

Yield and forage quality in maize (*Zea mays* L.) inbred lines

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ABSTRACT

Objective: To analyze the genetic diversity (agronomic attributes, forage yield, and fiber content) of 100 maize inbred lines and to identify genotypes with potential for the breeding of plants with the said traits.

Design/methodology/approach: One-hundred maize inbred lines were evaluated in two environments, in a 10×10 complete block experimental design, with two repetitions. Days to male flowering, days to female flowering, ear height, plant height, stem diameter, green forage yield, neutral detergent fiber, and acid detergent fiber data were recorded.

Results: Significant differences were observed in the environmental sources of variation and genotypes for all the traits evaluated. The first four components account for 81% of the total variation observed and the first two account for 66% of the variation. The variables which have the highest absolute value and which strongly influence the dispersion of the genotypes, as well as the formation of groups, were: plant height, forage yield, and acid detergent fiber (component one) and days to female flowering and days to male flowering (component two). Consequently, the genotypes were dispersed in four groups.

Study limitations/implications: It was not possible to conduct genetic diversity studies using molecular markers.

Findings/conclusions: The grouping pattern of inbred lines shows the genetic diversity present; this can be an important tool in genetic improvement programs aimed at obtaining hybrids with specific characteristics.

Keywords: Forage yield, plant breeding, forage quality, principal component analysis.

Citation: Gayosso-Barragán, O., Chávez-Aguilar, G., Tirado-González, D. N., Marroquín-Morales, J. A., & López-Benítez, A. (2021). Yield and forage quality in maize (*Zea mays* L.) inbred lines. *Agro Productividad*. <https://doi.org/10.32854/agrop.v14i11.1975>

Editor in Chief: Dr. Jorge Cadena Iniguez

Received: March 19, 2021.

Accepted: October 15, 2021.

Published on-line: November 26, 2021.

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INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereals in the world, since it supplies nutritional elements for human diets and animal feed, and it provides the industry with a basic raw material. It is used as fresh forage, silage or stubble for animal consumption, mainly during the dry season (Luna *et al.*, 2013).

The maize plant, transformed into silage, has been used with great success by the dairy and beef industries. In addition, after the grain is harvested, the dry leaves and



the upper part (including the tassel) are also used to provide relatively good forage for ruminants (Hassan *et al.*, 2018); this activity is carried out by many small farmers in various agricultural regions of Mexico.

In 2019, 553,095 ha were sown with maize for forage purposes in Mexico, achieving a total production of 15,101,860 t and an average yield of 31.1 t ha⁻¹. Based on the area in which maize for green forage is cultivated, the most important states are: Jalisco, Zacatecas, Aguascalientes, Durango, Chihuahua, State of Mexico, and Coahuila with 211,712 ha, 101,231 ha, 53,327 ha, 51,164 ha, 37,868 ha, 22,471 ha, and 22,382 ha, respectively (SIAP, 2019).

In genetic improvement programs, the genetic knowledge of the base populations facilitates the selection of superior lines. To choose the improvement scheme, the type of inheritance or specific gene action must be determined in order to establish quantitative traits of agronomic interest (Peyman *et al.*, 2009). Studying genetic diversity is a useful tool to select appropriate genotypes for hybridization, because divergent parents can produce highly heterotic effects (Falconer, 1960), with a significant impact on the genetic improvement of the crop (Hallauer *et al.*, 1988). Maize breeders have constantly emphasized the significant contribution of diverse parental genotypes for heterotic hybrids (Xia *et al.*, 2005).

The objective of this work was to analyze the genetic diversity (agronomic attributes, forage yield, and fiber content) of 100 maize inbred lines and to identify genotypes with potential for the genetic improvement of the said traits.

MATERIALS AND METHODS

Genetic material

One-hundred maize lines with different levels of inbreeding and diverse origins were evaluated. The seed was obtained from the genetic improvement programs for the tropics and subtropics of the germplasm bank of the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT).

Experimental sites and growing cycles

The field experiment was established at the Dr. Ernest W. Sprague experimental station, Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) in Agua Fría, Puebla, Mexico (20° 27' N, 97° 38' W, 812 masl) and at the Rancho Santa Margarita, Puerto Vallarta, Jalisco (20° 44' N, 105° 10' W, 731 masl). In both locations, cultural management practices common to the region were carried out, keeping the crop free from pests and diseases; sowing and harvesting were done manually.

Design and experimental unit

The experiments were established based on an experimental design with 10×10 complete blocks, with two repetitions, which included the 100 accessions. The experimental unit consisted of two 5-m long furrows, with 0.80 m between furrows and 0.15 m between plants, resulting in a planting density of 83,000 plants per hectare.

Traits measured

Regarding flowering, the days from the sowing to the female (FF) and male (FM) flowering were recorded: the first when 50% of the plants of the experimental unit had exposed stigmas and the second when 50% of the plants presented pollen emission.

The following characteristics were measured in five randomly selected plants from the useful plot: ear height (AMZ) was measured from the soil surface to the insertion of the first ear; plant height (AP) was measured from the union of the root and the stem to the base of the male inflorescence; diameter of the stem base (DT) was measured using a vernier caliper and the results were reported in centimeters.

To determine green forage yield (RFV), when the plants reached physiological maturity, ten plants were harvested from each experimental unit and the total weight per genotype was recorded. The forage was harvested from two central furrows of each useful plot, discarding the plants that grew one meter from both ends of the furrow, to avoid edge effect and to obtain fully competitive plants. A 30-kg Eura Brand M2/50 digital scale was used.

To determine neutral detergent fiber (FDN) and acid detergent fiber (FDA), the internodes were collected: second to fifth above the ground from five plant stems for each inbred line. With these samples, the determinations were made based on the principle of van Soest (1991), using an ANKOM 220 fiber analyzer.

Statistical analysis

A combined analysis of variance was performed across localities. An analysis of the principal components was applied based on the correlation matrix; the dispersion of the accessions in the plane determined by the first two biplot components was plotted; and a cluster analysis—in which the Euclidean distance coefficient and the unweighted pair group method with arithmetic (UPGMA) were used—was carried out. The Statistical Analysis System (SAS) version 9.3 for Windows and the Infostat statistical packages were used. All effects were considered as random effects.

RESULTS AND DISCUSSION

Analysis of variance

Significant differences were observed for the environmental variation sources and the genotypes for all the characteristics evaluated; meanwhile, in the genotypes \times environments interaction, significant differences were only observed for the plant height, ear height, stem diameter, and forage yield variables (Table 1). The significant differences observed between environments can be attributed to the effects of temperature and relative humidity, which were different in the evaluation environments. As a consequence of the random fluctuation of the environment, the agronomic evaluation of maize germplasm must be carried out in different environments, allowing a more accurate estimation of the value of the genetic components and the separation of the environmental genetic effect (Gutiérrez *et al.*, 2004; Alejos *et al.*, 2006).

The mean values for the plant and ear height (AP and AMZ) variables were 173.81 and 87.61 cm, respectively. Alfaro (2009) reported similar results for these variables describing the morphology of maize cultivars from southern Venezuela. Bejarano *et al.* (2000)

Table 1. Mean squares of the analysis of variance for yield traits and forage quality of 100 inbred maize lines evaluated in Agua Fría, Puebla and Puerto Vallarta, Jalisco, Mexico.

Variable	Variation source				Mean	SV (%)
	Localities (Loc)	Genotype (Gen)	Interaction Gen × Loc	Error		
DF	1	99	99	399		
Days to female flowering	4134.3**	80.1**	13.4 ^{NS}	13.60	78.7	4.7
Days male flowering	3795.1**	56.5**	13.1 ^{NS}	11.88	79.2	4.3
Plant height (cm)	2654.5**	2654.5**	249.8**	43.95	173.8	3.8
Ear height (cm)	25973.8**	1208.9**	144.1**	19.15	87.6	4.9
Stem diameter (mm)	148.1**	87.1**	5.1**	1.07	20.5	5.1
Forage yield (t ha ⁻¹)	10031.6**	702.1**	117.1**	0.30	40.2	10.3
Acid detergent fiber (%)	1337.1**	146.9**	0.1 ^{NS}	0.95	46.6	12.1
Neutral detergent fiber (%)	3911.7**	162.6**	1.3 ^{NS}	1.04	69.9	11.5

SV: Variation source, DF: Degree of freedom.

evaluated the AP and AMZ variables in simple maize crosses from lines with three different inbreeding levels and reported significant differences. In general, the lines evaluated in this study are short, as a consequence of the inbreeding depression that occurs when the lines are subjected to a successive self-fertilization in order to achieve homogeneity (Poehlman and Sleper, 1995).

The genotypes with the highest forage yield potential exceeded the national average of 31.1 t ha⁻¹ (SIAP, 2019). The lines with the highest values were: CLWN701, CLWN345, CML472, CML216, CLWQ232, and CML528, with 74.8, 72.2, 67.7, 65.8, 63.8, and 62.5 t ha⁻¹, respectively. These genotypes could be used in subsequent genetic improvement-oriented evaluations and for the generation of maize hybrids for forage production. These results are similar to those recorded by Peña *et al.* (2008) and Castillo *et al.* (2009), with 70-95 t ha⁻¹ yields.

The average acid detergent fiber (FDA) value was 46.62%. However, the CLRCY017, CLWQH2N75, CSL1612, CSL1620, CLYN548, CLYN482, CSL1732, and CLYN631 lines are the genotypes with the lowest FDA concentration, with 34.8, 36.9, 37.7, 38.612, 39.4, 39.8, 40.1, and 40.2%, respectively. In this regard, De la Cruz *et al.* (2007) report 25-35% FDA values in maize lines from two locations. The acid detergent fiber value (FDA) is used to estimate the energy value of maize silage. Forage digestibility is mainly linked to FDA, because it consists of cellulose, lignin, and proteins (Castillo *et al.*, 2009): as FDA increases, plant digestibility decreases. Forage crop quality depends on <28% FDA values. However, this percentage depends on factors such as days to harvest after sowing, sowing density, and fertilization, among others (Gallegos *et al.*, 2012).

The average neutral detergent fiber (FDN) value was 69.91% (with 59.01-87.48% values). The CLYN482, CSL1612, CSL1612, CLRCY017, CLYN531, CML427, CLWQH2N75, CLYN540, and CLYN548 genotypes had the lowest FDN concentration, with 59.01, 59.12, 60.52, 60.87, 61.65, 61.98, 62.27, 62.58, and 63.746%, respectively. In contrast, De la Cruz *et al.* (2007) reported 41-61% determinations of FDN in a group of maize inbred

lines. Núñez *et al.* (2001) evaluated the FDN concentration of eleven maize hybrids of temperate origin and seven of tropical origin and obtained values of 59.80 to 44.70%. This study identified inbred lines with a lower FDN content than those reported by Gallegos-Ponce *et al.* (2012), Amodu *et al.* (2014), and Fortis *et al.* (2009) for diverse maize genotypes evaluated under contrasting environmental conditions.

FDN content in forages is negatively correlated with intake and digestibility (Oramas and Vivas, 2007); therefore, obtaining forages with high energy value requires the use of maize hybrids with less than 50.0% of this type of fiber (Gallegos *et al.*, 2012).

Main component analysis

The result of the main components analysis indicated that the four components account for 81% of the total variation observed and the first two account for 66% of the variation. Ferraz *et al.* (2013) mention that a high proportion—explained by a low number of components— allows a better interpretation of the variation and the studies about the traits related to maize yield and shows that the models that involve biplot graphs are useful to determine the intensity and the nature of maize populations under certain environmental conditions (Martínez *et al.*, 2016). The classification of the main effects obtained through a biplot graphic representation can be easily interpreted, allowing a better observation of the relationship of the genotypes and the variables evaluated (Akinwale *et al.*, 2014).

In this study, the variables which have the highest absolute values—in the coefficients of the eigenvectors of the first two components—and which strongly influence the dispersion of the genotypes, as well as the formation of groups, were: plant height, forage yield, and acid detergent fiber (component one) and days to female flowering and days to male flowering (component two) (Table 2).

The dispersion of the accessions according to the first two main components enables the differentiation of four groups (Figure 1). The first group consists of the CLWN701,

Table 2. Eigenvalues and eigenvectors associated with the first four main components of the analysis of eight agronomic and forage quality variables in 100 maize inbred lines.

Variable	Eigenvector			
	CP 1	CP 2	CP 3	CP 4
Acid detergent fiber (FDA)	0.41	-0.07	0.54	-0.08
Neutral detergent fiber (FDN)	0.39	-0.01	0.55	-0.27
Plant height (AP)	0.46	-0.02	-0.33	-0.21
Ear height (AMZ)	0.40	0.01	-0.42	-0.43
Stem diameter (DT)	0.35	-0.13	0.06	0.76
Forage yield (RF)	0.42	-0.02	-0.34	0.32
Days to female flowering (DFF)	0.03	0.70	0.05	0.07
Days male flowering (DFM)	0.10	0.69	0.01	0.07
Eigenvalues	3.37	1.95	1.18	0.81
Proporción de la varianza explicada (%)	42	24	15	10

CP1, CP2, CP3, and CP4: Main components 1, 2, 3, and 4, respectively.

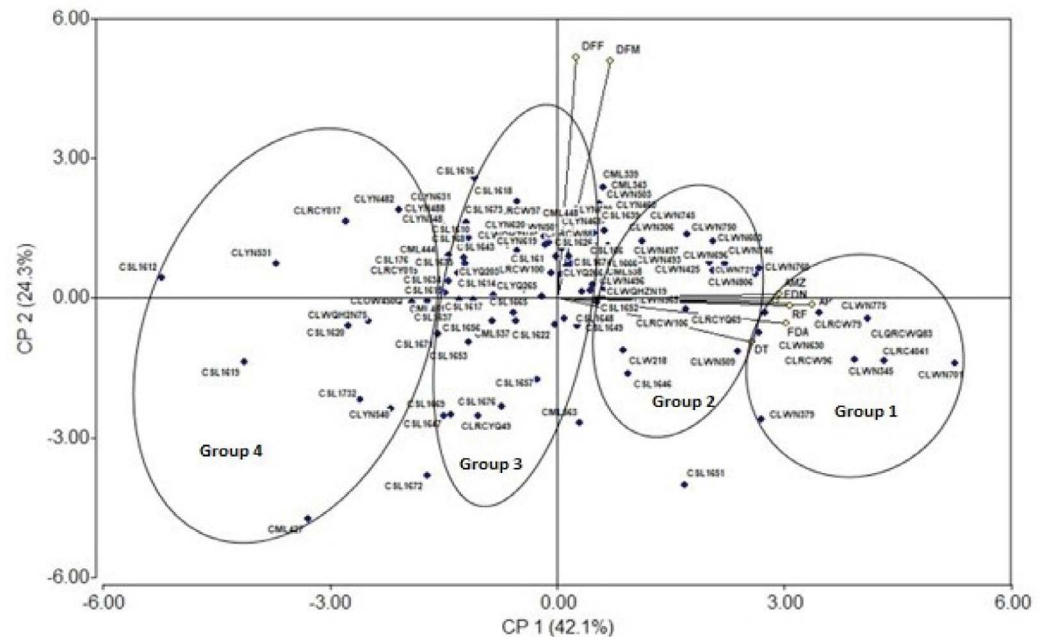


Figure 1. Dispersion of 100 maize inbred lines based on the first two main components.

CLWN630, CLWN775, CLWN345, CLWN379, CLRCW96, CLRC4041, and CLRCW79 genotypes and is mainly associated with the plant height, ear height and forage yield variables. Group two consists of the CLWN746, CLWN721, CLWN745, CLWN750, CLWN760, CSL166, CSL1652, CSL1648, and CSL1551 genotypes which are, associated with days to male flowering and days to female flowering variables. Group three shows the greatest association in the neutral detergent fiber and acid detergent fiber variables. Group four includes the CLYN631, CLYN482, CLYN531, CLYN548, CLYN540, CLYN488, CSL1612, CSL1620, CSL1619, and CSL1732 genotypes, which are mainly associated with stem diameter and forage yield.

Estimating genetic diversity and relationships between germplasm accessions facilitates the selection of parents with diverse genetic backgrounds, which is an essential component of the breeding program (Chinyere *et al.*, 2020). The considerable morphological variation found in this study was mainly due to genetic factors, although environmental factors were also involved (Table 2, Figure 1). Several works about the genetic diversity of maize inbred lines have also reported variability in different morphological traits (Enoki *et al.*, 2002; Azad *et al.*, 2012; Boakyewaa *et al.*, 2019).

CONCLUSIONS

The grouping pattern of inbred lines in this study shows the presence of genetic diversity. This diversity is an important tool in genetic improvement programs aimed to obtain maize hybrids with specific characteristics. The variability we detected can potentially contribute to the efficient use of inbred lines in the exploitation of heterosis and the formation of genetically diverse populations in maize breeding programs.

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