

Morphological characterization of native maize varieties (*Zea mays* L.)

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ABSTRACT

Objective: To characterize the traditional *milpa* agricultural system and evaluate the phenotypic variation of native maize ears in the coastal region of Oaxaca.

Design/methodology/approach: Thirty semi-structured interviews were conducted with local farmers, and morphological traits were evaluated in 100 ears from seven native maize types. Tests for normality (Shapiro-Wilk) and homoscedasticity (Kolmogorov-Smirnov) were performed, followed by ANOVA. Where significant differences were found, Tukey's test was applied ($\alpha < 0.05$).

Results: The *milpa* is a polyculture system, and seven native maize types were documented. Significant differences were found in traits such as peduncle and ear length, number of kernel rows, and grain dimensions.

Findings/conclusions: The *milpa* is a system that harbors biological diversity, contributing to the conservation of genetic resources and food security.

Keywords: diversity, maize, phenotypic variation, traditional agricultural system.

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INTRODUCTION

The *milpa* system is a traditional agricultural practice that has been used since pre-Hispanic times in Mesoamerica. It is characterized by the association of maize (*Zea mays* L.) with other crops such as common bean (*Phaseolus vulgaris* L.) and squash (*Cucurbita argyrosperma*) (Chávez-Servia *et al.*, 2011; Mateos-Maces *et al.*, 2016). This system is key to food security and the conservation of agrobiodiversity, as it allows for the coexistence of multiple species that provide agronomic and ecological benefits (Acosta-Díaz *et al.*, 2014).

In Mexico, maize represents a central element in rural culture and economy, with approximately 65 registered races, 59 of which are native. Of these, 35 are found in Oaxaca, establishing the state as a center of genetic diversity for the crop (Santillán-Fernández *et al.*, 2021; CONABIO, 2011). The *milpa* system is fundamental to food security and ecological sustainability, as it maintains soil fertility through practices such as the incorporation of organic matter and crop rotation (Marcial-Medina *et al.*, 2024). However, it faces various

threats, including the introduction of commercial hybrid maize seeds, intensive use of agrochemicals, and changes in land use, all of which jeopardize the conservation of native varieties and the autonomy of local farmers (Márquez-Sánchez, 2008; Moreno-Calles *et al.*, 2013).

In the coastal region of Oaxaca, Mixtec communities cultivate native maize and other useful species within *milpa* systems; however, their cultivation practices have not been documented. The objective of this study was to characterize the traditional agricultural system in Santiago Tetepec and to evaluate the phenotypic variation of native maize ears cultivated within this system, in order to contribute to the appreciation of traditional agriculture and to inform conservation strategies and breeding programs.

MATERIALS AND METHODS

Study Area

Santiago Tetepec (Figure 1) is located in the Jamiltepec district of Oaxaca. It is a Mixtec community with a warm sub-humid climate and annual precipitation ranging from 1,000 to 2,500 mm. Agriculture is the main economic activity, with maize, beans, and chili peppers being the principal crops (INEGI, 2020).

Characterization of the *milpa* system

A semi-structured interview was designed and applied to local farmers to document the *milpa* production cycle. The questionnaire included detailed sections on the crop's phenological stages, cultural practices, tools used, preferred maize types, yields obtained, and uses of the grain, among other aspects. It also included some sociodemographic

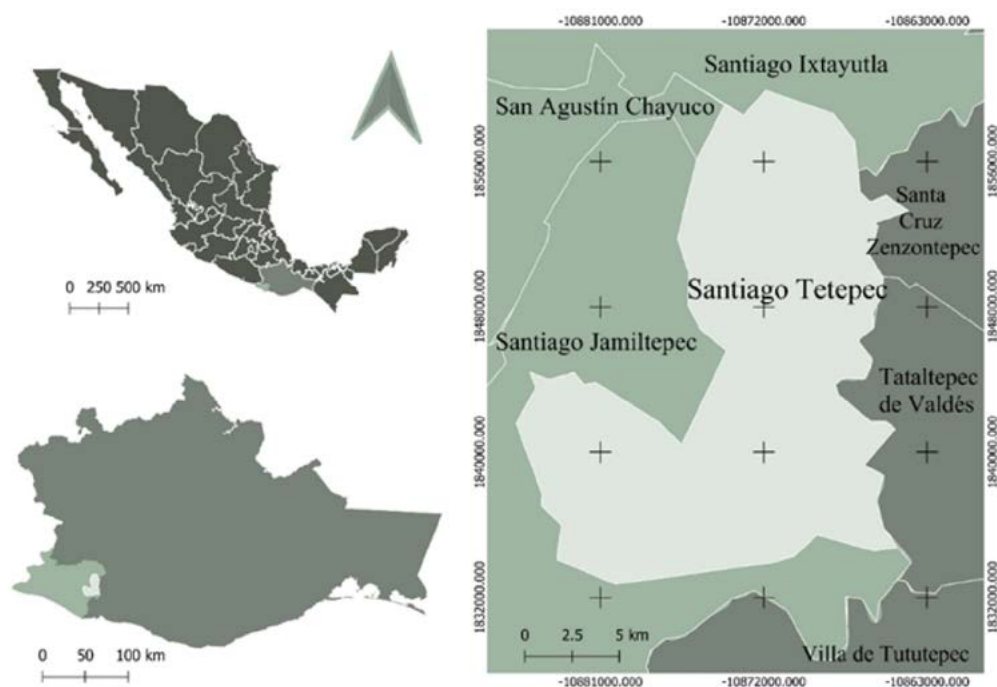


Figure 1. Geographic location of Santiago Tetepec, Oaxaca.

questions to gain insight into their work and educational background. The *milpas* of the interviewees were visited, and the information was complemented through participant observation, photographic documentation, and open-ended interviews.

Phenotypic characterization of maize

Ears from seven types of native maize were collected from the farmers interviewed in the community. An experimental plot was established in collaboration with a cooperating producer. The plot was divided into several subplots, each planted with 25 maize rows.

Planting was rainfed and carried out by making holes in the soil using a tool locally called *enduyo*. Four to five mixed seeds were placed in each hole, with a spacing of 1 m between rows. For weed control, two applications of Gramoxone[®] Super were made at different periods. Pest and disease control was carried out using agrochemicals. Fertilization was also chemical, with two applications of Yara[®] fertilizer: the first at the V5 growth stage and the second at the V10 stage.

Samples were taken from five central rows of each of the seven maize types. From each row, 20 plants were randomly selected, resulting in a total of 100 ears per maize type. Each collected sample was labeled and recorded, indicating the maize type, date, and row of origin. A total of 21 morphological traits were evaluated, of which 17 were quantitative and four qualitative. The number of ears per plant, the number and shape of the bracts, the color of the grain and cob, the number of rows, and the arrangement of the rows on the ear were recorded. A vernier caliper was used to measure the diameter of the cob, pith diameter, rachis diameter, peduncle diameter, and ear diameter at $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ of its length. A ruler was used to measure the length of the peduncle and the ear. The length, width, and thickness of the grain were also recorded. Ear weight and the weight of 100 grains were obtained using a scale.

Statistical analysis

The information obtained from the interviews was systematized and analyzed using descriptive statistics. To evaluate phenotypic variability, normality tests (Shapiro-Wilks) and homoscedasticity tests (Kolmogorov) were applied, as well as ANOVA to determine significant differences among the measured variables. Tukey's test ($\alpha < 0.05$) was used for mean comparisons.

RESULTS AND DISCUSSION

Characterization of the traditional milpa agricultural system

The planting of native maize is a tradition among the inhabitants of the Mixtec community of Santiago Tetepec. It is an activity inherited from parents to children, as the maize is used for food preparation, ensuring their food security. Maize production is carried out under a polyculture system called *milpa (itu savi)*, where other species are also established, such as common bean (*Phaseolus vulgaris* L.), squash (*Cucurbita argyrosperma* K. Koch), sesame (*Sesamum indicum* L.), hibiscus (*Hibiscus sabdariffa* L.), cassava (*Manihot esculenta* Crantz), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), cucumber (*Cucumis* sp.), chili pepper (*Capsicum annum* L.), jícama (*Pachyrhizus erosus* (L.) Urb.), and

bottle gourd (*Lagenaria* sp.). The maize types include short-cycle varieties, which take two to two and a half months, and long-cycle varieties, which take three to three and a half months. Various management activities are carried out in the cultivation, as shown in Figure 2.

The land preparation (slash-and-burn) — *Kañiyoo, cha’yayo yutu, Ka’miyoo chiin*

Land preparation begins between late April and early May and consists of slash, clear, and burn. Tall trees are cut with a chainsaw for firewood, while shrubs and grasses are removed with a machete and left to dry before burning. Currently, most producers only slash and burn, meaning they cut only shrubs and grasses, let them dry, and then burn them. To prevent uncontrolled fire spread, a firebreak is established by clearing vegetation along the edges of the area to be burned. The burning is carried out in the afternoon when winds are calmer. Subsequently, a minimum of three consecutive days of rain is awaited to ensure adequately moist soil for planting. Previous studies have documented this practice in different communities. Ayala-Enríquez *et al.* (2019) reported it in Santa Catarina, Morelos, including the use of harrows and fallow periods. Bastida-Francisca *et al.* (2024) mention it in the Mazahua agricultural calendar, where it begins in December, with fallow in January and harrowing in February. Carrera-García *et al.* (2012) describe a similar process in the Mazatec agricultural calendar, carried out with machetes and hoes.

Planting – *Tachiyoo*

It is carried out after the first rains and before noon. The tool used is known as *enduyo*, placing 3 to 4 maize grains per hole at a distance of one meter between rows

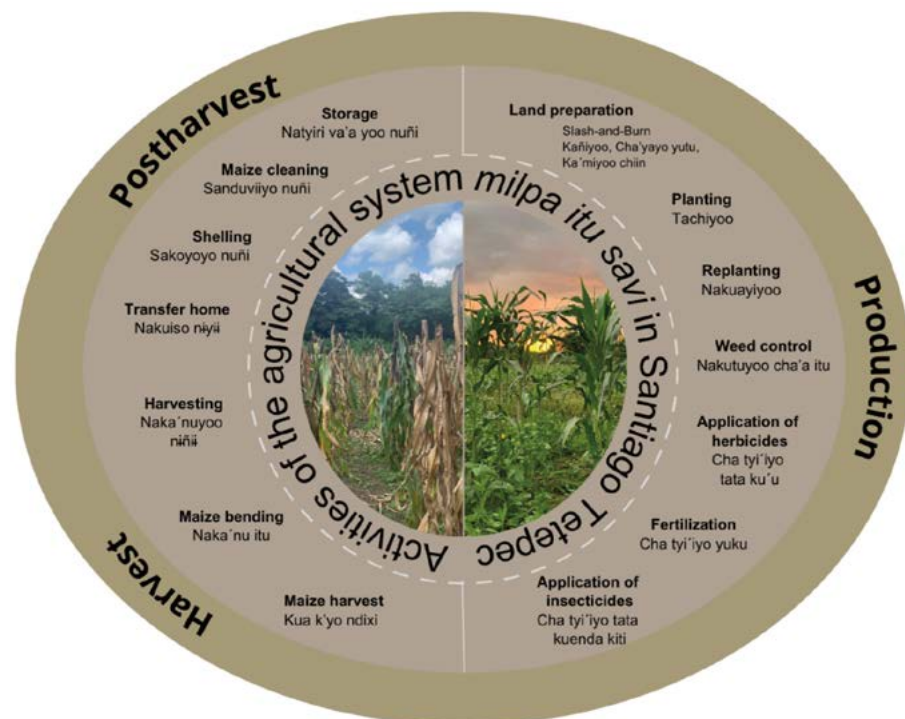


Figure 2. Agricultural activities carried out during the milpa production cycle.

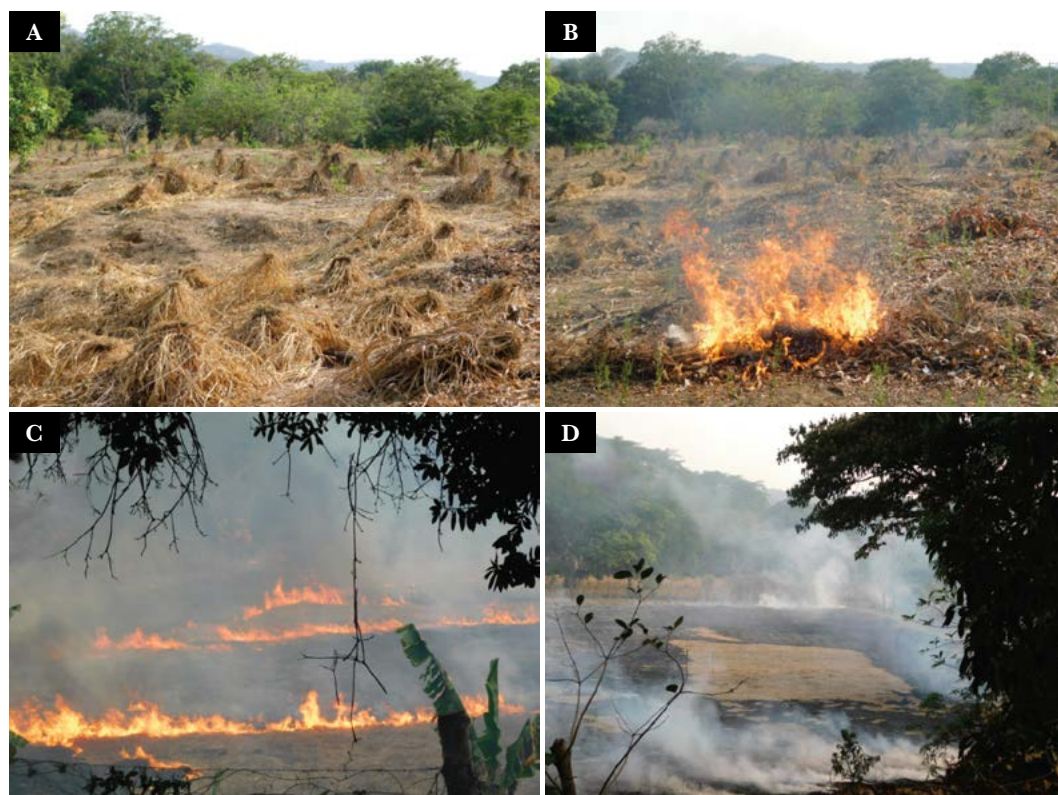


Figure 3. Land preparation. A) Slashing, B) Burning, C) Fire spreading, D) End of burning.

and between plants. Associated crops are planted on different dates. Ayala-Enríquez *et al.* (2019) documented that in Santa Catarina, Morelos, this activity is performed after eight consecutive days of rain, with a spacing of 70-80 cm between rows and one step between plants. Unlike the Mazahua agricultural calendar reported by Bastida-Francisca *et al.* (2024), where planting begins in March and April, the Mazatec calendar carries out planting in February and March (Carrera-García *et al.*, 2012).

Replanting – *Nakuayiyoo*

This is done three or four days after planting, in the spots where coleoptile emergence did not occur. Ayala-Enríquez *et al.* (2019) mention that this activity is carried out eight days after replanting in Santa Catarina, Morelos; and Bastida-Francisca *et al.* (2024) report that planting takes place in May.

Fertilization – Applying fertilizer – *Cha tyi'iyoyuku*

Fertilization is carried out using chemical products, the most commonly used being urea. The first application is made 15 days after planting, and the second when 50% of the plants are in male inflorescence. Ayala-Enríquez *et al.* (2019) agree with the use of chemical fertilizers; however, they also describe an accompanying activity called *primera mano* performed in the community of Santa Catarina, Morelos, which consists of loosening the soil.

Herbicide application – *Cha tyi´iyo tata ku´u*

Herbicides are applied using 20 L backpack sprayers; application doses depend on the recommendations from agrochemical store personnel, and some farmers perform a second application. In other regions such as Morelos (Ayala-Enríquez *et al.*, 2019), the State of Mexico (Bastida-Francisca *et al.*, 2024), and Oaxaca (Carrera-García *et al.*, 2012), manual weeding is performed using tools like the hoe and machete.

Insecticide application – *Cha tyi´iyo tata kuenda kiti*

The main pests affecting maize are the fall armyworm, which attacks the whorl, and the wireworm, which damages the roots. Daily monitoring is carried out, and insecticides are applied using backpack sprayers. In other regions, these pests are not controlled with agrochemicals (Ayala-Enríquez *et al.*, 2019; Bastida-Francisca *et al.*, 2024; Carrera-García *et al.*, 2012).

First harvest (Green maize) – *Kua k´yo ndixi*

The harvest of maize takes place when the kernels are still tender. They are consumed boiled or used to prepare tamales and tortillas made from fresh maize. This practice coincides with harvest dates reported in Santa Catarina, Morelos, and in the Mazahua agricultural calendar (Ayala-Enríquez *et al.*, 2019; Bastida-Francisca *et al.*, 2024). In Mazatec tradition, the green maize harvest occurs in August (Carrera-García *et al.*, 2012).

Maize bending – *Naka´nu itu*

This practice is carried out in October or early November to facilitate the drying of the maize and to protect it from pests and birds such as parakeets and grackles. Its implementation is not mentioned in Santa Catarina, Morelos, the State of Mexico, or Oaxaca (Ayala-Enríquez *et al.*, 2019; Bastida-Francisca *et al.*, 2024; Carrera-García *et al.*, 2012).

**Post-harvest (Harvesting – *Naka´nuyoo niñii*; Shelling – *Sakoyoyo nuñi*;
Cleaning – *Sanduviiyo nuñi*; Storage – *Natyri va´a yoo nuñi*)**

Harvesting is the activity in which the maize ears are collected. It takes place in January and February, when the ears are dry enough to detach easily. Shelling is done at home, followed by cleaning (removal of plant residues), inspecting for weevils, and storing the grain in sacks, drums, or silos. In the Mazahua agricultural calendar, post-harvest occurs in October and November, while in Santa Catarina, Morelos, it is carried out in December, after the feast of Our Lady of Guadalupe (Ayala-Enríquez *et al.*, 2019; Bastida-Francisca *et al.*, 2024). In the Mazatec culture, harvesting takes place between September and October (Carrera-García *et al.*, 2012).

Phenotypic characterization of native maize ears

The variables cob peduncle diameter, number of husk leaves per ear, number of ears per plant, and kernel thickness showed no significant differences (Table 1). The variables ear diameter at $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ length, cob (rachis) diameter, pith diameter, rachis diameter,

and ear weight exhibited variability (Table 1). Morphometric variables of native maize from Santiago Tetepec, Oaxaca.

In terms of ear length, the Conejo and Amarillo types are similar to each other but differ from the other maize types. In comparison with the study by Sánchez-Hernández *et al.* (2015), where only collection number three from the community of Chilapa showed greater ear length than the other collections in the state of Tabasco, Cabrera-Toledo *et al.* (2019) found that among 18 populations of the Zapalote Chico race, the collection OAX-834 from the locality of Santiago Laollaga presented the greatest ear length compared to other collections from the Isthmus of Tehuantepec. In the study by Toxtle-Flores *et al.* (2023), among four groups formed, only one group exhibited shorter ear length among the types collected in the municipality of Coronango, Puebla. Similarly, in the work of González-Martínez *et al.* (2020), which analyzed two groups and three subgroups, one group and one subgroup had shorter ear length and were significantly different from the rest, based on 98 maize populations from Tamaulipas.

In terms of the number of rows per ear, the Conejo type showed a significant difference compared to the Rojo type, and both differed from the other maize types. Guillen-De la Cruz *et al.* (2014) reported highly significant differences among 71 populations of native maize from the state of Tabasco. Cabrera-Toledo *et al.* (2019) found that only one collection from the locality of San Pedro Comitancillo showed a higher number of rows among the 18 populations of the Zapalote Chico race collected in the Isthmus of Tehuantepec. Similarly, in the study by Villalobos-González *et al.* (2019), only one accession had a higher average number of rows per ear among the thirteen collected in a community in the state of

Table 1. Morphometric variables of native maize from Santiago Tetepec, Oaxaca, Mexico.

Variables	p-value	Blanco A	Blanco B	Café	Rojo	Azul	conejo	Amarillo
Length of the peduncle	<0.0001	4.99 ^{bc}	5.55 ^{ab}	5.93 ^a	4.34 ^{cd}	5.22 ^b	4.12 ^d	3.90 ^d
Peduncle diameter	<0.0001	1.12 ^a	1.02 ^b	1.00 ^b	1.00 ^b	1.14 ^a	0.96 ^b	0.93 ^b
Length of the ear	<0.0001	17.32 ^a	16.23 ^{bc}	16.74 ^{ab}	15.66 ^c	15.93 ^{bc}	14.04 ^d	13.78 ^d
Ear diameter (1/4)	<0.0001	4.19 ^a	3.99 ^b	4.00 ^b	3.90 ^{bc}	3.99 ^b	3.96 ^{bc}	3.81 ^c
Ear diameter (1/2)	<0.0001	4.05 ^a	3.89 ^b	3.86 ^b	3.80 ^b	3.87 ^b	3.80 ^b	3.62 ^c
Ear diameter (3/4)	<0.0001	3.85 ^a	3.61 ^b	3.60 ^b	3.64 ^b	3.68 ^{ab}	3.59 ^b	3.35 ^c
Cob diameter	<0.0001	2.35 ^a	2.04 ^d	1.92 ^d	2.20 ^{bc}	2.41 ^a	2.20 ^c	2.32 ^{ab}
Pith diameter	<0.0001	0.62 ^a	0.50 ^{bcd}	0.47 ^d	0.49 ^{cd}	0.58 ^{ab}	0.56 ^{abc}	0.55 ^{bc}
Rachis diameter	<0.0001	1.05 ^a	0.88 ^c	0.87 ^c	0.88 ^c	1.01 ^{ab}	0.93 ^{bc}	0.94 ^{bc}
Number of rows	<0.0001	9.35 ^{bc}	8.61 ^{de}	8.72 ^{de}	8.25 ^e	9.84 ^{ab}	10.13 ^a	9.19 ^{cd}
Length of the grain	<0.0001	1.08 ^{bcd}	1.13 ^{ab}	1.18 ^a	1.12 ^{abc}	1.07 ^{cde}	1.05 ^{de}	1.02 ^e
Width of the grain	<0.0001	1.09 ^b	1.11 ^b	1.07 ^{bc}	1.15 ^a	1.03 ^{cd}	1.01 ^a	1.04 ^{cd}
Thickness of the grain	0.8181	0.41 ^a	0.41 ^a	0.41 ^a	0.40 ^a	0.40 ^a	0.40 ^a	0.40 ^a
Number of the bracts	<0.0001	9.69 ^a	9.78 ^a	10.28 ^a	8.55 ^b	8.47 ^b	10.16 ^a	8.27 ^b
Ear weight	<0.0001	132.97 ^a	121.87 ^a	125.39 ^a	102.83 ^b	102.49 ^b	93.03 ^{bc}	83.69 ^c
Weight of 100 grains	<0.0001	35.14 ^b	38.44 ^a	36.44 ^{ab}	36.29 ^{ab}	30.44 ^c	29.16 ^c	29.25 ^c
Number of ears	<0.0001	1.02 ^b	1.04 ^b	1.20 ^a	1.00 ^b	1.00 ^b	1.02 ^b	1.08 ^b

Campeche. In the work of Toxtle-Flores *et al.* (2023), one group exhibited a lower number of rows than the other groups collected in the community of Coronango, Puebla, Mexico.

Regarding grain width, the Conejo type showed a significant difference compared to the Rojo type; however, both types were similar to the other maize types. In comparison, Ángeles-Gaspar *et al.* (2020) reported that four accessions out of 23 collected in three localities of the municipality of Molcaxac, Puebla, exhibited greater grain width. Guillen-De la Cruz *et al.* (2014) also observed significant differences in native maize among 71 populations from the state of Tabasco. Similarly, Montes-Hernández *et al.* (2014) classified 18 populations of the Jala race and seven reference varieties into three groups based on differences in grain width in the municipality of Jala, Nayarit, Mexico.

The various studies conducted by different researchers in several locations across the State of Mexico report significant differences in morphological traits, primarily due to the climatic conditions under which native maize develops. Maize plants adapt to different climatic conditions and can either accelerate or delay their production cycle. The research carried out in Santiago Tetepec, Oaxaca, highlights the importance of the traditional milpa agricultural system as an integrated production model that combines polycropping, biodiversity conservation, and practices adapted to local conditions. In this Mixtec community, the milpa serves as both a means of subsistence and a reflection of the interaction between traditional knowledge and agrobiodiversity.

CONCLUSIONS

The milpas of Santiago Tetepec host a wide variety of useful species, with maize standing out as the main crop, accompanied by beans, squash, sesame, hibiscus, bush sweet potato, chili, and others. This system supports the coexistence of native maize varieties with both short and long growth cycles, which exhibit significant differences in phenotypic traits such as cob peduncle length, grain length and width, number of rows, and the weight of 100 grains. These differences highlight the genetic variability present in these plant genetic resources.

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