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Agronomic characteristics and stability in bioethanol production of sweet sorghum in northeastern Mexico

Características agronómicas y estabilidad en la producción de bioetanol de sorgo dulce en el noreste de México

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ABSTRACT

The sweet sorghum has great potential for renewable energy production (bioethanol). The present study aimed to determine the agronomic characteristics and stability in bioethanol production of seven sweet sorghum genotypes, in nine environments in northeastern Mexico. The evaluation was performed from 2013 to 2017: in five environments in Estación Cuauhtémoc, Tamaulipas state, and four environments in Marín, Nuevo León state. The genotypes were: Dulcina, Keller, Urja, and RB-Cañero (commercial varieties); (SBB-25 x Keller) 17-1-2-1 and (SBB-25 x Keller) 31-2-1-2 (experimental varieties) and Potrillo (commercial hybrid). A randomized complete block experimental design with three repetitions was used. For the stability parameters estimations, the GGE biplot model was used, where 85.93% of the total variability was explained. The agronomic characteristics of the materials were acceptable, except RB-Cañero, which exhibited the lowest value of °Brix (9.81) and bioethanol production (1237 L ha^{-1}). The most stable genotypes with the highest bioethanol yield were Keller (2935 L ha^{-1}); followed by (SBB-25 x Keller) 31-2-1-2 (2521 L ha^{-1}), and Urja (3214 L ha^{-1}); It was observed that the best genotype was Keller due to its stability, bioethanol production, and precocity.

KEY WORDS: Biomass; renewable energy; varieties *Sorghum bicolor*.

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Revista Bio ciencias
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R E S U M E N

El sorgo dulce presenta gran potencial para la producción de energía renovable (bioetanol). El objetivo de este estudio fue determinar las características agronómicas y estabilidad en la producción de bioetanol de siete genotipos de sorgo dulce, en nueve ambientes del noreste de México. La evaluación se realizó durante los años 2013 a 2017 en cinco ambientes en Estación Cuauhtémoc, Tamaulipas y cuatro ambientes en Marín, Nuevo León. Los genotipos fueron: Dulcina, Keller, Urja y RB-Cañero (variedades comerciales); (SBB-25 x Keller) 17-1-2-1 y (SBB-25 x Keller) 31-2-1-2 (variedades experimentales) y Potrillo (híbrido comercial). Se utilizó un diseño experimental de bloques completos al azar con tres repeticiones. Para estimar los parámetros de estabilidad, se utilizó el modelo GGE biplot, que explicó el 85.93 % de la variabilidad total. Las características agronómicas de los materiales fueron aceptables, excepto RB-Cañero; que obtuvo el menor valor de ^{0}Brix (9.81) y producción de bioetanol (1237 L ha^{-1}). Los genotipos más estables y con mayor rendimiento de bioetanol fueron: Keller (2935 L ha^{-1}); seguidas por (SBB-25 x Keller) 31-2-1-2 (2521 L ha^{-1}), y Urja (3214 L ha^{-1}): El mejor genotipo fue Keller, por su estabilidad, producción de bioetanol, y precocidad.

P A L A B R A S C L A V E : Biomasa; energías renovables; variedades; *Sorghum bicolor*.

Introduction

In Mexico, sorghum (*Sorghum bicolor* (L.) Moench) cultivation is highly significant due to its production volume (4.6 million tons) and planted area (1.4 million hectares); it ranks third after maize and beans (averages of the last five years). The Tamaulipas state, situated in northeastern Mexico, is the main sorghum producer, producing up to 43.0% of the national total (SIAP, 2023). Almost the entire production (95.0%) is used for animal feed (Reyes-Rodriguez *et al.,* 2017; SAGARPA, 2017).

The climate change problem, primarily caused by the indiscriminate use of fossil fuels and their constantly rising prices, has made renewable fuels increasingly acceptable and economically viable (Khawaja *et al.,* 2014). Plant biomass is a renewable energy source that can supplement fossil fuels (Ratnavathi *et al.,* 2010; Pabendon *et al.,* 2017). Sweet sorghum is gaining much attention since the juice from its stems can be directly fermented to produce bioethanol (Guden *et al.,* 2020), and it accumulates a large amount of fermentable sugars in the stalk, being closely as sugar-rich as sugarcane (*Saccharum officinarum* L.) (Silva *et al.,* 2021). It adapts to several production systems (López-Sandin *et al.,* 2021), can be grown with reduced inputs, responds

Revista Bio Ciencias
ISSN 2007-3380

favorably to water stress, and has outstanding biomass production potential (Ekefre *et al.,* 2017; Yücel *et al.,* 2022). Demand for bioethanol production is quickly increasing due to its use in automobile fuel mixtures (Prasad *et al.,* 2007), and sorghum bioethanol production helps mitigate the effects of climate change and environmental degradation (Williams-Alanís *et al.,* 2017).

Currently, the effects of climate change require plant breeders to develop genotypes adapted to mega-environments, which guarantees the best crop production (Maldonado-Moreno *et al.,* 2021). The evaluation of cultivars and identification of mega-environments are among the most important purposes of multi-environmental trials (MET), although their performance results from the combination of genotype (G), environment (E), and genotype × environment (GE) interactions. Only G and GE are appropriate for cultivar evaluation and mega-environment identification (Yan *et al.,* 2000).

The genotype × environment interaction is the factor that further restricts the identification of specific genotypes for specific environments (Snedecor & Cochran, 1980). This complicates the recommendation of cultivars for different environments, making stability analysis required (Silva *et al.,* 2021). Identifying sorghum varieties with better productivity and stability than existing cultivars has been one of the main research topics (Worede *et al.,* 2020). In Mexico, it is essential to develop sorghum genetic improvement programs to generate new varieties with higher fermentable sugar content and bioethanol yield, enabling the transition of the country to a sustainable energy system (Moreno-Hernández *et al.,* 2018). The National Institute of Forestry, Agricultural, and Livestock Research (INIFAP), Río Bravo Experimental Field, at Tamaulipas state, Mexico, began working on the genetic improvement of sorghum grain in 1973 (Williams-Alanís *et al.,* 2021; 2022). In the early 2000s, research on sweet sorghum began, and in 2010, the RB-Cañero variety was released, the first sweet sorghum genetic material in Mexico for producing biomass and sugars aimed at bioethanol production. Subsequently, sweet sorghum varieties RB-Pirulí, RB Tehua, and RB Cañaveral were released (Montes-García *et al.,* 2021). Hence, this work aimed to evaluate the agronomic traits and bioethanol production stability of seven commercial and experimental sweet sorghum genotypes in nine environments in northeastern Mexico.

Materials and Methods

The research was conducted from 2013 to 2017 across nine environments in northeastern Mexico: four in Marín, Nuevo León state (M); M OI 2013-2014, M V-2014, M V-2015, and M V-2017 and five in Estación Cuauhtémoc (EC), Tamaulipas state; EC V-2013, EC O-I-2013-2014, EC V-2014, EC V-2016, and EC O-I-2016-2017; (OI = fall-winter cycle; V = summer). Seven sweet sorghum genotypes were evaluated: Keller and Urja, commercial varieties introduced from the United States and India, respectively; three varieties, two experimental and one commercial, developed by the sweet sorghum genetic improvement program of INIFAP, Río Bravo Experimental Field; one variety and one commercial hybrid from the Autonomous University of Nuevo León (UANL) (Table 1). The experiments were conducted in uniform trials, utilizing a randomized complete block design with three replications.

Revista Bio ciencias
ISSN 2007-3380

Table 1. Genotypes used in the characterization and stability study of sweet sorghum.

N	Commercial name and/or genealogy	GN	Source
1	**Dulcina**	CV	Autonomous University of Nuevo Leon (FAUANL)
2	**(SBB-25 x Keller) 17-1-2-1**	EV	Río Bravo Experimental Field (INIFAP)
3	**(SBB-25 x Keller) 31-2-1-2**	EV.	Río Bravo Experimental Field (INIFAP)
4	**Keller**	CV	United States
5	**Urja**	CV	India
6	**RB-Cañero**	CV	Río Bravo Experimental Field (INIFAP)
7	**Potrillo**	CH	Autonomous University of Nuevo Leon (FAUANL)

N = Genotype number; GN = Genotype type; CV = Commercial variety; EV = Experimental variety; CH= Commercial hybrid; SBB-25 = Parent fertility maintaining line of the INIFAP grain sorghum hybrid RB-4000.

The Marín Campus, part of the Faculty of Agronomy at the Autonomous University of Nuevo León (FAUANL), located in the Marín municipality, Nuevo León state, located at coordinates 25° 53’ N and 100° 02’ W, at an altitude of 355 m. The climate corresponds to BS1 (hwe), described as a warm steppe dry climate with summer rains, an average annual precipitation of 595 mm, and an average annual temperature of 22°C (García, 2004). The soil type is a thin vertisol with a high clay and calcium carbonate content, low organic matter content, and a pH range of 7.5 to 8.5. In Marín, the experiments were conducted under irrigation conditions, and 2.0 t ha^{-1} of chicken manure was broadcast applied to the soil before planting.

The Estación Cuauhtémoc environment, located in the Altamira municipality, Tamaulipas state, at the INIFAP Las Huastecas Experimental Field, located at coordinates 22° 33’ N / 98° 09’ W, at an altitude of 20 m. It features a subhumid warm climate (Aw0) with summer rainfall and 5-10% winter rainfall (García, 2004), an average annual temperature of 24.5°C, and 842 mm of precipitation. The soil type is a deep clayey pellic vertisol, with a pH range of 7.5 to 7.8. In Estación Cuauhtémoc, the experiments were conducted under rainfed conditions, with planting done on moist soil, and seeds were manually sowed in furrow bottoms. In both locations, Estación Cuauhtémoc and Marín, the crop was managed according to the recommendations for sweet sorghum cultivation for the Tamaulipas state (Montes-García *et al.,* 2013).

Data collection

Data were collected on days to flowering (DF, when 50 % of the plants had flowered) and plant height (PH in cm, from the soil to the apex of the panicle). When the plants reached the milk and dough grain stage (Prasad *et al.,* 2007), when the plants reached the maximum sugar content

Revista Bio ciencias ISSN 2007-3380

in the stem juice, a one-meter linear section of the useful plot was harvested, taking plants in full competition, cutting the base of the stem 3 to 4 cm from the soil. The following data were then taken: total fresh plant weight (FPW). After removing the leaves and panicle, fresh stem weight (SW) was recorded. Brix values were measured using a manual Atago refractometer. The dry stem weight (DSW) was obtained by drying the fresh stem weight in an oven at 65°C for three days. The resulting weight of the sample was recorded as the DSW. The values in grams were converted to kg ha^{-1}. Juice weight (kg ha^{-1}) and theoretical bioethanol production (L ha^{-1}) were estimated using the formulas described by Rakhmetova *et al.* (2020).

Juice production (kg ha^{-1}) = (Fresh stem weight/kg ha^{-1} - Dry stem weight/kg ha^{-1}) x 0.866.

Theoretical bioethanol production (L ha^{-1}) = (Brix sugar/100) x (0.65 L bioethanol/1 kg sugar) x (0.85) x (stem weight/kg ha^{-1}).

The above formula for estimating bioethanol production in the trials was also used by Williams-Alanís *et al*. (2023) to estimate the combinatory ability of sweet sorghum genotypes. The effect of the environment and genotypes on yield and agronomic characteristics was quantified through an ANOVA combined with data from nine experiments (environments) and seven genotypes. Mean comparisons for agronomic traits were performed using Tukey's range test ($p \leq 0.05$). The IGA interaction analysis was interpreted using a graphical dispersion analysis based on principal component analysis (PCA) with the GGE biplot model (Yan et al., 2000). The ANOVA was performed using the SAS system (SAS, 2006), while the GGE biplot model and Gollob test (1968) were conducted using the GEA-R program (Pacheco *et al*., 2015).

Results and discussion

Table 2 shows the results of the mean agronomic traits of sweet sorghums planted in nine environments in northeastern Mexico. Regarding bioethanol production (BP), the varieties Urja (5), (SBB-25 x Keller) 17-1-2-1 (2), Keller (4), Dulcina (1), Potrillo (7), and (SBB-25 x Keller) 31-2-1-2 (3) were equal in bioethanol production, with a range of (2521 to 3214 L ha^{-1}), and were superior to RB-Cañero (6) (1237 L ha^{-1}). These results align with the trends reported by Williams-Alanís *et al.* (2017) in experiments conducted in southern Tamaulipas state, where RB-Cañero showed the lowest bioethanol production. In a study conducted in Turkey, where 53 sweet genotypes selected from 551 accessions and nine cultivars were evaluated across two contrasting environments over two years, the genotypes originating from the United States exhibited the highest values for bioethanol production and associated agronomic traits (Guden *et al.,* 2020). This aligns with the results in this study for Keller, a variety from the U.S., which showed excellent bioethanol production (2935 L ha^{-1}). Yucel *et al.* (2022) studied 21 sweet sorghum varieties in Turkey during 2016 and 2017 and found a theoretical bioethanol production range between 2020 and 5302 L ha^{-1}. The results of this study align with those obtained here, with bioethanol production ranging from 2521 to 3214 L ha^{-1}, except for the RB-Cañero variety, which had a lower bioethanol production (1237 L ha^{-1}).

Revista Bio ciencias
ISSN 2007-3380

Table 2. Mean comparisons for the traits measured in the experiment of nine environments in northeastern México.

V	GENEALOGY	BP L ha^{-1}	DF	PH (cm)	° Brix	FPW kg ha^{-1}	SW kg ha^{-1}	JW kg ha^{-1}
5	Urja	3214^{a}	86^{a}	251^{a}	17.25^{a}	41881^{ab}	32914^{ab}	17422^{ab}
2	Exp 17-1-2-1	3139^{ab}	80^{bc}	243^{ab}	16.53^{a}	43629^{ab}	34100^{ab}	17773^{ab}
4	Keller	2935^{ab}	77^{c}	235^{bc}	16.97^{a}	40412^{abc}	31083^{ab}	16280^{b}
1	Dulcina	2842^{ab}	80^{b}	231^{c}	16.26^{a}	41382^{ab}	32099^{ab}	17927^{ab}
7	Potrillo	2725^{ab}	74^{c}	229^{c}	14.66^{b}	47030^{a}	34921^{a}	20410^{a}
3	Exp 31-2-1-2	2521^{ab}	86^{a}	235^{bc}	16.50^{a}	36681^{bc}	28386^{bc}	15358^{b}
6	RB-Cañero	1237^{c}	79^{bc}	217^{c}	9.8^{1} c	33055^{c}	23532^{c}	14237^{c}
	Me	2669	80.26	235	15.43	40606	31005	17052
	CV	28.11	4.56	5.66	11.93	23.06	23.93	28.27

V = Number of varieties; BP = Bioethanol production L/ha^{-1}; DF = days to flowering; PH = Plant height; ° Brix = Sugar content; FPW = Total fresh plants weight; SW = Fresh stem weight; JW = juice weight, Me = Means; CV = coefficient of variation.

Significant differences were found between varieties for days to flowering, with Urja and (SBB-25 x Keller) 31-2-1-2 being the latest to flower compared to the others. The earliest varieties were Potrillo, Keller, and RB-Cañero. Early flowering is an important agronomic trait, as it allows for faster production. This can be useful for reducing the interval between crops (Williams-Alanís *et al.,* 2017). Zhang and Wang (2015) found that late genotypes in sweet sorghum typically have higher biomass production. Williams- Alanís *et al.* (2017) did not find any advantages for late sorghums in bioethanol production. On the other hand, it is indicated that when the growing season is short, early-cycle sorghums have advantages in drought and high-temperature conditions (Murphy *et al.,* 2014).

Plant height is an important agronomic component affecting biomass production, and its development depends on factors such as climate, soil, crop management, and genotype (Williams-Alanís *et al.,* 2017). Sweet sorghums often reach heights above 300 cm (Sylvester *et al.,* 2015). In these experiments, no genotypes of this plant height were found, perhaps because the agrological conditions of soil and climate in the environments were not optimal; thin and poor soils in Marín (García, 2004) and dry land conditions in Estación Cuauhtémoc. A positive relationship between plant height and bioethanol production has been found (Williams-Alanís *et al.,* 2017; Sylvester *et al.,* 2015). In this study, significant differences were found. Urja (5) with a height of 251 cm showed a bioethanol production of 3214 L ha^{-1}, while Exp 17-1-2-1 (2) with 243 cm produced

Revista Bio ciencias ISSN 2007-3380

3139 L ha^{-1}; both were superior to RB-Cañero (6) with 1237 L ha^{-1}, which had a significantly lower plant height (217 cm).

Significant differences were found in °Brix, with Urja (17.25), Exp 17-1-2-1 (16.53), Keller (16.97), Dulcina (16.26), and Exp 31-2-1-2 (16.50) being statistically better than Potrillo (14.66) and RB-Cañero (9.81). Selecting genotypes for good juice production, °Brix, and total soluble sugars is critical (Elangovan *et al.,* 2014). Sweet sorghums typically have juice sugar values between 12.40 and 24 °Brix (Elangovan *et al.,* 2014) and 12.55 to 20.00 °Brix (Yucel *et al.,* 2022). In this study, the genotypes fell within these ranges, except for RB-Cañero, which had a value of 9.81 °Brix, 2.67° lower than the minimum average considered suitable by these researchers. Considering that sweet sorghums are characterized by tall plants (2.00 m or more), high biomass production, stems, juice, and sugar, the low °Brix value of RB-Cañero suggests that it does not meet this classification.

The best sweet sorghums for bioethanol production are tall plants (Williams-Alanís *et al.,* 2017; Rono *et al.,* 2016; Murray, 2008), with high biomass production (Pothisoong and Jaisil, 2011; Elangovan *et al.,* 2014; Mishra *et al.,* 2015), high juice weight and °Brix values (Murray, 2008), higher stem weight (Rono *et al.,* 2016), and higher juice volume and weight (Murray, 2008). These traits are crucial and determinants for sweet sorghums used in bioethanol production.

Environments

The results obtained by environment are shown in Table 3, where it is observed that the environments evaluated in Estación Cuauhtémoc (EC) generally presented higher bioethanol production and had the highest values (ECV-2014 and ECOI-2016-2017). The environments in Marín (M) showed the lowest values (MOI-2013-2014 and MV-2017). In Estación Cuauhtémoc, the soil is deep, with a pH ranging from 7.5 to 7.8 . The environment plays an important role in sugar production, which aligns with the findings of other researchers (Rooney & Serna, 2000; Almodares *et al.,* 2007; Alhajturki *et al.,* 2012). The environment with the highest value for bioethanol production, ECV-2014 (3845 L ha^{-1}), also had the highest values for plant height (PH), total plant weight (FPW), and stem weight (SW).

Revista Biociencias
ISSN 2007-3380

Table 3. Mean comparisons of the traits measured in the experiment evaluated in the nine locations in northeastern México.

No Loc.	AM	BP L ha^{-1}	DF	PH cm	°Brix	FPW kg ha^{-1}	SW kg ha^{-1}
5	**ECV-2014**	3845^{a}	76^{c}	266^{a}	15.2^{bcd}	59782^{a}	45526^{a}
7	**ECOI-2016 2017**	3522^{ab}	80^{b}	252^{b}	18.0^{a}	45908^{bc}	34535^{b}
2	**MV-2014**	3110^{bc}	84^{ab}	235^{cd}	14.3^{de}	51041^{ab}	38343^{ab}
6	**ECV-2016**	2898^{bcd}	71^{d}	244^{bc}	14.9^{bcde}	46314^{bc}	34521^{b}
4	**ECOI-2013-2014**	2396^{cde}	71^{d}	229^{de}	13.1^{e}	38133^{cd}	31404^{bc}
9	**MV-2015**	2303^{dc}	84^{ab}	235^{cd}	16.1^{c}	34494^{de}	26024^{cd}
3	**ECV-2013**	2243^{dc}	87^{a}	198^{f}	16.0^{bcd}	32783^{de}	25248^{cd}
1	**MOI-2013-2014**	1931^{c}	87^{a}	216^{e}	14.7^{cde}	30127^{de}	23885^{d}
8	**MV-2017**	1769^{c}	84^{ab}	235^{cd}	16.5^{ab}	26874^{e}	19559^{d}
	Me	2669	80	235	15.4	40605	31605
	CV	28.11	4.57	5.66	11.93	23.06	23.93

No. loc = Number of Locations; AM = Locations; BP = Bioethanol production L/ha^{-1}; DF = days to flowering; PH = Plant height; ° Brix = Sugar content; FPW = Total fresh plant weight; PT = Fresh stem weight, Me = Means; CV = coefficient of variation; EC = Cuauhtémoc station; M = Marín; V = Summer; O-I = Winter.

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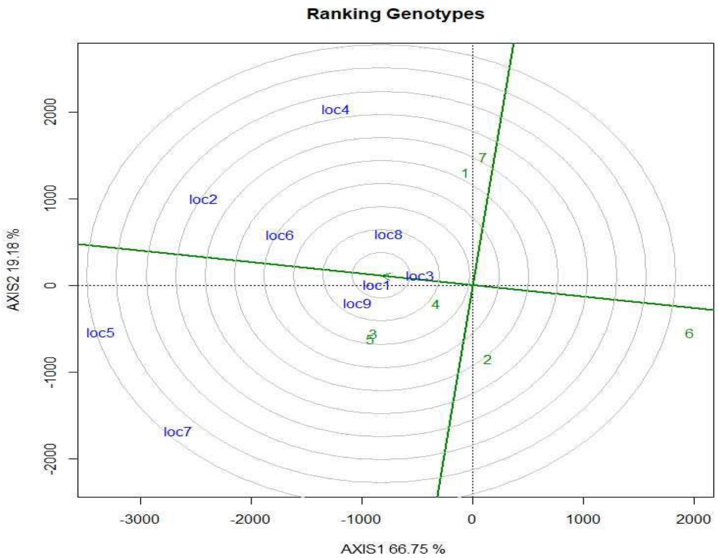

Figure 1. Genotype and Genotype x Environment graph (GGE biplot) that indicates the representativeness of nine environments for stability evaluation of seven sweet sorghum genotypes.

In Figure 1, the dispersion points of environments and genotypes are observed, along with an arrow drawn in a calculated environment, where the points are obtained from the average values of genotypes and environments for both principal components. The points are then connected by a line that locates the theoretical average environment, with the direction of the arrow indicating the placement of the best genotypes (Yan and Kang, 2003). This axis helps to define the behavior of genotypes; By referencing the abscissa axis (X) of average environment, it can be inferred that the best variety was Keller (4), followed by (SBB-25 x Keller) 31-2-1-2 (3) and Urja (5).

Revista Bio ciencias
ISSN 2007-3380

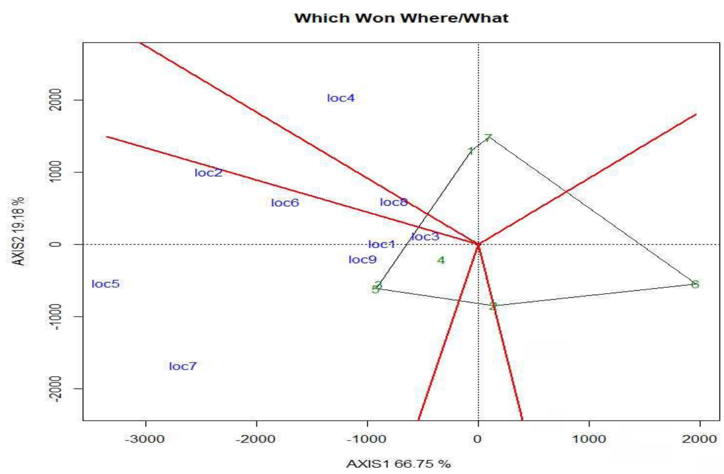

Figure 2. Environment evaluation for stability and adaptability of seven sweet sorghum genotypes evaluated in nine environments, represented in the Genotype and Genotype x Environment graph (GG biplot).

For the interpretation of genotypes, the response pattern “Which-Won-Where” is used, which aids in identifying the genotype that achieved the highest performance and also the environment. This concept is based on the fact that genotypes with higher performance vectors are those located farther from the origin of the biplot. When these genotypes are connected with straight lines, a polygon is formed (Figure 2) that identifies the most stable genotypes. This outer polygon is complemented by drawing perpendicular lines from the origin of the biplot, intersecting each line of the polygon, dividing the polygon into five parts, each with one vertex of the outer polygon where a genotype is placed. With this data, the outer polygon shows the behavior pattern of the genotypes, called “Which-Won-Where,” indicating the winning genotypes in each environment. For quadrant III, it is specified that among the environments loc 5, loc 7, loc 2, loc 6, loc 9, loc 1, loc 3 (Table 3), the genotype with the highest performance was Urja (5) > (SBB-25 x Keller) 31-2-1-2 (3) > Keller (4). For (SBB-25 x Keller) 17-1-2-1 (2) and RB-Cañero (6), which are located in quadrants where no environment was placed, it is interpreted that these genotypes exhibited low yields and did not stand out in any environment.

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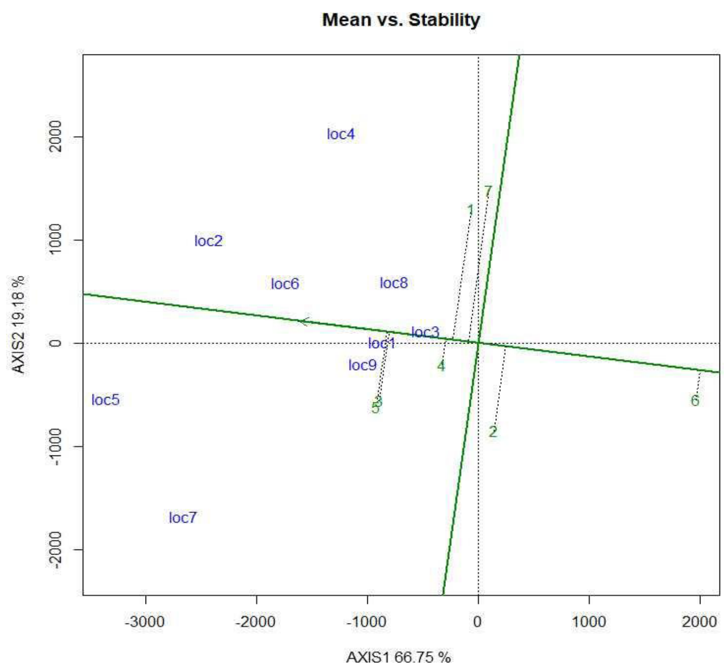

Figure 3. Stability of seven white sorghum genotypes evaluated in nine environments, represented in the Genotype and Genotype x Environment graph (GGE biplot), indicating the ordering of varieties based on the average yield and stability obtained for each environment.

The other perpendicular axis (Figure 3) allows for assessing the interaction degree between each genotype and the average environment. In this case, the most stable variety was Keller (4), as it had the smallest deviation or distance from the axis of the average environment. On the other hand, varieties Potrillo (7) and Dulcina (1), which exhibited the largest deviation from the axis, were the least stable or predictable. In Figures 1, 2, and 3, the model used explains 85.93% of the total variability (PC1 = 66.75% and PC2 = 19.18%), which justifies the application of the stability parameter method for evaluating varieties across environments. A value greater than 75% is considered acceptable (Williams-Alanís *et al.,* 2021). It is important to highlight that when the genotypes were distributed across the four quadrants, as in Figure 3, this indicates the genetic diversity they possess, which is essential in a successful breeding program (Maldonado *et al.,* 2021). The results show that Keller was the best variety due to its bioethanol production, stability, and greater precocity (77DF), making it the most suitable for recommending cultivation in northeastern Mexico.

Revista Bio ciencias
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Conclusions

The model used to estimate stability parameters, GGE biplot, explained 85.93% of the total variability. The agronomic traits associated with the bioethanol production of the evaluated genotypes were adequate, except for RB-Cañero, which exhibited the lowest °Brix value (9.81) and the lowest bioethanol production (1237 L ha^{-1}). The most stable genotypes with the highest bioethanol yield were: Keller (2935 L ha^{-1}), followed by (SBB-25 x Keller) 31-2-1-2 (2521 L ha^{-1}), and Urja (3214 L ha^{-1}). The best genotype was Keller, due to its good bioethanol production, stability, and precocity.

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