02943757

Agrociencia

EPIDEMIOLOGY OF THE ZOONOTIC NEMATODES PARASITING TILAPIES OF THE GENUS *Oreochromis* (Perciformes: Cichlidae) WITH SOCIOECONOMIC IMPORTANCE

Víctor Johan **Acosta-Pérez**[1], Fabián Ricardo **Gómez-De Anda**[1], Vicente **Vega-Sánchez**[1],
Nydia Edith **Reyes-Rodríguez**[1], Armando **Peláez-Acero**[1],
Jesús Benjamín **Ponce-Noguez**[2], Jorge Luis **de la Rosa-Arana**[3]*

[1]Instituto de Ciencias Agropecuarias. Área Académica de Medicina Veterinaria y Zootecnia. Avenida Universidad km 1, Rancho Universitario, Ex-Hacienda de Aquetzalpa, Tulancingo de Bravo, Hidalgo, Mexico. C. P. 43600.
[2]Universidad Autónoma de Chiapas. Facultad Maya de Estudios Agropecuarios. Carretera Catazajá-Palenque km 4, Catazajá, Chiapas, Mexico C. P. 29980.
[3]Universidad Nacional Autónoma de México. Facultad de Estudios Superiores Cuautitlán. Avenida 1o. de Mayo S/N, Campo 1, Cuautitlán Izcalli, State of Mexico, Mexico. C. P. 54743.
* Author for correspondence: jorgeluis.delarosa.arana@cuautitlan.unam.mx

Citation: Acosta-Pérez VJ, Gómez-De Anda FR, Vega-Sánchez V, Reyes-Rodríguez NE, Peláez-Acero A, Zepeda-Velázquez AP, Ponce-Noguez JB, de la Rosa-Arana JL. 2024. Epidemiology of the zoonotic nematodes parasiting tilapies of the genus *Oreochromis* (Perciformes: Cichlidae) with socioeconomic importance. **Agrociencia** 58(8): 943-953. https://doi.org/ 10.47163/ agrociencia.v58i8.2843

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: July 13, 2022.
Approved: October 23, 2024.
Published in Agrociencia:
December 02, 2024.

ABSTRACT

This work aimed to review the main epidemiological data on zoonotic nematodes parasitizing tilapia of the genus *Oreochromis* (Perciformes: Cichlidae) with socioeconomic importance. The literature review was conducted in six specialized search engines, and a total of 320 articles were analyzed. After eliminating documents with duplicated information or those that did not refer to zoonotic nematodes in cichlids, 10 bibliographic sources were processed. The analysis showed that the available documentation refers mainly to the genera *Gnathostoma* in America and *Contracaecum* in Africa, while data are scarce in Europe, Asia, and Oceania. This work includes information on prevalence, distribution, and diagnosis, both from natural and artificial environments. In conclusion, data on the epidemiology of zoonotic nematodes that parasitize tilapia of the genus *Oreochromis* are scarce, so attention should be paid to monitoring economically important fish to prevent parasitological problems in the production chain.

Keywords: *Oreochromis*, *Gnathostoma*, *Contracaecum*, aquaculture, fish meat, parasites.

INTRODUCTION

The development of new and improved technologies in the production of fish meat has economically benefited the industry, which had sustained growth during the last two decades. Currently, more than 173 million Mg of fish are produced (Quiazon, 2015). Tilapia, which refers to 70 species belonging to the Cichlidae family, is of great socioeconomic importance worldwide. The taxonomic classification of tilapia is complex due to the constant changes resulting from the hybridization that takes place naturally. However, there are three main genera: *Oreochromis*, *Sarotherodon*, and *Tilapia*, the first being the most relevant since the 32 species it comprises are resistant to diseases, reproduce rapidly, and adapt easily to changes in environment, food, and water quality (Vega-Villasante *et al*., 2010).

Tilapia has been introduced for aquaculture purposes in more than 90 countries around the world (Vega-Villasante *et al.*, 2010); however, some management practices are limited, resulting in the presence and spread of parasites (Shechonge *et al*., 2019). In Mexico, the production of *Oreochromis* spp. is equivalent to 60 % of national production (Domínguez-May *et al*., 2020), so food safety programs have been carried out to ensure the health and sustainable expansion of production chains. However, the information that exists on parasitic transmission from fish to humans is scarce, becoming a latent risk factor in public and veterinary health (Garrido-Olvera *et al*., 2017), which implies a challenge in the search for the integral management approach in the meat production and consumption chain.

Zoonotic nematodes are those helminths that can potentially be transmitted to humans by the consumption of raw or insufficiently cooked meat harboring parasite larvae. Most of the records on fish-transmitted nematode larvae correspond to the genera *Gnathostoma* (Carod-Artal *et al*., 2017) and *Contracaecum* (Ogutu-Ohwayo *et al*., 2016). Thus, the objective of this study was to make a literature review on the epidemiology of zoonotic nematodes parasitizing tilapia of the genus *Oreochromis* with socioeconomic importance.

MATERIALS AND METHODS

The literature review was performed with search engines using the keywords "zoonotic parasite," "foodborne parasites," "*Oreochromis,*" and "tilapia," together with variants that refer to the parasites or the disease they produce, such as *Gnathostoma* spp. (gnathostomiasis) and *Anisakis* spp. (anisakosis). From these words, the inclusion "*Gnathostoma* or gnathostomiasis and *Oreochromis* or tilapia" was structured for each parasitic organism or disease. Six specialized literature search engines were used (ScienceDirect, PubMed, Primo, CONRICyT, LILAES, and AJOL), from which a total of 320 search results were obtained.

Subsequently, articles referring to taxonomic groups of parasites and fish outside the scope of this review and duplicate bibliographic sources were eliminated, and those with epidemiological data on the zoonotic nematode-typhoid binomial were selected. This resulted in a total of 10 bibliographic sources for the information analyzed in this review. Then, information was captured for the presentation of data and the preparation of the geographical distribution map based on the background information available by country, using the open access program RStudio (Boston, MA, USA).

RESULTS AND DISCUSSION

Prevalence and distribution

Human activities have a direct impact on the incidence and spread of parasites that can be transmitted zoonotically. The main activities associated with zoonotic transmission of nematodes are the culinary habit of consuming raw or undercooked meat, tourism, migratory flows (Williams *et al*., 2020), and, of course, the introduction of different fish species for zootechnical purposes. The global distribution of zoonotic transmission nematodes (Figure 1) indicates the presence of *Gnathostoma* spp. in the Americas and *Contracaecum* spp. in Africa, involved in the tilapia production and consumption chain. It is important to consider that nematodiasis associated with the consumption of raw or undercooked meat of cichlid fish (species other than tilapia) is also reported in the Americas and in Mexico (DOF, 2009). In particular, gnathostomiasis, which is associated with the consumption of raw tilapia meat as the second intermediate host of *Gnathostoma spinigeru*, *G. turgidum,* and *G. binucleatum* (Diaz-Camacho *et al*., 2002; Mosqueda-Cabrera *et al*., 2009), is the subject of epidemiological surveillance (DOF, 2009).

Gnathostoma spp. are distributed in the states of Campeche, Chiapas, Guerrero, Jalisco, Nayarit, Oaxaca, Tabasco, and Veracruz; an outbreak of gnathostomiasis was even recorded due to the introduction of cichlids (species different from tilapia) in the Miguel Alemán dam in the state of Oaxaca (Martínez-Cruz *et al*., 1989). *G. binucleatum* has been recorded in *Oreochromis aureus*, *O. mossambicus*, and *O. niloticus* in the Temascal dam

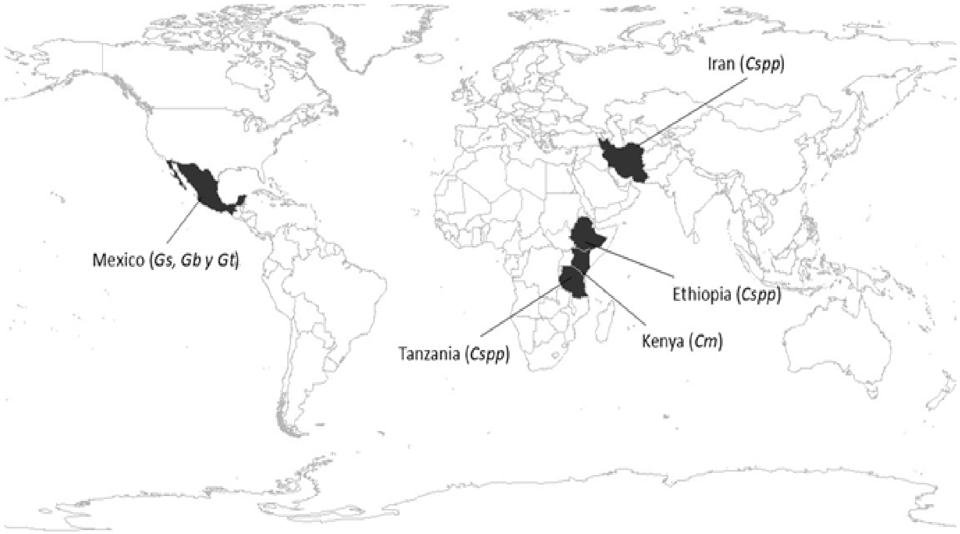


Figure 1. Distribution of nematodes with zoonotic potential associated with the production and consumption of raw or undercooked tilapia meat. Nematodes are identified as *Gnathostoma spinigerum* (*Gs*), *G. binucleatum* (*Gb*), *G. turgidum* (*Gt*), *Contracaecum* spp. (*Cspp*), and *C. multipapillatum* (*Cm*).

in Oaxaca, with a prevalence of 1.2, 1.7, and 2.1 %, respectively (Salgado-Maldonado *et al*., 2005). In Veracruz, *G. binucleatum* was found with a prevalence of 26 % in *O. massambicus* and 7 % in *O. niloticus* (León-Règagnon *et al*., 2005).

The presence of *Contracaecum* spp. has been documented in Kenya, Tanzania, and Ethiopia (Yimer, 2000; Adugna, 2020; Chibwana *et al*., 2020). *Oreochromis niloticus* and *Tilapia galileae* from Lake Nasser in Egypt have been documented with a prevalence of 35.6 and 0.14 %, respectively (Motamedi *et al*., 2019), while in Lake Turkana in Kenya, the nematode was found with a prevalence of 15.9 to 18.2 % in *Tilapia zillii* (Otachi *et al*., 2015). *O. amphimelas* from the Nyumba ya Mungu dam in Tanzania was documented with a prevalence of 47.3 % in commercially sized fish (20 to 30 cm in length); the prevalence in males was 46.7 %, and in females, 33.3 % (Chibwana *et al*., 2020). However, in the Gojam zone of Ethiopia, a prevalence of 58.8 % was reported for *Contracaecum* spp. in tilapia production farms (Adugna, 2020).

In Europe, the consumption of fish meat is recorded at 24.33 kg per capita per year, placing the inhabitants of the continent as one of the populations at highest risk. *Gnathostoma* spp. has been reported in England, where its presence has been documented in humans who acquired the infection in Botswana, a landlocked country in southern Africa (Herman *et al*., 2009). Asia is the continent with the highest aquaculture and fishery production worldwide, where China is one of the main tilapia producers. However, the only reports of *Contracaecum* spp. are from Iran (Table 1) (Motamedi *et al*., 2019).

Unfortunately, in different geographic areas, the monitoring of fish-borne zoonotic agents is low profile. The incidence of clinical cases associated with the consumption of raw tilapia meat from aquaculture systems is absent, largely due to the sanitary

Table 1. Summary of the prevalence and location of zoonotic nematode larvae in tilapias of socioeconomic value.

Country	Parasites	Prevalence (%)	Host	Anatomical distribution	Reference
Mexico	*Gnathostoma spinigerum,* *G. binucleatum,* *G. turgidum*	1.2–80	*Oreochromis niloticus,* *O. mossambicus,* *O. aureus*	Muscle	Diaz-Camacho *et al*. (2002); León-Règagnon *et al*. (2005); Mosqueda-Cabrera *et al*. (2009); Salgado-Maldonado *et al*. (2005)
Ethiopia	*Contracaecum* spp.	5.5–27.4	*Oreochromis niloticus,* *Tilapia zillii*	Gills, mesentery, heart	Gulelat *et al*. (2013)
Kenya	*Contracaecum multipapillatum*	-51.8–19.6	*Oreochromis leucostictus,* *Tilapia zillii*	Gills, mesentery, heart, intestine, liver	Otachi *et al*. (2014a, 2014b, 2015)
Tanzania	*Contracaecum* spp.	55.6	*Oreochromis aureus*	Mesentery	Chibwana *et al*. (2020)
Iran	*Contracaecum* spp.	0.1–35.6	*Oreochromis niloticus,* *Tilapia galileae*	Mesentery	Motamedi *et al*. (2019)

control system of each fish farm as well as the sanitary inspections carried out by the meat distribution companies. Despite the high risk of infection due to the consumption of fish over which there is no sanitary control and which are often consumed in "artisanal" culinary dishes based on raw or insufficiently cooked meat, the epidemiological report of the clinical case is ambiguous because it considers the clinical manifestation as "food poisoning." Few are the cases that conclude with a laboratory diagnosis (Acosta-Pérez *et al*., 2022); at least in Mexico, epidemiological surveillance of other zoonoses, such as taeniasis and cysticercosis, transmitted by pork consumption, have continuous monitoring and well-defined diagnostic strategies (Hernández-Ramírez *et al*., 2023).

Life Cycles

Nematodes of the genus *Gnathostoma* belong to the Gnathostomidae family. Their life cycle begins with dioecious adults lodged in the intestine of the definitive host (dogs, cats, pigs). The female oviposits eggs, which are released with the feces, which must be deposited in a freshwater environment so that the larvae of the first stage of development (L1) are released and consumed by a copepod (Crustacea), which acts as the first intermediate host where the L2 larvae develop (Diaz-Camacho *et al*., 2002). Secondary intermediate hosts such as tilapia become infected by ingesting L2 larvae lodged in the copepods. Subsequently, the L3 stage develops in muscle tissue.

Consumption of raw fish meat constitutes the source of infection for three different types of hosts. First, for transport or paratenic hosts (piscivorous birds). Secondly, for the definitive host (carnivorous mammal), which may consume the infected fish or the transport host. In the third case, humans become an accidental host, where L3, the etiological agent of gnathostomiasis, will migrate to the brain, eyes, viscera, or skin (Figure 2) (Martínez-Cruz *et al*., 1989; Leroy *et al*., 2017), but will not reach the adult stage.

Contracaecum is a nematode of the family Anisakidae that uses fish as intermediate hosts in its life cycle (Figure 3). The eggs hatch in the marine environment with the feces of the definitive host; in the water and inside the egg, the larval stages L1 and L2 develop. The egg hatches, and the L2s are ingested by a copepod, which is the first intermediate host where L3s develop. When infected copepods are ingested by fish, these become transport hosts. L3 can be transmitted several times from fish to fish until they are ingested by a piscivorous bird or marine mammal. Humans or other organisms with ichthyophagous habits become accidental hosts by the ingestion of raw or undercooked fish meat (Shamsi, 2019). In humans, L3s do not develop in adults, but parasite antigens provoke anaphylactic reactions.

Definitive hosts

Parasitic prevalence is rarely described in all hosts involved in the food web where tilapia participate, causing the diagnosis to occur fortuitously. In definitive hosts that are infected by ichthyophagous habits, parasitological evaluation is systematized and

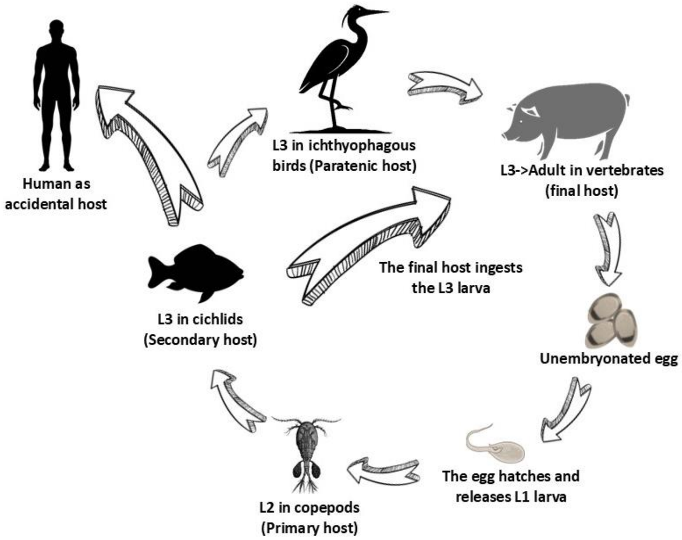


Figure 2. Life cycle of *Gnathostoma* spp. Humans become infected by eating raw or undercooked fish meat (tilapia, for example), where viable L3 larvae are found. Adapted from Moore *et al*. (2003).

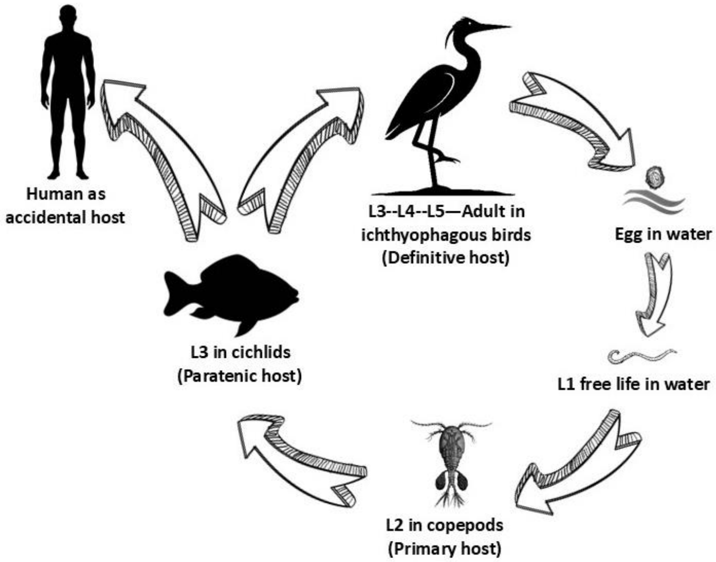


Figure 3. Life cycle of *Contracaecum* spp. L1, L2, and L3 refer to the larval stages in the different hosts involved in the life cycle. Adapted from Valles-Vega *et al*. (2017).

is usually associated with fauna living near water bodies (Pérez-Ponce de León *et al*., 2018).

In the life cycle of *Gnathostoma* spp., a wide variety of naturally infected definitive hosts have been identified, including herons (*Egretta alba* and *E. thula*), the white American pelican (*Pelecanus erythrorhynchos*), the blue heron (*Ardea herodias*), neotropical cormorant (*Phalacrocorax olivaceous*), the brown pelican (*Pelecanus occidentalis*), opossum (*Didelphis virginiana*) (Diaz-Camacho *et al*., 2002), turtles (*Kinosternon integrum*), and the amphibian leopard frog (*Lithobates zweifeli*) (Mosqueda-Cabrera *et al*., 2009). In addition, experimental infections by *Gnathostoma binucleatum* have been reported viable in dogs, evidencing that some domestic animals can act as definitive hosts (Álvarez-Guerrero *et al*., 2018). Likewise, humans are potential accidental hosts, where *Gnathostoma* spp. larvae do not develop into adults. Nevertheless, parasitic infections can lead to relevant conditions (Herman, 2009).

In Mexico, *Contracaecum microcephalum* has only been recorded in Mexico City on the birds *Nycticorax nycticorax*, *Pelecanus erythrorhynchus*, and *Anhinga anhinga*; in the State of Guerrero, *Pelecanus erythrorhynchus* has been identified, while *C. caballeroi* has only been recorded once on *Anhinga anhinga* in the Chapultepec Zoo (Fagerholm and Overstreet, 2009).

Diagnosis in tilapia

The diagnosis of parasites in cichlid fish is carried out by necropsy; in this procedure, different parasitic stages are recovered, including L3 larvae that can infect humans and other accidental hosts. The parasites recovered are processed by staining to show morphological structures that allow their identification by means of taxonomic keys (Sepulveda and Kinsella, 2013). In addition, Mexican regulations indicate the candling procedure for parasite identification (DOF, 2009). Likewise, microscopic analysis of larvae in muscle includes the observation of sectioned meat or organs between two glass plates using low magnification objectives (Diaz-Camacho *et al*., 2002). However, it is becoming increasingly relevant that observations are complemented with molecular diagnostic techniques. This is indispensable for the confirmation and identification of etiological agents in fish as well as in human clinical cases. For example, for the identification of nematodes of the genus *Contracaecum*, the amplification of the ITS2 regions, including the 5.8S rRNA gene (Motamedi *et al*., 2019), and the ITS1 region (Otachi *et al*., 2015), has been documented.

Tilapia as a transmitter of zoonotic nematodes and their impact on human health

The consumption of culinary dishes that include raw tilapia meat, such as sushi or sashimi, is a risk factor for the transmission of nematodes with zoonotic potential (Leroy *et al*., 2017). Some adulterations in marketing, such as species substitution in the meat industry, increase the risk for consumers. Previously, the presence of larvae of the nematode *Gnathostoma* spp. was reported, in addition to sporozoites of *Cryptosporidium parvum* and adults of the cestode *Diphyllobothrium latum* in tilapia, which is sold as a substitute for snapper fish (Williams *et al*., 2020), which is a traditional fish in the sushi trade, an internationally popular culinary preparation of Japanese gastronomy, based on rice and raw fish meat (Vicente-Pardo, 2016).

Frequently, human clinical diagnosis of nematodes is based on epidemiology, blood chemistry (hypereosinophilia and positive serological test), and isolation of larvae in skin biopsies (Leroy *et al*., 2017). For example, in England, two clinical cases of human gnathostomiasis were followed up, where at 2- and 5-weeks post-infection, intermittent abdominal discomfort was reported, along with pain in body mobility, spleen swelling, pruritus in the right axilla and groin, a subcutaneous lump at the level of the thorax, larval mobility, swelling, and pain and itching in the knee. Regarding eosinophilia, 0.69 × 109 and 0.9 × 109 L1 cells were recorded, respectively in each case (Herman, 2009).

The clinical manifestations caused by *Gnathostoma* spp. may include skin damage caused by the migratory larvae (Martínez-Cruz *et al*., 1989; Diaz-Camacho *et al*., 2002), as well as visceral disease, including hepatic, pulmonary, and gastrointestinal conditions, less frequently urogenital, and in the most severe cases, cerebral and ocular involvement (Carod-Artal *et al*., 2017) that can induce radiculomyelitis with paraplegia, myeloradiculoencephalitis, and subarachnoid or cerebral hemorrhage (Leroy *et al*., 2017).

Clearly, the next step after diagnosis is the therapeutic treatment of nematodiases, for which different anthelmintics such as diethylcarbamazine, ivermectin, albendazole, and praziquantel have been used, in some cases combined with dexamethasone (Herman *et al*., 2009; Leroy *et al*., 2017). However, the information analyzed indicates that there is no standardized treatment. Thus, the characterization and reporting of the parasitosis show areas of opportunity in the study of zoonotic diseases caused by the consumption of raw or insufficiently cooked tilapia meat.

CONCLUSIONS

Tilapia species act as a host and reservoir for parasites with zoonotic potential that can lead to sanitary problems in cichlid populations and public health. Considering that tilapia is a fish resistant to changes in diet and environment, it is often preferred over other species in aquaculture; however, it is also considered an invasive species that limits the growth of native populations (Mendoza-Alfaro and Koleff-Osorio, 2014). Human infections originate from the consumption of raw or undercooked tilapia meat that harbors viable larvae, which try to complete their life cycle, causing clinical problems. Thus, zoonotic parasites pose the need to develop and implement epidemiological surveillance programs within a comprehensive and unifying “one health” approach to balance and optimize the health of people, animals, and ecosystems.

ACKNOWLEDGEMENTS

Víctor Johan Acosta-Pérez, Fabián Ricardo Gómez-De Anda, Vicente Vega-Sánchez, Nydia Edith Reyes-Rodríguez, Andrea Paloma Zepeda-Velázquez, Jesús Benjamín Ponce-Noguez, and Jorge Luis de la Rosa-Arana are fellows of the *Sistema Nacional de Investigadoras e Investigadores* (SNII) (National System of Researchers) of the *Consejo Nacional de Humanidades, Ciencias y Tecnologías* (CONAHCYT) of Mexico.

REFERENCES

Acosta-Pérez VJ, Ángeles-Hernández JC, Vega-Sánchez V, Zepeda-Velázquez AP, Añorve-Morga J, Ponce-Noguez JB, Reyes-Rodríguez NE, de la Rosa-Arana JL, Ramírez-Paredes JG, Gómez-de Anda FR. 2022. Prevalence of parasitic infections with zoonotic potential in tilapia: A systematic review and meta-analysis. Animals 12 (20): 2800. https://doi.org/10.3390/ani12202800

Adugna M. 2020. The prevalence of fish parasites of Nile tilapia (*Oreochromis niloticus*) in selected fish farms, Amhara Regional State. Ethiopian Journal of Agricultural Sciences 30 (3): 119–128.

Álvarez-Guerrero C, Ramírez-Valle A, de la Cruz-Moreno O, González-Morteo C. 2018. Infección experimental de *Gnathostoma binucleatum*, en *Canis familiaris* del municipio de Tepic, Nayarit, México. Abanico Veterinario 8 (1): 53–58. https://doi.org/10.21929/abavet2018.81.5

Carod-Artal FJ, García HH, Winkler AS, Ajzenberg D. 2017. Neurological syndromes or diseases caused by parasites in tropical areas. *In* Pierre-Marie P, Michel D. (eds.), Neuroepidemiology in Tropical Health. Elsevier: London, UK, pp: 233–246. https://doi.org/10.1016/B978-0-12-804607-4.00018-6

Chibwana FD, Mshana JG, Katandukila JV. 2020. A survey of fish parasites from Pangani Catchment and Lake Kitangiri in Singida, Tanzania. Tanzania Journal of Science 46 (1): 42–52.

Diaz-Camacho SP, Willms K, Ramos M, de la Cruz-Otero MC, Nawa Y, Akahane H. 2002. Morphology of *Gnathostoma* spp. isolated from natural hosts in Sinaloa, Mexico. Parasitology Research 88 (7): 639–645. https://doi.org/10.1007/s00436-002-0636-1

DOF (Diario Oficial de la Federación). 2009. NORMA Oficial Mexicana NOM-242-SSA1-2009, productos y servicios. Productos de la pesca frescos, refrigerados, congelados y procesados. Especificaciones sanitarias y métodos de prueba. Gobierno de México. Secretaría de Salud. Ciudad de México, México. https://www.dof.gob.mx/normasOficiales/4295/salud2a/salud2a.htm (Retrieved: September 2024).

Domínguez-May R, Poot-López GR, Hernández J, Gasca-Leyva E. 2020. Dynamic optimal ration size in tilapia culture: Economic and environmental considerations. Ecological Modelling 420 (7): 108930. https://doi.org/10.1016/j.ecolmodel.2020.108930

Fagerholm HP, Overstreet RM. 2009. Ascaridoid nematodes: *Contracaetum*, *Porrocaecum*, and *Baylisascaris*. *In* Atkinson CT, Thomas NJ, Hunter DB. (eds.), Parasitic Diseases of Wild Birds. Wiley-Blackwell: Ames, IA, USA, pp: 413–433. https://doi.org/10.1002/9780813804620.ch24

Garrido-Olvera L, Benavides-González F, Rábago-Castro JL, Pérez-Castañeda R, García-Prieto L. 2017. Endohelminths of fishes of commercial importance from Vicente Guerrero Reservoir, Tamaulipas, Mexico. Comparative Parasitology 84 (2): 194–200. https://doi.org/10.1654/1525-2647-84.2.194

Gulelat Y, Yimer E, Asmare K, Bekele J. 2013. Study on parasitic helminths infecting three fish species from Koka reservoir, Ethiopia. SINET: Ethiopian Journal of Science 36 (2): 73–80.

Herman JS, Wall EC, van Tulleken C, Godfrey-Faussett P, Bailey RL, Chiodini P. 2009. Gnathostomiasis acquired by British tourists in Botswana. Emerging Infectious Diseases 15 (4): 594–597. https://doi.org/10.3201/eid1504.081646

Hernández-Ramírez CV, Sánchez-García DC, Carpio-Pedroza JC, Alcántara-Anguiano I, Meza-Lucas A, Tapia-Romero R, de la Rosa-Arana JL. 2023. *Taenia solium*: Socioeconomic changes and prevalence evolution in four endemic communities in northwestern Mexico. Zoonoses Public Health 70 (5): 434–444. https://doi.org/10.1111/zph.13034

León-Règagnon V, Osorio-Sarabia D, García-Prieto L, Lamothe-Argumedo R, Bertoni-Ruiz F, Oceguera-Figueroa A. 2005. New host records of the nematode *Gnathostoma* sp. in Mexico. Parasitology International 54 (1): 51–53. https://doi.org/10.1016/j.parint.2004.10.001

Leroy J, Cornu M, Deleplancque AS, Loridant S, Dutoit E, Sendid B. 2017. Sushi, ceviche and gnathostomiasis - a case report and review of imported infections. Travel Medicine and Infectious Disease 20: 26–30. https://doi.org/10.1016/j.tmaid.2017.10.010

Martínez-Cruz JM, Bravo-Zamudio R, Aranda-Patraca A, Martínez-Marañón R. 1989. La gnatostomiasis en México. Salud Pública de México 31 (4): 541–549.

Mendoza-Alfaro R, Koleff-Osorio P. 2014. Especies acuáticas invasoras en México. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. Ciudad de México, México. 555 p.

Moore DAJ, McCroddan J, Dekumyoy P, Chiodini PL. 2003. Gnathostomiasis: An emerging imported disease. Emerging Infectious Diseases 9 (6): 647–650. https://doi.org/10.3201/eid0906.020625

Mosqueda-Cabrera MÁ, Miranda ES, Calderón LC, Ortiz-Nájera HE. 2009. Finding advanced third-stage larvae of *Gnathostoma turgidum* Stossich, 1902 in Mexico from natural and experimental host and contributions to the life cycle description. Parasitology Research 104 (5): 1219–1225. https://doi.org/10.1007/s00436-008-1318-4

Motamedi M, Iranmanesh A, Teimori A, Sadjjadi SM, Nasibi S. 2019. Detection of *Contracaecum multipapillatum* (Nematoda: Anisakidae) in the indigenous killifish *Aphanius hormuzensis* (Teleostei: Aphaniidae) and its histopathological effects: A review of Iranian *Aphanius* species parasites. Journal of Applied Ichthyology 35 (2): 558–569. https://doi.org/10.1111/jai.13848

Ogutu-Ohwayo R, Natugonza V, Musinguzi L, Olokotum M, Naigaga S. 2016. Implications of climate variability and change for African lake ecosystems, fisheries productivity, and livelihoods. Journal of Great Lakes Research 42 (3): 498–510. https://doi.org/10.1016/j.jglr.2016.03.004

Otachi E, Körner W, Avenant-Oldewage A, Fellner-Frank C, Jirsa F. 2014. Trace elements in sediments, blue spotted tilapia *Oreochromis leucostictus* (Trewavas, 1933) and its parasite *Contracaecum multipapillatum* from Lake Naivasha, Kenya, including a comprehensive health risk analysis. Environmental Science and Pollution Research 21 (12): 7339–7349. https://doi.org/10.1007/s11356-014-2602-8

Otachi E, Magana A, Jirsa F, Fellner-Frank C. 2014. Parasites of commercially important fish from Lake Naivasha, Rift Valley, Kenya. Parasitology Research 113 (3): 1057–1067. https://doi.org/10.1007/s00436-013-3741-4

Otachi E, Szostakowska B, Jirsa F, Fellner-Frank C. 2015. Parasite communities of the elongate tigerfish *Hydrocynus forskahlii* (Cuvier 1819) and redbelly tilapia *Tilapia zillii* (Gervais 1848) from Lake Turkana, Kenya: Influence of host sex and size. Acta Parasitologica 60 (1): 9–20. https://doi.org/10.1515/ap-2015-0002

Pérez-Ponce de León G, Lagunas-Calvo O, García-Prieto L, Briosio-Aguilar R, Aguilar-Aguilar R. 2018. Update on the distribution of the co-invasive *Schyzocotyle acheilognathi* (=*Bothriocephalus acheilognathi*), the Asian fish tapeworm, in freshwater fishes of Mexico. Journal of Helminthology 92 (3): 279–290. https://doi.org/10.1017/s0022149x17000438

Quiazon KMA. 2015. Updates on aquatic parasites in fisheries: Implications to food safety, food security and environmental protection. Journal of Coastal Zone Management 18: 396.

Salgado-Maldonado G, Aguilar-Aguilar R, Cabañas-Carranza G, Soto-Galera E, Mendoza-Palmero C. 2005. Helminth parasites in freshwater fish from the Papaloapan river basin, Mexico. Parasitology Research 96 (2): 69–89. https://doi.org/10.1007/s00436-005-1315-9

Sepulveda MS, Kinsella JM. 2013. Helminth collection and identification from wildlife. Journal of Visualized Experiments 82: e51000. https://doi.org/10.3791/51000

Shamsi S. 2019. Parasite loss or parasite gain? Story of *Contracaecum* nematodes in antipodean waters. Parasite Epidemiology and Control 4: e00087. https://doi.org/10.1016/j.parepi.2019.e00087

Shechonge A, Ngatunga BP, Bradbeer SJ, Day JJ, Freer JJ, Ford AGP, Kihedu J, Richmond T, Mzighani S, Smith AM *et al*. 2019. Widespread colonisation of Tanzanian catchments by introduced *Oreochromis* tilapia fishes: The legacy from decades of deliberate introduction. Hydrobiologia 832 (1): 235–253. https://doi.org/10.1007/s10750-018-3597-9

Valles-Vega I, Molina-Fernández D, Benítez R, Hernández-Trujillo S, Adroher FJ. 2017. Early development and life cycle of *Contracaecum multipapillatum* from a brown pelican *Pelecanus occidentalis* in the Gulf of California, Mexico. Diseases of Aquatic Organisms 125 (3): 167–178. https://doi.org/10.3354/dao03147

Vega-Villasante F, Cortés-Lara M, Zúñiga-Medina LM, Jaime-Ceballos B, Galindo-López J, Basto-Rosales MER, Nolasco-Soria H. 2010. Small-scale culture of tilapia (*Oreochromis niloticus*), alimentary alternative for rural and peri-urban families in Mexico? Revista Electrónica de Veterinaria 11 (4): 177–185.

Vicente-Pardo JM. 2016. El anisakis y sus enfermedades como enfermedad profesional. Medicina y Seguridad del Trabajo 62 (244): 223–240.

Williams M, Hernandez-Jover M, Shamsi S. 2020. Fish substitutions which may increase human health risks from zoonotic seafood borne parasites: A review. Food Control 118: 107429. https://doi.org/10.1016/j.foodcont.2020.107429

Yimer E. 2000. Preliminary survey of parasites and bacterial pathogens of fish at Lake Ziway. SINET: Ethiopian Journal of Science 23 (1): 25–33. https://doi.org/10.4314/sinet.v23i1.18154

Agrociencia

VIABILITY OF VITRIFIED EMBRYOS FROM ROMOSINUANO AND TROPICAL MILKING CATTLE OVER TWO SEASONS OF THE YEAR IN VERACRUZ, MEXICO

Sara del Refugio **Ávila-Rueda**[1], Carlos Miguel **Becerril-Pérez**[1,2*,] Adalberto **Rosendo-Ponce**[1],
Oscar Enrique **Zárate-Guevara**[3], Alejandra **Ramírez-Martínez**[1],
Alberto **Riaño-Gaya**[4], Froylan **Rosales-Martínez**[5]

[1]Colegio de Postgraduados Campus Veracruz. Carretera Xalapa-Veracruz km 88.5, Tepetates, Manlio Fabio Altamirano, Veracruz, Mexico. C. P. 91690.
[2]Colegio de Postgraduados Campus Montecillo. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, Estado de Mexico, Mexico. C. P. 56264.
[3]Universidad Veracruzana. Facultad de Medicina Veterinaria y Zootecnia. Miguel Ángel de Quevedo s/n, esquina Yáñez, Veracruz, Veracruz, México. C. P. 91710.
[4]Reproducción Genética Avanzada RGA *In vitro*. Cornuda 264, Fracc. Costa de Oro, Boca del Río, Veracruz, México. C. P. 94299.
[5]Universidad Autónoma de Chiapas. Facultad Maya de Estudios Agropecuarios. Carretera Catazajá-Palenque km 4, Catazajá, Chiapas, México. C. P. 29980.
* Author for correspondence: color@colpos.mx

ABSTRACT

The seasons of the year in tropical climates affect the behavior of female cattle of different breeds. The aim of this study was to determine the effect of the weather season on the viability of vitrified embryos on the Tropical Milking (TM) and Romosinuano (RM) breeds. Ultrasound-guided follicular aspiration sessions, *in vitro* fertilization, and embryo vitrification were carried out during two seasons: hot-dry (HD) and hot-humid (HH). A total of 31 RM and 27 TM donors were used, and data were collected on the viability of oocytes and embryos. The response variables observed in the devitrified embryos were the capacity of eclosion (CE), blastocele reconstitution (BL), trophoblast (TR), zona pellucida (ZP), and cell mass (CM). For cell viability, the number of live cells (LIV) and dead cells (DEC) were considered. The data were analyzed using a generalized linear model with the GENMOD procedure of SAS®. Multiple comparison tests and adjusted mean differences were performed with the Tukey test. An effect on the season × breed interaction ($p \leq 0.0001$) was observed in the characteristics studied. The TM breed displayed higher values in five characteristics ($p \leq 0.0001$), and the HH season ($p \leq 0.001$) in four characteristics. No breed effect was found on cell viability. However, in the HH season, the number of dead cells was lower ($p \leq 0.04$). Seasonal climatic variation in Southeastern Mexico had effects on the vitrified embryos.

Keywords: *Bos taurus* L., criollo breeds, genetic resources, hot climates, intertropical region, reproductive biotechnologies.

Citation: Ávila-Rueda SR, Becerril-Pérez CM, Rosendo-Ponce A, Zárate-Guevara OE, Ramírez-Martínez A, Riaño-Gaya A, Rosales-Martínez F. 2024. Viability of vitrified embryos from Romosinuano and Tropical Milking cattle over two seasons of the year in Veracruz, Mexico. **Agrociencia** 58(8): 954-967. https://doi.org/ 10.47163/ agrociencia.v58i8.3153

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: January 22, 2024.
Approved: September 26, 2024.
Published in Agrociencia: November 27, 2024.

INTRODUCTION

In 2021, the global production of *in vivo* bovine embryos was 386 374, while *in vitro* was 1 521 018 (Stringfellow and Seidel, 2000; Viana, 2022), of which 65 % were transferred fresh and 35 % cryopreserved. One of the most important advances in bovine reproduction technology has been the vitrification of embryos. The percentages of gestation using devitrified embryos range from 65 to 27 % (Youngs, 2011; Naranjo-Chacón *et al.,* 2016). Therefore, the reproductive biotechnology of vitrified embryos is improving to obtain greater percentages of gestation.

The total percentage of eclosion observed 72 h after cultivation following devitrification is variable, ranging from 29 to 51.8 % (Cho *et al.,* 2002; Silva *et al.,* 2013). These differences may be influenced by factors external to the *in vitro* production process in the laboratory, including the climatic seasons of the year. These variations affect the reproduction capability of the bovine female, particularly the ovarian follicular dynamics and the embryo viability (Tomita *et al.*, 2023). Crossbred cows (*Bos taurus x Bos indicus*) showed more follicles during the rainy season (15 ± 1.3) than during the dry season (7.6 ± 0.9) (Cansino-Arroyo *et al.,* 2014). There is also evidence that the quality of collected embryos varies according to the season of the year. In Saudi Arabian cows, embryos were of excellent quality in 59 % of cases in the winter and 27 % in the summer (Rayn *et al.*, 1992).

The rise in the ambient temperature and the different tolerance to it in females affect their reproductive behavior by reducing the concentrations of hormones such as estrogen and the luteinizing hormone (LH), which negatively impacts the follicular dynamic (Rodrigues *et al.,* 2016). Heat stress activates the hypothalamus-pituitary-adrenal (HPA) axis, increasing cortisol levels, inhibiting the hypothalamus-pituitary-gonadal (HPG) axis, and reducing the release of the gonadotropin releasing hormone (GnRH), luteinizing hormone (LH), and follicle stimulating hormone (FSH). This results in lower estrogen production by the ovaries, which in turn affects the follicular recruitment, selection, and dominance process and has unfavorable effects on the quality of the oocytes (Roth and Wolfenson, 2016). Different studies have indicated that the bovine oocyte is sensitive to heat stress during maturation, which affects its quality and survival (Roth and Wolfenson, 2016; Rodrigues *et al.,* 2016).

On the other hand, the animal genotype also influences the reproductive behavior. In South America, particularly in Brazil, the production of embryos by *in vitro* fertilization has been promoted in *B. indicus* breeds due to their greater ability to provide viable oocytes (Gimenes *et al.,* 2015). The *B. taurus* criollo breeds, descendants of cattle brought to the New World in the 15th century, can tolerate temperatures from 25 to 40 °C (de Alba-Martínez, 2011). In hot tropical climates, the annual average temperature is greater than 22 °C, and the average of the coldest month is 18 °C. The differential volume of precipitation throughout the year affects the seasonal availability of forages and influences the reproductive behavior of cattle, especially in non-adapted breeds (Rosales-Martínez *et al*., 2021).

More research and biotechnological improvements are needed for adapted *B. taurus* in hot tropical climates. The criollo Tropical Milking and Romosinuano breeds are adapted to the hot climates of the Americas, including Mexico. These breeds would benefit from oocyte recovery, *in vitro* fertilization, and cryopreservation to increase their population sizes and participate in conservation and genetic breeding programs (de Alba-Martínez, 2011; Rosendo-Ponce and Becerril-Pérez, 2015). Therefore, the aim of this study was to evaluate the effect of the season of the year on the viability of embryos vitrified *in vitro* of the criollo Tropical Milking and Romosinuano breeds.

MATERIALS AND METHODS

Area of study

The study was carried out in Veracruz, Mexico (18° 58′–19° 11′ N and 96° 16′–96° 20′ W). Altitude varies between 20 and 100 m (SMN, 2020). The predominant climate is hot subhumid (Aw), with high temperatures throughout the year and rainfalls between June and October (García, 2004). For this study, two seasons of the year were defined and used: hot-dry season (HD) from March to May, with mean maximum temperatures of 31.6 ± 1.0 °C and 77.2 ± 1.1 % relative humidity, and hot-humid season (HH) from June to October, with mean maximum temperatures of 31.4 ± 0.4 °C and a relative humidity of 84.7 ± 1.0 % (Rosales-Martínez *et al*., 2021).

Experimental animals

Criollo Tropical Milking (TM; 27) and Romosinuano (RM; 31) cows with up to three calves delivered and the presence of corpus luteum of follicular dynamics were used, aged 54.2 ± 4.9 to 95.9 ± 5.5 months and with a body condition scale from 2.6 ± 0.1 to 2.9 ± 0.1 (scale of 1–5) according to Edmonson *et al.* (1989). The annual vaccination against rabies (Rabisin B®, Merial, Mexico) was also given. Additionally, parasites were removed (1 mL 50 kg^{-1}; Virbamec® Platinum, Virbac, Mexico), and phosphorous was given four times per month (10 mL; Phospho®, Virbac, Mexico), as well as 100 g of mineral salt (Veramin 8 bovines in grazing®, Virbac, Mexico) every day for 30 days. Feeding was carried out at pará grass (*Brachiaria mutica* Forssk) and native grass (*Paspalum* spp.) pastures with free access to water. From each cow, data on the age (EDD), respiratory frequency (RF), cardiac frequency (CF), body temperature (BT), serum glucose (GLU), basal insulin (INS), and blood cortisol (COR) were collected.

In vitro embryo production

During both seasons, the oocytes were obtained using the ultrasound-guided follicular aspiration process (Filipiak and Larocca, 2012). The oocytes were classified by the morphological characteristics of the *Cumulus* (Bó and Mapletoft, 2018). Viable oocytes were selected, placed in maturation media (MIV-Salt Biotech, Salt Biotech®, Brazil), and transported to the lab (Advanced Genetic Reproduction: AGR *In vitro*®, Veracruz). They were incubated (Eve incubator, WTA®, Denmark) for 24 h at 38.7 °C, 20.9 % oxygen, 5.5 % carbon dioxide, 73.6 % nitrogen, and 46 % relative humidity (Bó and Mapletoft, 2018).

For the *in vitro* fertilization, the oocytes (maximum of 28) were placed in 50 μL *in vitro* fertilization medium drops (FIV-Salt Biotech®, Salt Biotech, Brazil). To fertilize the oocytes, semen straws from TM and RM breeds were used. After thawing in a water bath at 36 °C, the straws were released into 1.5 mL Eppendorf tubes that were conditioned using the Percoll gradient method. The number of spermatozoa in each drop varied from 2 to 5 μL, depending on the concentration (at least 25x10^6 spermatozoa) and individual motility (at least 60 % progressive motility of spermatozoa). After 18 to 20 h, the cumulus cells of the fertilized oocytes were removed using a 100 μL micropipette. Once the fertilized oocytes were denuded, they were placed in Petri dishes (35×10 mm; Corning Incorporated®, USA) with 100 μL of culture medium (CIV-Salt Biotech, Salt Biotech®, Brazil) supplemented with 10 % fetal bovine serum (FBS) (Gibco™, Thermo Fisher Scientific®, USA), 0.25 μL of antibiotic (Gentamicina, Pfizer®, USA) with pH of 7.2–7.4 and 4 mL of mineral oil (Ovoil, Vitrolife®, Sweden). The fertilized oocytes were incubated (Eve incubator, WTA®, Denmark) for 7 days at 38.7 °C, 5 % oxygen, 5 % carbon dioxide, 90 % nitrogen, and a relative humidity of 46 %. A total of 632 fertilized oocytes were recorded, with structures with 2 to 16 blastomeres found.

Cryopreserved embryos

A total of 109 embryos were cryopreserved in a blastocyst stage with 90 to 100 cells, considered excellent in quality (quality 1) according to their morphology, integrity of the inner cell mass and zona pellucida, homogeneous cytoplasm, blastomeres of uniform size, and no signs of pyknosis. The open pulled straw (OPS vitrification protocol) was used (Romo *et al.*, 2007; Youngs, 2011).

Vitrification

The base medium (BM) was prepared with 6 mL cell culture medium (TCM 199-HEPES: Gibco™ Medium 199 (1X), liquid, with HEPES, Thermo Fisher Scientific®, USA) plus 1.5 mL FBS. For the LL 0.5 M solution, 3 mL of BM plus ethylene glycol (LL; Freeze, ARBiotech, Mexico). The vitrification solution 1 (VS1) consisted of 425 μL of BM, 37.5 μL of dimethyl sulphoxide (DMSO; PRIME-XV FreezIS DMSO-Free, Irvine Scientific, USA) plus 37.5 μL 5-methoxymethylfurfural (MMF, Sigma-Aldrich, Mexico); and the vitrification solution 2 (VS2) consisted of 670 μL of LL 0.5 M solution, 165 μL of DMSO, and 165 μL of MMF.

The selected and identified embryos were placed using the pipette and washed in two drops of 100 μL of the base medium. They were then moved in groups of at least five into 200 μL VS1 drops, where they remained for 3 min. With the micropipette, they were placed into the VS2 drop, where they remained for 15 min. They were then placed in a 0.25 mL straw for embryos cut in the shape of a spear (previously prepared), removing the excess VS2 medium and submerging the spear into the liquid nitrogen (LN2) between 25 and 30 s, from the placement of the embryo into VS2 and vitrification (Romo *et al.*, 2007, Youngs, 2011).

Heating of the vitrified embryos

To heat the embryos vitrified *in vitro*, the media were prepared using the following solution concentrations: 0.5 M LL solution; 0.25 M LL solution; 7.5 mL 0.5 M LL solution plus 7.5 mL of MB; 0.15 M LL solution: 2 mL 0.25 M LL solution plus BM; and transportation medium: 4.5 mL of TCM 199-HEPES, 500 µL FBS, 10 µL pyruvate (Gibco™ Pyruvate Solution, Thermo Fisher Scientific®, USA) plus 25 µL of antibiotic (Gentamicin, Pfizer®, USA). All solutions were adjusted to a pH of 7.1–7.2 to ensure proper heating and manipulation of cryopreserved embryos.

The media were stabilized at room temperature. The spears that contained the embryos were identified and taken out of the LN2 to submerge them in a dish with 0.25 M LL solution for 1 min, raising and lowering the embryos with the pipette for 30 s, and grouping them at the bottom of the dish. Then, the embryos were placed in a 100 µL drop with 0.15 M LL solution, where they were washed and removed for 30 s, and grouped at the bottom for 5 min. Later, they were transferred to 100 µL of the MB medium and placed in Petri dishes (35×10 mm; Corning®, USA) with 3 mL of transportation medium (Romo *et al.,* 2007, Youngs, 2011).

Viability of the cryopreserved embryos

To determine the viability of cryopreserved embryos, as well as the functionality of their structures, techniques such as *in vitro* embryo eclosion, eclosion ability, and cell viability (live and dead cells) were used.

Capacity of *in vitro* eclosion (structural viability)

The heated embryos were planted for 72 h and transferred into Petri dishes with 100 µL drops of culture medium (CIV-Salt Biotech, Salt Biotech®, Brazil), supplemented with 10 % of FBS, 0.25 µg mL^{-1} of gentamicin, and 0.5 % of non-essential amino acids, with a pH of 7.2–7.4 and 4 mL of mineral oil. They were incubated (Eve incubator, WTA®, Denmark) at 38.7 °C, 5.5 % carbon dioxide, 20.9 % oxygen, 76.6 % nitrogen, and 46 % relative humidity. To estimate the structural viability of the embryos, they were counted and their *in vitro* capacity of eclosion (CE) after 72 h was observed, along with the blastocele reconstitution (BL), trophoblast (TR), zona pellucida (ZP), and cell mass (CM). The embryos were evaluated following the technique described by Bó and Mapletoft (2018).

Cell viability, double staining technique with Hoechst/propidium iodide

For the staining with propidium iodide (P-4170, Sigma®, USA) and bisbenzimide (BIS, Hoechst 33342, Sigma®, USA), the media were prepared using the following solution concentrations: bisbenzimide staining: 1 mg of bisbenzimide stock solution plus 1 mL of sodium citrate (2.3 %); bisbenzimide working solution (10 µg mL^{-1}): 750 µL of sodium citrate (2.3 %), 250 µL ethanol (100 %) plus 10 µL of bisbenzimide stock solution; propidium iodide staining: iodide stock solution (5 mg mL^{-1}), 5 mg propidium iodide (P-4170, Sigma®, USA) and 1 mL PBS; iodide working solution

(0.05 mg mL^{-1}): 10 μL of iodide stock solution plus 990 μL of PBS; and washing solution (PBS + 0.025 % PVP): PBS (phosphate buffered saline, Sigma®, USA) 100 mL plus PVP (polyvinylpyrrolidone, Sigma®, USA) 25 mg.

After 24 h of having planted the embryos, they were stained, extracted from the culture medium, and double washed in a saline solution buffered with phosphate with 0.025 % PVP for 1 min. Afterwards, the embryo with a volume of 10 μL of washing medium was placed on a slide. The excess medium was removed and 10 μL of working bisbenzimide solution was added (10 μg mL^{-1}) for 1 min; the sample was kept away from direct light. After the allotted time, the bisbenzimide was removed and washed with 10 μL of solution, pipetting gently without losing sight of the embryo. The washing medium was then removed, and 10 μL of the iodide working solution (0.05 mg mL^{-1}) was added for 1 min. The excess propidium iodide was removed and washed, followed by the addition of 10 μL of antifade solution (S7114, Sigma®, USA) to prepare the slide for the embryo evaluation, with four dots made of a paraffin-petroleum jelly mixture (1:10 p/p) placed in the four corners to create space and prevent the embryo from losing its shape.

Serial sections were observed and obtained using a confocal microscope TCS-SP8+STED (Leica Microsystems GmbH, Wetzlar, Germany), with a HCX PL APO 40x0.75 DRY lens and the use of a filter with the ability to read the intensity of fluorochromes, the Hoechst (excitation at 405 nm and emission at 433–473 nm), and the propidium bromide (excitation at 488 nm and emission at 620 nm). Both fluorochromes were intercalated into the DNA. Hoechst enters all cells, while propidium iodide only enters dead or necrotic cells with membrane damage. The precise count and target of live cells (CVI) and dead cells (CMU) of the embryo was carried out using the software CaptaVision® (version 5.1), acknowledging the state and the viability of the embryo under study (Chatzimeletiou *et al.,* 2021). From the counts, the percentage of live cells (PCVI) and dead cells (PCMU) were calculated.

Statistical analysis

The data were analyzed with a fixed-effect generalized linear model, which included the effects of the season (EST), the breed (RA), the season × breed interaction (EST*RA), the variables of blastocele reconstitution (BL), trophoblast (TR), zona pellucida (ZP), cell mass (CM), live cells (CVI), dead cells (CMU), percentages of live cells (PCVI) and of dead cells (PCMU), and the covariable of age (EDD), respiratory frequency (FR), cardiac frequency (FC), body temperature (TR), serum glucose (GLU), basal insulin (INS), and blood cortisol (COR). A $P(\lambda)$ distribution of the experimental error was considered, with a logit link function, using the GENMOD procedure of SAS® (SAS Institute, 2013). The comparison of means was performed using Tukey's honest test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Capacity of *in vitro* eclosion

The EST*RA interaction had a significant effect ($p \leq 0.001$) on the evaluated characteristics. In addition, the main effect of EST on the capacity of eclosion (CE) was observed 72 h after devitrification, which favored HD by more than 13 % ($p \leq 0.001$). RA significantly increased TM values by over 16 % ($p \leq 0.001$) (Figure 1).

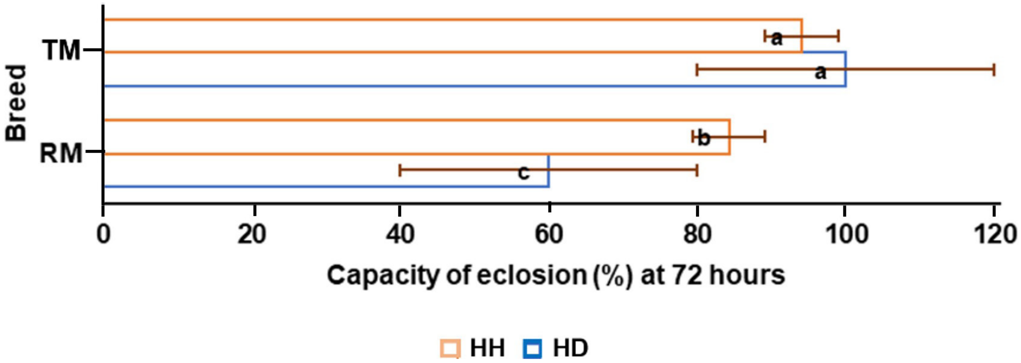


Figure 1. Effect of the hot-dry (HD) and hot-humid (HH) seasons on the capacity of eclosion observed at 72 h of planting after the devitrification of Tropical Milking (TM) and Romosinuano (RM) criollo cows in Veracruz, Mexico. ^{abc} Different letters indicate statistical differences ($p \leq 0.001$).

For the effects of EST and RA on BL, TR, ZP, and CM (Table 1), BL showed a 30 % reduction for RM in HD and a 12.5 % reduction in HH. The TM breed displayed a similar response to BL in both seasons, with a global difference with the RM breed of over 16 % ($p \leq 0.001$) and between seasons of over 11 % in favor of HH ($p \leq 0.001$). For TR, a 32 % reduction was estimated for RM in HD and 12 % for HH. The TM responded similarly to TR in both seasons, with a 20 % global difference with RM ($p \leq 0.001$) and more than 15 % in favor of HH ($p \leq 0.001$) between seasons.

In ZP, a 25 % reduction was estimated for RM in HD and 12 % in HH compared to TM, which displayed a similar response in both seasons, with a global difference with the RM breed of 17 % ($p \leq 0.001$) and between seasons of 13 % in favor of HH ($p \leq 0.001$). On CM, RM was estimated to be reduced by 25 % in HD and by more than 10 % in HH. The TM breed responded similarly to the CM breed, with a global difference of over 16 % ($p \leq 0.001$) and a seasonal difference of over 10 % favoring HH by 13 % ($p \leq 0.001$). For CE and the reconstitution variables BL, TR, ZP, and CM, the TM breed presented the highest values, which were similar in the HD and HH seasons, indicating that TM embryos are more resistant to seasonal change. Likewise, the RM breed consistently responded positively, moving from a less favorable condition in

Table 1. Functional characteristics of the devitrified embryos of the Tropical Milking (TM) and Romosinuano (RM) breeds during the hot-dry (HD) and hot-humid (HH) seasons in Veracruz, Mexico.

		Season of year		
Characteristic	Breed	HD	HH	Global
BL	RM	62.6 ± 2.0^{c}	82.7 ± 1.3^{b}	75.0 ± 1.1^{B}
	TM	92.6 ± 2.4^{a}	95.2 ± 1.3^{a}	91.1 ± 1.3^{A}
	Global	77.3 ± 1.5^{Y}	88.5 ± 0.9^{X}	
TR	RM	62.6 ± 2.0^{c}	84.0 ± 1.3^{b}	75.8 ± 1.1^{B}
	TM	92.6 ± 2.4^{a}	95.2 ± 1.3^{a}	90.9 ± 1.3^{A}
	Global	77.3 ± 1.6^{Y}	89.1 ± 0.9^{X}	
ZP	RM	67.9 ± 2.1^{c}	85.4 ± 1.3^{b}	78.6 ± 1.2^{B}
	TM	92.6 ± 2.4^{a}	95.8 ± 1.3^{a}	91.9 ± 1.3^{A}
	Global	80.0 ± 1.6^{Y}	90.3 ± 0.9^{X}	
CM	RM	67.9 ± 2.1^{c}	85.9 ± 1.3^{b}	78.9 ± 1.2^{B}
	TM	92.6 ± 2.4^{a}	95.8 ± 1.3^{a}	91.8 ± 1.3^{A}
	Global	80.0 ± 1.6^{Y}	90.5 ± 0.9^{X}	

BL: blastocele reconstitution; TR: trophoblast; ZP: zona pellucida; CM: cell mass. ^{AB} Different letters per row indicate statistical differences ($p \leq 0.001$). ^{XY} Different letters per column indicate statistical differences ($p \leq 0.001$). ^{abc} Different letter per row and column indicate statistical differences ($p \leq 0.001$).

HD to a more favorable one in HH, although at a lower response level than the TM breed.

The impact of the maternal environment on the reproductive behavior of the criollo breeds has been discussed (Guerrero *et al*., 2011; Rosales-Martínez *et al*., 2021). Prostaglandin application has been shown to cause an estrous manifestation of 52–100 % for TM (Guerrero *et al*., 2011; Rosales-Martínez *et al*., 2021). Fertility was similar in TM (81 %) and RM (83 %) (Parra-Cortés *et al*., 2019); the TM breed had gestation percentages between 40 and 77 % (Guerrero *et al*., 2011; Parra-Cortés *et al*., 2019), whereas RM had a value of 85 % (Martínez y Pérez, 2006). In terms of birth rate, TM had 85 % (Parra-Cortés *et al*., 2019), and RM, from 85 to 89 % (Ossa-Saraz, 2013; Parra-Cortés *et al*., 2019). In addition, the RM calving interval ranges from 442 to 553 d, while the TM calving interval ranges from 389 to 455 d (Ossa-Saraz, 2013; Rosendo-Ponce and Becerril-Pérez, 2015; Parra-Cortés *et al*., 2019; Martínez-Rocha *et al*., 2021).

For over 500 years, natural selection and cattle farmer selection during various historical periods have led to the adaptation of the TM and RM Criollo breeds. While the RM has been focused on producing meat, the TM has been more focused on producing dairy (de Alba-Martínez, 2011). In terms of adaptation, it is reasonable to assume that

TM embryos are very well adapted and more stable during the transition from HD to HH season. This may, in turn, have a positive impact on its ability to reconstitute after vitrification. Naranjo-Chacón *et al.* (2016) found that TM cows had 28.6 % ($p \leq 0.5$) more pregnancies with vitrified embryos than crossbred cows, which had only 5.6 %. When analyzing climatology in both seasons, there is no evidence of a significant difference in the mean maximum temperature, with 31.6 °C in HD and 31.4 °C in HH. However, there is a significant difference in relative humidity (RH), with 77.2 % in HD and 84.7 % in HH, as well as annual rainfall (5.4 % in HD and 89.5 % in HH), which is manifested in a larger reproductive response of the cattle (Rosales-Martínez *et al.*, 2021).

The feed of the bovines and their nutritional condition are relevant in the reproductive behavior (Tomita *et al.*, 2023). Malnutrition, understood as a deficient nutritional stage due to scarcity, inadequate absorption, an increase in needs, or an excessive loss of nutrients, is related to a reduction in the number of recruited follicles, a low ovulation rate, a reduction in the duration and return to estrus, a reduction in embryonic development, and an increase in embryonic and fetal mortality, which have a bearing on longer calving intervals and, generally, lower fertility (Henao and Trujillo, 2003; Tomita *et al.*, 2023).

Previous studies found that the percentage of gestation in artificially inseminated heifers decreased during the HD season, which was characterized by lower forage quality than the HH season (50 and 65 %, respectively) (Rosales-Martínez *et al.*, 2021). Bovines with a body condition < 2.5 had a higher embryonic mortality rate of 11 %, compared to bovines with a condition > 2.5, where embryonic mortality was 5 % (Rhind, 2004). These findings suggest that weather and diet have an effect on reproductive behavior and physiological mechanisms.

Cell viability

No effect of RA or EST was observed in CVI, PCVI, or PCMU ($p > 0.05$) (Figure 2). EST reduced HH in CMU by over 38 % ($p \leq 0.04$) (Figure 3). According to de Barros and Paula-Lopes (2018), the embryonic development *in vitro* is susceptible to heat stress before the activation of the embryonic genome. Silva *et al*. (2013) observed that embryos in their first stages displayed alterations in gene expression (caudal-type homeobox transcription factor 2; *CDX2*) associated with embryonic survival, implantation, and placental development. Sakatani (2015) and Paula-Lopes and Hansen (2002) found that expanded embryos undergo DNA fragmentation at 41 °C. Rodrigues *et al*. (2016) reported that blastocysts produced from heat-stressed oocytes had a lower abundance of transcripts related to cell growth and differentiation, whereas Sakatani *et al*. (2013) found a decrease in cell division rate in oocytes exposed to heat stress at 41 °C for 12 h. The increased expression of heat stress protective genes (*HSP70*; heat shock protein 70, *HSP90*; heat shock protein 90, *HSP27*; heat shock protein 27, *HSP60*; heat shock protein 60, *Hsf1*; heat shock factor 1, *BAG3*; and *BCL2*-associated athanogene 3) reduces apoptosis and increases embryonic survival (Silva *et al*., 2013). TM and RM breeds

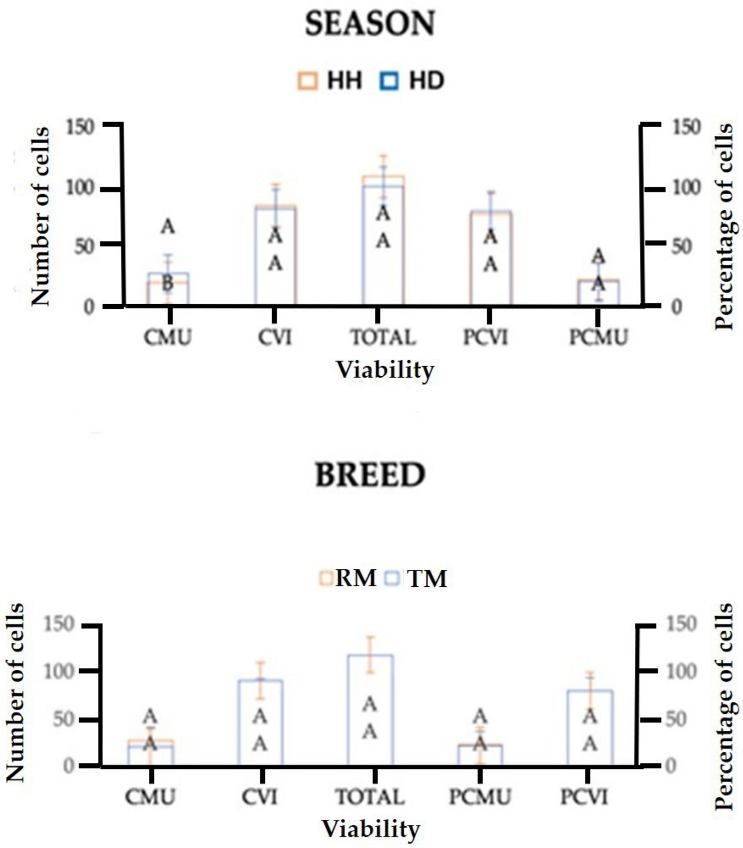


Figure 2. Cell viability of devitrified embryos of Tropical Milking (TM) and Romosinuano (RM) criollo cows in Veracruz, Mexico, under hot-dry (HD) and hot-humid (HH) seasons. CVI: number of live cells; CMU: number of dead cells; PCVI: percentage of live cells; PCMU: percentage of dead cells. ^{AB} Different lines indicate a statistical difference ($p \leq 0.05$).

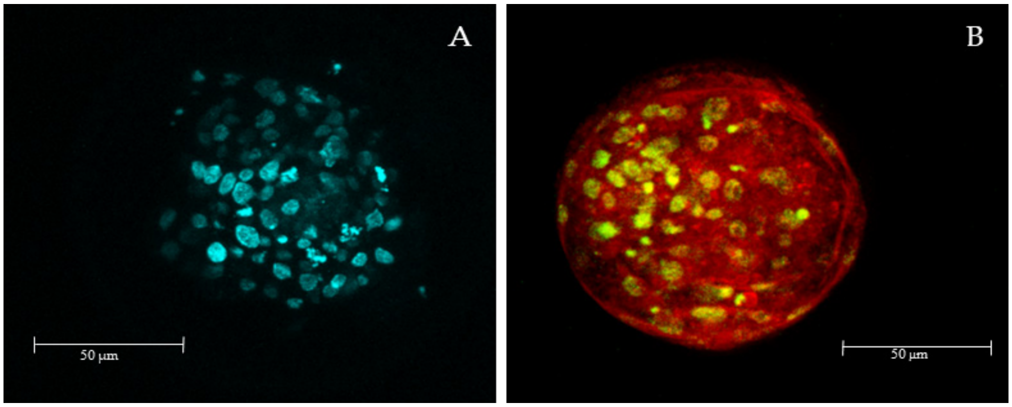


Figure 3. Embryos of the Tropical Milking Criollo breed captured using a TCS-SP8+STED confocal microscope (Leica Microsystems GmbH, Wetzlar, Germany) and the HCX PL APO 40x/0.75 DRY lens. A: Image with a high content of live cells; B: image with a high content of dead cells.

have developed genetic and physiological mechanisms to maintain homeostasis during extreme heat (Martínez-Rocha *et al*., 2021). These adaptation mechanisms assist in their survival and reproduction in high-temperature environments, including a greater resistance to apoptosis caused by heat stress in embryos.

Seasonal effects may have an impact on the viability of cells in vitrified embryos, with a higher number of dead cells during the HD season compared to the HH season. According to Sakatani *et al*. (2015), the critical stage in which heat stress may negatively affect embryonic development in humans is between fertilization and the stage of 4–8 cells, whereas in bovines it is produced at the stage of 8–16 cells. High temperatures induce DNA fragmentation and affect RNA; therefore, if the cumulus-oocyte complexes (COCs) are exposed to high temperatures in warm seasons, the number of cells in the few embryos that reach the blastocyst stage is reduced, resulting as well in greater damage in the blastomeres (de Barros and Paula-Lopes, 2018; Paula-Lopes and Hansen, 2002; Rodrigues *et al.,* 2016). These damages have an impact of heat stress on embryo survival (Roth and Wolfenson, 2016).

Sakatani *et al.* (2013) observed that the morula stage is more resistant to higher temperatures (40–41 °C) in culture than the two-cell embryo. In addition, Paula-Lopes and Hansen (2002) and Sakatani *et al*. (2015) found an increase in the concentration of intracellular reactive oxygen species (ROS) in the embryo. These studies indicate underlying mechanisms that may contribute to embryo apoptosis caused by high temperatures. One of them is an increase in the production of ROS in embryonic cells; ROSs are highly reactive molecules that may cause oxidative damage to cell structures such as DNA, proteins, and lipids, which leads to the activation of apoptotic signaling pathways and, eventually, cell death.

Heat stress-induced apoptosis may have a significant impact on embryonic viability and development if the transcription of stress-related genes is not carried out correctly during embryonic development, whether *in vitro* or *in vivo*, and may jeopardize the result in the final stages of development and thus affect the embryo after vitrification (Paula-Lopes and Hansen, 2002; Sakatani *et al*., 2015; de Barros and Paula-Lopes, 2018). This indicates the importance of rigorously controlling the temperature during the oocyte recovery, IVF, and embryo cryopreservation in assisted reproduction procedures.

CONCLUSIONS

The effect of the season × breed interaction was observed in the characteristics of the devitrified embryos. In the Romosinuano breed, a greater negative impact of the hot-dry season was observed on the eclosion capacity of the embryos, unlike the Tropical Milking breed, whose embryos had a greater reconstitution and development capacity in both seasons, hot-dry and hot-humid. No main effect of the breed was discovered on embryo viability. These results highlight the importance of considering the effect of the season on embryo vitrification when planning assisted reproduction programs, reproductive technologies and feeding, with special reference to the hot-dry season.

ACKNOWLEDGEMENTS

The authors wish to thank the Consejo Nacional de Humanidades, Ciencia y Tecnología (CONAHCYT) for the scholarship granted for the corresponding author's PhD studies. Thanks also go to the Programa en Agroecosistemas Tropicales, Veracruz Campus and the Colegio de Postgraduados for their support to carry out the research project. To the Reproducción y Genética Avanzada (RGA *In vitro*) Laboratory, Boca del Río, Veracruz, for the facilities and use of the cutting-edge equipment to produce and observe the embryos in this study. To the Instituto Nacional de Ecología (INECOL), the Advanced Microscopy Laboratory, Xalapa, Veracruz, for their collaboration in the cell studies.

REFERENCES

Bó GA, Mapletoft RJ. 2018. Evaluation and classification of bovine embryos. Animal Reproduction 10 (3): 344–348.

Cansino-Arroyo G, Ávila-Rueda S, Ruiz-Cruz, JL, Arias-de la Cruz D, Urbina-Cortes R, Piña-Gutiérrez JM, Ramírez-Vera S. 2014. La adición de ácidos grasos poliinsaturados a la dieta incrementa el número y tamaño de los folículos en vacas alimentadas bajo sistema de pastoreo tropical. Tropical and Subtropical Agroecosystems 17 (2): 303–307.

Chatzimeletiou K, Sioga A, Petrogiannis N, Panagiotidis Y, Prapa M, Patrikiou A, Tarlatzis BC, Grimbizis G. 2021. Viability assessment using fluorescent markers and ultrastructure of human biopsied embryos vitrified in open and closed systems. Reproductive BioMedicine Online 43 (5): 833–842. https://doi.org/10.1016/j.rbmo.2021.05.011

Cho SK, Cho SG, Bae IH, Park CS, Kong IK. 2002. Improvement in post-thaw viability of *in vitro*-produced bovine blastocysts vitrified by glass micropipette (GMP). Animal Reproduction Science 73 (3–4): 151–158. https://doi.org/10.1016/S0378-4320(02)00132-X

de Alba-Martínez J. 2011. El libro de los bovinos criollos de América. Fundación Colegio de Postgraduados en Ciencias Agrícolas A.C.: San Luis Huexotla, México. 464 p. https://doi.org/10.1017/S2078633612000227

de Barros ROF, Paula-Lopes FF. 2018. Cellular and epigenetic changes induced by heat stress in bovine preimplantation embryos. Molecular Reproduction and Development 85 (11): 810–820. https://doi.org/10.1002/mrd.23040

Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G. 1989. A body condition scoring chart for Holstein dairy cows. Journal of Dairy Science 72 (1): 68–78. https://doi.org/10.3168/jds.S0022-0302(89)79081-0

Filipiak Y, Larocca C. 2012. Utilización del azul tripán para diferenciar ovocitos bovinos vivos y muertos en fertilización *in vitro*. Archivos de Zootecnia 61 (234): 309–312.

García E. 2004. Modificaciones al sistema de clasificación climática de Köppen. Universidad Nacional Autónoma de México: Ciudad de México, México. 90 p.

Gimenes LUL, Ferraz MP, Fantinato-Neto R, Chiaratti M, Mesquita GLF, Sá Filho, M, Baruselli SP. 2015. The interval between the emergence of pharmacologically synchronized ovarian follicular waves and ovum pickup does not significantly affect *in vitro* embryo production in *Bos indicus, Bos taurus*, and *Bubalus bubalis*. Theriogenology 83 (3): 385–393. https://doi.org/10.1016/j.theriogenology.2014.09.030

Guerrero HLJ, Pérez-Hernández P, López-Ortiz S, Montiel Palacios F, Estrella-García A, Ahuja-Aguirre C. 2011. Sincronización del estro con PGF2*α* y conducta estrual en vacas Criollo Lechero Tropical. Archivos de Zootecnia 60 (23): 829–832.

Henao G, Trujillo LE. 2003. Dinámica folicular y función lútea durante la gestación temprana. Estudio de un caso en *Bos indicus*. Revista de la Facultad Nacional Agraria 56 (1): 1779–1788.

Martínez-Rocha RE, Ramírez-Valverde R, Núñez-Domínguez R, García-Muñiz JG, Parra-Bracamonte GM. 2021. Comportamiento de crecimiento y reproductivo en hembras bovinas Romosinuano. Revista MVZ Córdoba 26 (1): e2033. https://doi.org/10.21897/rmvz.2033

Martínez RA, Pérez JE. 2006. Parámetros y tendencias genéticas para características de crecimiento en el ganado criollo colombiano Romosinuano. Revista de Investigaciones Pecuarias, 2(1), 21-33.

Naranjo-Chacón F, Becerril-Pérez CM, Canseco-Sedano R, Zárate-Guevara OE, Soto-Estrada A, Rosendo-Ponce A, Rosales-Martínez F. 2016. Comparación de dos métodos de transferencia de embriones en el ganado criollo Lechero Tropical. Ecosistemas y Recursos Agropecuarios 3 (7): 113–120.

Ossa-Saraz G, Hinestroza AD, Santana-Rodríguez M, Reza-García S, Pérez-García J, Abuabara PY. 2013. Formación, desarrollo y caracterización fenotípica de los caracteres productivos y reproductivos del hato Romosinuano del banco de germoplasma de Colombia. Ciencia y Tecnología Agropecuaria 14 (2): 231–243.

Parra-Cortés RI, Magaña-Magaña MÁ. 2019. Características técnico-económicas de los sistemas de producción bovina basados en razas criollas introducidas en México. Ecosistemas y Recursos Agropecuarios 6 (18): 535–547. https://doi.org/10.19136/era.a6n18.2160

Paula-Lopes FF, Hansen PJ. 2002. Apoptosis is an adaptive response in bovine preimplantation embryos that facilitates survival after heat shock. Biochemical and Biophysical Research Communications 295 (1): 37–42. https://doi.org/10.1016/S0006-291X(02)00619-8

Rayn DEJ, Halbach TV, Armstrong D. 1992. Season and lactation number effects on milk production and reproduction in dairy cattle in Arizona. Journal Dairy Science 75 (11): 2976–2983. https://doi.org/10.3168/jds.S0022-0302(92)78061-8

Rhind SM. 2004. Effects of maternal nutrition on fetal and neonatal reproductive development and function. Animal Reproduction Science 83: 169–181. https://doi.org/10.1016/j.anireprosci.2004.04.003

Rodrigues TA, Ispada J, Risolia PH, Rodrigues MT, Lima RS, Assumpção ME, Visintin JÁ, Paula-Lopes FF. 2016. Thermoprotective effect of insulin-like growth factor 1 on *in vitro* matured bovine oocyte exposed to heat shock. Theriogenology 86 (8): 2028–2039. https://doi.org/10.1016/j.theriogenology.2016.06.023

Romo SH, Pryor JR, Looney C, Walter DE, Seidel GF, Hasler JC, Kraemer D. 2007. Comparación entre congelación convencional y la vitrificación para la criopreservación de embriones de ganado Brahman. Memorias. Congreso Nacional y Latinoamericano de Buiatría: Acapulco, México, pp: 205–207.

Rosales-Martínez F, Rosendo-Ponce A, Cortez-Romero C, Gallegos-Sánchez J, Cuca-García JM, Becerril-Pérez CM. 2021. Relation of the maximum temperature and relative humidity close to the insemination with the Tropical Milking criollo heifer's gestation in three seasons. Tropical Animal Health and Production 53 (1): 27. https://doi.org/10.1007/s11250-020-02430-3

Rosendo-Ponce A, Becerril-Pérez CM. 2015. Avance en el conocimiento del bovino criollo Lechero Tropical de México. Ecosistemas y Recursos Agropecuarios 2 (5): 233–243.

Roth Z, Wolfenson D. 2016. Comparing the effects of heat stress and mastitis on ovarian function in lactating cows: Basic and applied aspects. Domestic Animal Endocrinology 56: S218–S227. https://doi.org/10.1016/j.domaniend.2016.02.013

Sakatani M, Bonilla L, Dobbs KB, Block J, Ozawa M, Shanker S, Hansen PJ. 2013. Changes in the transcriptome of morula-stage bovine embryos caused by heat shock: Relationship to developmental acquisition of thermotolerance. Reproductive Biology and Endocrinology 11 (3). https://doi.org/10.1186/1477-7827-11-3

Sakatani M, Yamanaka K, Balboula AZ, Takenouchi N, Takahashi M. 2015. Heat stress during *in vitro* fertilization decreases fertilization success by disrupting anti-polyspermy systems of the oocytes. Molecular Reproduction and Development 82 (1): 36–47. https://doi.org/10.1002/mrd.22441

SAS Institute. 2013. SAS 9.1.3 Help and documentation, Cary, NC, USA.

Silva CF, Sartorelli ES, Castilho ACS, Satrapa RA, Puelker RZ, Razza EM, Ticianelli ES, Eduardo HP, Loureiro B, Barros CM. 2013. Effects of heat stress on development, quality and survival of *Bos indicus* and *Bos taurus* embryos produced *in vitro*. Theriogenology 79 (2): 351–357. https://doi.org/10.1016/j.theriogenology.2012.10.003

SMN (Sistema Meteorológico Nacional). 2020. Sistema de información climatológica y geográfica del estado de Veracruz, México. Gobierno de México. Comisión Nacional del Agua. Sistema Meteorológico Nacional. Ciudad de México, México. https://smn.conagua.gob.mx/es/ (Retrieved: November 2023).

Stringfellow DA, Seidel SM. 2000. Manual de la Sociedad Internacional de Transferencia de Embriones. International Embryo Transfer Society: Champaign, IL, USA. 185 p.

Tomita K, Ishii T, Endo N, Tanaka T. 2023. Effects of short-term dietary supplementation on the number of ovarian follicles, quantity and quality of oocytes, and *in vitro* embryo production in Japanese Black cows. Journal of Reproduction and Development 69 (2): 65–71. https://doi.org/10.1262/jrd.2022-103

Viana HMJ. 2022. 2021 statistics of embryo production and transfer in domestic farm animals: World embryo industry grows despite the pandemic. Embryo Technology Newsletter 40 (4): 8–25.

Youngs CR. 2011. Cryopreservation of preimplantation embryos of cattle, sheep, and goats. Journal of Visualized Experiments 54: e2764. https://doi.org/10.3791/2764

Agrociencia

ESTIMATION OF LAND COVER CHANGE USING LANDSAT SATELLITE IMAGERY AND THE RANDOM FOREST CLASSIFIER

José **Rodríguez-Rosales**[1], Juan Manuel **González-Camacho**[1*],
Antonia **Macedo-Cruz**[2], Yolanda M. **Fernández-Ordoñez**[1]

[1]Colegio de Postgraduados Campus Montecillo. Posgrado en Socioeconomía, Estadística e Informática-Cómputo Aplicado. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, State of Mexico, Mexico. C. P. 56264.
[2]Colegio de Postgraduados Campus Montecillo. Posgrado en Hidrociencias. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, State of Mexico, Mexico. C. P. 56264.
* Author for correspondence: jmgc@colpos.mx

ABSTRACT

The quantification of land cover change facilitates natural resource management. In general, these changes are determined at large scales but less frequently at a regional level. In this research, changes in land area covered by vegetation (*V*), agricultural use (*A*), grassland (*G*), and urban-rural (*U*) were estimated for the period 2002–2021 in the municipality of Huehuetla, in the Northern Sierra of Puebla, Mexico (39.5 km^2), based on Landsat satellite images and the random forest classifier (RF). The latter was trained and evaluated with two datasets consisting of three spectral bands (red, green, and blue) and seven vegetation indices. RF performed well in classifying the four cover types at the beginning and end of the evaluation period. RF obtained an overall correct classification accuracy of 92.5 % in 2002 and 92.3 % in 2021. At the land cover level, RF identified vegetation cover with an F1 score of 100 % in 2002 and 98.2 % in 2021; however, it identified the urban-rural cover less effectively, with an F1 of 71.5 % in 2002 and 81.8 % in 2021. In the period analyzed, the urban area increased from 1.7 to 6.4 % (an increase of 4.7 % of the total area), and the vegetation area from 48.1 to 68.7 % (an increase of 15.6 %), at the expense of a reduction in the grassland area (19.8 %), while the agricultural area remained stable (reduction of 0.5 %). This study illustrates the importance of using machine learning techniques and satellite images to assess land cover changes at the regional level as a viable and low-cost alternative.

Keywords: Machine learning, decision trees, land use, natural resources, resource management.

Citation: Rodríguez-Rosales J, González-Camacho JM, Macedo-Cruz A, Fernández-Ordoñez YM. 2024. Estimation of land cover change using Landsat satellite imagery and the random forest classifier.
Agrociencia 58(8): 968-984.
https://doi.org/ 10.47163/agrociencia.v58i8.2846

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: July 13, 2022.
Approved: November 12, 2024.
Published in Agrociencia:
December 20, 2024.

INTRODUCTION

The integrated management of natural and environmental resources represents one of the central problems in the development of communities in the Northern Sierra of the state of Puebla, Mexico. Changes in land use and vegetation reflect the transformation of the environment because of the interaction of natural and human factors (Guevara-Romero and Montalvo-Vargas, 2015). Loera-Martínez *et al*. (2017) pointed out that coffee cultivation is the main activity that generates income for smallholders in the region; however, the coffee crisis in the 1990s led to the development of cattle ranching in the area, and new pastures were opened in areas of forest and perennial or annual crops. The municipality of Huehuetla, Puebla, is geographically divided into two main areas: one with an area of 39.5 km² (studied area) and the other with 8.1 km² (INEGI, 2010), which distribute their land use in agriculture (52.6 %), urban area (1.6 %), grassland (33.9 %), jungle (11.5 %), and forest (0.38 %).

Ardila-López et al. (2005) reported that in the 1990s, satellite image classification and analysis models included classical statistical techniques such as maximum likelihood or minimum distance classification; however, these methods have low accuracy for solving problems that are nonlinearly separable between classes. In contrast, Eisavi *et al*. (2015) reported the use of the random forest (RF) model to estimate land cover change from digital images, with an overall classification accuracy of 95.1 %. Similarly, Baeza *et al*. (2014) obtained an overall accuracy of 89.6 % in the classification of four land covers (perennial forage resources, afforestation and bush, summer crops, and double cropping) in Uruguay, based on decision trees, normalized difference vegetation index (NDVI-MODIS) time series in the period from May 2011 to March 2012, and Landsat satellite images.

In Spain, Paredes-Gómez *et al*. (2019) reported the monitoring and evaluation of irrigated arable crops from Sentinel 2 satellite imagery and decision trees from 2016 to 2018, with an overall classification accuracy of 84, 79.5, and 83.7 %, respectively, in the three years of study. Chucos-Baquerizo and Vega-Ventocilla (2022) conducted a study in the Peruvian Amazon to evaluate four land covers (water bodies, crops, urban, and forests) based on three machine learning algorithms from Landsat 8, Sentinel 2 multispectral satellite images (support vector machine, SVM), and Bayes-naive, and obtained an accuracy of 90.9 % and RF of 86.4 %. The RF classifier is an efficient and simple method that has been successfully used in forest species mapping (Chuvieco-Salinero, 2010).

The objective of this study was to evaluate changes in vegetation cover and land use in the period from 2002 to 2021 in the municipality of Huehuetla, in the Northern Sierra of Puebla, Mexico, by means of a comparative analysis of Landsat images classified with the RF classifier.

MATERIALS AND METHODS

Image collection

The Landsat 7 images (LE07_L1TP_025046_20020115_20170201201_01_T1), acquired on January 15, 2002, and Landsat 8 images (LC08_L1TP_025046_20211229_20220106_01_T1) from December 29, 2021, were obtained from the United States Geological Survey (USGS) website. Both images have a spatial resolution of 30 m, and the panchromatic band has a spatial resolution of 15 m (USGS, 2019). The images were obtained in the original sensor format, which is known as product level 1 (L1), without any preprocessing. The images were selected in such a way that they did not present cloudy conditions in the study area. The municipality files were downloaded in shapefile format from the Geo Information 2021 portal of the National Biodiversity Information System (CONABIO, 2021).

Computer resources

The creation of a database of samples of the types of soil cover existing in the study area, based on color characteristics, was carried out in the Python 3.10 programming language and the Scikit-learn machine learning software library. The computer hardware used in the image preprocessing was a Lenovo Ideapad 320 with a Windows 11 operating system, AMD A12-9720P RADEON R7 processor, 12 compute cores 4C+8G, 2.7 GHz, a 1 Tb hard disk, and 12 Gb RAM. Training and validation of the model were carried out on the Google Collaboratory Service platform to take advantage of high-performance computational resources.

Image processing

Image processing was performed with the freely available QGIS desktop 3.24.1 software and its add-on (SCP, semi-automatic classification plugin) (Congedo, 2016). In this research, the following satellite image processing steps were applied (Hantson *et al*., 2011):

Geometric correction

A geometric correction was made to each Landsat image to assign to each pixel the UTM coordinates corresponding to the UTM coordinate reference system WGS84 zone 14N. The images provided by the USGS were georeferenced with the QGIS software.

Atmospheric correction

Atmospheric correction was performed to eliminate the scattering and absorption effect of the atmosphere and to obtain the surface reflectance in the image using the dark object subtraction method of the QGIS software based on the image. This allowed improving the visual quality of the satellite image and eliminating the intrusive components of the atmosphere.

Pansharpening

This procedure combines information from the red, green, and blue (RGB) spectral bands and the panchromatic band. As a result, a multispectral image was obtained with the RGB bands with 15 m spatial resolution of the panchromatic band; for this, a Brovey transformation was applied based on intensity values of the bands (Congedo, 2016).

Histogram equalization

This procedure generates a uniform distribution of pixels in the image to improve the contrast of the satellite images to appreciate the details. The routines available in the Scikit-image library were applied according to the methodology described by Dey (2020). The correction was applied individually to each chromatic channel to obtain an image with optimized local contrast.

Processed Landsat images

Landsat images were cropped to the size of the municipality of Huehuetla and exported to jpg format for color feature extraction (Figure 1).

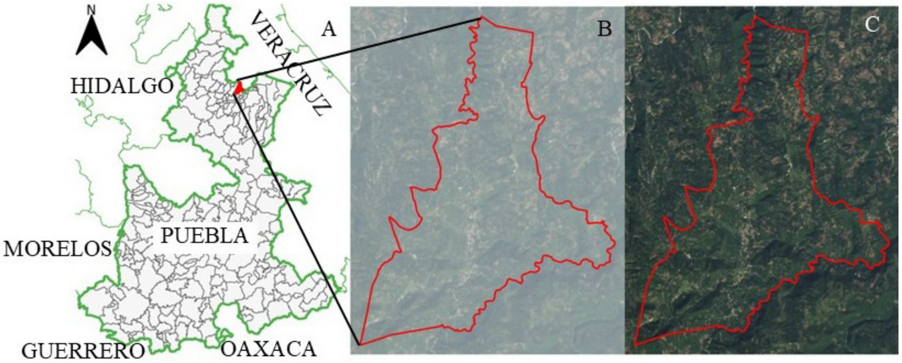


Figure 1. Combination of RGB bands from Landsat 8 satellite image in 2021. A: Location of the municipality of Huehuetla, in the northern Sierra of Puebla, Mexico; B: Landsat 8 image without corrections (spatial resolution 30 m); C: Landsat 8 image with geometric, atmospheric, and pansharpening corrections (spatial resolution 15 m).

Satellite image sampling

Image samples for each land cover and land use class were obtained using the region selection algorithm, which consists of extracting the three RGB spectral color bands of each pixel and their location coordinates (x, y) in the image. The region selection algorithm allows selecting an initial seed pixel and then comparing nearby (neighboring) pixels by means of a similarity criterion. The success of the extraction depends on the similarity criterion and the selection threshold (Valdés *et al*., 2015).

The selection of a seed pixel for each cover category was made based on information from the shapefile format of land use and vegetation of series I (INEGI, 2001) and VII (INEGI, 2021) of the geographic metadata catalogs containing vegetation cover, human settlements, grassland, and agricultural areas. These files were contrasted with the images to allow the identification of each cover category and to determine the precise location of the seed pixels.

After extracting similar pixels in each target class, four subsets of sample data were created corresponding to the target classes: agricultural (*A*), grassland (*G*), vegetation (*V*), and urban-rural (*U*). The total set of extracted pixel samples was processed to remove repeated samples within classes and then between classes to obtain an input dataset with three features or predictor variables of RGB spectral color bands associated with their corresponding target classes.

Vegetation indices

Vegetation indices are combinations of spectral bands. They are used as proxies to estimate the "greenness", density, vigor, or health status of vegetation based on its spectral response. Attenuated with other components such as soil, vegetation development is estimated by radiation intensity values (Mayorga-Arias *et al*., 2019). The seven vegetation indices used in this research were calculated based on the RGB spectral bands of Landsat satellite images (Table 1).

Input datasets

Input datasets E2002 and E2021 were created as a result of image sample extraction and calculation of vegetation indices. Each set consisted of 10 predictor variables from

Table 1. Vegetation indices calculated based on red (R), green (G), and blue (B) spectral bands.

Vegetation index	Formula
Excess green (ExG)	$E \text{ x } G = 2G - R - B$ (Ribeiro *et al*., 2005)
Excess red (ExR)	$E \text{ x } R = 1.4\ \mathrm{R} - \mathrm{G}$ (Meyer *et al*., 1998)
Difference between green and red excess (ExGR)	$E \text{ x } GR = exG\ - exR$ (Meyer and Neto, 2008)
Vegetation index extraction (CIVE)	$CIVE = 0.44R - 0.81G + 0.39B + 18.79$ (Kataoka *et al*., 2003)
Vegetative (VEG)	$VEG = \frac{G}{R^{a}B^{1-a}} con\ a = 0.667$ (Hague *et al*., 2006)
Normalized green-red difference (NGRDI)	$NGRDI = \frac{G - R}{G + R}$ (Gitelson *et al*., 2002)
Normalized difference (NDI)	$NDI = \left(\frac{G - R}{G + R} + 1\right) * 128$ (Gitelson *et al*., 2002)

three spectral bands (red, green, and blue) and seven vegetation indices calculated from these bands and was used to train and test the random forest classifier. E2002 consisted of 7370 observations, grouped into four target classes: *A* (1773), *G* (1653), *V* (3093), and *U* (851). E2021 consisted of 10 143 observations grouped into four target classes: *A* (2672), *G* (2246), *V* (3507), and *U* (1718).

Random forest classifier

The random forest (RF) classifier is an ensemble model that consists of calculating the average response of multiple decision trees to reduce the variance of individual trees to obtain better predictive performance and avoid the overfitting of the model (Breiman, 2001). The algorithm selects observations or replacement samples from the training set. Then, a decision tree grows with a subset of features or variables that are randomly selected. After training the decision trees, the prediction of a target class is performed with the majority vote.

The model seeks to minimize an impurity measure such as the entropy criterion or the Gini impurity criterion. The entropy criterion $I_E(t)$ for non-empty target classes is defined as:

$$I_E(t) = -\sum_{i=1}^{C} p(i|t) log_2\big(p(i|t)\big)$$

where $p(i|t)$ is the proportion of samples belonging to the target class i for a node t, and C is the number of classes. If all samples at a node belong to the same class, the entropy value $I_E(t) = 0$, but if there is a uniform class distribution, there is maximum entropy $I_E(t) = 1$.

The Gini impurity criterion $I_G(t)$ is defined as:

$$I_G(t) = 1 - \sum_{i=1}^{C} p(i|t)^2$$

where $I_G(t)$ reaches its maximum value ($I_G(t) = 1$) when the samples have a uniform distribution within classes (Raschka and Mirjalili, 2019).

The best RF model is obtained by means of a hyperparameter grid search and a cross-validation procedure. The most common hyperparameters of an RF model are the number of estimators or decision trees (*ne*) of the model, the node splitting criterion (*cr*), the maximum depth of decision trees (*md*), and the maximum number of input features (*mf*), which are randomly selected to split the nodes of a decision tree.

Performance metrics

To evaluate classifier performance, the metrics accuracy (*ACC*), precision (*P*), sensitivity (*S*), *F1* score, *F1-macro*, area under the ROC curve (AUC_{roc}) and area under the P-S curve ($AUC_{P\text{-}S}$) were used.

These metrics are derived from a confusion matrix that describes the performance of a classification model. This matrix contains four values: true positives (*TP*) are true responses predicted correctly; true negatives (*TN*) are negative responses predicted correctly; false positives (*FP*) are negative responses predicted as positive; and false negatives (*FN*) are positive responses predicted as negative (Table 2).

Table 2. Confusion matrix for the case of a binary classification of the random forest classifier.

		Predicted classes	
		Positive	Negative
True classes	Positive	*TP*	*FN*
	Negative	*FP*	*TN*

TP: true positives; *FP*: false positives; *FN*: false negatives; *TN*: true negatives.

ACC is defined as:

$$ACC = \frac{TP + TN}{TP + TN + FP + FN}$$

where *P* is the ratio of *TP* to total positive predictions, and is calculated by:

$$P = \frac{TP}{TP + FP}$$

S is the proportion of *TP* in relation to the total number of positive observed responses, that is:

$$S = \frac{TP}{TP + FN}$$

The *F1* score is the harmonic mean of *P* and *S*, and is defined as:

$$F1 = \frac{2\,P \times S}{P + S}$$

ACC, *P*, *S,* and *F1* take values between 0 and 1. A value close to zero indicates a low classifier performance, while a value close to one indicates high performance.

The overall *F1-macro* performance metric is the mean of the *F1* scores of each target class, and is calculated as:

$$F1\text{-}macro = \frac{1}{C}\sum_{i=1}^{C} F1_i$$

where $F1_i$ is the *F1* score of the *i*-th target class, and *C* is the total number of target classes. *F1-macro* is an appropriate metric when the target classes are unbalanced (Lipton *et al*., 2014).

For diagnosing classifier bias and variance problems, learning and validation curves were used. These curves are plotted as a graph of the *ACC* of training and validation against the number of samples of the input data. This plot shows whether the model has high variance or high bias, and whether collecting more data samples helps to reduce these problems.

The curve graph AUC_{ROC} associates the *TP* rate on the vertical axis against the *FP* rate on the horizontal axis, and each point on the curve represents a pair corresponding to a given decision level. The curve AUC_{ROC} takes values between 0 and 1; the closer to one, the better the performance of the classifier (Armesto, 2011). In binary classification, a AUC_{ROC} curve is obtained, while in multiclass classification, a AUC_{ROC} curve is obtained for each predicted class of the model.

The precision-sensitivity curve ($AUC_{P\text{-}S}$) identifies which values of *S* result in a reduction of *P* and vice versa. In an ideal model, the values of *P* and *S* are equal or very close to one. Analyzing the performance of a model using the curves AUC_{ROC} and $AUC_{P\text{-}S}$ provides more information about the performance of a classifier, especially when having unbalanced classes (Raschka and Mirjalili, 2019).

Random forest classifier training

Selection of the optimal characteristics and hyperparameters

The RF classifier was trained with the total set of 10 features. Subsets were selected based on the relative importance of the features obtained with the model (Raschka and Mirjalili, 2019). The selection of optimal hyperparameters of the RF classifier was performed by a lattice search and a cross-validation procedure with $k = 10$ folds of the training set.

The training and validation used 90 % of the total dataset. To evaluate the performance of the classifier in training, validation curves were generated showing the variation of the *ACC* metric from the training and validation sets as a function of the number of samples in the input dataset. The final model configuration was obtained by partitioning the data that generated the maximum *ACC* value after testing the possible combinations of hyperparameters (Table 3).

Table 3. Values of the hyperparameters that were combined in the grid search to obtain the best random forest model to estimate land cover change.

Model	Hiperparameter	Interval
RF	*ne*	50, 100, 120, 150, and 200
	cr	Entropy or Gini
	mf	Square root or Log_2
	md	8, 10, 11, 12, 13, 14, and 15

RF: random forest classifier; *ne*: number of estimators; *cr*: splitting criterion; *mf*: maximum number of features or input variables; *md*: maximum depth.

Predictive performance evaluation

The RF test for prediction was performed with the optimal hyperparameter set, with the full data set and cross-validation with $k = 10$ folds. In addition to *ACC*, all average RF performance metrics were obtained for each target class.

Calculation of land cover changes

The quantification of areas and rates of land cover change consisted of the detection and digital cartographic identification of vegetation and land use changes. Spatial analysis was used to evaluate land cover changes. Prediction with the optimal RF model was performed for each pixel of each satellite image delimiting the study area in both 2002 and 2021.

The agricultural target class included the area corresponding to bare soil in preparation; the grassland category included primary vegetation (herbaceous) and paddocks; the vegetation class included forests, secondary shrub vegetation, and shade crops; and the urban-rural class included the area occupied by buildings and infrastructure (highways, paved roads, houses, and streets) of the main population settlements.

RESULTS AND DISCUSSION

Selected characteristics or predictor variables

Based on the evaluation results of the importance of the characteristics or input variables with RF, five predictor variables with the highest importance were selected for the E2002 input dataset (*R, G, B*, *ExG*, and *VEG*). For the E2021 input dataset, the variables *R, G, B*, *ExR*, and *NGRD*I were selected (Figure 2).

Optimal hyperparameters and validation curves

The values of the optimal RF classifier hyperparameters selected for E2002 were *ne* = 120, *cr* = entropy, *mf* = square root, and *md* = 8. For E2021, *ne* = 200, *cr* = Gini, *mf* = log_2,

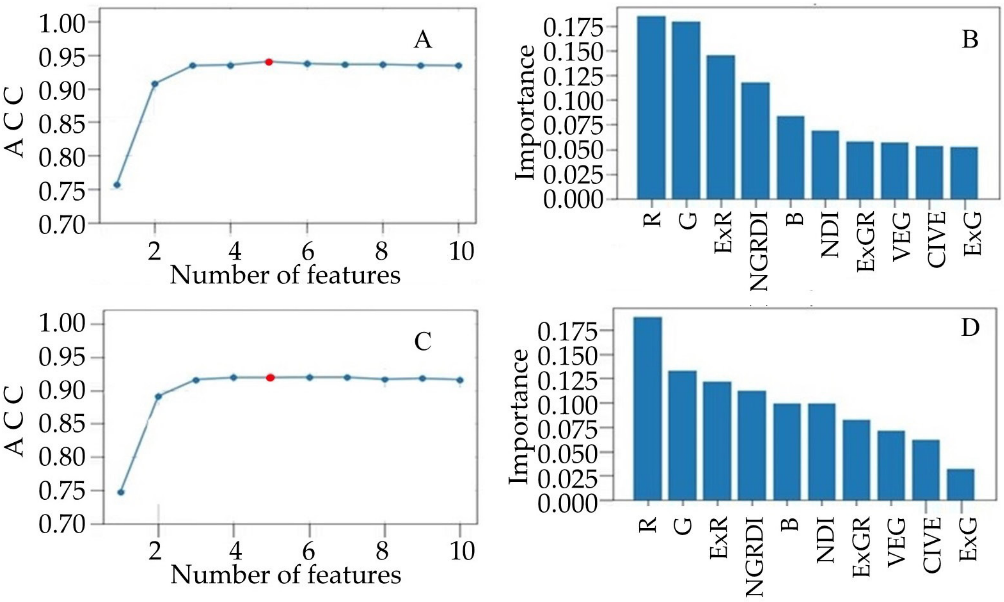


Figure 2. Optimal number and relative importance of input characteristics or predictor variables. A: E2002 performance curve; B: relative importance (E2002 scenario); C: E2021 performance curve; D: relative importance (E2021 scenario). *ACC*: accuracy; R: red spectral band; G: green spectral band; ExR: excess red; NGRDI: normalized green-red difference; B: blue spectral band; NDI: normalized difference; ExGR: difference between green and red excess; CIVE: vegetation index extraction; ExG: excess green.

and *md* = 10. The RF validation curve for E2002 showed a better fit of the data at 6000 samples, where the training and validation curves converge (Figure 3A). For E2021, the RF model was a better fit at 6500 samples, where the training and validation curves converge and stabilize (Figure 3B).

Evaluation in prediction of the random forest classifier

For the E2002 input data, RF obtained an *ACC* in prediction of 92.5 %, and for E2021, an *ACC* of 92.3 %; RF performance by target class showed that class *V* was the best ranked in both input scenarios, while class *U* was the worst ranked in terms of the F1 score metric (Table 4).

Confusion matrices

The predictive performance of RF in classifying the four target classes was analyzed with the normalized confusion matrices for scenarios E2002 and E2021. In both scenarios, class *V* was the best classified; however, RF in E2002 presented problems of confusion of class *U* with agricultural and grassland covers (classes *A* and *G*), and in E2021, it presented problems with class *A* (Figure 4).

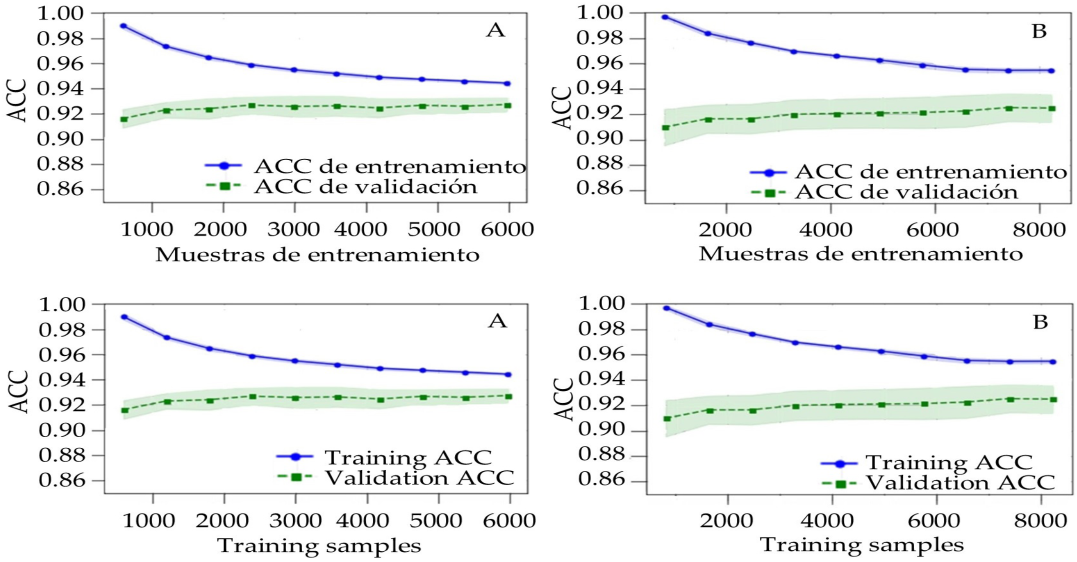


Figure 3. Learning curves of the random forest (RF) classifier for predicting target classes (agricultural, grassland, vegetation, and urban-rural). A: E2002 scenario; B: E2021 scenario. *ACC*: accuracy.

Table 4. Average prediction performance metrics of the random forest (RF) classifier for E2002 scenario and E2021 scenario.

RF	*ACC*	*F1-macro*	Class	*P*	*S*	*F1*
E2002	0.925 +/- 0.004	0.882+/- 0.006	*A*	0.898	0.898	0.898
			G	0.906	0.933	0.919
			V	1.000	1.000	1.000
			U	0.738	0.694	0.715
E2021	0.924 +/- 0.005	0.911 +/- 0.006	*A*	0.906	0.849	0.877
			G	0.969	0.960	0.965
			V	0.980	0.983	0.981
			U	0.773	0.869	0.818

A: agricultural; *G*: grassland; *V*: vegetation; *U*: urban-rural. *F1-macro* metrics: F1 global score; *P*: precision; *S*: sensitivity.

Performance curves

The receiver operating characteristic curves (AUC_{ROC}) for each target class of the RF classifier in E2002 are: class *V*, with a perfect score AUC_{ROC}= 1; classes *A* and *G*, with a very high score AUC_{ROC}= 0.99; and class *U*, with the smallest area AUC_{ROC}= 0.97. In the case of the classifier in E2021, classes *G* and *V* are equivalent to one, while classes *U* and *A* present an area of 0.98. However, all these values are very close to one (Figure 5). This shows that RF performed very well in both scenarios in identifying the four target classes according to this metric.

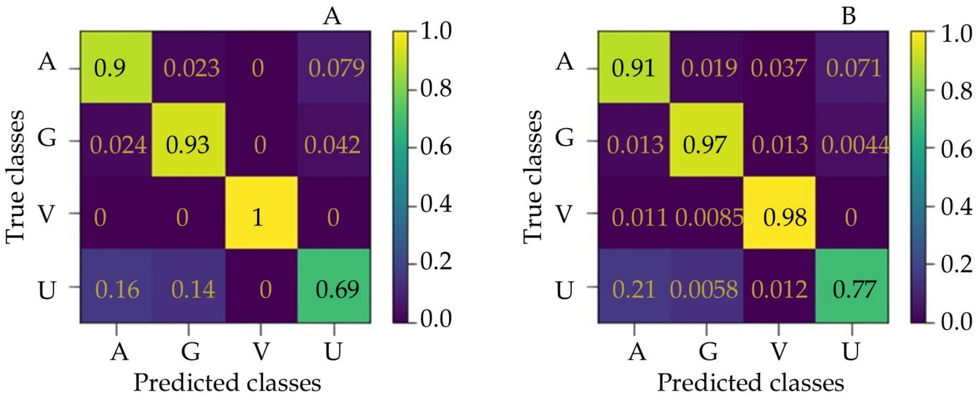


Figure 4. Normalized confusion matrix of the random forest classifier to predict agricultural (*A*), grassland (*G*), vegetation (*V*) and urban-rural (*U*) target classes. A: E2002 scenario; B: E2021 scenario.

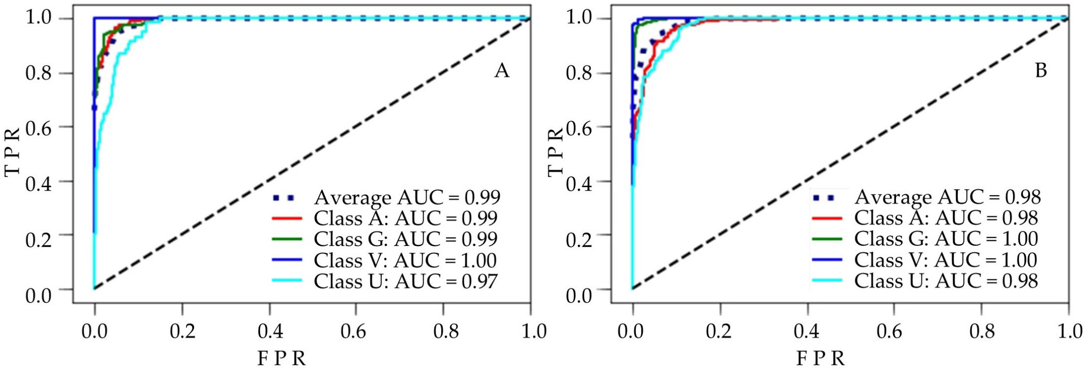
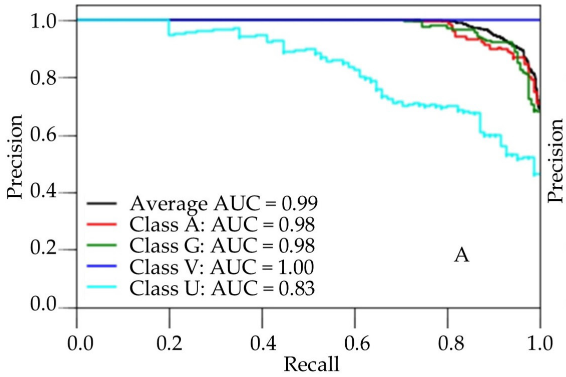


Figure 5. Random forest (RF) classifier AUC_{ROC} curves to predict agricultural (*A*), grassland (*G*), vegetation (*V*), and urban-rural (*U*) target classes. A: E2002 scenario; B: E2021 scenario. TPR: true positive rate; FPR: false positive rate; AUC: area under the curve.

The areas under the curve of precision versus sensitivity plot (AUC_{P-S}) show a good RF performance in identifying the target classes. In both scenarios (E2002 and E2021), RF correctly identified class *V*, and with lower performance, class *U* (AUC_{P-S}) = 0.83 and 0.9, respectively) (Figure 6).

The AUC_{ROC} and AUC_{P-S} areas are very close to one, so the RF performance is high for classes *A*, *G*, and *V* in both input scenarios; however, class *U* presented more problems for identification. This is explained by the smaller number of data samples used to identify this class and the confusion of *U* class samples with *A* and *G* class samples (*FP*), or the reverse, *A* or *G* class samples identified as *U* class (*FN*). In unbalanced classes, the performance metric AUC_{P-S} provides a more conservative estimator of classifier quality than AUC_{ROC} (Murphy, 2012).



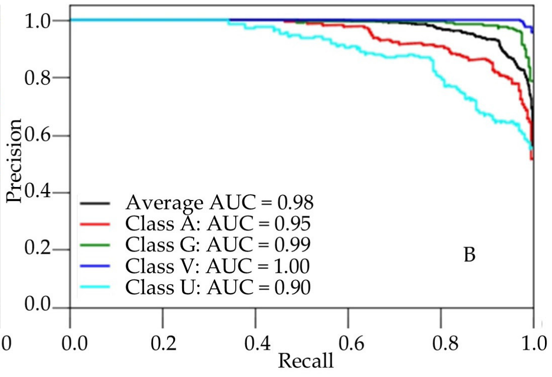


Figure 6. Accuracy-sensitivity curves (AUC_{P-S}) of the random forest (RF) classifier to predict agricultural (*A*), grassland (*G*), vegetation (*V*) and urban-rural (*U*) target classes; A: E2002 scenario; B: E2021 scenario. *AUC*: Area under the curve.

In this study it was not possible to obtain more pixel samples that would uniquely identify the *U* class to obtain a better balance between target classes, since in the urban-rural sector there are areas with backyards for agricultural use or grassland in the localities.

Quantification of land area and land cover change rate

With the optimal RF models for the years 2002 and 2021, a pixel classification of the images covering the study area was performed (Figure 7). During this period, an increase in class *V* and a decrease in class *G* were observed, while class *A* decreased slightly, and class *U* showed a notable increase in 2021.

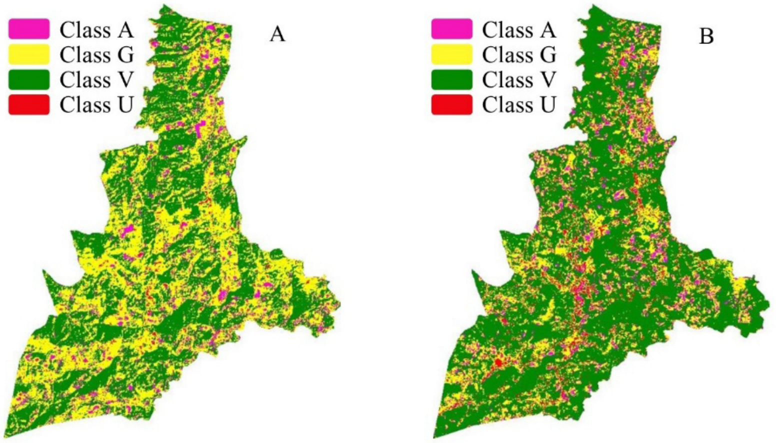


Figure 7. Land cover classification of the municipality of Huehuetla, in the Northern Sierra of Puebla, Mexico, carried out with the random forest (RF) classifier of the target classes (agricultural (*A*), grassland (*G*), vegetation (*V*), and urban-rural (*U*)). A: E2002 scenario; B: E2021 scenario.

The areas of classes *V* and *U* increased 617.3 and 186.3 ha, respectively; while classes *A* and *G* decreased 19.3 and 784.1 ha, respectively (Table 5). The decrease in areas of agricultural cover and grasslands (classes *A* and *G*) was due to their conversion to vegetation and urban-rural areas (classes *V* and *U*).

Table 5. Quantification of land cover changes (in area and relative change in relation to the total area) between 2002 and 2021 in the municipality of Huehuetla, in the Northern Sierra of Puebla, Mexico, using the random forest (RF) classifier.

Class	2002		2021		Change	
	Number of Pixels	Area (ha)	Number of Pixels	Area (ha)	Area (ha)	%
Agricultural	13 070	294.1	12 211	274.8	-19.3	-0.5
Grassland	75 064	1688.9	40 214	904.8	-784.1	-19.8
Vegetation	84 481	1900.8	111 916	2518.1	617.3	15.6
Urban-rural	3015	67.8	11 291	254.0	186.2	4.7

The increase in vegetation cover in Huehuetla is partially due to the expansion of forests and shade crops, driven by the Sowing Life Program that began in 2019 (Cotler-Ávalos *et al*., 2020). This program has promoted reforestation and the transition of agricultural and grassland areas to vegetation. In addition, an analysis by Apodaca-González *et al*. (2023) reveals that the perception of the effects of hurricanes influences the decision of producers to continue with agricultural and livestock activities. The negative impact of hurricanes such as Stan in 2005 and Dean in 2007 on the municipality's agricultural sector caused significant losses and affected the livelihoods of farmers (CENAPRED, 2009). The conversion of agricultural and grassland areas into vegetation and urban-rural zones reflects an adaptation to these environmental and socioeconomic challenges during this period.

CONCLUSIONS

The random forest machine learning classifier trained from Landsat 7 (2002) and Landsat 8 (2021) satellite images representative of the Huehuetla region, in the Northern Sierra of Puebla, Mexico, achieved an accuracy of 92.5 % in prediction to classify the four land cover types analyzed (agricultural, grassland, vegetation, and urban-rural). The most relevant features (predictors) were the red, green, and blue spectral bands in both images, as well as the excess green and vegetative plant indices for the 2002 Landsat 7 image, and the excess red and normalized green-red difference indices for the 2021 Landsat 8 image. Cover changes showed an increase in vegetation and urban-rural areas, while a decrease in agricultural and grassland areas was observed from 2002 to 2021.

REFERENCES

Apodaca-González C, Juárez-Sánchez J, Ramírez-Valverde B, Méndez-Espinoza J. 2023. Estrategias de adaptación campesina ante la variabilidad climática. Caso del café, municipio de Huehuetla, Estado de Puebla, México. Revista Geográfica Venezolana 64 (1): 73–84. https://doi.org/10.53766/rgv/2022.64.01.03

Ardila-López JP, Espejo-Valero OJ, Herrera-Escorcia JL. 2005. Validación de una metodología de clasificación de imágenes satelitales en un entorno orientado a objetos. Ingeniería 10(1): 61–69.

Armesto D. 2011. Pruebas diagnósticas: curvas ROC. Electronic Journal of Biomedicine 2011 (1): 77–82.

Baeza S, Baldassini P, Bagnato C, Pinto P, Paruelo J. 2014. Caracterización del uso/cobertura del suelo en Uruguay a partir de series temporales de imágenes MODIS. Agrociencia 18 (2): 95–105. https://doi.org/10.31285/agro.18.470

Breiman L. 2001. Random forests. Machine Learning 45 (1): 5–32. https://doi.org/10.1023/a:1010933404324

CENAPRED (Centro Nacional de Prevención de Desastres). 2009. Características e impacto socioeconómico de los principales desastres ocurridos en la República Mexicana en el año 2007. Gobierno de México. Sistema Nacional de Protección Civil. Centro Nacional de Prevención de Desastres. Ciudad de México, México. 624 p.

Chucos-Baquerizo N, Vega-Ventocilla EJ. 2022. Evaluation of machine learning algorithms in the classification of multispectral satellite images, case: Peruvian Amazon. Ciencia Latina Revista Científica Multidisciplinar 6 (1): 4946–4963. https://doi.org/10.37811/cl_rcm.v6i1.1843

Chuvieco-Salinero E. 2010. Teledetección ambiental: La observación de la Tierra desde el espacio. Digital Reasons: Ariel, España. 600 p.

CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad). 2021. Geoportal del Sistema Nacional de Información sobre Biodiversidad. http://www.conabio.gob.mx/informacion/gis/ (Retrieved: May 2021).

Congedo L. 2016. Semi-Automatic Classification Plugin: A Python tool for the download and processing of remote sensing images in QGIS. Journal of Open Source Software 6 (64): 3172. https://doi.org/10.21105/joss.03172

Cotler-Ávalos H, Manson R, Nava-Martínez JD. 2020. Evaluación de la focalización del Programa Sembrando Vida. Gobierno de México. Centro de Investigación en Ciencias de Información Geoespacial. Instituto de Ecología A.C. Consejo Nacional de Humanidades, Ciencias y Tecnologías. Ciudad de México, México. 53 p.

Dey S. 2020. Python Image Processing Cookbook: Over 60 recipes to help you perform complex image processing and computer vision tasks with ease. Packt Publishing: Birmingham, UK. 438 p.

Eisavi V, Homayouni S, Yazdi AM, Alimohammadi A. 2015. Land cover mapping based on random forest classification of multitemporal spectral and thermal images. Environmental Monitoring Assessment 187 (5): 291. https://doi.org/10.1007/s10661-015-4489-3

Gitelson AA, Kaufman YJ, Stark R, Rundquist D. 2002. Novel algorithms for remote estimation of vegetation fraction. Remote Sensing of Environment 80 (1): 76–87. https://doi.org/10.1016/S0034-4257(01)00289-9

Guevara-Romero ML, Montalvo-Vargas R. 2015. Cambio de uso de suelo y vegetación derivados de la dotación de infraestructura: Sierra Norte del Estado de Puebla. Nova Scientia 7 (13): 314–336.

Hague T, Tillet ND, Wheeler H. 2006. Automated crop and weed monitoring in widely spaced cereals. Precision Agriculture 7 (1): 21–32. https://doi.org/10.1007/s11119-005-6787-1

Hantson S, Chuvieco E, Pons X, Domingo C, Cea C, Moré G, Tejeiro JA. 2011. Cadena de pre-procesamiento estándar para las imágenes Landsat del Plan Nacional de Teledetección. Revista de Teledetección 36 (7): 51–61.

INEGI (Instituto Nacional de Estadística y Geografía). 2001. Uso del suelo y vegetación, escala 1:250000, serie I. http://geoportal.conabio.gob.mx/metadatos/doc/html/usv250s6gw.html (Retrieved: April 2022).

INEGI (Instituto Nacional de Estadística y Geografía). 2010. Compendio de información geográfica municipal 2010, Huehuetla, Puebla. Ciudad de México, México. https://www.inegi.org.mx/contenidos/app/mexicocifras/datos_geograficos/21/21072.pdf (Retrieved: April 2022).

INEGI (Instituto Nacional de Estadística y Geografía). 2021. Uso del suelo y vegetación, escala 1:250000, serie VII. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. Ciudad de México, México. http://geoportal.conabio.gob.mx/metadatos/doc/html/usv250s6gw.html (Retrieved: April 2022).

Kataoka T, Kaneko T, Okamoto H, Hata S. 2003. Crop growth estimation system using machine vision. *In* Proceedings 2003 IEEE/ASME International Conference on Advanced Intelligent Mechatronics (AIM 2003). Institute of Electrical and Electronics Engineers. Kobe, Japan. https://doi.org/10.1109/aim.2003.1225492

Lipton ZC, Elkan C, Naryanaswamy B. 2014. Optimal thresholding of classifiers to maximize F1 measure. *In* Calders T, Esposito F, Hüllermeier E, Meo R. (eds.), Machine Learning and Knowledge Discovery in Databases. ECML PKDD 2014. Lecture Notes in Computer Science 8725. Springer: Berlin, Germany. https://doi.org/10.1007/978-3-662-44851-9_15

Loera-Martínez J, Sepúlveda-Jiménez D, Sepúlveda-Robles DE. 2017. Propuesta para mejorar la productividad y rentabilidad en el medio rural del Municipio de Huehuetla, Puebla. ECORFAN 2: 1–13.

Mayorga-Arias D, Pazos-Roldán M, Uvidia-Vélez M. 2019. Uso del índice normalizado de vegetación para la elaboración de planos de cultivo. Opuntia Brava 11 (2): 261–265. https://doi.org/10.35195/ob.v11i2.760

Meyer GE, Metha T, Kocher M, Mortensen D, Samal A. 1998. Textural imaging and discriminant analysis for distinguishing weeds for spot spraying. American Society of Agricultural and Biological Engineers 41 (4): 1189–1197. https://doi.org/10.13031/2013.17244

Meyer GE, Neto JC. 2008. Verification of color vegetation indices for automated crop imaging applications. Computers and Electronics in Agriculture 63 (2): 282–293. http://doi.org/10.1016/j.compag.2008.03.009

Murphy KP. 2012. Machine learning: A probabilistic perspective. MIT Press: Cambridge MA, USA. 184 p.

Paredes-Gómez V, del Blanco-Medina V, Gutiérrez-García A, Nafría-García D. 2019. Seguimiento y evaluación de la capacidad de discriminación de cultivos herbáceos en regadío a partir de imágenes de satélite en el periodo 2016-2018. *In* Teledetección: Hacia una Visión Global del Cambio Climático. Asociación Española de Teledetección. Madrid, España, pp: 15–18.

Raschka S, Mirjalili V. 2019. Python machine learning (Third edition). Packt Publishing: Birmingham, UK. 770 p.

Ribeiro A, Fernández-Quintanilla C, Barroso J, García-Alegre MC. 2005. Development of an image analysis system for estimation of weed pressure. Precision Agriculture 2005: 169–174.

USGS (United States Geological Survey). 2019. Landsat satellite missions. United States Geological Survey. U.S. Department of the Interior. Reston, VA, USA. https://www.usgs.gov/landsat-missions/landsat-satellite-missions (Retrieved: May 2021).

Valdés SD, Mesejo LD, León MA. 2015. Selección de píxel semilla mediante wavelets para crecimiento por regiones difuso. Revista Internacional de Gestión del Conocimiento y la Tecnología 3 (1): 14–25.

Agrociencia

GENETIC DIVERSITY OF *Prunus persica* (L.) Batsch AND OTHER SPECIES OF *Prunus* USING MICROSATELLITES

Adriana Isabel **Pérez-Luna**[1], Guillermo **Calderón-Zavala**[1], Martha **Hernández-Rodríguez**[*2], Ángel **Villegas-Monter**[1], Salvador **Pérez-González**[3]

[1]Colegio de Postgraduados Campus Montecillo. Recursos Genéticos y Productividad, Fruticultura. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, State of Mexico, Mexico. C. P. 56264.

[2]Colegio de Postgraduados Campus Montecillo. Recursos Genéticos y Productividad, Genética. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, State of Mexico, Mexico. C. P. 56264.

[3]Investigador independiente del Centro de Recursos Genéticos y Mejoramiento de *Prunus*. Loma Pinal de Amoles 3, Vista Dorada, Huimilpan, Querétaro, Mexico. C. P. 76060.

* Author for correspondence: hernandez.martha@colpos.mx

ABSTRACT

Mexico has three genetic breeding programs for peaches and nectarines (*Prunus persica* (L.) Batsch). The diversity of its germplasm at a DNA level is unknown. Therefore, the aims of this work were: 1) to characterize the diversity of three *P. persica* populations using microsatellites or simple sequence repeats (SSRs); 2) to genetically compare two *P. persica* populations from the *Prunus* breeding programs of the College of Postgraduates and the Center for Genetic Resources and Improvement; and 3) to extrapolate the use of these SSRs to other *Prunus* species. DNA was extracted from leaves of 162 individuals from three locations: Montecillo and Coatepec Harinas, State of Mexico, and Huimilpan, Querétaro. A CTAB-based protocol was used. The variation of six SSRs was examined in polyacrylamide, and the diversity statistics were obtained for loci and populations. On average, 10.8 alleles were found per locus, a polymorphic information content (PIC) of 0.66, genic diversity of 0.69, and heterozygosity of 0.49. The most polymorphic locus was UCDCH15, with a PIC of 0.84 for *P. persica*, and UCDCH14, with 0.9 for the other three *Prunus* species. Regarding *P. persica* populations, only five SSRs were polymorphic. The expected heterozygosity was 0.512, 0.719, and 0.621, and the observed heterozygosity was 0.334, 0.716, and 0.768 for Montecillo, Querétaro, and Coatepec Harinas, respectively, indicating that Querétaro is the most diverse, and Montecillo, the most homogenous. The similarity between Montecillo and Coatepec Harinas was 0.543, followed by Montecillo and Querétaro (0.47) and Coatepec Harinas and Querétaro (0.183). For the other *Prunus* species, the six SSRs were polymorphic, confirming that their use can be extrapolated to related species such as apricot (*Prunus armeniaca* L.), plums (*P. salicina* L.), and cherries (*P. avium* L.). This is the first report on diversity in *P. persica* and some related species by SSRs in Mexico.

Keywords: *Prunus persica* (L.) Batsch, *Prunus armeniaca* L., *Prunus avium* L., *Prunus salicina* L., molecular markers, genetic variability.

Citation: Pérez-Luna AI, Calderón-Zavala G, Hernández-Rodríguez M, Villegas-Monter A, Pérez-González S. 2024. Genetic diversity of *Prunus persica* (L.) Batsch and other species of *Prunus* using microsatellites. **Agrociencia** 58(8): 985-998. https://doi.org/ 10.47163/ agrociencia.v58i8.3092

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: October 18, 2023.
Approved: October 28, 2024.
Published in Agrociencia:
December 18, 2024.

INTRODUCTION

Peach (*Prunus persica (*L.) Batsch) is the third most important deciduous fruit tree in the world after apples and table grapes (FAO, 2023). In Mexico, in 2022, Zacatecas was the main peach-producing state, with almost 11 800 ha planted and a production of over 87 000 Mg out of the country's total of 239 133 Mg of fruit harvested in 33 000 ha (SIAP, 2023). Peach (*P. persica*), apricot (*P. armeniaca*), plum (*P. salicina*), and cherry trees (*P. avium*) are Asian in origin (Li and Wang, 2020) and have a significant impact on human health and the economy.

Hybridization is used to increase the variability in the *Prunus* species in genetic fruit breeding programs. In Mexico, several *Prunus* breeding programs have stood out, led by the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP), Postgraduate College (COLPOS), and the *Prunus* Genetic Resources and Breeding Center (CRGyM), currently active and run by Dr. Salvador Pérez González, which have focused on obtaining new peach and nectarine cultivars (*P. persica*) (Pérez-González, 2007; Calderón-Zavala *et al*., 2019).

Diverse studies have been carried out using molecular markers to know the degree of kinship between individuals, to identify cultivars, and to protect intellectual property (Firas and Abdulkareem, 2015). In genetic diversity studies carried out on *P. persica* and other *Prunus* species, microsatellite or SSR (Simple Sequence Repeat) markers have been used (Sosinski *et al*., 2000; Struss *et al.,* 2003; Trifonova *et al*., 2021). These markers are characterized for being highly polymorphic, codominant, and informative, as well as for the possibility of being extrapolated to other species (García-Gómez *et al*., 2018; Butiuc-Keul *et al*., 2022).

Due to the interest in generating new peach, nectarine, and other *Prunus* species materials that satisfy farmers' demands, it is relevant to characterize the genetic diversity at the molecular level of the germplasm at COLPOS and CRGyM to know the existing degree of divergence and genetic similarity. Therefore, the aims of this work were: 1) to characterize the diversity of three *P. persica* populations using SSRs; 2) to genetically compare two *P. persica* populations from the *Prunus* breeding programs of the COLPOS and the CRGyM; and 3) to extrapolate the use of these SSRs to other *Prunus* species.

MATERIALS AND METHODS

Plant material and location of the experiment

For the genetic diversity study of *P. persica*, a total of 162 individuals were evaluated, out of which 85 were from COLPOS, 58 from CRGyM, and 19 from the Salvador Sánchez Colín Foundation. The individuals from COLPOS Campus Montecillo (19° 30′ N, 98° 53′ W, 2250 m altitude) were from an F_2 population composed of 35 peach trees and 50 nectarine trees. The individuals from the CRGyM established in Huimilpan, Querétaro (20° 22′ N, 100° 16′ W, 2294 m altitude) were 26 peach trees, 12 nectarine trees (derived from an F_2 and advanced selections), and 20 trees from three *Prunus* species that were used to validate the functionality of the SSRs: 11 plum trees (*P. salicina*), seven apricot trees (*P. armeniaca*), and two cherry trees (*P. avium*). Finally, 18 peach trees and one nectarine tree were among the 19 cultivars from the Salvador Sánchez Colín Foundation that came from the COLPOS breeding program.

It was not possible to obtain parental plant material from all individuals, since in some cases they were unknown and in others, the plants had died. Therefore, the sampled plants corresponded to advanced selections, some cultivars, and F_2 segregants. Before sampling, the plants were identified as nectarines or peaches when they were in production. Approximately 15 to 20 leaves were collected per tree at a height of 2 m, distributed in the four cardinal points (the second and third leaves completely extended), which were labeled and transported to the COLPOS Genetics Laboratory. The leaves were cleaned, and a sample was prepared per tree by collecting fragments of the leaves until 0.3 g was obtained, which was then stored at -80 °C until DNA extraction.

DNA extraction

The genomic DNA of peaches and nectarines was extracted following a protocol based on CTAB (cetrimonium bromide) (Doyle, 1991). The samples were homogenized using liquid nitrogen and 8.5 cm-long polypropylene pestles, directly in 2 mL conical-bottom tubes (Axygen). The tissue of each sample was pretreated with 1 mL of saline buffer (0.1 M Tris HCl, 0.001 M EDTA, 2 M NaOH, and 0.05 % bovine albumin). A Vortex Genie 2 shaker (Scientific Industries) was used to homogenize the sample, which was centrifuged at 13 000 rpm at 4 °C for 5 min. The supernatant was discarded, and the tissue was lysed with 1 mL of 2 % CTAB plus 5 μL of β-mercaptoethanol. The mixture was shaken again and incubated for 30 min at 55 °C. After incubation, 400 μL of 25:24:1 phenol:chloroform:isoamyl alcohol were added. The mixture was shaken for 5 min and centrifuged at 13 000 rpm at 4 °C for 10 min. A total of 500 μL of the supernatant was extracted and placed in a new 1.5 mL tube to precipitate the DNA with 50 μL of 7.5 M ammonium acetate and 500 μL of absolute cold isopropanol, leaving the mixture at -20 °C for 2 h. The pellet was sedimented by centrifuging at 13 000 rpm at 4 °C for 10 min. The supernatant was discarded, and the pellet was washed with 1 mL of 70 % cold ethanol. Next, it was centrifuged as in the previous step; the supernatant was discarded, and the pellet was left to dry at room temperature for 2 h. Finally, the DNA pellet was hydrated in 50 μL of TE (Tris-EDTA) pH 8.

The DNA of apricots, cherries, and plums was extracted based on the method described by Struss *et al*. (2003), with some modifications. First, the homogenized tissue was washed two or three times in saline buffer. Then, 800 μL of a lysis buffer, freshly prepared and preheated at 65 °C (2 % CTAB, 100 mM Tris-HCl pH 8, 1.4 M NaCl, 20 mM EDTA, 1 % PVP 40, and 10 mM DTT) were added to each sample, along with 10 μL of proteinase K. The mixture was incubated at 65 °C for 30 min. After incubation, the mixture was purified three times using 500 μL of chloroform:isoamyl alcohol

(24:1), followed by centrifuging at 13 500 rpm at 4 °C for 10 min in each purification, retrieving the supernatant in a new 1.5 mL tube. After the final purification, the DNA in the supernatant was precipitated with 0.6 volumes of cold isopropanol. The mixture was centrifuged at 13 500 rpm at 4 °C for 10 min. The pellet formed in the bottom of the tube was washed with 1 mL of 70 % ethanol, and it was recovered by centrifuging at 13 500 rpm at 4 °C for 5 min. The washed pellet was left to dry at room temperature and resuspended in 50 µL of RNase-free water for 1 h at 4 °C. Then, it was precipitated once again with 5 µL of 3 M sodium acetate and 2 volumes of 100 % ethanol for 1 h at -20 °C. Finally, the DNA was recovered by centrifuging at 13 500 rpm at 4 °C for 15 min, washed twice with 1 mL of 70 % ethanol, air-dried at room temperature, and rehydrated with 50 µL of TE pH 8.

PCR and marker electrophoresis

Six pairs of microsatellites reported in *P. persica* studies (Struss *et al*., 2003; Sosinski *et al*., 2000) were selected based on their ability to detect polymorphism (Table 1).

The annealing temperature of each marker was determined with a 50–60 °C temperature gradient test to eliminate the excess accessory bands using a BioRad C1000 thermocycler (USA). Once the annealing temperatures were determined (Table 1), each marker was amplified in 15 µL reactions with the following components: 10 ng of DNA, 0.25 µM of each primer, 0.2 mM of dNTPs, 1 U of Taq Polymerase (GoTaq Flexi, PROMEGA), and 1.5 mM $MgCl_2$. An Axygen MaxyGen II thermocycler (USA)

Table 1. Flanking sequences, size, and repeat unit of simple sequence repeat (SSR) markers used to determine diversity in *Prunus* species.

SSR/UR	Sequence 5′-3′	RFS	OS	AT
UCDCH14[1] /(CT)18	F-GTACACGGACCCAATCCTG R-TCTAACATCATGTTAAACATCG	155–185	139–185	51 °C
UCDCH15[1] /(CT)15	F-TCACTTTCGTCCATTTTCCC R-TCATTTTGGTCTTTGAGCTCG	78–120	82–140	54 °C
UCDCH19[1] /(CA)12	F-GTACAACCGTGTTAACAGCCTG R-ACCTGCACTACATAAGCATTGG	100–115	125[†] 117–143[¶]	59 °C
UCDCH21[1] /(CA)18	F-TTGTTGACCATCGAATATGAAG R-GAAGGTACATGGCGTGCC	140	86–110	55 °C
PCHGMS1[2] /(AC)12(AT)6	F-GGGTAAATATGCCCATTGTGCAATC R-GGATCATTGAACTACGTCAATCCTC	194	178–208	60 °C
PCHGMS2[2] /(CT)24	F-GTCAATGAGTTCAGTGTCTACACTC R-AATCATAACATCATTCAGCCACTGC	163	147–161	60 °C

[1]Struss *et al*. (2003); [2]Sosinski *et al*. (2000). RFS: reported fragment size; UR: repetition unit; AT: annealing temperature; OS: observed size; F: forward; R: reverse. [†]*P. persica*; [¶]other species.

was used with the following amplification program: one denaturation cycle at 94 °C for 5 min, followed by 35 cycles with denaturation at 94 °C for 1 min, a defined annealing temperature for each marker for 1 min, and extension at 72 °C for 2 min. The protocol ended with an extension cycle at 72 °C for 5 min (Struss *et al*., 2003).

For markers PCHGMS1 and PCHGMS2, the reaction volume was of 10 μL, mixing 4 ng of DNA, 0.12 μM of each primer, 0.2 mM of dNTPs, 0.5 U of Taq Polymerase (GoTaq Flexi, PROMEGA), and 1.5 mM of $MgCl_2$. For these markers, the PCR program consisted of a cycle of denaturation at 94 °C for 5 min, followed by 32 cycles with denaturation at 94 °C for 45 s, the annealing temperatures estimated for each primer for 30 s, and extension at 72 °C for 30 s, followed by a final extension cycle at 72 °C for 5 min (Sosinski *et al*., 2000). At the end of the PCR program, the reactions were chilled at 12 °C and their amplification signal was evaluated in 3 % agarose gels (Sigma, St. Louis, MO, USA), diluting the reactions with loading buffer (Tris 50 mM, EDTA 50 mM pH 8.0, 25 % saccharose, 0.25 % bromophenol blue) in a 2:1 ratio.

The polymorphism of each marker was defined by vertical electrophoresis using 3 μL of reaction mixture in a non-denaturing polyacrylamide gel using the MG33-1063 system (CBS Scientific, USA). Markers UCDCH19, UCDCH21, PCHGMS1, and PCHGMS2 were separated in 8 % 19:1 polyacrylamide gel, while markers UCDCH14 and UCDCH15 were separated in 10 % 29:1 polyacrylamide gel. Electrophoresis was performed for 2–3 hours at 250 V, using a 20 bp ladder (Sigma-Aldrich) as a molecular weight reference. The fragments were then detected by staining with $AgNO_3$ (Sigma, USA) according to the description by CIMMYT (2006) and documented with the MiniBis Pro 16 mm transilluminator (Bio Imaging Systems, Israel). The weight of each band was calculated using the GelAnalyzer software version 19.1 to generate the molecular data matrix. These amplification and electrophoresis conditions were applied to all the *Prunus* species evaluated.

Data analysis

Using the information obtained from the SSR markers, a data matrix was created in Excel. The number of alleles (Na) was calculated, along with the number of genotypes, the observed heterozygosity (*Ho*), the expected heterozygosity (*He*), the polymorphic information content (PIC), and the number of effective alleles (Ne) using the PowerMarker V3.25 (Liu and Muse, 2005) and GenAlEx V6.503 programs (Peakall and Smouse, 2012). The genetic distance was calculated following Nei (1972), and a dendrogram was obtained using the unweighted pair group method average (UPMGA). The analysis of molecular variance (AMOVA) was carried out using GenAlEx V6.503 (Peakall and Smouse, 2012), as well as the calculation of Wright's *F* values for five of the six loci examined, due to one of them being monomorphic.

RESULTS AND DISCUSSION

Diversity of *Prunus persica*

A total of 17 alleles were identified, with a mean of 5.7 per population. The germplasm from each location differed in terms of the allelic variability it carries. For example, 7.8 different alleles were found in the germplasm from Querétaro, while there were 4.8 alleles in the population from Montecillo and 4.4 alleles in Coatepec Harinas (Table 2). The germplasm from Querétaro displayed the highest gene diversity. A study carried out in La Palma, Spain, found that, when comparing peaches from this island with collections from Spain and other countries, the number of alleles per locus ranged between 3.2 and 4.7, with the one from La Palma being the lowest. The authors suggest that this lower diversity in La Palma may be due to its geographic isolation and to the process of hybridization between a limited number of genotypes (Pérez *et al*., 2020).

Based on the diversity indicators between populations, 50 % of the alleles were observed to be inherited from one generation to the next (number of alleles (Na) values in regard to the effective number of alleles (Ne)) (Table 2). However, these Ne values are higher than those reported by other authors, whose values fluctuate between 1.19 and 2.84 (Pérez *et al*., 2020).

Table 2. Diversity indicators of the populations studied based on five polymorphic simple sequence repeats (SSRs) in *Prunus persica* (L.) Batsch.

Populations		N	Na	Ne	Ho	He	F
Montecillo	Mean	85	4.8	2.636	0.334	0.512	0.273
	SE	0	1.5	0.700	0.088	0.119	0.132
Querétaro	Mean	38	7.8	4.439	0.716	0.719	-0.016
	SE	0	1.1	0.933	0.094	0.073	0.123
Coatepec Harinas	Mean	19	4.4	2.777	0.768	0.621	-0.248
	SE	0	0.8	0.275	0.076	0.049	0.098

N: total number of samples per population; Na: number of alleles; Ne: effective number of alleles; *Ho*: observed heterozygosity; *He*: expected heterozygosity; *F*: fixation index; SE: standard error.

In genetic diversity studies, two key concepts are found: allele richness and genetic uniformity. The latter is expressed via the expected heterozygosity (*He*), defined as the probability of two randomly chosen alleles in a sample being different. Their values range between 0 and 1 (Hoban *et al*., 2022). In this study, the values of *He* were 0.512 for the Montecillo population, 0.719 for the Querétaro population, and 0.621 for Coatepec Harinas. These results highlight the population from Querétaro as the one with the highest adaptive advantage. However, in this study, all three populations displayed high *He* values, suggesting that together they have a reservoir of alleles capable of producing offspring with greater advantages (Table 2).

These results contrast with those from other investigations, in which the values for *He* were below 0.5 when analyzing three peach tree populations from La Palma (*He* = 0.280), Spain (*He* = 0.398), and other countries (*He* = 0.476) (Pérez *et al.,* 2020). Meanwhile, in three apple tree (*Malus* x *domestica*) populations, a high gene diversity was found, with *He* values greater than 0.8 in populations in Galicia (0.853), Portugal (0.828), and the Canary Islands (0.834) (Pereira-Lorenzo *et al.,* 2018).

Regarding observed heterozygosity (*Ho*), the population from Coatepec Harinas displayed the greatest proportion of heterozygotic individuals, followed by populations from Querétaro and Montecillo (Table 2). The population from Montecillo was the least variable in terms of *Ho*, with a higher proportion of individuals that share the same allele, indicating a tendency towards a particular homozygotic state, since this population is an F_2. This result was corroborated with the fixation index (*F*), which was 0.273 for Montecillo, indicating a high homozygosity in comparison with the populations from Querétaro and Coatepec Harinas, whose *F* values were -0.016 and -0.248, respectively, indicating their tendency towards heterozygosity (Table 2). On average, the *F* value of the three populations was 0.003, indicating an absence of significant genetic differentiation between them.

Differences and similarities between populations

The Nei (1972) genetic distance revealed that the most similar populations were Montecillo and Coatepec Harinas (0.543), followed by Montecillo and Querétaro (0.47) and Coatepec Harinas and Querétaro (0.183). The similarity between Montecillo and Coatepec Harinas is explained because the individuals evaluated come from the same genetic breeding program (COLPOS), which implies the presence of alleles from the same common paternal origin expressed in two different environments. The similarity between the populations from Montecillo and Querétaro suggests that they shared parental origins, a hypothesis that still requires confirmation. On the other hand, the noticeable genetic difference between the populations from Coatepec Harinas and Querétaro is a significant finding, as it indicates favorable genetic diversity due to their belonging to two different breeding programs (COLPOS and CRGyM).

At the subpopulation level (nectarines and peaches), a very similar tendency was found to the one found between populations. The peaches from Montecillo are genetically very similar to those from Coatepec Harinas (0.512), followed by peaches from Montecillo and Querétaro (0.427) and Coatepec Harinas and Querétaro (0.192). In the case of the nectarines, the differences were not noticeable. The genetic distances indicated that the nectarines from Montecillo and Coatepec Harinas share a high level of similarity (0.629), followed by Montecillo and Querétaro (0.524) and Coatepec Harinas and Querétaro (0.384). When comparing peach and nectarine trees, obvious differences were found due to the expression of the pubescence gene. The nectarine and peach trees within Montecillo were highly different (0.076), the same as in Querétaro

(0.073). In the population from Coatepec Harinas (0.24), there were no broad results due to the limited number of nectarines within the population studied (one nectarine and 18 peaches).

The variation distribution by the molecular variance analysis (AMOVA) showed that 63 % of the variation is found within the individuals, 25 % among the three populations, and the lowest part of the variation (12 %), between individuals. These values are similar to those found in a genetic diversity study in five peach tree populations from Asia, the Americas, and Europe, with different degrees of domestication (wild and cultivars), in which the greatest diversity was explained within individuals (68 %), followed by the variation between populations (27 %) (Li *et al*., 2013).

The differences in the AMOVA indicate variability among groups (Laurentin, 2009). In this study, the main difference comes from the individuals that make up each population, highlighting their value within the germplasm population. The value for *Fis*, which represents the inbreeding coefficient and is derived from the *Ho* and *He* values within the populations, indicated a trend towards homozygosity (0.161). This trend could be overestimated, since one of the populations (Montecillo) is an F_2 with a lower *Ho* value than the other two populations (Table 2). This aspect requires greater research in order to understand the history of these populations and why the value of *Fis* pointed out this trend. The analysis without the population from Montecillo (F_2) gave a negative *Fis* (-0.0119), suggesting that the evaluated populations are genetically heterogenous and diverse as a result of different allelic combinations, unlike a positive *Fis* value that indicates that the populations are highly endogamic (Pérez *et al*., 2020).

Diversity of the six loci

The amplification of the six SSR loci showed that the size of the alleles was within the intervals reported in the literature. These markers, reported by Struss *et al.* (2003) for cherry trees and extrapolated to peach trees, revealed that the marker UCDCH15 was the most polymorphic, while marker UCDCH19 was monomorphic in the three populations with a 125 bp band.

The number of alleles varied between 8 and 16, with an average of 10.8 (Table 3). This average is greater in comparison with other investigations: 4.0 in Chen and Okie (2022), 5.67 in Trifonova *et al*. (2021), and 6.41 in Chavez *et al*. (2014). This variation may be due to the diverse origin of the three populations and the SSR selection in this study, which were chosen for their high informative value, based on a panel of 15 cherry tree cultivars, one plum cultivar (‘Santa Rosa’), one apricot cultivar (‘Patterson’), and one peach (‘Elegant Lady’) (Struss *et al*., 2003).

Per marker, the number of alleles was 14, 16, and 8 for UCDCH14, UCDCH15, and UCDCH21, respectively, which contrasts with reports by Struss *et al*. (2003), who found three alleles for UCDCH14 and UCDCH15 and four alleles for UCDCH21. Regarding the markers PCHGMS1 and PCHGMS2, eight alleles were found for each marker among the 142 materials evaluated, in comparison with the four and two alleles, respectively, reported by Sosinski *et al*. (2000) for a population of 28 peach

Table 3. Diversity parameters calculated for five simple sequence repeats (SSR) markers in 142 *Prunus persica* (L.) Batsch samples.

Marker	Heterozygosity	Number of genotypes	Number of alleles	Gene diversity	PIC
UCDCH21	0.37	12	8	0.64	0.58
UCDCH14	0.70	20	14	0.78	0.76
UCDCH15	0.57	21	16	0.86	0.84
PCHGMS1	0.38	8	8	0.63	0.58
PCHGMS2	0.45	6	8	0.55	0.53
Media	0.49	13	10.8	0.69	0.66

PIC: polymorphic information content.

cultivars. The number of alleles per marker helped identify 13 genotypes out of an average of 64 possible combinations, revealing specific allele combinations in five of the six positions of the peach and nectarine germplasm genome (Table 3).

The variation in the number of alleles is congruent with the discriminant capacity of the selected markers. This parameter is evaluated with the polymorphic information content (PIC), which had an average of 0.66. Marker UCDCH15 stood out as the most informative, with a PIC of 0.84, followed by UCDCH14 with 0.76 (Table 3). PIC values above 0.5 indicate highly informative markers. The five markers used in this work surpassed this criterion, as well as the values reported in other studies. Trifonova *et al*. (2021), who evaluated the genetic diversity of 85 peach materials from the Nikita Botanical Garden in Russia, reported an average PIC of 0.49 for 12 SSRs. Chen and Okie (2022) informed of a PIC of 0.32 in 112 peach materials from genetic breeding programs in the United States using 20 SSRs. Chavez *et al*. (2014) reported a PIC of 0.44 in 164 peach and nectarine materials with 36 SSRs, whereas Demirel *et al.* (2024) obtained a PIC of 0.2 using inter-simple sequence repeat markets (ISSR) to evaluate the genetic diversity in 54 genotypes of *P. persica*. Koohi *et al.* (2020) reported a PIC of 0.8 for 67 EST-SSRs evaluated in 293 *P. persica* individuals.

The heterozygosity (*H*), expected heterozygosity (*He*), and observed heterozygosity (*Ho*) of the markers were 0.49, 0.6, and 0.61, respectively. The value of *H* was greater than that reported by Chen and Okie (2022) (0.33) and similar to the one reported by Sosinski *et al*. (2000) (0.45). The *He* and *Ho* of the evaluated loci were similar to each other (0.61 and 0.6), in comparison to other works in which *Ho* is lower than *He*. For example, Trifonova *et al*. (2021) obtained values of 0.52 for *He* and 0.31 for *Ho*; Chavez *et al*. (2014) reported 0.49 and 0.41 for *He* and *Ho*, respectively; and Koohi *et al.* (2020) obtained 0.83 for *He* and 0.36 for *Ho*.

Heterozygosity reveals both the diversity and the genetic status in which two alleles of the same locus could be in. In this study, approximately 60 % of the individuals were heterozygotic. This can be attributed to both samples belonging to two genetic breeding programs in which one of the goals has been the generation of peaches and nectarines, some of which are F_2 segregating for the trait of pilosity. The gene responsible for the lack of pubescence is recessive (Vendramin *et al.,* 2014). Its expression is visible in 25 % of the second generation, where the heterozygotic expression is double, which led to the values of heterozygosis values found per marker.

Extrapolation of the SSRs used in *P. persica* to other *Prunus* species

The protocol used for DNA extraction in peaches and nectarines displayed limitations when applied to other *Prunus* species (plum, apricot, and cherry trees). Therefore, a new extraction protocol was standardized based on the methodology by Struss *et al*. (2003), with some modifications, which helped reach the purity, concentration, and integrity standards needed to carry out the PCRs and test the standardized microsatellites in peaches and nectarines. In this context, the six SSRs turned out to be polymorphic in the three *Prunus* species different from *P. persica*; that is, there was amplification in cherry, plum, and apricot trees, unlike peach and nectarine trees, where only five were polymorphic (except UCDCH19).

These results are consistent with those reported by Dettori *et al.* (2015), who mentioned the low level of polymorphism of *P. persica* in comparison with other species of the *Prunus* genus. In this study, the averages of the genetic diversity indicators were higher for the other species of *Prunus* than for *P. persica*. At least five markers displayed *H* values greater than 0.5. The three most informative markers were UCDCH14, UCDCH15, and UCDCH19 (Table 4). The latter marker contrasts in its discriminant power between species, since in *P. persica* it was monomorphic and had no informative power, whereas in the other *Prunus* species it did prove to be polymorphic and highly informative.

Table 4. Diversity parameters calculated for six simple sequence repeats (SSRs) in other *Prunus* species.

SSR	Number of genotypes	Number of alleles	Gene diversity	Heterozygosity	PIC
UCDCH21	13.0	12.0	0.71	0.54	0.70
UCDCH14	17.0	16.0	0.91	0.77	0.90
UCDCH15	16.0	13.0	0.90	0.35	0.89
UCDCH19	14.0	10.0	0.83	0.38	0.81
PCHGMS1	7.0	7.0	0.56	0.50	0.53
PCHGMS2	2.0	2.0	0.49	0.85	0.37
Mean	11.5	10.0	0.73	0.56	0.70

The extrapolation of markers developed for a species and applied to another of the same genus or even of different genera has been reported in the literature. Sosinski *et al*. (2000) not only tested *Prunus* but also SSR markers in *Arabidopsis thaliana*, while Bedö *et al.* (2018) managed to extrapolate six SSRs from *P. persica* to *P. armeniaca,* although only five turned out to be polymorphic, unlike our investigation, in which the six SSRs were extrapolated in *P. armeniaca.* This ability to extrapolate markers in species within the same genus helps broaden the use of the markers developed in one species for comparative genomics purposes. Using the six SSRs, the formation of three groups is observed: one including nectarines and peaches, another one with apricots and plums, and the third group containing only cherry trees (Figure 1).

The clustering of species was different when using five markers and omitting PCHGMS2 for being less informative (PIC = 0.37) (Figure 2). This omission helped separate groups by species more clearly, forming four groups: *P. persica, P. armeniaca, P. salicina*, and *P. avium*, with cherries as the furthest species from peaches and nectarines. These results coincide with reports by Dettori *et al.* (2015), who mention that cherry is the most phylogenetically distant species from *P. persica.*

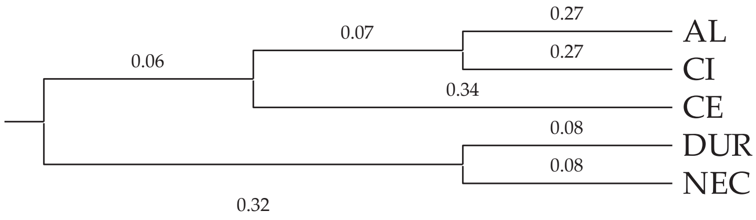


Figure 1. Dendrogram (UPGMA) based on the Nei genetic distances (1972) from six microsatellites and 26 samples of different species. AL: apricot (*Prunus armeniaca* L.); CI: plum (*P. salicina* L.); CE: cherry (*P. avium* L.); DUR: peaches (*P. persica* (L.) Batsch); NEC: nectarines (*P. persica* (L.) Batsch*)*.

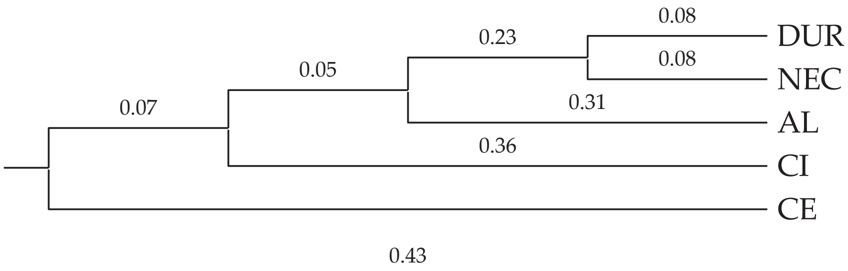


Figure 2. Dendrogram (UPGMA) based on the Nei genetic distances (1972) from five microsatellites and 26 samples of different species. DUR: peaches (*P. persica* (L.) Batsch*)*; NEC: nectarines (*P. persica* (L.) Batsch*)*; AL: apricot (*Prunus armeniaca* L.); CI: plum (*P. salicina* L.); CE: cherry (*P. avium* L.).

CONCLUSIONS

In this work, three collections of peach and nectarine germplasm (*Prunus persica* (L.) Batsch) were characterized using six microsatellites, five of which were polymorphic (UCDCH14, UCDCH15, UCDCH21, PCHGMS1, and PCHGMS1). The populations displayed a high heterozygosity, with the population from Querétaro being the most diverse. At a locus level, a trend towards homozygosity was observed in the population from Montecillo. The genetic distances showed that the populations are more related due to their founder origin. The efficiency of the six markers used in *P. persica* for comparative genomics purposes was shown. This extrapolation revealed that peaches and nectarines are genetically more distant from cherries (*P. avium* L.), followed by plums (*P. salicina* L.) and apricots (*P. armeniaca* L.).

ACKNOWLEDGEMENTS

To the National Council of Humanities, Sciences, and Technologies (CONAHCYT) for funding scholarship No. 746232, to the Postgraduate College for funding this research through project E01-57-09.02 under the "CONV_RGAA_2023" call, and to the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP) for the support given through the "Research Staff Professional Training and Development" program from 2019 to 2023.

REFERENCES

Bedö J, Baris E, Szani Z, Surányi D, Kiss E, Veres A. 2018. SSR based characterization of peach (*Prunus persica* L.) and apricot (*Prunus armeniaca* L.) varieties cultivated in Hungry. Acta Agraria Debreceniensis 74: 17–24. https://doi.org/10.34101/actaagrar/74/1659

Butiuc-Keul A, Coste A, Postolache D, Laslo V, Halmagyi A, Cristea V, Farkas A. 2022. Molecular characterization of *Prunus* cultivars from Romania by microsatellite markers. Horticulturae 8 (4): 291. https://doi.org/10.3390/horticulturae8040291

Calderón-Zavala G, Rodríguez-Alcazar J, Espíndola-Barquera MC, García-Ávila A. 2019. Variedades mejoradas de durazno (*Prunus persica* L.). Agroproductividad 12 (9): 81–83. https://doi.org/10.32854/agrop.v12i9.1492

Chavez DJ, Beckman TG, Werner DJ, Chaparro JX. 2014. Genetic diversity in peach [*Prunus persica* (L.) Batsch] at the University of Florida: Past, present and future. Tree Genetics and Genomes 10 (5): 1399–1417. https://doi.org/10.1007/s11295-014-0769-2

Chen C, Okie WR. 2022. Population structure and phylogeny of some U.S. peach cultivars. Journal of the American Society for Horticultural Science 147 (1): 1–6. https://doi.org/10.21273/JASHS05117-21

CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo). 2006. Protocolos de laboratorio: Laboratorio de Genética Molecular Aplicada del CIMMYT (Tercera edición). Centro Internacional de Mejoramiento de Maíz y Trigo: Ciudad de México, México. 92 p.

Demirel S, Pehluvan M, Aslantas R. 2024. Evaluation of genetic diversity and population structure of peach (*Prunus persica* L.) genotypes using inter-simple sequence repeat (ISSR) markers. Genetic Resources and Crop Evolution 71 (3): 1301–1312. https://doi.org/10.1007/s10722-023-01691-9

Dettori MT, Micali S, Giovinazzi J, Scalabrin S, Verde I, Cipriani G. 2015. Mining microsatellites in the peach genome: Development of new long-core SSR markers for genetic analyses in five *Prunus* species. Springer Plus 4 (1): 337. https://doi.org/10.1186/s40064-015-1098-0

Doyle J. 1991. DNA Protocols for plants. *In* Hewitt GM, Johnston AWB, Young JPW. (eds.), Molecular Techniques in Taxonomy. Springer: Berlin, Heidelberg, pp: 283–293. https://doi.org/10.1007/978-3-642-83962-7_18

FAO (Food and Agriculture Organization). 2023. FAOSTAT. FAO Database on food and agriculture. United Nations Food and Agriculture Organization. Rome, Italy. https://www.fao.org/faostat/es/#data/QCL (Retrieved: April 2023).

Firas RS, Abdulkareem A. 2015. Molecular markers: An introduction and applications. European Journal of Molecular Biotechnology 9 (3): 118–130. https://doi.org/10.13187/ejmb.2015.9.118

García-Gómez B, Razi M, Salazar JA, Prudencio AS, Ruiz D, Dondini L, Martínez-Gómez P. 2018. Comparative analysis of SSR markers developed in exon, intron, and intergenic regions and distributed in regions controlling fruit quality traits in *Prunus* species: genetic diversity and association studies. Plant Molecular Biology Reporter 36 (1): 23–35. https://doi.org/10.1007/s11105-017-1058-7

Hoban S, Archer FI, Bertola LD, Bragg JG, Breed MF, Bruford MW, Coleman MA, Ekblom R, Funk WC, Grueber CE *et al*. 2022. Global genetic diversity status and trends: Towards a suite of essential biodiversity variables (EBVs) for genetic composition. Biological Reviews 97 (4): 1511–1538. https://doi.org/10.1111/brv.12852

Koohi DM, Beigzadeh T, Sorkheh K. 2020. Novel in silico EST-SSR markers and bioinformatic approaches to genetic variation among peach (*Prunus persica* L.) germplasm. Journal of Forestry Research 31 (4): 1359–1370 https://doi.org/10.1007/s11676-019-00922-z

Laurentin H. 2009. Data analysis for molecular characterization of plant genetic resources. Genetic Resources and Crop Evolution 56 (2): 277–292. https://doi.org/10.1007/s10722-008-9397-8

Li X, Meng X, Jia H, Yu M, Ma R, Wang L, Cao K, Shen Z, Niu L, Tian J *et al*. 2013. Peach genetic resources: Diversity, population structure and linkage disequilibrium. BioMed Central Genetics 14 (1): 84. https://doi.org/10.1186/1471-2156-14-84

Li Y, Wang L. 2020. Genetic resources, breeding programs in China, and gene mining of peach: A review. Horticultural Plant Journal 6 (4): 205–215. https://doi.org/10.1016/j.hpj.2020.06.001

Liu K, Muse SV. 2005. PowerMarker: Integrated analysis environment for genetic marker data. Bioinformatics 21 (9): 2128–2129. https://doi.org/10.1093/bioinformatics/bti282

Nei Mi. 1972. Genetic distance between populations. The American Naturalist 106 (949): 283–292. https://doi.org/10.1086/282771

Peakall R, Smouse PE. 2012. GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28 (19): 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Pereira-Lorenzo S, Ramos-Cabrer AM, Ferreira V, Díaz-Hernández MB, Carnide V, Pinto-Carnide O, Rodrigues R, Velázquez-Barrera ME, Rios-Mesa D, Ascasíbar-Errasti J *et al.* 2018. Genetic diversity and core collection of *Malus* x *domestica* in northwestern Spain, Portugal and Canary Islands by SSRs. Scientia Horticulturae 240: 49–56. https://doi.org/10.1016/j.scienta.2018.05.053

Pérez-González S. 2007. Duraznero. Ecofisiología, mejoramiento genético y manual para su cultivo. Universidad Autónoma de Querétaro: Querétaro, México. 104 p.

Pérez V, Larrañaga N, Abdallah D, Wünsch A, Hormanza JI. 2020. Genetic diversity of local peach (*Prunus persica*) accessions from La Palma Island (Canary Islands, Spain). Agronomy 10 (4): 457. https://doi.org/10.3390/agronomy10040457

SIAP (Servicio de Información Agroalimentaria y Pesquera). 2023. Cierre de la producción agrícola (1980–2023). Gobierno de México. Servicio de Información Agroalimentaria y Pesquera. Ciudad de México, México. https://nube.siap.gob.mx/cierreagricola/ (Retrieved: April 2023).

Sosinski B, Gannavarapu M, Hager LD, Beck LE, King GJ, Ryder CD, Rajapakse S, Baird WV, Ballard RE, Abbott AG. 2000. Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. Theorical and Applied Genetics 101 (3): 421–428. https://doi.org/10.1007/s001220051499

Struss D, Ahmad R, Southwick SM. 2003. Analysis of sweet cherry (*Prunus avium* L.) cultivars using SSR and AFLP markers. Journal of the American Society for Horticultural Science 128 (6): 904–909. https://doi.org/10.21273/jashs.128.6.0904

Trifonova AA, Boris KV, Mesyats NV, Tsiupka VA, Smykov AV, Mitrofanova IV. 2021. Genetic diversity of peach cultivars from the collection of the Nikita Botanical Garden based on SSR markers. Plants 10 (12): 2609. https://doi.org/10.3390/plants10122609

Vendramin E, Pea G, Dondini L, Pacheco I, Dettori MT, Gazza L, Scalabrin S, Strozzi F, Tartarini S, Bassi D *et al*. 2014. A unique mutation in a MYB gene cosegregates with the nectarine phenotype in peach. PLOS ONE 9 (3): e90574. https://doi.org/10.1371/journal.pone.0090574

Agrociencia

GENOTYPE-ENVIRONMENT INTERACTION ON THE SEED QUALITY OF MAIZE HYBRID PROGENITORS (*Zea mays* L.)

Rocío Edelmira **Hernández-Caldera**[1], Alfredo Josué **Gámez-Vázquez**[1],
Gabino **García-de los Santos**[2], Rosalba **Zepeda-Bautista**[3],
José Luis **Arellano-Vázquez**[4], Miguel Ángel **Ávila-Perches**[1*]

[1]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Bajío. Carretera Celaya-San Miguel de Allende km 6.5, Celaya, Guanajuato, Mexico. C. P. 38110.
[2]Colegio de Postgraduados Campus Montecillo. Carretera México-Texcoco km 36.5, Texcoco, State of Mexico, Mexico. C. P. 56264.
[3]Instituto Politécnico Nacional. Escuela Superior de Ingeniería Mecánica y Eléctrica Zacatenco. Unidad Profesional Adolfo López Mateos, Colonia Lindavista, Mexico City, Mexico. C. P. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Valle de México. Carretera Los Reyes-Texcoco km 13.5, Coatlinchán, Texcoco, State of Mexico, Mexico. C. P. 56250.
*Author for correspondence: avila.miguel@inifap.gob.mx

ABSTRACT

The environmental conditions at the multiplication site have an impact on seed quality. Parents capable of counteracting changes in production environments are required to maintain and conserve the excellent attributes of maize (*Zea mays* L.) seed. This study sought to determine the effects of genotype and environment on the physical and physiological traits of single crosses and inbred lines, which are the progenitors of trilineal maize hybrids adapted to the High Valleys of Mexico. During the spring-summer 2014 and 2015 cycles, nine inbred lines, three single crosses, and three hybrids were evaluated in Coatlinchán, Celaya, and San Luis de la Paz, Mexico, in a randomized complete block design with three replications. The hectoliter and thousand-seeds weight, commercial seed percentage, standard germination, and after-cold test were evaluated. A pooled analysis of variance and multiple comparison of means with Tukey ($p \leq 0.05$) were performed. The site regression model (SREG) was used to account for the genotype-environment interaction. The best environments for physical quality were San Luis de la Paz 2014; for a thousand-seeds weight (295.3 g), Coatlinchán 2015; for hectoliter weight (74.7 kg hL^{-1}), Coatlinchán 2014; and for commercial seed percentage (76 and 73.3 %), San Luis de la Paz 2014. With the exception of Celaya 2014, the environments were statistically equal for physiological quality in standard germination, and San Luis de la Paz 2015 (82 %) was the best environment for germination after cold exposure. Regarding the genotype-environment interaction, parents M-47xM-46 and M-55xM-54 performed best for physical quality and M-43xM-44 for physiological quality at San Luis de la Paz; thus, seed production from these parents is possible in this location.

Key words: stability, High Valleys, Transition Zone, physical quality, germination.

Citation: Hernández-Caldera RE, Gámez-Vázquez AJ, García-de los Santos G, Zepeda-Bautista R, Arellano-Vázquez JL, Ávila-Perches MA. 2024. Genotype-environment interaction on the seed quality of maize hybrid progenitors (*Zea mays* L.). **Agrociencia** 58(8): 999-1012. https://doi.org/ 10.47163/ agrociencia.v58i8.3062

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: September 05, 2023.
Approved: October 24, 2024.
Published in Agrociencia:
December 16, 2024.

INTRODUCTION

The global maize production is 1423 million Mg, with an average yield of 4.9 Mg ha^{-1} (FAO, 2022). Maize is the foundation of food in Mexico, and 27.5 million Mg are produced, equivalent to 1.9 % of world production, with average yields of 3.9 Mg ha^{-1} (SIAP, 2022). One of the alternatives to increase yields is the adoption of certified seed; however, only 30 % of the area planted in Mexico with this crop is covered (Luna-Mena *et al*., 2012).

Seeds are one of the most important inputs in production, marking the beginning of the life cycle of each plant, so ensuring its quality is a priority and a requirement to achieve high yields in all plant species (Miloševic *et al.*, 2015). It is estimated that approximately 80 % of economically important crops are established with the direct or indirect use of seeds (Filho, 2015). Seed quality is the sum of genetic, physical, physiological, and sanitary attributes that affect the ability to perform vital functions related to germination, vigor, and longevity (Carranza-González *et al*., 2022).

In any cropping system, seed germination and vigor are important attributes to ensure uniform emergence and establishment of a crop (Finch-Savage and Bassel, 2016); however, these factors are greatly influenced by the physical characteristics of the seed (Sulewska *et al*., 2014). Seed size and the volumetric weight of a thousand seeds are considered indicators of physical quality, which is related to agronomic management and weather conditions during the development of the crop (Fernández-Sosa *et al*., 2015). Quality is highly influenced by environmental factors such as parent plant nutrition (Filho, 2015), temperature (Hatfield and Prueger, 2015), moisture, soil fertility, and the presence of pathogens (Akinnuoye-Adelabu and Modi, 2018), which have an effect on flowering, ripening, and harvest.

The evaluation of parents is critical for successful seed multiplication. It is important to understand the environmental impact on seed quality; therefore, the germination test is an essential component of seed production. Although it does not have a strong correlation with seedling emergence in the field (Navarro *et al*., 2015), it is the fastest and most commonly used labeling assessment and is required for seed sales.

Due to the importance of quality in the production of registered and certified seed from simple progenitor lines, crosses, and maize hybrids, the objective of the study was to determine the effect of the genotype-environment interaction on the physical and physiological quality characteristics of inbred lines and simple crosses, which are the progenitors of trilinear maize hybrids adapted to High Valleys of Mexico that were previously evaluated exclusively in that area (Virgen-Vargas *et al.*, 2014; 2016). The present work also involves the Transition and El Bajío regions to evaluate the response in these alternative zones for seed multiplication.

MATERIALS AND METHODS

Germplasm, evaluation environments, and agronomic management

Fifteen genotypes, nine inbred lines, three single crosses, and three trilinear hybrids used as controls (Table 1) were evaluated in three contrasting environments by altitude:

Table 1. Genealogy of maize (*Zea mays* L.) genotypes evaluated in five environments in the State of Mexico, and Guanajuato, Mexico.

Hybrid	Simple crossbreeding ♀[1]	Genealogy	Line ♂[2]	Genealogy
H-70[a]	M-55[a]xM-54[a]	(Tlax 151 SFC1-11-2-2-2) (Mich. 21 Comp.I-7-2-14-1-3)	CML-242[b]	Bat 8785 MH 10-1-1-2TL-1-3TL-3-1TL-b
H-66[a]	M-43[a]xM-44[a]	(Mich 21–181-14-1-16-5) (Mich21 Comp I-7-2-11-9)	M-52[b]	CML 169 BPVC-159-B-1-1-1-2-BTL
H-44[a]	M-47[a]xM-46[a]	(Tlax.77-2-1-2) (Mich 21-Comp I-7-2-14-5)	M-48[a]	Pob87xSIB/-1-4-3

[a] Inbred lines obtained by the Instituto National Forestry, Agricultural, and Livestock Research Institute (INIFAP); [b] inbred lines obtained by the International Maize and Wheat Improvement Center (CIMMYT). [1] ♀: Female; [2] ♂: Male.

1) High Valleys: Coatlinchán (COA), State of Mexico, located at 19° 29′ 05“ N, 98° 53′ 11″ W, and 2250 m altitude; 2) Transition Zone: San Luis de la Paz (SLP), Guanajuato, located at 20° 34′ 40“ N, 100° 49′ 37″ W, and 2004 m altitude; and 3) El Bajío: Celaya (CEL), Guanajuato, located at 21° 13′ 11“ N, 100° 29′ 41″ W, and 1765 m elevation. The first two were evaluated during 2014 and 2015, and the last one in 2014.

A randomized complete block experimental design with three replications was used. The experimental unit consisted of four 5-m long furrows. A total of five environments were studied, which were established under irrigated conditions. Fertilization was carried out using the 160-80-40 NPK formula, respectively. The stocking density was 65 000 plants ha^{-1}, and the rest of the agronomic management was carried out according to the recommendations of the National Forestry, Agricultural, and Livestock Research Institute (INIFAP) for each region.

Weather conditions of the test environments

The COA 2014 environment presented the highest precipitation (834 mm), as opposed to CEL 2014, which had the lowest value (461 mm). Precipitation for the 2015 agricultural cycle was lower than that of 2014 for the COA (9.8 %) and SLP (12.4 %) localities. Temperatures were similar in the COA and SLP environments, with maximum values of 23.8–25 °C and minimum values of 9.5–10.6 °C. CEL 2014 recorded the highest value for maximum (28.8 °C) and minimum (12.8 °C) temperatures. The High Valleys and Transition Zone environments presented similar climatic characteristics, unlike El Bajío, which was the most contrasting with higher temperature and precipitation at a lower altitude (Table 2).

For the inbred lines, the controlled pollination method was used with fraternal crosses to increase seed yield. The evaluation of the physical and physiological quality was

Table 2. Temperature and rainfall values recorded during the crop growth cycle at the five evaluation environments in the State of Mexico and Guanajuato, Mexico.

Localities	Temperature (°C)			Rainfall (mm)
	Maximum	Minumum	Mean	
COA-14	24	10	17	834
SLP-14	24	10	17	476
CEL-14	29	13	21	461
COA-15	25	10	13	752
SLP-15	25	11	18	417

COA: Coatlinchán; CEL: Celaya; SLP: San Luis de la Paz.

carried out at the seed analysis laboratory of the Genetic Resources and Productivity program of the Postgraduate College Montecillo Campus and the facilities of the Valley of Mexico Experimental Field (CEVAMEX) of the National Institute of Forestry, Agriculture and Livestock Research (INIFAP), both located in Texcoco, State of Mexico, Mexico.

Physical seed quality

The thousand-seeds weight (TWS) was evaluated according to the recommendations of the International Seed Testing Association (ISTA, 2016), expressed in grams. The hectoliter weight (HW) was determined and expressed in kg hL^{-1}. The commercial seed percentage (PCS) was obtained by grading 1 kg of seed per experimental plot and refers to the amount of seed that remained on top of the sieve with a round perforation of 7 mm diameter.

Physiological quality

Seed from hand pollination was used to increase lines and form single crosses and hybrids. Standard germination (SG) was determined as a percentage according to ISTA (2016) recommendations, except that four replicates of 25 seeds per experimental plot were used.

To determine germination after-cold test (GACT), the coils were placed in a refrigerator for 7 d at 10 °C. After this time, they were placed in a germination chamber with light at a temperature of 25 °C and relative humidity of 95±1 %. Seven days later, germination evaluation (GE) was performed using the same methodology for the standard germination test. For statistical analysis, data in percent were transformed with the arcsine function.

Statistical analysis

A combined analysis of variance was performed when environments were considered as random effects and genotypes as fixed effects. Means were compared using Tukey's test ($p \leq 0.05$), and Pearson's correlation analysis was performed (SAS Institute Inc., 2011). The GEA-R program (Pacheco *et al*., 2015) with the site regression model (SREG) was used for genotype-environment interaction analysis (GEI), which integrates analysis of variance and principal component analysis.

RESULTS AND DISCUSSION

Combined analysis of variance and comparison of means

The results of the combined analysis of variance (Table 3) for the thousand-seeds weight (TSW), hectoliter weight (HW), commercial seed (CS), standard germination (SG), and germination after-cold test (GACT) showed statistical differences ($p \leq 0.01$) among environments (E), genotypes (G), and for the genotype x environment interaction (GEI) for all variables. Based on the magnitude of the mean squares for the physical quality variables, the most important source of variation was G, followed by E and GEI. Of the total variance for the expression of the TWS variable, G presented a greater importance by contributing 60.7 %, followed by the effect of E with 32.1 % and GEI with 7.3 %. For HW expression, G determined 57.1 % of the variance, followed by 39.2 % of A, while GEI contributed only 3.7 %. For CS expression, G influenced 67.6 %, E 29.1 %, and GEI 3.4 %.

Table 3. Mean squares of the combined variance and principal components analysis of the site regression model (SREG) for physical and physiological quality variables of maize (*Zea mays* L.) parents and hybrids evaluated in five environments in the State of Mexico and Guanajuato, Mexico.

S.V.		Physical quality			Physiological quality		
	DF	TSW	HW	CS	DF	SG	GACT
Environment (E)	4	10 098.2*	136.1*	1 095.8*	4	1 012.6*	2 313.5*
Genotype (G)	14	19 116.4*	198.3*	2 548.7*	14	598.1*	635.3*
G×A	56	2 284.6*	12.7*	126.6*	56	163.5*	127.5*
CP1	17	17 174.8*	165.5*	5 073.4*	17	507.0*	1 315.8*
CP2	15	4 118.2*	25.1*	409.8*	15	382.3*	460.3*
Error	33	3643	24.7	524.7	33	264.5	483.4*
CV (%)		9.2	2.5	12.3		9.5	10.4

*Significance at 1 % probability; S.V.: source of variation; DF: degrees of freedom; TSW: thousand-seeds weight; HW: hectoliter weight; CS: commercial seed; SG: standard germination; GACT: germination after-cold test.

As for physiological quality (germination before and after-cold test), unlike physical quality, the most important source of variation was A, followed by G and GEI. For GE, A presented greater importance with 57.1 % of the variance, followed by A with 33.7 %, while GEI contributed 9.2 %. For GACT, A contributed 75.2 %, followed by G with 20.6 % and GEI with 4.2 %. According to the coefficients of variation, the results are considered acceptable.

Evaluation of environments

In the test of means (Table 4), statistical differences ($p \leq 0.05$) were observed for the physical and physiological quality variables. For physical quality, the highest value was obtained for TWS in E of SLP 2014, where a lower value of maximum temperature was presented in an interval between 10.3 and 23.8 °C during the crop cycle, in contrast to CEL 2014, which had the lowest TWS and A, with the highest temperature in an interval between 12.8 and 28.8 °C. In general, it was observed that the environments that presented better conditions for TWS expression were those with lower temperatures (High Valleys and Transition Zone). For the expression of HW, among environments, COA 2015 recorded the highest HW (74.7 kg hL^{-1}), while Celaya 2014 presented the lowest value (70 kg hL^{-1}), confirming the relationship that exists between the prevailing environmental conditions during crop development and the expression of seed HW (Fernández-Sosa *et al*., 2015).

In general, the High Valleys and Transition Zone environments presented the highest values (71.2–74.7 kg hL^{-1}), unlike El Bajío. For the commercial seed variable, the highest values were presented in the 2014 cycle (76 %), and the best environments were COA 2014 and SLP 2014 (76 and 73.3 %, respectively). Seed size is determined by the production environment, cropping practices, and variety genetics; in general, larger

Table 4. Test of means between evaluation environments for physical and physiological quality variables of maize (*Zea mays* L.) parents and hybrids evaluated in five environments in the State of Mexico and Guanajuato, Mexico.

Environment	Physical quality			Physiological quality (% germination)	
	Weight of a thousand seeds (g)	Hectolitric weight (kg hL^{-1})	Commercial seed (%)	SG	GACT
SLP 2014	295.3a	71.4b	73.3a	87a	76c
COA 2015	272.5b	74.7a	62.0b	90a	80b
COA 2014	270.4b	71.2b	76.0a	90a	76c
SLP 2015	267.8c	72.2b	61.0b	91a	82a
CEL 2014	253.7d	70.0c	65.1b	81b	57d

SG: standard germination; GACT: germination after-cold test; COA: Coatlinchán; CEL: Celaya; SLP: San Luis de la Paz. Means with the same letter in each column are statistically equal ($p \leq 0.05$).

seeds perform better in the field (Ambika *et al*., 2014). Seed development is dependent on nutrient accumulation, which is directly correlated with vigor, i.e., seed size and mass (Miloševic *et al*., 2015). In this way, the nutrients stored in the seed provide the necessary elements for seedling establishment.

The expression of physiological quality presented statistical differences ($p \leq 0.05$) for standard germination, where the SLP 2015 environment (91 %) showed the highest values; however, it was statistically equal to the SLP 2014 and COA 2014 and 2015 environments, unlike the Celaya locality, which had the lowest germination percentages. The standard germination test is the most commonly used for labeling seeds for sale, so it is recommended to perform it under ideal conditions. However, it is a poor predictor of field emergence because it takes place under favorable environmental conditions (Navarro *et al*., 2015). However, it can be used as a reference point for seed marketing to compare the physiological quality of the lots and to determine sowing density. This allows to obtain comparable results and to define seed buying and selling relationships (ISTA, 2004).

The germination after-cold test (GACT) correlated negatively with the minimum average temperature (r = -0.89) and the altitude of the localities (m), with r = 0.81. The environments of the Transition Zone with maximum, minimum, and mean average temperatures of 24.5, 10, and 15 °C coincided with the expression of this character, followed by that of High Valleys. The lowest expression was obtained in the locality of El Bajío. For GACT, the 2015 cycle presented the best values, while A of SLP 2015 had the highest average (82 %) and A of CEL 2014 (57 %) had low germination percentages. The response of GACT expression was correlated with the parameters of maximum temperature (r = -0.87) and the altitude of the locations (r = 0.73), which coincided with the correlation parameters of the SG percentage, but with a different degree of correlation. Other reports mention that germination, vigor, and viability are highly influenced by environmental factors in the field (Jyoti and Malik, 2013; Hatfield and Prueger, 2015). These attributes should be evaluated under stress conditions (e.g., after-cold test) to ensure uniform emergence and crop establishment (Navarro *et al*., 2015). In general, the best conditions for the expression of these characters were found in the High Valleys and Transition Zone environments and were negatively correlated with temperature.

Genotype evaluation

Among the female inbred lines, the one with the highest TWS was M-55, which is the female parent of the single female cross (SFC) of H-70, with 247.2 g (Table 5). Higher values (270.83 g) have been reported in progenitor lines of maize hybrids in the High Valleys of Mexico (Zepeda-Bautista *et al*., 2021), which contrasts with the present study as different environments were assessed. The female with the lowest TWS was M-47, sire of H-44. Of the six male lines, the highest TWS (286.5) was M-48, parent of H-44, followed by M-46 and M-54; the male lines with the lowest TWS were M-52 and M-44. Likewise, the SFC M-43xM-44 outperformed its H-70 and H-44 equivalents in TWS.

Table 5. Comparison of physical and physiological quality variables of maize (*Zea mays* L.) parents and hybrids evaluated in five environments in the State of Mexico and Guanajuato, Mexico.

Genotype	Physical quality			Physiological quality (% germination)	
	Weight of a thousand seeds (g)	Hectolitric weight (kg hL^{-1})	Commercial seed (%)	SG	GACT
M-43	243.1 g	69.3 j	48.6 d	85 e	68 g
M-47	222.3 h	74.1 d	20.3 e	89 c	71 f
M-55	247.2 f	70.3 i	55.8 d	88 c	68 g
M-48	286.5 d	75.9 b	73.8 b	90 c	83a
M-46	266.6 e	68.5 k	79.3 b	83 f	71 f
M-54	250.5 f	64.0 m	71.7 c	78 g	57 i
M-52	214.1 i	71.0 h	29.4 e	87 c	76 d
M-44	243.6 g	66.5 l	82.4 b	86 d	69 g
CML-242	250.3 f	72.9 e	73.9 b	76 h	65 h
M-47xM-46	295.4 c	73.0 e	74.8 b	89 c	73 e
M-43xM-44	311.9 b	72.6 f	76.4 b	92 c	81 b
M-55xM-54	305.2 b	72.6 g	83.4 b	93 b	79 c
H-44	313.9 b	76.5a	75.0 b	95a	84a
H-66	309.4 b	75.0 c	81.1 b	92 c	82a
H-70	319.0a	76.0 b	86.5a	94a	85a

GE: standard germination; GACT: germination after cold test. Means with the same letter in each column are statistically equal ($p \leq 0.05$).

In hybrid seed production, the number of seeds per kilogram is important to determine the seeding density of the female and male parents (Virgen-Vargas *et al*., 2014), and it also serves as an indicator of seed size. The female line M-47 expressed higher HW (74.1 kg hL^{-1}), followed by M-55 and M-43, while the male line M-48 presented higher HW (75.9 kg hL^{-1}) and outperformed its similar lines. Virgen-Vargas *et al*. (2016), using progenitor lines of maize hybrids from High Valleys in locations in the State of Mexico and Tlaxcala, reported a lower HW (71.75 kg hL^{-1}) than those obtained in this evaluation. When evaluating the physicochemical quality of maize for the dough and tortilla industry in hybrids from the central High Valleys of Mexico, Vázquez-Carrillo *et al*. (2020) reported a higher HW (77.2 and 78.4 kg hL^{-1}) than that obtained in this study for H-66 and H-70 (75 and 76 kg hL^{-1}).

For the commercial seed variable, the female lines M-55 and SFC M-55xM-54, progenitors of the hybrid H-70, had the highest value, followed by H-66 and H-44. The male line M-44, progenitor of the SFC of H-66, had the highest commercial seed value (82.4 %), presenting a high correlation (r = 0.75) with the TWS.

Regarding the percentage of SG, the female line with the highest germination was M-47 (89 %), while M-48 was the highest from the male inbred lines (90 %), both progenitors of H-44; the male lines CML-242 (76 %) and M-54 (78 %) presented the lowest germination values, a response associated with the first signs of disorganization and loss of integrity of the cell membrane system caused by lipid peroxidation, which reduces the physiological potential of the seed (Filho, 2015) as a consequence of inbreeding depression due to the increased frequency of homozygotes (de Farias and de Miranda, 2000), unlike hybrids. In terms of the germination percentage, the SFC of H-70 performed better than that of its H-44 and H-66 counterparts.

In seed production, the standard laboratory germination criteria for line certification are based on field inspections and tolerances. The producer is responsible for the quality of the line; however, a minimum of 90 % germination is recommended for the production of single crosses and hybrids in the basic and registered category. According to the evaluation results, all genotypes meet this criterion, with the exception of M-47xM-46 (SNICS, 2020). In GACT, the female line M-47 (71 %) and the male line M-48 (83 %), progenitors of H-44, showed the highest values, coinciding with GE. Therefore, these lines are considered as genotypes with good physiological potential to perform their vital functions under favorable and unfavorable environmental conditions (Filho, 2015). The SFC of H-66 outperformed its equivalents, while the female line M-43 (68 %) and the male line M-54 (57 %) showed low germination values in the after-cold test.

Genotype-environment interaction

By decomposing the sum of squares of the GEI into principal components (CP), the SREG analysis showed five components. The first three were significant ($p \leq 0.01$) for the parameters of physical and physiological quality. However, most of the variation is explained by the first two (Figures 1 and 2), accumulating values greater than 89 % for the evaluated variables, so they are considered valid parameters for the study of the GEI of maize hybrids (Crossa, 1990; Vázquez-Carrillo *et al*., 2012). In the Cartesian planes, the behavior of E, G, and the GEI is observed, while the environments are located in quadrants I and IV (Figures 1 and 2).

Weight of a thousand seeds

Two major components explain 89.43 % of the total variation (Figure 1A) due to the interaction expressed in TWS. The environments were concentrated in two sectors (mega-environments): sector one, located in quadrants I and IV, which concentrate four environments, and sector two, where the CEL 2014 environment is observed, being positioned in a different sector as it has contrasting altitude, temperature, and precipitation, unlike the rest of the environments.

In terms of genotypes, two classification groups can be seen, considering the trilinear hybrids (H-70, H-44, and H-66), simple crosses (M-47xM-46, M-43xM-44, and M-55xM-54), and the M-48 line as the first group, located in the first sector, and the remaining progenitor lines located in sectors 2, 3, 4, 5, and 6. The expression response

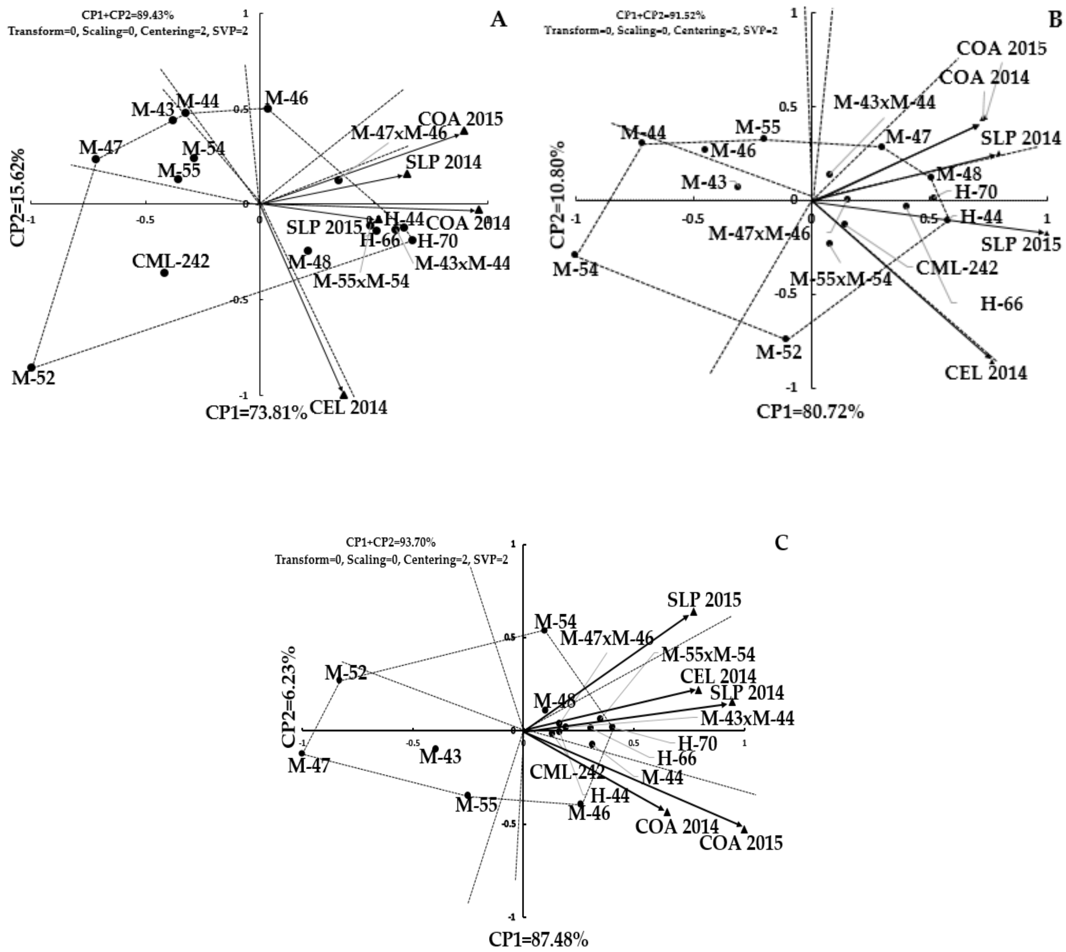


Figure 1. Genotype and environment dispersion based on the first two principal components of the site regression model (SREG) for physical quality in seed production of maize (*Zea mays* L.) parents and hybrids evaluated in five environments in the State of Mexico and Guanajuato, Mexico. A: thousand-seeds weight; B: hectoliter weight; C: commercial seed.

of line M-48 was associated with its vigor as a result of deriving lines from the cross between genotypes with different geographical origins (High Valleys × temperate and subtropical weather). The response of the second group (inbred lines) is related to the negative effect of inbreeding on the reduction of the mean of quantitative traits, as a consequence of recessive deleterious alleles known as inbreeding depression (de Farias and de Miranda, 2000).

The genotype located at the vertex with the highest TWS was H-70 in the mega environment formed by the localities of Coatlinchán and San Luis de la Paz. This hybrid presented adaptability to High Valleys and Transition Zone for the expression of this character, followed in magnitude by genotypes H-66, H-44, M-43xM-44, and

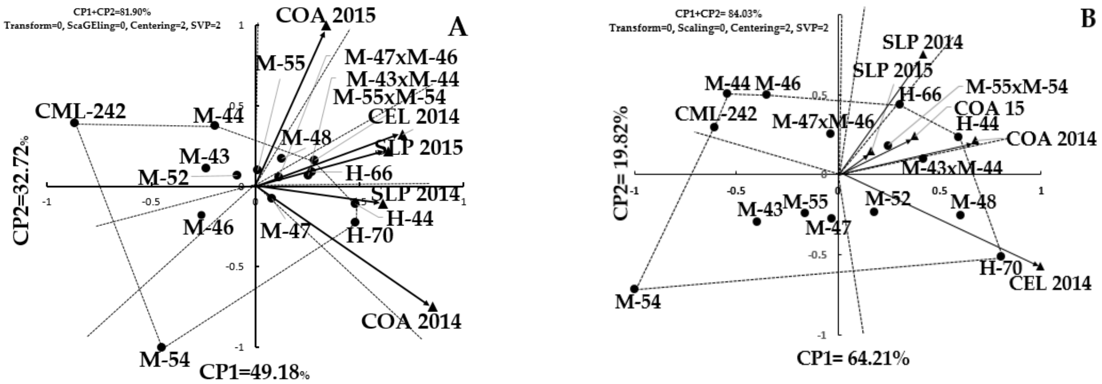


Figure 2. Cartesian plane of the genotype and environment contribution to the interaction using the first two principal components of the site regression model (SREG) on seed yield of maize (*Zea mays* L.) parents and hybrids evaluated in five environments in the State of Mexico and Guanajuato, Mexico. A: standard germination percentage; B: after-cold test.

M-55xM-54 that adapted to SLP 2015, given that the temperature and altitude conditions were similar to the environment from which they were generated (High Valleys). Line M-52 outperformed CML-242 in TWS at the CEL 2014 location, while lines M-46, M-44, M-43, M-54, M-55, and M-47 did not perform well in any environment.

Hectoliter weight

The environments were concentrated in three sectors (mega-environments) (Figure 1B). The COA 2015 and COA 2014 environments presented similar conditions for HW expression, unlike CEL 2014, which presented the best behavior. Genotype M-47 showed adaptation and better HW response to the COA 2014 and COA 2015 environments. In the second sector, the apex genotype with the highest HW (76.5 kg hL^{-1}) was H-44, which presented greater adaptability to San Luis de la Paz 2015 and 2014, followed by H-70 and H-66, while M-48 had better adaptability to SLP 2014, followed by H-70 at that location. The genotypes with the lowest HW values were lines M-54, M-44, M-46, M-43, and M-55, which did not perform well in the environments evaluated.

Commercial seed

For this parameter, the environments were concentrated in three sectors (mega-environments) (Figure 1C). The best environment was COA 2015, unlike COA 2014, which had the lowest percentage of commercial seed. In terms of genotype response, in general, no differentiation between hybrids and lines was observed, as lines M-48, M-54, M-44, and CML-242 grouped with trilinear hybrids and single crosses. The genotype with the highest expression in commercial seed production was H-70 in the CEL and SLP environments, both in 2014. Genotypes M-54 and M-48 were best adapted to SLP 2015; CML-242, H-44, M-44, H-66, M-47xM-46, and M-55xM-54 were best adapted to CEL 2014 and SLP 2014, while genotype H-70 presented the highest value in all environments. The M-46 genotype showed specific behavior in the COA environment. Lines M-55, M-43, M-47, and M-52 did not perform well.

Standard germination

In this case, the environments were concentrated in three sectors (mega-environments), where the best was COA 2014, in contrast to SLP 2015 (Figure 2A). The genotypes that presented the highest germination were H-70 in COA 2014 and H-44 in SLP 2014, while M-54, CML-242, and M-44 did not excel in any environment; M-55xM-54 presented its best response in COA 2015. The M-55 line showed a specific adaptation to COA 2015. The single female crosses M-55xM-54, M-43xM-44, and M-47xM-46 showed higher adaptability to CEL 2014 and SLP 2015, and line M-47 and hybrids H-44 and H-70 in COA 2014 and SLP 2014 environments. The apex genotypes H-44 and H-70 showed higher germination than M-47 in COA-15 and CEL-14, which could be an effect of inbreeding in the parents and vigor among hybrids (Guo *et al*., 2013; Omar *et al*., 2022).

Germination after-cold test

For this variable, the environments were concentrated in three mega-environments (Figure 2B), showing that the best environment was CEL 2014, unlike SLP 2015, which presented greater variation in the variable. No definite differentiation was observed in the response between hybrids and lines; the hybrids evaluated, the single crosses, M55xM54 and M43xM44, as well as the lines M48 and M52, were the ones that presented higher germination after low-temperature stress, so their genotype-environment interaction improved their response in the cold test. Genotypes H-66, M-55xM-54, and H-44 showed adaptability to COA 2015, COA 2014, and SLP 2015 environments. Hybrid H-70 presented the highest germination percentage after-cold test in CEL 2014, and genotype M-48 showed specific behavior in CEL 2014, while the most unstable genotype was line M-54.

CONCLUSIONS

Environmental conditions influenced physical and physiological quality characteristics of maize parents, especially hectoliter weight and marketable seed percentage. The parents M-47xM-46 and M-55xM-54 showed the best performance for physical quality and the latter for physiological quality, so that seed production from these parents can be carried out at San Luis de la Paz, Guanajuato. The production of hybrids H-66 and H-44 is suggested to be carried out in locations with altitudes of 2000 m, since they showed specific adaptation to the SLP 2015 environment in terms of physical quality.

REFERENCES

Akinnuoye-Adelabu BD, Modi TA. 2018. The effect of soil fertility and harvesting stage on maize seed quality under rain-fed conditions. Journal of Plant Nutrition 41 (17): 2170–2182. https://doi.org/10.1080/01904167.2018.1484133

Ambika S, Manonmani V, Somasundaram G. 2014. Review on effect of seed on seedling vigour and seed yield. Research Journal of Seed Science 7 (2): 31–38. https://doi.org/10.3923/rjss.2014.31.38

Crossa J. 1990. Statistical analyses of multilocation trials. Advances in Agronomy 44: 55–85. https://doi.org/10.1016/S0065-2113(08)60818-4

Carranza-González S, Carballo-Carballo A, Villaseñor-Mir HE, Hernández-Livera A, Ramírez ME. 2022. Calidad física de semilla en 24 variedades mejoradas de trigo liberadas en México. Revista Mexicana de Ciencias Agrícolas 13 (5): 827–840. https://doi.org/10.29312/remexca.v13i5.3003

de Farias NAL, de Miranda FJB. 2000. Inbreeding in two maize subpopulations selected for tassel size. Scientia Agricola 57 (3): 487–490. https://doi.org/10.1590/S0103-90162000000300018

FAO (Food and Agriculture Organization of the United Nations). 2022. Crop production. Rome, Italia. https://www.fao.org/faostat/es/#data/QCL (Retrieved: November 2022).

Fernández-Sosa R, Carballo-Carballo A, Villaseñor-Mir HE, Hernández-Livera A. 2015. Calidad de la semilla de trigo de temporal en función del ambiente de producción. Revista Mexicana de Ciencias Agrícolas 6 (6): 1239–1251. https://doi.org/10.29312/remexca.v6i6.573

Finch-Savage WE, Bassel GW. 2016. Seed vigour and crop establishment: Extending performance beyond adaptation. Journal of Experimental Botany 67 (3): 567–591. https://doi.org/10.1093/jxb/erv490

Guo B, Chen Y, Zhang G, Xing J, Hu Z, Feng W, Yao Y, Peng H, Du J, Zhang Y, Ni Z, Sun Q. 2013. Comparative proteomic analysis of embryos between a maize hybrid and its parental lines during early stages of seed germination. PLoS ONE 8 (6): e65867. https://doi.org/10.1371/journal.pone.0065867

Hatfield JL, Prueger JH. 2015. Temperature extremes: Effect on plant growth and development. Weather and Climate Extremes 10: 4–10. https://doi.org/10.1016/j.wace.2015.08.001

ISTA (International Seed Testing Association). 2004. International rules for seed testing. ISTA Editions: Zurich, Switzerland. 243 p.

ISTA (International Seed Testing Association). 2016. International rules for seed testing, ISTA Editions: Zurich, Switzerland. 192 p.

Jyoti, Malik CP. 2013. Seed deterioration: A review. International Journal of Life Sciences. Biotechnology and Pharma Research 2 (3): 374–385.

Luna-Mena BM, Hinojosa-Rodríguez MA, Ayala-Garay OJ, Castillo-González F, Mejía-Contreras JA. 2012. Perspectivas de desarrollo de la industria semillera de maíz en México. Revista Fitotecnia Mexicana 35 (1): 1–7.

Filho JM. 2015. Seed vigor testing: An overview of the past, present and future perspective. Scientia Agricola 72 (4): 363–374. https://doi.org/10.1590/0103-9016-2015-0007

Miloševic M, Vujakovic M, Karagic D. 2015. Vigour test as indicators of seed viability. Genetika 42 (1): 103–118. https://doi.org/10.2298/gensr1001103m

Navarro M, Febles G, Herrera RS. 2015. Vigor: Essential element for seed quality. Cuban Journal of Agricultural Science 49 (4): 447–458.

Omar S, Tarnawa A, Kende Z, Abd GR, Kassai MK, Jolánkai M. 2022. Germination characteristics of different maize inbred hybrids and their parental lines. Cereal Resspikech Communications 50 (4): 1229–1236. https://doi.org/10.1007/s42976-022-00250-9

Pacheco A, Vargas M, Alvarado G, Rodríguez F, Crossa J, Burgueño J. 2015. GEA-R (Genotype x environment analysis with R for Windows) Version 2.0. International Maize and Wheat Improvement Center. https://hdl.handle.net/11529/10203 (Retrieved: November 2022).

SAS Institute Inc. 2011. SAS/STAT®9.3 user´s guide. Software Release V 9.3. Cary, NC, USA.

SIAP (Servicio de Información Agroalimentaria y Pesquera). 2022. Panorama agroalimentario 2020. Gobierno de México. Secretaría de Agricultura y Desarrollo Rural. Ciudad de México, México. 196 p.

SNICS (Servicio Nacional de Inspección y Certificación de Semillas). 2020. Regla para la calificación de semillas de maíz. Servicio Nacional de Inspección y Certificación de Semillas. Ciudad de México, México. 29 p.

Sulewska H, Śmiatacz K, Szymańska G, Panasiewicz K, Bandurska H, Głowicka-Wołoszyn R. 2014. Seed size effect on yield quantity and quality of maize (*Zea mays* L.) cultivated in South East Baltic region. Zemdirbyste-Agriculture 101 (1): 35–40. https://doi.org/10.13080/z-a.2014.101.005

Vázquez-Carrillo MG, Mejía-Andrade H, Tut-Couoch C, Gómez-Montiel N. 2012. Características de granos y tortillas de maíces de alta calidad proteínica desarrollados para los Valles Altos Centrales de México. Revista Fitotecnia Mexicana 35 (1): 23–31. https://doi.org/10.35196/rfm.2012.1.23

Vázquez-Carrillo MG, Martínez-Gutiérrez A, Zamudio-González B, Espinosa-Calderón A, Tadeo-Robledo M, Turrent-Fernández A. 2020. Estabilidad de rendimiento y características fisicoquímicas de grano de híbridos de maíz de Valles Altos de México. Revista Mexicana de Ciencias Agrícolas 11 (8): 1803–1814. https://doi.org/10.29312/remexca.v11i8.1990

Virgen-Vargas J, Zepeda-Bautista R, Ávila-Perches MA, Espinosa-Calderón A, Arellano-Vázquez JL, Gámez-Vázquez AJ. 2014. Producción de semillas de líneas progenitoras de maíz: densidad de población e interacción. Agronomía Mesoamericana 25 (2): 323–335. https://doi.org/10.15517/am.v25i2.15439

Virgen-Vargas J, Zepeda-Bautista R, Avila-Perches MA, Espinosa-Calderón A, Arellano-Vázquez JL, Gámez-Vázquez AJ. 2016. Producción y calidad de semilla de maíz en Valles Altos de México. Agronomía Mesoamericana 27 (1): 191–206. https://doi.org/10.15517/am.v27i1.21899

Zepeda-Bautista R, Virgen-Vargas J, Suazo-López F, Arrellano-Vázquez JL, Ávila-Perches M. 2021. Nitrógeno en fertirriego para producir semilla de líneas progenitoras y cruzas simples de maíz: dosis y distribución. Revista Fitotecnia Mexicana 44 (2): 191–200. https://doi.org/10.35196/rfm.2021.2.191

Agrociencia

UNDERSTANDING THE ADOPTION AND IMPACT OF INFORMATION AND COMMUNICATION TECHNOLOGIES ON CLIMATE CHANGE AWARENESS: EVIDENCE FROM UNIVERSITY GRADUATES IN PAKISTAN

Manan **Aslam**[1,2], Zhiwen **Li**[1*], Muhammad **Naeem**[3], Abdullah Ahmed **Al-Ghamdi**[4],
Shabir **Ahmad**[5], Khujanov **Alisher**[6], Aymuratov **Rapat**[7], Abduraimov **Ozodbek**[8]

[1] Jiangsu University, School of Management. Zhenjiang, Jiangsu. China (212002)
[2] Muhammad Nawaz Shareef University of Agriculture Multan. Department of Agribusiness and Entrepreneurship Development, Multan. Pakistan (60000)
[3] Government College University, Faisalabad, Faculty of Economics and Management Sciences, Lyallpur Business School (LBS), Faisalabad. Pakistan (37000).
[4] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, P.O. 2455, Saudi Arabia (11451).
[5] Department of Plant Sciences, Quaid- i- Azam University Islamabad. Islamabad. Pakistan (45320).
[6] Institute of Biochemistry of Samarkhand State university named after Sharof Rashidov, Department of Plant Physiology and Microbiology, Samarkhand. Uzbekistan (141500).
[7] Instituto de Investigación Científica de Ciencias Naturales de Karakalpak de la Rama Karakalpak de la Academia de Ciencias de la República de Uzbekistán, Nukus. Uzbekistán (230100).
[8] Institute of Botany Academy Sciences Republic of Uzbekistan, Tashkent. Uzbekistan (100000).
* Author for correspondence: zhiwenli@ujs.edu.cn

ABSTRACT

Climate change is a pressing issue that significantly impacts agriculture, health, biodiversity, water resources, forests, and the socioeconomic well-being of populations, particularly in developing and least developed nations. Raising public awareness of climate change through accurate knowledge is a key strategy to encourage proactive mitigation and adaptation efforts. This study investigates the use of Information and Communication Technologies (ICTs) by university graduates to enhance climate change awareness, focusing on the role of both educators and students in knowledge transfer within academic environments. The research was conducted across 15 major universities in Pakistan, using a structured questionnaire to collect data on demographic, socio-cultural, technological, and economic factors influencing graduates' awareness and perceptions of climate change. A binary logistic regression analysis was used to assess the impact of ICTs on climate change awareness. The results indicate that access to smartphones, agricultural magazines, news bulletins, newspapers, television, and internet connectivity significantly enhances climate change awareness. However, access to radio and computers or laptops did not show a significant impact. Universities should integrate ICT-based training and outreach activities into their curricula, promoting technological solutions that address climate change challenges. Furthermore, collaboration between technological solution providers, environmental departments, and academic institutions is recommended to design ICT-driven strategies for raising climate change awareness among the youth.

Keywords: ICTs adoption, biodiversity, learning environment, climate change awareness.

Citation: Aslam M, Li Z, Naeem M, Al-Ghamdi AA, Ahmad S, Alisher K, Rapat A, Ozodbek A. 2024. Understanding the adoption and impact of information and communication technologies on climate change awareness: Evidence from university graduates in Pakistan. **Agrociencia** 58(8): 1013-1022. https://doi.org/ 10.47163/agrociencia.v58i8.3091

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: October 18, 2023.
Approved: October 23, 2024.
Published in Agrociencia:
December 05, 2024.

INTRODUCTION

Climate change represents a significant obstacle to global development in the 21st century (Naylor and Ford, 2023). Rising global temperatures are outpacing the natural rate of climate change (Aslam and Li, 2024), driven by a variety of natural processes over time. However, over the past 200 years, human activities have also contributed significantly to global warming. Despite ongoing debates, there is a broad consensus among researchers that human actions, such as deforestation, fossil fuel combustion, industrial processes, and water, air, and land pollution, play a critical role in accelerating climate change (IPCC, 2022). This has led many scholars to describe climate change as a human-induced alteration of the environment, primarily due to the increased concentration of greenhouse gases (Dwivedi *et al*., 2022; Santos *et al*., 2022; Malik and Ford, 2024).

During the 20th century, the average global temperature rose by approximately 0.6 °C, and it is expected to increase by an additional 2–3 °C by the end of the 21st century (IPCC, 2022). Like many other Asian nations, the majority of Pakistan's population remains unaware of the implications of climate change. However, the country faces critical challenges such as food shortages and frequent flooding, exacerbating its already struggling economy (Ahmad *et al*., 2024; Shakoor *et al*., 2011; Rahman *et al*., 2023; Aslam and Li, 2024). The global impact of climate change has become more evident with its increasing frequency and intensity (Lilavanichakul and Pathak, 2024; Rahman *et al*., 2023). Despite this, climate change is still not perceived as a critical issue in many developing nations (Leiserowitz *et al*., 2015).

Educating the public on the consequences of climate change and its effects on investments and livelihoods becomes essential (Meath *et al*., 2016; Thinda *et al*., 2020; Stelvia *et al*., 2022). According to empirical findings from the latest climate change index, Pakistan ranks fifth among the countries most vulnerable to climate change (Adnan *et al*., 2024). The Pakistani government has recognized climate change as a critical threat to the nation's development. In 2002, the Global Climate-Change Impact Studies Centre (GCISC) was established to analyze both global and national climate trends. GCISC is also responsible for informing the public and evaluating the impact of climate change on Pakistan's key socioeconomic sectors (Shahid and Piracha, 2010; Rasul, 2011).

Climate change has had a significant impact in Pakistan, particularly on agriculture (Shakoor *et al*., 2011; Rasul, 2011; Göpfert *et al*., 2019). Changes in rainfall patterns and temperature fluctuations have impacted crop yields and farmers' incomes (Thinda *et al*., 2020; Santos *et al*., 2022; Lilavanichakul and Pathak, 2024). In recent years, the adoption of technological advancements has become increasingly important for enhancing economic viability and agricultural productivity. The integration of Information and Communication Technologies (ICTs) facilitates more efficient exchange of information (Aslam and Li, 2024; Lilavanichakul and Pathak, 2024). By accessing valuable information on environmental conditions, climatic patterns, and market opportunities, farmers can make informed decisions. Internet-based technological solutions help mitigate information asymmetry by delivering reliable information rapidly and affordably (Aslam and Li, 2024).

In response to climate change, both mitigation and adaptation strategies are essential to benefit the public and minimize adverse outcomes (Jamal *et al*., 2022). Educator capacity building is critical for increasing their subject-matter expertise, as they play a pivotal role in raising public awareness about climate change (UNDP, 2008). Raising awareness through comprehensive education and training empowers individuals at all levels of society to actively engage in climate change mitigation and adaptation efforts.

University graduates, in particular, require a solid understanding of climate change impacts, and this awareness can be influenced by the adoption of ICTs. Providing university graduates with critical knowledge about climate change increases their ability to contribute to climate action and adaptation initiatives. Therefore, this research aims to explore the impact of ICT adoption on climate change awareness among university graduates from major universities and higher educational institutions in Pakistan.

MATERIALS AND METHODS

Data collection

The study was conducted in three stages. In the first stage, 15 prominent public sector universities in Pakistan were selected from different regions (Table 1). In the second phase, data was collected through interviews with 20 graduates from each selected university, resulting in a total sample size of 300 respondents. These interviews assessed the socio-personal profiles and awareness levels of the participants. In the final phase, the study identified key ICT-based factors influencing climate change awareness among university graduates. These factors were evaluated through hypotheses designed to test their significance.

The following hypotheses were constructed to test the impact of various ICTs on climate change awareness: H1, access to television has a significant effect on climate change awareness; H2, access to smartphones significantly impacts climate change awareness; H3, access to agricultural magazines, news bulletins, and newspapers has a significant effect on climate change awareness; H4, access to radio has a significant link with climate change awareness; H5, access to computers or laptops significantly influences climate change awareness; and H6, access to internet connectivity has a strong relationship with climate change awareness.

A purposive sampling technique was used to select 300 university graduates from the selected universities. Data collection took place between August 20 and October 17, 2023, using face-to-face interviews conducted by trained enumerators. Both open-ended and close-ended questions were used to gather comprehensive data.

Table 1. Distribution of selected universities by region in Pakistan.

Region	Universities
Federal Region (3 out of 13)*	- Quaid-i-Azam University, Islamabad - National University of Sciences and Technology (NUST) - COMSATS University, Islamabad
Punjab Region (4 out of 22)*	- University of the Punjab, Lahore - MNS University of Agriculture, Multan - University of Agriculture, Faisalabad - University of Veterinary and Animal Sciences, Lahore
Sindh Region (3 out of 15)*	- Sindh Agriculture University - Mehran University of Engineering and Technology - University of Karachi
Khyber Pakhtunkhwa (KPK) Region (3 out of 16)*	- University of Agriculture, Peshawar - Ghulam Ishaq Khan Institute of Engineering Sciences and Technology (GIKI) - Military Academy, Abbottabad
Balochistan Region (3 out of 5)*	- Balochistan University of Information Technology, Engineering and Management Sciences (BUITEMS)
Azad Jammu and Kashmir (AJK) Region (1 out of 2)*	- University of Azad Jammu and Kashmir
Gilgit Baltistan Region (1 out of 1)*	- Karakoram International University

*Number of selected universities for this study out of the total number of existing universities per region.

Statistical Analysis

Descriptive statistics were used to analyze the socio-personal characteristics of the respondents. To assess the impact of ICTs on climate change awareness, binary logistic regression was applied. The data were analyzed using SPSS 26.0 (IBM Statistics).

The probability of adopting ICTs to understand the impacts of climate change was modeled using a logistic regression framework. The adoption probability, denoted by *Pi*, and the odds of adoption, represented by:

$$Y = \left(\frac{Pi}{1 - Pi}\right)$$

where *Pi* is the probability of a respondent adopting ICTs to become aware of climate change, and *1-Pi* is the probability of not adopting ICTs.

The odds ratio for ICT adoption is given by:

$$ln(Odds) = \ln\left(\frac{p}{1-p}\right) = \beta 0 + \beta i$$

where $\beta 0$ is the intercept, and βi represents the coefficients of the independent variables.

The odds ratio in this equation represents the probability that an individual will use ICTs to understand climate change versus the likelihood that they will not. If the odds ratio is greater than one, it means that increasing a continuous variable or categorizing an independent variable increases the likelihood of ICT adoption.

ICT adoption is measured as a binary outcome:

$P = 1$ if a university graduate utilizes ICTs to learn about climate change, and
$P = 0$ if the individual does not use ICTs for this purpose.

The specific logistic regression model used to estimate the odds of ICT adoption is:

$$\ln\left(\frac{p}{1-p}\right) = \beta 0 + \beta 1W1 + \beta 2W2 + \beta 3W3 + \beta 4W4 + \beta 5W5 + \beta 6W6 + \varepsilon$$

where $W1$ denotes access to television; $W2$ access to smartphones; $W3$ access to agricultural bulletins, magazines, or newspapers; $W4$ access to radio; $W5$ access to computers; and $W6$ access to the internet. In the model, $\beta 0$ serves as the intercept term, while $\beta 1$ to $\beta 6$ are the coefficients for the independent variables (W1 to W6). Finally, $\in$ represents the error term in the equation, accounting for the variation not explained by the independent variables.

The independent variables were measured using a five-point Likert scale: strongly agree (5), agree (4), neutral (3), disagree (2), and strongly disagree (1). These variables measure access to various ICT tools that may influence an individual's decision to adopt technologies for understanding the impacts of climate change.

RESULTS AND DISCUSSION

Awareness level of climate change

Out of the 300 individuals surveyed, 230 graduates (77 %) had a minimal level of awareness about climate change. A smaller group of 30 graduates (10 %) had medium knowledge or awareness, while 40 graduates (13 %) exhibited the highest level of awareness on the subject. This distribution reveals a significant gap in climate change awareness among the sample population (Table 2).

Table 2. Respondent distribution based on climate change awareness levels.

Awareness level of climate change	Frequency	Percentage
Low	230	77
Medium	30	10
High	40	13
Total	300	100

Importance of climate change for respondents

To make well-informed decisions, the general public must be aware of the issue. When asked about the importance of climate change, 160 graduates (53 %) expressed that climate change is highly important, while 20 graduates (7 %) stated it was fairly important. On the other hand, 50 graduates (17 %) considered it not highly important, and 70 graduates (23 %) felt it was not important at all (Table 3). This indicates that while a majority are concerned, there is still a notable proportion of the population that does not recognize the severity of the issue.

Table 3. Respondent distribution based on the significance of climate change.

Climate change importance	Frequency	Percentage
Highly important	160	53
Fairly important	20	7
Not highly important	50	17
Not important at all	70	23
Total	300	100

Climate change as a threat

Climate change is expected to have a significant impact on both sustainable incomes and livelihoods. Among the respondents, 200 graduates (66 %) viewed climate change as a significant threat to sustainable livelihoods and global development. A total of 50 graduates (17 %) expressed neutrality, while another 50 (17 %) did not see climate change as a concern (Table 4). These results are consistent with the findings of Aslam and Li (2024), showing a general consensus on the dangers of climate change, although some graduates remain indifferent.

Table 4. Distribution of respondents regarding climate change as a threat.

Climate change is perceived as a threat	Frequency	Percentage
Yes	200	66
No	50	17
In-between	50	17
Total	300	100

Binary logistic regression for ICTs adoption and climate change awareness

A binary logistic regression model was employed to examine the influence of ICT adoption on climate change awareness. The Cox and Snell R^2 value of 0.689 indicates that 69 % of the variation in climate change awareness can be explained by the factors studied, while the Nagelkerke R^2 value of 0.813 shows that 81.3 % of the variation is explained by the model (Table 5).

Table 5. Model summary for the binary logistic regression on ICT adoption and climate change awareness.

Metric	Value
-2 log likelihood	11.59
Value of Cox and Snell R^2	0.689
Value of Nagelkerke R^2	0.813

The Hosmer and Lemeshow test yielded a chi-square value of 1.29 ($p > 0.05$), confirming that the model is a good fit for the data (Table 6). University graduates had adequate access to communication, information, and technology, which increased awareness of climate change and its consequences for society. Of the information they know, 92.9 % is correct. The overall correction estimate was 97.5 %. This classification table was most appropriate when the primary goal of our analysis was categorization; however, it should only be used in conjunction with a more precise method for determining model fitness.

Table 6. Hosmer and Lemeshow test results.

Metric	Value
Chi-square value	1.29
Degrees of freedom	9
Significance level	0.1

Access to television had an odds ratio of 1.945, suggesting that for every unit increase in television accessibility, there is a 1.945 times higher likelihood of increased climate change awareness among university graduates (Table 7). Similarly, access to smartphones, with an odds ratio of 0.584, was found to have a significant effect, indicating that smartphone use contributes to awareness at a 10 % significance level. Access to agricultural magazines, news bulletins, and newspapers also showed a positive impact on climate change awareness with an odds ratio of 1.209. This finding is consistent with the results of Naylor and Ford (2023), Rahman *et al*. (2023), Ahmad *et al*. (2024), and Karki *et al*. (2022).

The odds ratio for internet connectivity was 0.172, revealing that access to the internet has a substantial positive impact on raising climate change awareness (Table 7). This finding is consistent with Nyoni *et al*. (2024), Yang *et al*. (2024), and Jamal *et al*. (2022). In contrast, radio and computers or laptops were found to be non-significant factors in influencing awareness levels, with an odd ratio of 3.869 (Table 7). This result is in line with studies of Shakoor *et al*. (2011), Rasul (2011), and Göpfert *et al*. (2019).

Table 7. Analysis of the impact of ICT adoption on climate change awareness using binary logistic regression.

Factors	B-value	Wald test	*p*-value	exp(B)
Access to television	0.665	3.027	0.082**	1.945
Access to smart phone	1.257	3.221	0.073**	0.584
Access to agricultural magazine, news bulletin or newspaper	18.404	4.448	0.056*	1.209
Access to radio	0.178	0.965	0.326 NS	1.194
Access to computer/laptop	10.539	6.69	0.589 NS	3.869
Access towards internet connectivity	2.627	3.919	0.033*	0.172
Constant	24.243	0.000	1.000	3.377

[*]Significance level at $p \leq 0.01$; [**]significance level at $p \leq 0.1$.

CONCLUSION

Climate change and global warming are accelerating at an alarming rate, with consequences felt worldwide, but especially in developing economies like Pakistan. The country is dealing with both traditional and nontraditional security threats, making climate change a critical issue. Raising awareness about climate change is critical to mitigating its negative consequences. Effective awareness campaigns, which use Information and Communication Technologies (ICTs) such as social media, television, smartphones, radio, agricultural news bulletins, and other agricultural tools, are critical for educating the public, particularly university graduates, about the climate threat. The findings of the study reveal that access to smartphones, agricultural magazines, news bulletins, newspapers, internet connectivity, and television have a significant impact on climate change awareness among university graduates. In contrast, access to radio and computers or laptops had no significant effect on raising awareness in the study area.

The public and private sectors must become more actively involved in this issue. Collaborations among research organizations, academic institutions, and other stakeholders can help to raise climate change awareness and action. Digital platforms, such as blogs, podcasts, and social media videos (such as those on TikTok), can be effective tools for informing the public about climate change and the role of ICTs in combating it. By combining these strategies and leveraging ICTs, we can not only raise awareness about climate change but also take meaningful steps to mitigate its negative effects.

ACKNOWLEDGEMENTS

The authors extend their appreciation to the Researchers Supporting Project number (RSP2024R483), King Saud University, Riyadh, Saudi Arabia.

REFERENCES

Adnan M, Xiao B, Bibi S, Xiao P, Zhao P, Wang H. 2024. Addressing current climate issues in Pakistan. An opportunity for a sustainable future. Environmental Challenges 15: 100887. https://doi.org/10.1016/j.envc.2024.100887

Ahmad MI, Ma H, Shen Q, Rehman A, Oxley L. 2024. Climate change variability adaptation and farmers decisions of farm exit and survival in Pakistan. Climate Services 33: 100437. https://doi.org/10.1016/j.cliser.2023.100437

Aslam M, Li Z. 2024. A multinomial approach for organic agricultural production practices adoption vis-à-vis socio-economic and ICT determinants. Journal of Animal and Plant Sciences 34 (3).

Dwivedi YK, Hughes L, Kar AK, Baabdullah AM, Grover P, Abbas R, Andreini D, Abumoghli I, Barlette Y, Bunker D *et al*. 2022. Climate change and COP26: Are digital technologies and information management part of the problem or the solution? An editorial reflection and call to action. International Journal of Information Management 63: 102456. https://doi.org/10.1016/j.ijinfomgt.2021.102456

Göpfert C, Wamsler C, Lang W. 2019. A framework for the joint institutionalization of climate change mitigation and adaptation in city administrations. Mitigation and Adaptation Strategies for Global Change 24 (1). https://doi.org/10.1007/s11027-018-9789-9

IPCC (Intergovernmental Panel on Climate Change). 2022. Climate change 2022: Impacts, adaptation, and vulnerability. Working group II contribution to the sixth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press: Cambridge, UK. 3056 p.

Jamal S, Malik IH, Ahmad WS. 2022. Dynamics of urban land use and its impact on land surface temperature (LST) in Aligarh City, Uttar Pradesh. *In* Re-envisioning Advances in Remote Sensing. CRC Press: Boca Raton, FL, USA, pp: 25–40.

Karki G, Bhatta B, Devkota NR, Acharya RP, Kunwar RM. 2022. Climate change adaptation (CCA) research in Nepal: Implications for the advancement of adaptation planning. Mitigation and Adaptation Strategies for Global Change 27 (3). https://doi.org/10.1007/s11027-021-09991-0

Leiserowitz A, Kates RW, Parris TM. 2015. Do global attitudes and behaviors support sustainable development? Environment 47 (9): 22–38.

Lilavanichakul A, Pathak TB. 2024. Thai farmers' perceptions on climate change: Evidence on durian farms in Surat Thani province. Climate Services 34: 100475. https://doi.org/10.1016/j.cliser.2024.100475

Malik IH, Ford JD. 2024. Addressing the climate change adaptation gap: Key themes and future directions. Climate 12 (2): 24. https://doi.org/10.3390/cli12020024

Meath C, Linnenluecke M, Griffiths A. 2016. Barriers and motivators to the adoption of energy savings measures for small- and medium-sized enterprises (SMEs): The case of the Climate Smart Business Cluster program. Journal of Cleaner Production 112 (5): 3597–3604. https://doi.org/10.1016/j.jclepro.2015.08.085

Naylor AW, Ford J. 2023. Vulnerability and loss and damage following the COP27 of the UN Framework Convention on Climate Change. Regional Environmental Change 23 (1): 38. https://doi.org/10.1007/s10113-023-02033-2

Nyoni RS, Bruelle G, Chikowo R, Andrieu N. 2024. Targeting smallholder farmers for climate information services adoption in Africa: A systematic literature review. Climate Services 34: 100450. https://doi.org/10.1016/j.cliser.2024.100450

Rahman MF, Falzon D, Robinson SA, Kuhl L, Westoby R, Omukuti J, Schipper ELF, McNamara KE, Resurrección BP *et al*. 2023. Locally led adaptation: Promise, pitfalls, and possibilities. Ambio 52 (10): 1543–1557. https://doi.org/10.1007/s13280-023-01884-7

Rasul G. 2011. An analysis of knowledge gaps in climate change research. Pakistan Journal of Meteorology 7 (13): 1–9.

Santos FD, Ferreira PL, Pedersen JST. 2022. The climate change challenge: A review of the barriers and solutions to deliver a Paris solution. Climate 10 (5): 75. https://doi.org/10.3390/cli10050075

Shahid Z, Piracha A. 2010. Climate change impacts in Pakistan: Awareness and adaptation. International Journal of Climate Change: Impacts and Responses 2 (1): 119–130.

Shakoor U, Saboor A, Ali I, Mohsin AQ. 2011. Impact of climate change on agriculture: Empirical evidence from arid region. Pakistan Journal of Agricultural Sciences 48 (4): 327–333.

Stelvia M, Viardot E, Sovacool BK, Geels FW, Xiong Y. 2022. Innovation and climate change: A review and introduction to the special issue. Technovation 117: 102612. https://doi.org/10.1016/j.technovation.2022.102612

Thinda KT, Ogundeji AA, Belle JA, Ojo TO. 2020. Understanding the adoption of climate change adaptation strategies among smallholder farmers: Evidence from land reform beneficiaries in South Africa. Land Use Policy 99: 104858. https://doi.org/10.1016/j.landusepol.2020.104858

UNDP (United Nations Development Program). 2008. Fighting climate change: Human solidarity in a divided world. New York, NY, USA. 384 p.

Yang Y, Zhang Y, Zhu BX, Zhou J, Liu Y, Gao D, Sauer J. 2024. ICT promotes smallholder farmers' perceived self-efficacy and adaptive action to climate change: Empirical research on China's economically developed rural areas. Climate Services 33: 100431. https://doi.org/10.1016/j.cliser.2023.100431

Agrociencia

ENTOMOFAUNA ASSOCIATED WITH AGROFORESTRY SYSTEMS IMMERSED IN THE MESOPHILIC MOUNTAIN FOREST OF ATZALAN, PUEBLA, MEXICO

Claudio **Romero-Díaz**[1], Eduardo **Valdés-Velarde**[1], Saúl **Ugalde-Lezama**[1],
Armando **Equihua-Martínez**[2*], Luis Antonio **Tarango-Arámbula**[3], Jesús **Romero-Nápoles**[2]

[1]Universidad Autónoma Chapingo. Departamento de Fitotecnia. Carretera México-Texcoco km 38.5, Chapingo, Texcoco, State of Mexico, Mexico. C. P. 56227.
[2]Colegio de Posgraduados Campus Montecillo. Departamento de Entomología y Acarología. Carretera Mexico-Texcoco km 36.5, Montecillo, Texcoco, State of Mexico, Mexico. C. P. 56264.
[3]Colegio de Posgraduados Campus San Luis Potosí. Calle Iturbide 73, Salinas de Hidalgo, San Luis Potosí, Mexico. C. P. 78620.
* Author for correspondence: equihuaa@colpos.mx

ABSTRACT

Agroforestry systems contribute to entomological conservation by allowing the development of interactions that maintain the stability of the agroecosystem. To determine the diversity of insect families and their trophic guilds associated with agroforestry systems immersed in the mountain mesophyll forest, two agricultural systems (agroforestry and agrosilvopastoral) were studied in Atzalan, in the municipality of Xochiapulco, Puebla, Mexico. Insect collections were conducted at 10 sites from November 2021 to October 2022. The frequencies of observation (FO), the richness estimator (Jackknife test), and the indices of relative abundance (RAI), diversity (Shannon-Wiener), equity, and evenness (Jaccard) were determined. Kruskal-Wallis, χ^2 and cluster analysis tests were performed. The frequencies of observation showed high values for the families Drosophilidae (34.15 %), Tachinidae (9.45 %), and Sciaridae (4.95 %). The RAI had higher values for Drosophilidae (0.68), Tachinidae (0.18), and Sciaridae (0.09). The Jackknife test presented average values of 70.1, 91.7, and 94.77. The Shannon-Wiener index exhibited an average diversity of H ´ = 3.78, 4.12, and 4.18. The Jaccard index displayed values of 45, 53, and 55 %, respectively. The Kruskal-Wallis test exhibited significant differences for abundance, richness, and diversity. The χ^2 test showed that abundance and richness for the agrosilvicultural condition were not as expected by the model. Cluster analysis showed the formation of two, two, and three clusters for abundance and five, two, and three, respectively, for richness. The evaluated systems harbor a high diversity of insect families that apply the differential use of the available trophic resource and allow the ecological balance of the analyzed agroecosystems. It is recommended to broaden the structural complexity of agroforestry systems in order to contribute to the conservation of entomological resources.

Keywords: agroforestry, agrosilvopastoral, bioindicators, biological control, diversity, monophagy.

Citation: Romero-Díaz C, Valdés-Velarde E, Ugalde-Lezama S, Equihua-Martínez A, Tarango-Arámbula LA, Romero-Nápoles J. 2024. Entomofauna associated with agroforestry systems immersed in the mesophilic mountain forest of Atzalan, Puebla, Mexico. **Agrociencia** 58(8): 1023-1041. https://doi.org/ 10.47163/ agrociencia.v58i8.3221

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: May 17, 2024.
Approved: October 28, 2024.
Published in Agrociencia:
December 14, 2024.

INTRODUCTION

Globally, the entomological group represents a taxon of great evolutionary success, with wide diversity, abundance, and distribution. Despite not having a real figure, it is estimated that there are about 5.5 million species, pointing out that around 80 % of the total remain to be described (Stork, 2018). This diversity places these organisms as an omnipresent resource that makes use of the different ecological niches of the planet (Scudder, 2009). However, anthropogenic factors such as overexploitation of resources, expansion of the agricultural frontier, and irrational use of chemical inputs have brought different taxonomic groups to the brink of extinction, and many others have depleted their populations (Raven and Wagner, 2020). To date, it is estimated that between 250 000 and 500 000 insect species have become extinct, representing between 5 and 10 % of the entomological diversity of the planet (Cardoso *et al*., 2020).

In Mexico, knowledge about the current diversity and problems faced by the entomofauna in terms of conservation status is scarce (Castillo *et al.*, 2018). Almost 48 000 species of insects have been recorded, representing 66 % of the known fauna in the country (SEMARNAT, 2012). However, due to human factors such as increased agricultural production, mining companies, the exploitation of fossil fuels, and urbanization, an exponential reduction in biological diversity has been registered (Raven and Wagner, 2020).

Efforts to document the loss of diversity have focused on the study and conservation of vertebrates and large, charismatic organisms. Therefore, there is little work on smaller groups, such as insects (Falcón-Brindis *et al*., 2021). As an example, Morón and Terrón (1984) evaluated the diversity of necrophilous insects in tropical and mesophilic forests with different degrees of disturbance, showing that, despite the impact on these ecosystems, high levels of diversity and abundance of this entomological group are still maintained.

Agroforestry systems represent a sustainable alternative that contributes to mitigating the problems caused by environmental deterioration and ensuring food security (Villanueva-López *et al*., 2019). These systems are integrated through the deliberate combination of herbaceous, shrub, and tree species in association with animals, allowing for better productive development. At the same time, a broad vertical-horizontal multi-layer structure is generated, providing optimal light, temperature, and moisture conditions and creating differential habitats that allow the development of a wide variety of organisms (Bentrup *et al*., 2019). The latter favors the connection between vegetation remnants (patches) and allows the movement and dispersal of flora and fauna (Villanueva-López *et al*., 2019).

In this context, insects, being evolutionarily and ecologically versatile organisms, make differential use of a wide range of niches, contributing to the ecological stability of agroecosystems (Jankielsohn, 2018). Likewise, entomofauna (particularly the orders Hymenoptera and Hemiptera) play an important role in the pollination of productive components and provide a diverse range of products (honey, wax, royal jelly, propolis, silk, and dyes, among others) that contribute to the productive economy and constitute an element of the cultural identity of the communities.

The presence of parasites and predators contributes to biological pest control and reduces the use of chemical products, while decomposers favor the recycling of nutrients and soil fertility. The presence of ants and beetles improves the quality and structure of the edaphic component, in addition to being a source of food for various taxonomic groups (particularly birds, bats, and different mammals) and bioindicators of the health and sustainability of agroforestry systems (Ramos *et al*., 2020). However, despite the importance of these organisms, there are few studies that consider the diversity of entomofauna within these systems (Kaur *et al*., 2023).

In the town of Atzalan, which belongs to the municipality of Xochiapulco in the northern highlands of Puebla, Mexico, agriculture is practiced for subsistence purposes and, in most cases, through a multi-layered arrangement with an agroforestry system structure, immersed in the mesophilic mountain forest. Given the structure and floristic composition of these agroecosystems, it is inferred that they could harbor a considerable number of insects that could play an important role in the maintenance and ecological stability of these bioproductive environments. Therefore, the objective of this study was to determine the diversity of insect families and their trophic guild within agroforestry systems in this region.

MATERIALS AND METHODS

The study area is located at 19° 53′ 49′′ N and 97° 37′ 17′′ W, at an altitude of 1565 m. The natural vegetation is defined by mountain mesophilic forest (MMF), with pine-oak dominance, an average annual rainfall of 599 mm, average temperatures of 22 °C, and humidity of 62 %. The predominant soil types are Andosol and Luvisol. Ten study units (SU) were established, with each point representing a system (three agroforestry and seven agrosilvopastoral systems). These points were selected by systematic sampling, guaranteeing the independence of data to prevent insects recorded at a certain point from being recorded again in another SU (León-Burgos *et al*., 2019).

The agricultural components that integrated the evaluated systems (agroforestry and agrosilvopastoral) were maize fields (maize, beans, squash, and chili), fruit trees (avocado, peach, plum, coffee, apple, orange, lemon, banana, guava, capulin, custard apple, tangerine, and pomegranate, among others), and medicinal, aromatic, ornamental, and forage plants (grasses and legumes). The livestock component of the agrosilvopastoral system (free grazing and stabled) consisted of poultry (chickens, turkeys, and ducks), rabbits, sheep, cattle, pigs, and horses. The variables were monitored on a monthly basis from November 2021 to October 2022.

The collection of insects was carried out with a beating net on herbaceous, shrubby, and arboreal plants. For the latter stratum, only insects recorded at a height of less than 2 m were considered; in parallel, insects were collected with Malaise traps (Sánchez-Flores *et al*., 2019; Soca-Flores *et al*., 2022). These methodologies were applied and conducted in the first eight days of each month. Taxonomic keys were used to identify insects at the family level as proposed by Borror *et al*. (2005). The trophic guild of insects was determined through the insect guide proposed by Zumbado and Azofeifa (2018).

The frequency of observation (FO), relative abundance (RAI), richness (Jackknife test), and diversity (Shannon-Wiener) were determined, and a dissimilarity analysis (Jaccard index) was performed. To detect possible differences in the abundance, richness, and diversity of the insect families in different samplings, Kruskal-Wallis analyses were used. To determine whether the proportion of individuals recorded was adequate, a χ^2 analysis was applied. Both analyses were performed in JMP IN v.8.0.2 (SAS Institute Inc., Cary, NC, USA). For all cases, $\alpha = 0.05$ was used. Finally, to visualize the similarity between the abundance and richness of recorded families, cluster analyses were developed using the XLSTAT statistical software version 2018.7.5 (XLSTAT, 2018).

RESULTS AND DISCUSSION

A total of 15 363 insects were collected, and 11 orders and 92 families were determined. Of the total number of specimens, 10 995 were linked to the agrosilvopastoral system and 4368 to the agroforestry system. Moreover, two families were found to be exclusive to the agroforestry system, and 24 to the agrosilvopastoral system (Table 1).

Table 1. Frequency of insects per family recorded and their taxonomic classification based on Borror *et al*. (2005), in the agricultural systems studied in Atzalan, in the municipality of Xochiapulco, Puebla, Mexico.

				Recorded frequency		
Order	Suborder	Superfamily	Family	AS	ASP	General
Blattodea	NA	Blaberoidea	Ectobiidae	1	5	6
Coleoptera	Polyphaga	Curculionoidea	Attelabidae	2	3	5
			Curculionidae	13	83	96
		Buprestoidea	Buprestidae**	0	1	1
		Elateroidea	Cantharidae**	0	4	4
		Caraboidea	Carabidae	10	12	22
		Cerambycoidea	Cerambycidae**	0	21	21
		Chrysomeloidea	Chrysomelidae	80	243	323
		Coccinelloidea	Coccinellidae	10	19	29
		Elateroidea	Elateridae	17	109	126
		Tenebrionoidea	Oedemeridae	2	2	4
			Meloidae	1	17	18
			Tenebrionidae	10	20	30
		Cucujoidea	Erotylidae	1	10	11
		Elateroidea	Lampyridae	7	14	21
		Elateroidea	Lycidae	6	11	17
		Cucujoidea	Nitidulidae**	0	2	2
		Scarabaeoidea	Scarabaeidae	7	83	90

Table 1. Continue.

Order	Suborder	Superfamily	Family	Recorded frequency AS	 ASP	 General
		Cucujoidea	Silvanidae**	0	2	2
		Staphylinoidea	Staphylinidae	4	27	31
Dermaptera	Neodermaptera	Forficuloidea	Forficulidae	25	26	51
Diptera	Nematocera	Tipuloidea	Tipulidae	112	129	241
		Sciaroidea	Sciaridae	260	502	762
			Bibionidae**	0	1	1
	Brachycera	Muscoidea	Anthomyiidae**	0	1	1
		Asiloidea	Asilidae**	0	4	4
		Oestroidea	Calliphoridae	110	326	436
		Empidoidea	Dolichopodidae	3	4	7
		Ephydroidea	Drosophilidae	1616	3631	5247
		Empidoidea	Empididae**	0	3	3
		Lauxanioidea	Lauxaniidae*	1	0	1
		Muscoidea	Muscidae	1	18	19
		Tephritoidea	Ulidiidae	4	2	6
		Oestroidea	Sarcophagidae	8	1	9
			Stratiomyidae	1	1	2
			Syrphidae	33	76	109
		Oestroidea	Tachinidae	615	837	1452
		Tephritoidea	Tephritidae	3	6	9
		Asiloidea	Therevidae	1	7	8
Hemiptera	Auchenorrhyncha	Membracoidea	Membracidae	9	267	276
			Cicadellidae	40	119	159
		Cercopoidea	Cercopidae**	0	2	2
	Heteroptera	Coreoidea	Coreidae	47	418	465
		Pentatomoidea	Cydnidae	1	1	2
			Scutelleridae	4	2	6
		Lygaeoidea	Lygaeidae	3	10	13
		Coreoidea	Alydidae**	0	5	5
		Miroidea	Miridae	81	290	371
		Notonectoidea	Notonectidae**	0	1	1
		Pentatomoidea	Pentatomidae	17	84	101
		Pyrrhocoroidea	Pyrrhocoridae**	0	3	3
		Reduvioidea	Reduviidae	2	17	19
Hymenoptera	Apocrita	Apoidea	Andrenidae**	0	2	2
			Apidae	172	537	709
			Sphecidae	9	32	41
		Ichneumonoidea	Braconidae**	0	1	1
		Formicoidea	Formicidae	3	2	5
		Ichneumonoidea	Ichneumonidae	127	349	476
		Proctotrupoidea	Pelecinidae**	0	4	4
		Pompiloidea	Pompilidae	1	1	2
		Scolioidea	Scoliidae	12	51	63
		Vespoidea	Vespidae	160	421	581
Lepidoptera	NA	Bombycoidea	Apatelodidae**	0	1	1
		Pyraloidea	Crambidae**	0	7	7

Table 1. Continue.

Order	Suborder	Superfamily	Family	Recorded frequency AS	 ASP	 General
		Noctuoidea	Erebidae	1	15	16
		Gelechioidea	Gelechiidae	1	8	9
		Geometroidea	Geometridae	46	154	200
		Papilionoidea	Hesperiidae	12	37	49
		Lasiocampoidea	Lasiocampidae	2	2	4
		Zygaenoidea	Limacodidae*	2	0	2
		Papilionoidea	Lycaenidae	7	6	13
		Noctuoidea	Noctuidae	68	139	207
		Noctuoidea	Nolidae	1	3	4
		Noctuoidea	Notodontidae**	0	5	5
		Papilionoidea	Nymphalidae	17	53	70
		Papilionoidea	Papilionidae**	0	5	5
		Papilionoidea	Pieridae	56	187	243
		Pyraloidea	Pyralidae	142	411	553
		Bombycoidea	Saturniidae	2	6	8
			Sphingidae	1	2	3
		Tortricoidea	Tortricidae	56	325	381
Neuroptera	Hemerobiiformia		Chrysopidae**	0	1	1
			Hemerobiidae	10	48	58
Odonata	Anisoptera	Aeshnoidea	Aeshnidae	3	4	7
		Libelluloidea	Macromiidae**	0	6	6
Orthoptera	Caelifera	Acridoidea	Acrididae	196	556	752
		Pyrgomorphoidea	Pyrgomorphidae	59	72	131
		Acridoidea	Romaleidae	12	27	39
	Ensifera	Grylloidea	Gryllidae	20	23	43
		Stenopelmatoidea	Stenopelmatidae**	0	1	1
		Tettigonioidea	Tettigoniidae	2	8	10
Thysanoptera	Terebrantia		Adiheterothripidae**	0	1	1

AS: agroforestry system; ASP: agrosilvopastoral system; NA: not available. *Families exclusive to the agroforestry system; **families exclusive to the agrosilvopastoral system.

The frequency of observation (FO) analysis presented high values for the families Drosophilidae (34.15 %), Tachinidae (9.45 %), Sciaridae (4.95 %), Acrididae (4.89 %), and Apidae (4.61 %) (Table 2). The relative abundance index (RAI) showed higher values for the families Drosophilidae (0.68), Tachinidae (0.18), and Sciaridae (0.09) (Table 2). Therefore, it is evident that these insect families represent the greatest dominance of individuals present in the systems analyzed. The population structure of the family Drosophilidae, a major pest commonly known as fruit flies, is determined by biological control through parasitism by insects of the family Tachinidae (an

Table 2. Observation frequency index and relative abundance of the insect families registered in the agricultural systems evaluated in Atzalan, in the municipality of Xochiapulco, Puebla, Mexico.

Families	Frequency of observation (FO) index			Relative abundance index (RAI)		
	Agroforestry	Agrosilvopastoral	General	Agroforestry	Agrosilvopastoral	General
Acrididae	4.487	**5.057**	4.895	0.090	**0.101**	0.098
Adiheterothripidae	0.000	0.009	0.007	0.000	0.000	0.000
Aeshnidae	0.069	0.036	0.046	0.001	0.001	0.001
Alydidae	0.000	0.045	0.033	0.000	0.001	0.001
Andrenidae	0.000	0.018	0.013	0.000	0.000	0.000
Anthomyiidae	0.000	0.009	0.007	0.000	0.000	0.000
Apatelodidae	0.000	0.009	0.007	0.000	0.000	0.000
Apidae	3.938	4.884	4.615	0.079	0.098	0.092
Asilidae	0.000	0.036	0.026	0.000	0.001	0.001
Attelabidae	0.046	0.027	0.033	0.001	0.001	0.001
Bibionidae	0.000	0.009	0.007	0.000	0.000	0.000
Braconidae	0.000	0.009	0.007	0.000	0.000	0.000
Buprestidae	0.000	0.009	0.007	0.000	0.000	0.000
Calliphoridae	2.518	2.965	2.838	0.050	0.059	0.057
Cantharidae	0.000	0.036	0.026	0.000	0.001	0.001
Carabidae	0.229	0.109	0.143	0.005	0.002	0.003
Cerambycidae	0.000	0.191	0.137	0.000	0.004	0.003
Cercopidae	0.000	0.018	0.013	0.000	0.000	0.000
Chrysomelidae	1.832	2.210	2.102	0.037	0.044	0.042
Chrysopidae	0.000	0.009	0.007	0.000	0.000	0.000
Cicadellidae	0.916	1.082	1.035	0.018	0.022	0.021
Coccinellidae	0.229	0.173	0.189	0.005	0.003	0.004
Coreidae	1.076	3.802	3.027	0.022	0.076	0.061
Crambidae	0.000	0.064	0.046	0.000	0.001	0.001
Curculionidae	0.298	0.755	0.625	0.006	0.015	0.012
Cydnidae	0.023	0.009	0.013	0.000	0.000	0.000
Dolichopodidae	0.069	0.036	0.046	0.001	0.001	0.001
Drosophilidae	**36.996**	**33.024**	**34.153**	**0.740**	**0.660**	**0.683**
Ectobiidae	0.023	0.045	0.039	0.000	0.001	0.001
Elateridae	0.389	0.991	0.820	0.008	0.020	0.016
Empididae	0.000	0.027	0.020	0.000	0.001	0.000
Erebidae	0.023	0.136	0.104	0.000	0.003	0.002
Erotylidae	0.023	0.091	0.072	0.000	0.002	0.001
Forficulidae	0.572	0.236	0.332	0.011	0.005	0.007
Formicidae	0.069	0.018	0.033	0.001	0.000	0.001
Gelechiidae	0.023	0.073	0.059	0.000	0.001	0.001
Geometridae	1.053	1.401	1.302	0.021	0.028	0.026
Gryllidae	0.458	0.209	0.280	0.009	0.004	0.006
Hemerobiidae	0.229	0.437	0.378	0.005	0.009	0.008
Hesperiidae	0.275	0.337	0.319	0.005	0.007	0.006
Ichneumonidae	2.908	3.174	3.098	0.058	0.063	0.062
Lampyridae	0.160	0.127	0.137	0.003	0.003	0.003
Lasiocampidae	0.046	0.018	0.026	0.001	0.000	0.001
Lauxaniidae	0.023	0.000	0.007	0.000	0.000	0.000
Limacodidae	0.046	0.000	0.013	0.001	0.000	0.000

Table 2. Continue.

Families	Frequency of observation (FO) index			Relative abundance index (RAI)		
	Agroforestry	Agrosilvopastoral	General	Agroforestry	Agrosilvopastoral	General
Lycaenidae	0.160	0.055	0.085	0.003	0.001	0.002
Lycidae	0.137	0.100	0.111	0.003	0.002	0.002
Lygaeidae	0.069	0.091	0.085	0.001	0.002	0.002
Macromiidae	0.000	0.055	0.039	0.000	0.001	0.001
Meloidae	0.023	0.155	0.117	0.000	0.003	0.002
Membracidae	0.206	2.428	1.797	0.004	0.049	0.036
Miridae	1.854	2.638	2.415	0.037	0.053	0.048
Muscidae	0.023	0.164	0.124	0.000	0.003	0.002
Nitidulidae	0.000	0.018	0.013	0.000	0.000	0.000
Noctuidae	1.557	1.264	1.347	0.031	0.025	0.027
Nolidae	0.023	0.027	0.026	0.000	0.001	0.001
Notodontidae	0.000	0.045	0.033	0.000	0.001	0.001
Notonectidae	0.000	0.009	0.007	0.000	0.000	0.000
Nymphalidae	0.389	0.482	0.456	0.008	0.010	0.009
Oedemeridae	0.046	0.018	0.026	0.001	0.000	0.001
Papilionidae	0.000	0.045	0.033	0.000	0.001	0.001
Pelecinidae	0.000	0.036	0.026	0.000	0.001	0.001
Pentatomidae	0.389	0.764	0.657	0.008	0.015	0.013
Pieridae	1.282	1.701	1.582	0.026	0.034	0.032
Pompilidae	0.023	0.009	0.013	0.000	0.000	0.000
Pyralidae	3.251	3.738	3.600	0.065	0.075	0.072
Pyrgomorphidae	1.351	0.655	0.853	0.027	0.013	0.017
Pyrrhocoridae	0.000	0.027	0.020	0.000	0.001	0.000
Reduviidae	0.046	0.155	0.124	0.001	0.003	0.002
Romaleidae	0.275	0.246	0.254	0.005	0.005	0.005
Sarcophagidae	0.183	0.009	0.059	0.004	0.000	0.001
Saturniidae	0.046	0.055	0.052	0.001	0.001	0.001
Scarabaeidae	0.160	0.755	0.586	0.003	0.015	0.012
Sciaridae	**5.952**	4.566	**4.960**	**0.119**	0.091	**0.099**
Scoliidae	0.275	0.464	0.410	0.005	0.009	0.008
Scutelleridae	0.092	0.018	0.039	0.002	0.000	0.001
Silvanidae	0.000	0.018	0.013	0.000	0.000	0.000
Sphecidae	0.206	0.291	0.267	0.004	0.006	0.005
Sphingidae	0.023	0.018	0.020	0.000	0.000	0.000
Staphylinidae	0.092	0.246	0.202	0.002	0.005	0.004
Stenopelmatidae	0.000	0.009	0.007	0.000	0.000	0.000
Stratiomyidae	0.023	0.009	0.013	0.000	0.000	0.000
Syrphidae	0.755	0.691	0.709	0.015	0.014	0.014
Tachinidae	**14.080**	**7.613**	**9.451**	**0.282**	**0.152**	**0.189**
Tenebrionidae	0.229	0.182	0.195	0.005	0.004	0.004
Tephritidae	0.069	0.055	0.059	0.001	0.001	0.001
Tettigoniidae	0.046	0.073	0.065	0.001	0.001	0.001
Therevidae	0.023	0.064	0.052	0.000	0.001	0.001
Tipulidae	2.564	1.173	1.569	0.051	0.023	0.031
Tortricidae	1.282	2.956	2.480	0.026	0.059	0.050
Ulidiidae	0.092	0.018	0.039	0.002	0.000	0.001
Vespidae	3.663	3.829	3.782	0.073	0.077	0.076

entomological group mostly recognized for its role in biological pest control), which also controls the population dynamics of the family Sciaridae, which contains species that could become pests in agricultural systems. On the other hand, individuals of the Sciaridae family play an important role in nutrient recycling, since, being saprophytic organisms, they contribute to degrade and reincorporate nutrients into the soil.

This trend is in agreement with Armijos-Vásquez *et al*. (2020) and Huang *et al*. (2021), who indicate that the most representative specimens in agroforestry systems and natural forest areas correspond to the orders Diptera and Hymenoptera. These authors attribute these values to factors such as the physiological age of the vegetation, the productive stage of the crop, and the agro-climatic characteristics, which define the food availability and the reproductive period of the insects. In this context, Ambele *et al*. (2023) describe how agroforestry plantations present a mixed tree cover structure, similar to natural forests, providing differential habitats for a wide diversity of insects that contribute to key agroecosystem processes (pollination, soil fertilization, organic matter decomposition, nutrient cycling, and biological pest control).

Of the 92 families described, 17 trophic guilds were recorded, divided into monophagous, oligophagous, and polyphagous groups. Additionally, members of certain families include pest insects and biological controllers, seed dispersers, organic matter decomposers, and indicators of environmental quality (Table 3). The differential use of the trophic resource shows sympatric coexistence and highlights the ecological importance of insect diversity (at the family level) contributing to the ecological stability of the agroforestry systems analyzed.

Table 3. Feeding habits of insect families recorded in the agricultural systems evaluated in Atzalan, in the municipality of Xochiapulco, Puebla, Mexico.

Families	Phy	Sap	Par	Fun	Pre	Pol	Nec	Gran	Rhz	Xyl	Frug	Car	Hem	Necro	Suck	Omn	Myce	Mono	Oli	Poly	BiolC	Pest	EnQI	OorMD	Seedis
Acrididae	x															x			x	x					
Adiheterothripidae	x				x															x					
Aeshnidae					x															x					
Alydidae	x				x											x				x					
Andrenidae						x	x												x	x					
Anthomyiidae	x	x	x													x				x					
Apatelodidae	x																			x					
Apidae						x	x											x	x						
Asilidae			x		x															x					
Attelabidae	x	x													x	x		x							
Bibionidae	x	x				x	x									x									
Braconidae			x			x	x											x			x				
Buprestidae						x	x			x								x	x						
Calliphoridae		x	x		x		x			x				x		x				x					
Cantharidae	x				x	x	x									x				x	x				

Table 3. Continue.

Families	Phy	Sap	Par	Fun	Pre	Pol	Nec	Gran	Rhz	Xyl	Frug	Car	Hem	Necro	Suck	Omn	Myce	Mono	Oli	Poly	BiolC	Pest	EnQI	OorMD	Seedis
Carabidae		x			x									x					x						
Cerambycidae	x									x					x					x		x			
Cercopidae	x									x					x					x		x			
Chrysomelidae	x					x			x	x						x				x					
Chrysopidae					x	x	x									x			x		x				
Cicadellidae	x								x						x					x		x			
Coccinellidae				x	x	x	x												x		x				
Coreidae	x														x					x		x			
Crambidae	x					x	x	x	x											x		x			
Curculionidae	x			x				x	x	x						x		x		x		x			
Cydnidae	x								x						x					x		x			
Dolichopodidae					x											x				x	x				
Drosophilidae		x		x			x				x				x					x		x			
Ectobiidae		x												x		x				x		x			
Elateridae		x			x			x	x		x					x				x		x			
Empididae					x		x												x		x				
Erebidae	X																			X					
Erotylidae																									
Forficulidae		x			X									x		X				X	x				
Formicidae	x				x									x		x				x					x
Gelechiidae	x																		x			x			
Geometridae	x						x													x		x			
Gryllidae	x								x							x				x		x			
Hemerobiidae					x	x	x									x			x		x				
Hesperiidae	x						x											x	x				x		
Ichneumonidae			x				x												x		x		x		
Lampyridae					x	x	x												x		x		x		
Lasiocampidae	x																		x						
Lauxaniidae	x	x					x									x				x					
Limacodidae	x						x													x					
Lycaenidae	x						x											x							
Lycidae					x							x						x			x				
Lygaeidae	x				x										x					x		x			
Macromiidae					x							x							x				x		
Meloidae	x		x		x	x	x												x						
Membracidae	x																			x		x			
Miridae	x				x										x					x		x			
Muscidae		x			x								x							x	x				
Nitidulidae		x		x				x		x	x			x			x			x					
Noctuidae	x						x				x								x			x			
Nolidae	x																	x		x					
Notodontidae	x																			x					
Notonectidae					x													x			x				
Nymphalidae	x						x											x		x					
Oedemeridae	x	x				x	x													x					
Papilionidae	x						x											x	x				x		
Pelecinidae	x		x																x		x				

Table 3. Continue.

Families	Phy	Sap	Par	Fun	Pre	Pol	Nec	Gran	Rhz	Xyl	Frug	Car	Hem	Necro	Suck	Omn	Myce	Mono	Oli	Poly	BiolC	Pest	EnQI	OorMD	Seedis
Pentatomidae	x				x						x									x	x	x			
Pieridae	x						x												x			x			
Pompilidae			x		x		x											x			x				
Pyralidae	x								x		x								x			x			
Pyrgomorphidae	x																		x						
Pyrrhocoridae	x				x			x								x				x					
Reduviidae					x								x						x	x					
Romaleidae	x																	x		x					
Sarcophagidae		x	x		x		x					x		x										x	
Saturniidae	x																	x		x					
Scarabaeidae	x	x							x										x			x			
Sciaridae		x		x					x													x			
Scoliidae			x				x				x								x		x				
Scutelleridae	x														x			x		x					
Silvanidae								x											x			x		x	
Sphecidae					x		x											x		x	x				
Sphingidae	x						x				x								x						
Staphylinidae			x		x											x				x	x				
Stenopelmatidae		x												x					x					x	
Stratiomyidae		x																x						x	
Syrphidae					x															x	x				
Tachinidae			x																	x	x				
Tenebrionidae	x	x							x	x				x		x				x				x	
Tephritidae	x						x	x			x									x		x			
Tettigoniidae	x				x															x					
Therevidae					x														x		x				
Tipulidae		x			x								x						x					x	
Tortricidae	x						x	x			x									x					
Ulidiidae	x	x									x			x						x		x		x	
Vespidae					x		x												x		x				

Phy: phytophage; Sap: saprophage; Par: parasitoid; Fun: fungivore; Pre: predator; Pol: pollenophage; Nec: nectarivore; Gran: granivore; Rhz: rhizophage; Xyl: xylophage; Frug: frugivore; Car: carnivore; Hem: hematophage; Necro: necrophage; Suck: sucker; Omn: omnivore; Myce: mycetophagous; Mono: monophagous; Oli: oligophagous; Poly: polyphagous; BiolC: biological control; EnQI: environmental quality indicator; OorMD: organic matter decomposer; Seedis: seed disperser.

The mean observed values of insect family richness were 54.97, 75.3, and 79.33 for agroforestry, agroforestry, and general, respectively. The expected results using the Jackknife estimator showed means of 70.1, 91.7, and 94.77 families, respectively. Therefore, so far, with the sampling effort applied, 76, 82.69, and 85.2 % of the insect families theoretically present in the evaluated agroecosystems are known (Figure 1).

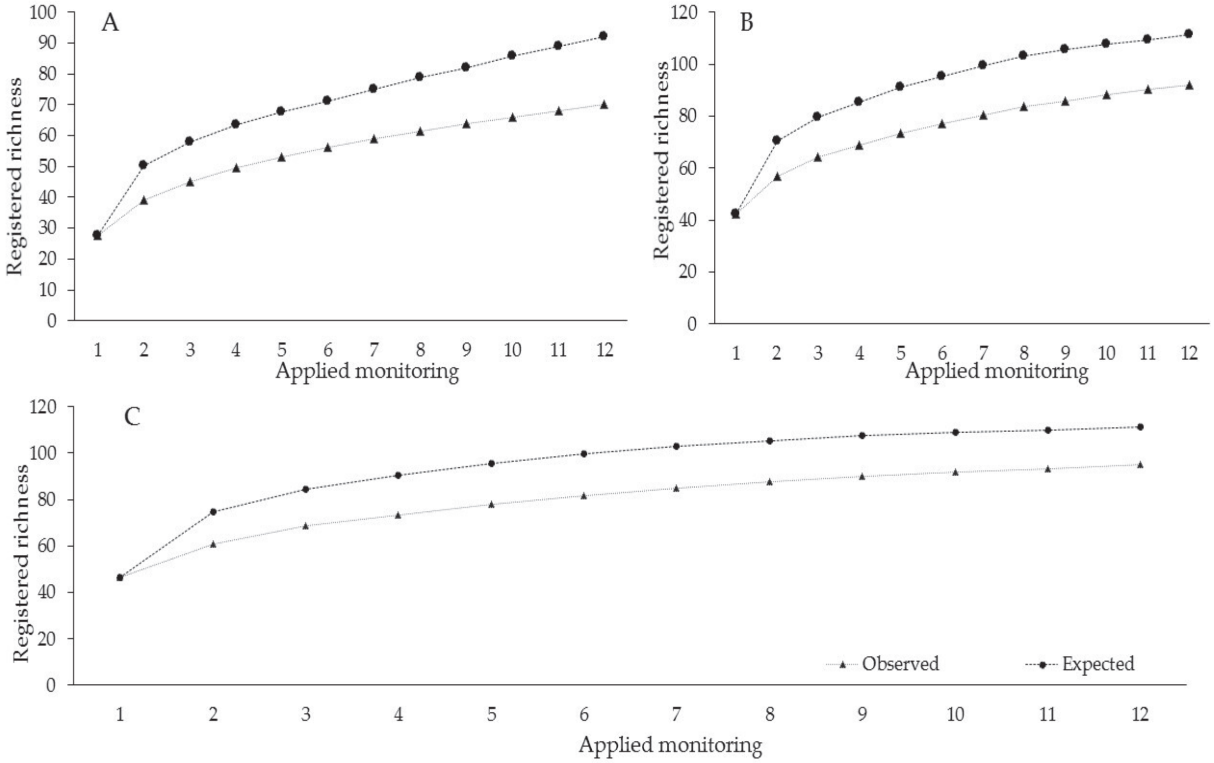


Figure 1. Richness (Jackknife test) of families recorded in the agroforestry systems analyzed. A: agroforestry; B: agroslivopastoral; C: general overview of systems.

The Shannon-Wiener index obtained minimum, maximum, and average values of H´= 3.3, 3.92, and 3.78 for the agroforestry system; H´= 3.74, 4.23, and 4.12 for the agrosilvopastoral system; and H´= 3.83, 4.29, and 4.18 for the general condition. These results represent the high value of the entomological diversity recorded, showing how the systems evaluated allow a differential use of the available trophic resources that allow ecological stability to be maintained. Furthermore, the systems analyzed represent a sustainable production alternative that contributes to the conservation of entomofauna at the local level.

The richness and diversity of insects with higher value in the agrosilvopastoral system was the result of a higher sampling effort (seven sites). It was also associated with ecological habitat variables that are influenced by the presence of the animal component. In these systems, the impact of livestock modifies the structural arrangement of the agricultural, forestry, and herbaceous resources (particularly in pastures). This creates greater habitat heterogeneity and offers a wide range of niches and microhabitats that can be occupied by diverse insect families. The structural diversity of physical-ecological and vegetation resources favors a greater complexity of ecological interactions, such as pollination, biological pest control, competition, and predator-prey relationships. The presence of livestock introduces new elements to the ecosystem, such as dung, which attracts decomposing insects such as dung beetles (Rigueiro-Rodríguez *et al*., 2010).

Martins *et al.* (2020) point out that agroforestry systems, unlike conventional crops, conserve a greater diversity of insects that maintain ecological stability in equilibrium. These authors point out that the heterogeneous structure of agroforestry systems determines the movement of insect herbivores, their duration, abundance, and mortality. This promotes ecological interactions (host-parasitoid-plant) that provide niches for feeding, nesting, shelter, and development, which have a wide range of microclimatic spaces that buffer against extremes of temperature and rainfall (Centeno-Alvarado *et al*., 2023).

Agroforestry systems are represented by the incorporation of woody plants (trees and shrubs) associated with crops and/or livestock in the same space, which promotes the conservation of different taxonomic groups, particularly insects (Bentrup *et al*., 2019). Silva-Neto *et al*. (2023) point out that the woody plant component provides alternative food for insects, particularly pollinators, by offering a food resource when agricultural crops are not flowering. Kingazi *et al*. (2024) also showed that agroforestry systems encourage pollinator presence because the woody plant cover increases floral availability, which draws a wider variety of insects.

Centeno-Alvarado *et al*. (2023) indicate that the vegetation structure of agroforestry systems, particularly trees, provides protection against extreme environmental events and agricultural disturbances. Under the same approach, Varah *et al*. (2020) have shown that floristic diversity in agroforestry systems increases insect diversity, particularly pollinators, which is attributed to the asynchronous floral diversity found in these agroecosystems. This phenomenon was corroborated in the present study. Throughout the monitoring period, pollinating insects (particularly of the order Hymenoptera) were recorded, which developed a differentiated spatio-temporal use of the available resource throughout the annual cycle.

According to the percentage values derived from Jaccard's index for the similarity of richness documented during the various surveys, agroforestry accounted for 45 %, agroforestry for 53 %, and general for 55 %. There is a great dissimilarity in the wealth of families recorded. This difference was determined by the annual seasonality of the plant component and the landscape-level structure that defines the physical-ecological ensemble of utilization niches, which determines the population dynamics of insects and other taxonomic groups over time. In contrast, Galbraith *et al.* (2020) and Tarigan *et al*. (2021) showed no significant differences in the richness and abundance of insects present in agroforestry systems. However, in some cases, insect abundance was lower compared to conventional production systems. However, Kingazi *et al.* (2024) mention that there is still very little work on this subject, so there is still a lack of research to generate conclusions and propose strategies for improvement at the agroforestry level.

The Kruskal-Wallis analysis showed statistically significant differences between the medians analyzed for abundance, richness, and diversity recorded in each of the agroforestry systems evaluated (Table 4).

Table 4. Kruskal-Wallis results for abundance, richness, and diversity recorded in the agroforestry systems evaluated in Atzalan, in the municipality of Xochiapulco, Puebla, Mexico.

Chi-square	Degrees of freedom	Prob > Chi-square
Agroforestry (abundance)		
21.64	11	0.02
Agrosilvopastoral (abundance)		
22.44	11	0.02
General (abundance)		
20.65	11	0.03
Agroforestry (richness)		
22.17	11	0.02
Agrosilvopastoral (richness)		
21.07	11	0.03
General (richness)		
20.06	11	0.04
Diversity		
20.07	2	<0.00

The χ^2 results demonstrate that the abundance in the agroforestry system and the recorded richness of families do not reflect what may exist in the agroecosystems evaluated. In turn, the diversity recorded corresponds to what could occur under the conditions studied (Table 5).

Table 5. χ^2 results for abundance, richness and diversity recorded in the agroforestry systems evaluated in Atzalan, in the municipality of Xochiapulco, Puebla, Mexico.

N	Degrees of freedom	Chi-square	Prob > Chi-square
Agroforestry (abundance)			
840	484	543.86	0.03
Agrosilvopastoral (abundance)			
1104	836	822.79	0.62
General (abundance)			
1140	1056	1053.36	0.51
Agroforestry (richness)			
840	11	22.19	0.02
Agrosilvopastoral (richness)			
1104	11	21.09	0.03
General (richness)			
1140	11	20.08	0.04
Diversity			
36	58	63.00	0.30

The cluster analysis revealed the formation of several clusters. For abundance, agroforestry = two, agrosilvopastoral = two, and general = three (Figure 2), and for richness: agroforestry = five, agrosilvopastoral = two, and general = three (Figure 3). Different percentages of dissimilarity were shown in the record of both parameters (abundance and richness) (Table 6). These results are attributed to the annual seasonality that defines the food availability and reproductive cycle of the insects, associated with the plant structure and physical-ecological factors present throughout the year.

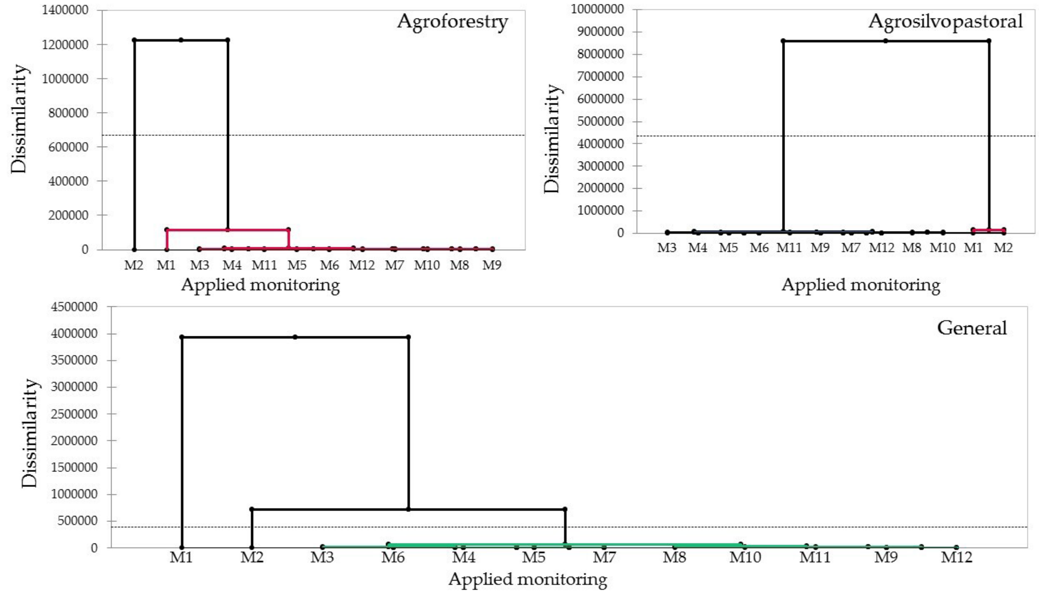


Figure 2. Cluster analysis of the abundance of insect families recorded in the different surveys. The horizontal line (Euclidean line) defines the number of clusters for each condition assessed; the colors show the grouping of the monitoring sites with the highest similarity in the abundance of families recorded.

This study provides information on agroforestry systems immersed in mesophilic mountain forests in relation to the incidence of insects; however, in order to propose better agroforestry management practices, it is necessary to develop specific works that allow visualizing the effect of these systems and the structure of the habitat on the population parameters of insects as a resource indicator of environmental quality, contributing to the conservation of insects and various taxonomic groups for this region in particular.

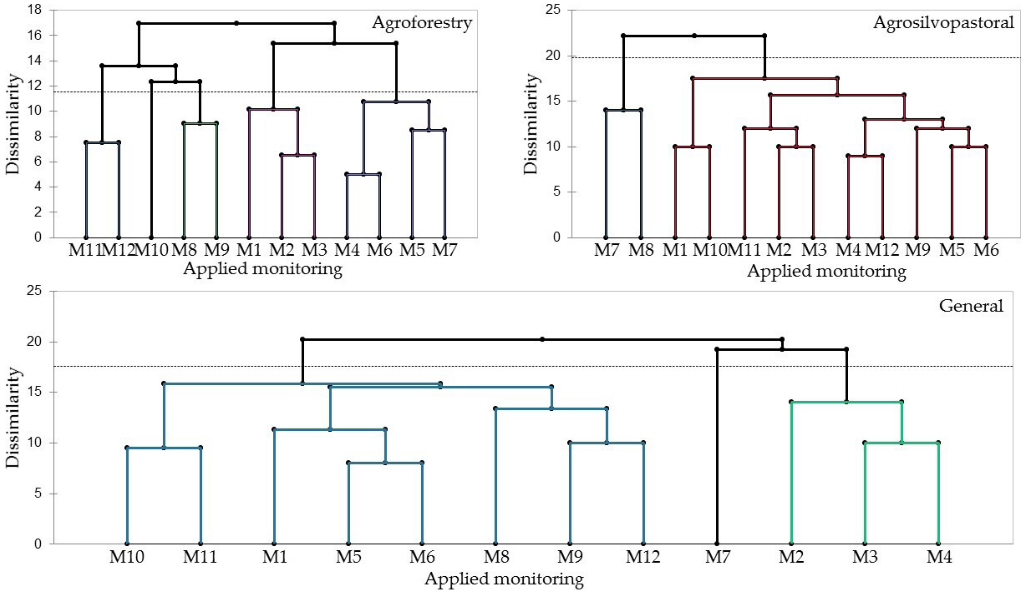


Figure 3. Cluster analysis of the richness of insect families recorded in each monitoring. The horizontal line (Euclidean line) defines the number of clusters for each condition assessed; the colors show the grouping of the monitoring sites with the highest similarity in richness of families recorded.

Table 6. Absolute and percentage value results of the clusters formed with the abundance and richness estimators of insect families recorded in the agroforestry systems evaluated in Atzalan, in the municipality of Xochiapulco, Puebla, Mexico.

Variance decomposition	Absolute	Percentage
	Agroforestry abundance	
Intraclass	136 098.72	10.01 %
Intraclasses	1 223 275.27	89.99 %
	Agrosilvopastoral abundance	
Intraclass	372956.30	4.16 %
Intraclasses	8581899.45	95.84 %
	General abundance	
Intraclass	181 959.00	3.77 %
Intraclasses	4 645 049.58	96.23 %
	Agroforestry richness	
Intraclass	57.41	49.64 %
Intraclasses	58.25	50.36 %
	Agrosilvopastoral richness	
Intraclass	123.10	84.75 %
Intraclasses	22.15	15.25 %
	General richness	
Intraclass	107.50	73.17 %
Intraclasses	39.41	26.83 %

CONCLUSIONS

The heterogeneous structure of agroforestry systems contributes to insect incidence by providing differential trophic niches. It is recommended to increase the diversity of plant strata (vertical-horizontal) to conserve insect provision and maintain balanced population dynamics and positive ecological interactions as indicators of environmental quality. The evaluated agroforestry systems conserve a wide diversity of insect families and represent an option in the face of current scenarios of habitat loss, degradation, and fragmentation in natural forests.

ACKNOWLEDGMENTS

To the National Council of Humanities, Sciences, and Technologies (CONAHCYT) for funding the development of this project. To the PhD in Multifunctional Agriculture for Sustainable Development program of the Autonomous University of Chapingo. To the producers of the community of Atzalan for allowing this work to be carried out in their production areas. To Armando Romero Díaz and Nanci Itzel Romero Díaz for their support in carrying out the fieldwork.

REFERENCES

Ambele CF, Bisseleua HDB, Djuideu CTL, Akutse KS. 2023. Managing insect services and disservices in cocoa agroforestry systems. Agroforestry Systems 97 (1): 965–984. https://doi.org/10.1007/s10457-023-00839-x

Armijos-Vásquez V, García-Cruzatty LC, Vera LD, Castro-Olaya J, Martínez M. 2020. Insectos polinizadores en sistemas de producción de *Theobroma cacao* L. en la zona central del litoral ecuatoriano. Ciencia y Tecnología 13 (2): 23–30. https://doi.org/10.18779/cyt.v13i2.389

Bentrup G, Hopwood J, Adamson NL, Vaughan M. 2019. Temperate agroforestry systems and insect pollinators: A review. Forests 10 (11): 981. https://doi.org/10.3390/f10110981

Borror DJ, Triplehorn CA, Johnson NF. 2005. An introduction to the study of insects (Seventh edition). Thomson Book/Cole: Belmont, CA, USA. 879 p.

Cardoso P, Barton PS, Birkhofer K, Chichorro F, Deacon C, Fartmann T, Fukushima CS, Gaigher R, Habel JC, Hallmann CA *et al*. 2020. Scientists' warning to humanity on insect extinctions. Biological Conservation 242 (1): 108426. https://doi.org/10.1016/j.biocon.2020.108426

Castillo M, Barba-Álvarez R, Mayorga A. 2018. Riqueza y diversidad de insectos acuáticos en la cuenca del río Usumacinta en México. Revista Mexicana de Biodiversidad 89: S45–S64.

Centeno-Alvarado D, Lopes AV, Arnan X. 2023. Fostering pollination through agroforestry: A global review. Agriculture, Ecosystems and Environment 351 (1): 108478. https://doi.org/10.1016/j.agee.2023.108478

Falcón-Brindis A, León-Cortés J, Montañez-Reyna M. 2021. How effective are conservation areas to preserve biodiversity in Mexico? Perspectives in Ecology and Conservation 19 (1): 399◎410. https://doi.org/10.1016/j.pecon.2021.07.007

Galbraith SM, Griswold T, Price WJ, Bosque-Perez N. 2020. Biodiversity and community composition of native bee populations vary among human-dominated land uses within the seasonally dry tropics. Journal of Insect Conservation 24 (1): 1045–1059. https://doi.org/10.1007/s10841-020-00274-8

Huang J, Miao X, Wang Q, Menzel F, Tang P, Yang D, Wu H, Vogler A. 2021. Metabarcoding reveals massive species diversity of Diptera in a subtropical ecosystem. Ecology and Evolution 12 (1): e8535. https://doi.org/10.1002/ece3.8535

Jankielsohn A. 2018. The importance of insects in agricultural ecosystems. Advances in Entomology 6 (2): 62–73. https://doi.org/10.4236/ae.2018.62006

Kaur G, Sarao PS, Chhabra N. 2023. Therapeutic use of insects and insect products. Indian Journal of Entomology 85 (3): 798–807. https://doi.org/10.55446/IJE.2023.964

Kingazi N, Temu R, Sirima A, Jonsson M. 2024. Tropical agroforestry supports insect pollinators and improves bean yield. Journal of Applied Ecology 61 (5): 1067–1080. https://doi.org/10.1111/1365-2664.14629

León-Burgos A, Murillo-Pacheco J, Bautista-Zamora D, Quinto J. 2019. Insectos benéficos asociados a plantas arvenses atrayentes en agroecosistemas del Piedemonte de la Orinoquia Colombiana. Cuadernos de Biodiversidad 56 (1): 1–14.

Martins AL, Lemes JRA, Lopes PR, Dias AMP. 2020. The Chrysidoidea wasps (Hymenoptera, Aculeata) in conventional coffee crops and agroforestry systems in Southeastern Brazil. Papeis Avulsos de Zoologia 60 (1): e20206058. http://doi.org/10.11606/1807-0205/2020.60.58

Morón M, Terrón R. 1984. Distribución altitudinal y estacional de los insectos necrófilos en la Sierra Norte de Hidalgo, México. Acta Zoológica Mexicana (Nueva Serie) 3: 1꩜47. https://doi.org/10.21829/azm.1984.132052

Ramos DL, Cunha WL, Evangelista J, Lira LA, Rocha MVC, Gomes PA, Frizzas MR, Togni PHB. 2020. Ecosystem services provided by insects in Brazil: What do we really know? Neotropical Entomology 49 (6): 783–794. https://doi.org/10.1007/s13744-020-00781-y

Raven PH, Wagner DL. 2020. Agricultural intensification and climate change are rapidly decreasing insect biodiversity. Proceedings of the National Academy of Sciences 118 (2): e2002548117. https://doi.org/10.1073/pnas.2002548117

Rigueiro-Rodríguez A, Rois-Díaz M, Mosquera-Losada MR. 2010. Integrating silvopastoralism and biodiversity conservation. *In* Lichtfouse E. (ed.), Biodiversity, Biofuels, Agroforestry and Conservation Agriculture. Sustainable Agriculture Reviews, vol 5. Springer: Dordrecht, Netherlands. https://doi.org/10.1007/978-90-481-9513-8_12

Sánchez-Flores P, Alvariño L, Iannacone J. 2019. Diversity of terrestrial insects in four vegetable communities of the regional conservation area (acr) Ventanilla wetland, Callao, Peru. The Biologist (Lima) 17 (1): 73–94.

Scudder G. 2009. The importance of insects. *In* Foottit RJ, Adler PH. (eds.), Insect Biodiversity: Science and Society. John Wiley and Sons: New York, NY, USA. https://doi.org/10.1002/9781118945568.ch2

SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales). 2012. Informe de la situación del medioambiente en México. Compendio de estadísticas ambientales indicadores clave y de desempeño ambiental. Ciudad de México, México. https://apps1.semarnat.gob.mx:8443/dgeia/informe_12/04_biodiversidad/cap4_1.html (Retrieved: January 2024).

Silva-Neto C de M, Santos LAC, de Souza WG, Martins T de O, Castro e Silva T, de Lima AA, Calil FN, de Souza MMO. 2023. Bees in agroforestry systems in the Cerrado. Journal of Apicultural Research 62 (4): 675–679. https://doi.org/10.1080/00218839.2021.1907977

Soca-Flores M, Vergara C, Callohuari Y, Chávez A. 2022. Insectos fitófagos asociados al cultivo de quinua (*Chenopodium quinoa* Willd) en invierno y sus controladores biológicos. Manglar 19 (2): 143–151. https://doi.org/10.17268/manglar.2022.018

Stork NE. 2018. How many species of insects and other terrestrial arthropods are there on Earth? Annual Review of Entomology 63 (1): 31–45. https://doi.org/10.1146/annurev-ento-020117-043348

Tarigan S, Buchori D, Siregar IZ, Azhar A, Ullyta A, Tjoa A, Edy N. 2021. Agroforestry inside oil palm plantation for enhancing biodiversity-based ecosystem functions. IOP Conference Series: Earth and Environmental Science 694 (1): 012058. https://doi.org/10.1088/1755-1315/694/1/012058

Varah A, Jones H, Smith J, Potts S. 2020. Temperate agroforestry systems provide greater pollination service than monoculture. Agriculture, Ecosystems and Environment 301 (1): 107031. https://doi.org/10.1016/j.agee.2020.107031

Villanueva-López G, Lara-Pérez LA, Oros-Ortega I, Ramírez-Barajas PJ, Casanova-Lugo F, Ramos-Reyes R, Aryal DR. 2019. Diversity of soil macro-arthropods correlates to the richness of plant species in traditional agroforestry systems in the humid tropics of Mexico. Agriculture, Ecosystems and Environment 286 (1): 106658. https://doi.org/10.1016/j.agee.2019.106658

XLSTAT. 2018. Statistical and data analysis solution, by Addinsoft, version 2018.7.5. https://www.xlstat.com (Retrieved: January 2024).

Zumbado MA, Azofeifa D. 2018. Insectos de importancia agrícola. Guía básica de entomología. Programa Nacional de Agricultura Orgánica (PNAO). Heredia, Costa Rica. 204 p.

VOLUME 58, NUMBER 8 | NOVEMBER 16 - DECEMBER 31, 2024 | MEXICO