









## Line tester analysis to estimate combining ability in sweet sorghum

### Análisis de línea x probador para estimar la aptitud combinatoria en sorgo dulce

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#### ABSTRACT

Sweet sorghum is important for bioethanol production, which constitutes a renewable energy source, capable of substituting fossil fuels. This study aimed to estimate general (GCA) and specific combining ability (SCA), in parents and hybrids of sweet sorghums, to identify the best genotypes for bioethanol production. The experiment consisted of five lines, six testers, and the 30 possible hybrids, planted under rainfed conditions, during the spring-summer 2017 cycle; at Las Huastecas Experimental Station (INIFAP) in a randomized complete block experimental design with three replicates. Combining ability was estimated using the Line X Tester Analysis. Obtained results showed that lines; Potranca and K.CollierA, and testers; 3-2-1 and 4-1-1 had highly significant values ( $p \leq 0.01$ ) of GCA for °Brix, total plant weight, stem weight, juice weight, and bioethanol production. This indicates the predominance of additive genes. For bioethanol production, the best hybrids were: Potranca\*2-1-2, K.CollierA\*3-2-1, K.CollierA\*4-1-1, and Potranca\*4-1-1, which are highly significant ( $p \leq 0.01$ ) in SCA for bioethanol production; also, they are the ones that presented the highest values.

**KEY WORDS:** Hybrids, bioethanol, combining ability, parents, *Sorghum bicolor*.



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## RESUMEN

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El sorgo dulce es importante para la producción de bioetanol, el cual constituye una fuente de energía renovable, capaz de sustituir los combustibles fósiles. El objetivo del estudio fue estimar la aptitud combinatoria general (GCA) y específica (SCA), en progenitores e híbridos de sorgos dulces, para identificar los mejores para la producción de bioetanol. El experimento consistió en cinco líneas, seis probadores y los 30 híbridos posibles, sembrados en condiciones de temporal, durante el ciclo primavera-verano 2017; en el Campo Experimental Las Huastecas (INIFAP) en un diseño experimental de bloques completos al azar con tres repeticiones. La aptitud combinatoria se estimó mediante el método de línea x probador. Los resultados mostraron que las líneas; Potranca y K.CollierA, y los probadores; 3-2-1 y 4-1-1, tuvieron valores altamente significativos ( $p \leq 0.01$ ) de GCA para: °Brix, peso total de planta, peso de tallo, peso de jugo y producción de bioetanol. Lo que indica la predominancia de los genes aditivos. Los mejores híbridos para producción de bioetanol fueron: Potranca\*2-1-2, K.CollierA\*3-2-1, K.CollierA\*4-1-1 y Potranca\*4-1-1, fueron altamente significativos ( $p \leq 0.01$ ) en SCA para producción de bioetanol; además fueron los que presentaron los valores más altos.

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**PALABRAS CLAVE:** Híbridos, bioetanol, aptitud combinatoria, progenitores, *Sorghum bicolor*.

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## Introduction

*Sorghum* (*Sorghum bicolor* (L.) Moench) is produced globally at around 60.4 Mton and is used as a source of human food, animal fodder, fiber, and fuel (SIAP, 2020). Rising world market prices for fossil fuels, increasing demand, and instability in producing regions now make renewable fuels economically viable (Khawaja *et al.*, 2014). As fossil energy sources are rapidly depleting, the search for different options to solve this problem is indispensable. Plant biomass is a favorable renewable energy source, which can be used for energy production to complement fossil fuels (Pabendon *et al.*, 2017; Ratnavathi *et al.*, 2010). Sweet sorghum has been developed for juice production, rather than grain. It has been identified as a potential feedstock crop for ethanol in rainfed areas in different parts of the world (Yücel *et al.*, 2022). Sweet and grain sorghums differ in many important features; including biomass production, stalk sugar accumulation, and juice (Kanbar *et al.*, 2021; Pabendon *et al.*, 2017). Sorghum stalk juice contains about 10 to 20 % sugar levels similar to those of sugarcane (Rakhmetova *et al.*, 2020) and exhibits adaptability to different production systems (López-Sandín *et al.*, 2021). It has become a promising feedstock since it can be grown with reduced inputs, responds to stress more efficiently than traditional crops, and

has excellent biomass production potential (Ekefre *et al.*, 2017; Yücel *et al.*, 2022). According to the Mexican Ministry of Energy (SENER) and the National Energy Balance databases, in 2021, 5.42 % of total energy was produced from biomass (SENER, 2021). Ali *et al.* (2008) indicate that there is a need to produce new sweet sorghum cultivars with high sugar content, in combination with other desirable agronomic characteristics.

The Line X Tester Analysis Method is simple, efficient, and commonly used in plant breeding (Aslam *et al.*, 2014). The Specific Combining Ability (SCA), reflects the effect of dominant and epistatic genes and is when some hybrid combinations are expressed favorably or not, concerning the average parental behavior. While the General Combining Ability (GCA), is defined as the superiority of a genotype in a series of hybridizations and is due to the effect of additive genes (Falconer, 1981; Kutlu & Sirel, 2019). In plant breeding, the estimation of genetic parameters helps in making the best decisions (De la Cruz-Lazaro *et al.*, 2010). The Line X Tester Analysis developed by Kempthorne (1957), is useful breeding strategy to predict the General Combining Ability (GCA) of parents and the Specific Combining Ability (SCA) of hybrids. In addition, it provides information on the inheritance and gene action of agronomic traits (Arzu, 2017). This methodology has been successfully used in several studies, to select the best parents and hybrids: (Aslam *et al.*, 2014; Kutlu & Sirel., 2019; Rachman *et al.*, 2022; Williams *et al.*, 2022). The study aims to estimate the General Combining Ability (GCA) and Specific Combining Ability (SCA) in sweet sorghum parents and hybrids to identify the best ones for bioethanol production.

## Material and Methods

### Genetic material used

Commercial lines A/B: Potranca and Kansas Collier. R lines: Keller. Experimental A/B lines 18-2-1, 11-1-1 and 14-18-1 and testers; 3-1-2, 4-1-1, 2-1-2, 3-2-1, and TS-3-2-1. Potranca is a male-sterile line that was generated at the Universidad Autónoma de Nuevo León (UANL) from Rox Orange and is the parent of the sweet hybrid Potrillo (Williams *et al.*, 2020). Rox Orange, Kansas Collier, and Keller are sweet sorghum varieties that originated in the USA (Ali *et al.*, 2008). The Kansas Collier male-sterile line was formed in 2015, at the Marín Campus (FAUANL) by sterilizing it with line 46038 originating from Nebraska, USA for six generations. SBB-25 is a fertility-maintaining grain line originated by INIFAP, Mexico (Williams *et al.*, 2004, Williams *et al.*, 2006). SPV 1411 commercial sweet sorghum variety, originated from the International Crops Research Institute for the Semi-Arid Tropics, India. RTx437, fertility restoring grain line, originated at Texas A&M University, USA (Rooney *et al.*, 2003).

### Increased genetic material

At Campus Marín, Facultad de Agronomía, Universidad Autónoma de Nuevo León (FAUANL), during the spring-summer 2016 and fall-winter 2016-2017 cycles; lines A, B, and R were increased and hybrids were formed under the line x tester crossing scheme (5 × 6).

## Experimental Design

The field phase was established under rainfed conditions during the spring-summer cycle (S-S) 2017 at the Las Huastecas Experimental Field (INIFAP); located at Estación Cuauhtémoc, municipality of Altamira, Tamaulipas, Mexico; Coordinates 22° 33' N/ 98° 09' W, with an altitude of 20 masl. It presents a climate (Aw0) warm sub-humid with summer rainfall and winter rainfall of 5-10 % (García, 2004); with an average annual temperature of 24.5 °C and 842 mm of precipitation (INIFAP, 2015). To determine the *per se* behavior of the maternal parent, the B lines or fertility maintainers were used. It consisted of 30 possible hybrid combinations, five fertility-maintaining B lines, and six restorer lines including the Keller control. The experimental design was a randomized complete block design with 41 treatments and three replications, in plots of one furrow 5 m long and 0.80 m apart. The sowing date was August 13<sup>th</sup>, 2017. The management of the experiment was carried out according to the recommendations for sweet sorghum cultivation of INIFAP, in Tamaulipas (Montes-García *et al.*, 2013).

## Data Collection

Data were collected on days to flowering (DF, at 50 % of the plants) and plant height (PH in cm, from the ground to the apex of the panicle). When the plants were at stage seven (milky grain), as described by Vanderlip (1993). Equivalent to three to five weeks after flowering, a period in which the plants reach maximum sugar in stem juice (Dávila *et al.*, 2011). A linear m (0.8 m<sup>2</sup>) of the plot was harvested in complete competition, cutting the base of the stem from 3 to 4 cm from the ground, where the following data (g) were taken: total plant fresh weight (PFW), after removing leaves and panicle from the plants, the stem fresh weight (SFW) and °Brix were obtained with a manual refractometer brand Atago. Stem dry weight (SDW) was obtained by drying the fresh weight in an oven at 65 °C for three days. Values in g were transformed to kg ha<sup>-1</sup>. The values of juice weight (JW) kg ha<sup>-1</sup> and theoretical bioethanol production l ha<sup>-1</sup> were estimated as described by Rakhmetova *et al.* (2020) using the following formulas:

$$\text{Juice yield/kg ha}^{-1} = (\text{fresh stem weight/kg ha}^{-1} - \text{dry stem weight/kg ha}^{-1}) \times 0.866.$$

$$\text{Theoretical bioethanol production/l ha}^{-1} = (\text{Brix sugar}/100) \times (0.65 \text{ l bioethanol}/1 \text{ kg sugar}) \times (0.85) \times (\text{stalk weight/kg ha})^{-1}$$

The combinational ability analysis was performed using the line x tester test method (Kempthorne, 1957). The analysis was performed using R software (R Core Team, 2020).

## Results and Discussion

The ANOVA (Table 1) revealed highly significant differences ( $p \leq 0.01$ ) among treatments, parents (except °Brix and JW), and Prog x Cru (except BIO), and for crosses significant and highly significant differences (except PH, and °Brix). Highly significant differences were also found for lines in days to flowering, juice weight, and significant differences for °Brix. Meena & Ranwah (2020), found the same results for treatments, parents, crosses, lines, and testers for DF, PH, and green forage production. Mohammed (2009), found significant differences in forage sorghum in lines for days to flowering. Takare *et al.* (2014) found highly significant differences for line x testers for plant height. The treatments (genotypes) were highly significant for the characteristics: DF, PH, °Brix, PFW, SFW, JW, and BIO. For bioethanol production, highly significant differences ( $p \leq 0.01$ ) were found for treatments, parents, crosses, and testers. These results indicate the existence of a broad base of genetic variability in the germplasm used, which favors appropriate selection. For lines, there were no significant differences, indicating that variability in this case was reduced.

### General Combining Ability (GCA)

The GCA values for lines are observed in Table 2, where it was found that K.CollierA, presented highly significant values for GCA, in the variables; DF, PH, °Brix, PFW, SFW, JW, and BIO. This coincides with reports indicating that late-cycle sweet sorghums (Williams *et al.*, 2017; Zhang & Wang 2015), taller plants (Anami *et al.*, 2015), higher PFW, SFW, and PW, (Williams *et al.*, 2017), higher juice weight and °Brix (Elangovan *et al.*, 2014), are associated with higher bioethanol production. Significant GCA values are related to the predominance of additive genes (Kutlu & Sirel, 2019). Hussien (2015), indicates that genotypes with adequate GCA, present favorable genetic combinations for hybrid production. Potranca resulted in highly significant GCA values for °Brix, PFW, SFW, JW, and BIO. Line 18-2-1-1 presented positive values for DF, PH, SFW, and JW. For GCA the testers 2-1-2, 3-2-1, and 4-1-1, presented the best results; they were highly significant for PFW, SFW, JW, and BIO. The experimental lines 11-1-1-1, 14-18-1-1, and 18-2-1-1 did not show significant differences in GCA for bioethanol production, which indicates that they are not suitable for hybrid formation.

**Table 1. Degrees of freedom and mean squares of the analysis of variance of the agronomic variables evaluated in the line x tester test for sweet sorghum genotypes.**

S.V	DoF	DF (days)	PH (cm)	°Brix	PFW (kg/ha) <sup>-1</sup>	SFW (kg/ha) <sup>-1</sup>	JW (kg/ha) <sup>-1</sup>	BIO (l/ha) <sup>-1</sup>
Repetitions	2	1.195	475.71	54.18**	170983481**	122763309**	97861.06	34725.72
Treatments	40	20.86**	1990.88**	15.40**	75593161**	37976928**	5635646.78**	363210.88**
Progenitors	10	26.09**	4950.42**	10.82	79421811**	52542150**	3159098.04	411055.57**
Prog vs Cru.	1	152.62**	12805.97**	161.13**	1339709920**	497766710**	41846667.22**	332085.53
Crosses	29	14.51**	597.41	11.95	30682704*	17099618*	5240973.22*	347785.99**
Lines	4	69.87**	588.59	19.25*	42153925	27807350	10688322.78**	638287.90
Testers	5	8.99	1128.06	25.53**	52488253*	30187703*	4062355.41	704054.94**
Lin. x Prob.	20	4.82	466.51	7.10	22937072	11686051	4446157.76*	200618.37
Error	80	2.92	389.51	8.87	22866616	10653159	2525193.26	142423.35
C.V		2.4	8.7	22.4	19.6	18.9	32.8	30.0

S.V: Sources of Variation; DoF: Degrees of Freedom; DF: days to flowering; PH: plant height; °Brix: juice sugar content; PFW: total plant weight; SFW: stem weight; JW: juice weight; BIO: bioethanol production; C.V: coefficient of variation. \*, \*\*: Significant at 0.05 and 0.01 % probability respectively.

### Specific Combining Ability (SCA)

The effects of SCA are shown in Table 3, where we found that, for DF, only one hybrid showed significant differences; for the variable PH, 11 crosses and for °Brix, only three crosses. This shows that dominance and epistasis genes were not important for DF and °Brix. The above agrees with what was observed for DF by Bunphan *et al.* (2015), Williams & Arcos (2015) and Williams *et al.* (2017) who reported that hybrids are generally earlier than their parents. For PFW, SFW, and JW, approximately half of the crosses were significant, and for bioethanol production, 11 out of 30 crosses were significant.

Hybrids that presented higher values of bioethanol production: Potranca\*2-1-2, K.CollierA\*3-2-1, K.CollierA\*4-1-1, and Potranca\*4-1-1 (Table 3), coincide in both lines; Potranca and K.CollierA, as well as the testers, were involved in their formation: 2-1-2, 3-2-1 and 4-1-1, had

significant GCA values for PFW, SFW, JW, and BIO (Table 2). In addition, these hybrids presented highly significant values ( $P \leq 0.01$ ) in the Specific Combining Ability (SCA) for PFW, SFW, JW, and BIO, except for hybrids Potranca\*3-2-1 and KCollierA\* 2-1-2 (Table 3). Bunphan *et al.* (2015) indicated that the use of hybrids instead of varieties can offer advantages, as they exhibit similar sugar yields as their parents, but in less time. In addition, hybrid seeds can be easily and more cheaply produced from androfertile plants; provided they are of lower plant height, which facilitates mechanical harvesting. The open-pollinated genotypes that presented the highest *per se* values in bioethanol production were the 2-1-2 and 4-1-1 testers and were similar to the Keller control (Table 4). Among the evaluated varieties, the two testers described above showed the highest values for bioethanol production and can be used for commercial production. Seed production from sweet varieties is complicated by the fact that they tend to have tall plants, which makes mechanical harvesting of the grain difficult and increases production costs.

**Table 2. Estimated values of General Combining Ability (GCA) effects for the agronomic variables studied.**

	DF (days)	PH (cm)	°Brix	PFW (kg/ha) <sup>-1</sup>	SFW (kg/ha) <sup>-1</sup>	JW (kg/ha) <sup>-1</sup>	BIO (l/ha) <sup>-1</sup>
Lines							
11-1-1-1	<b>0.878**</b>	-1.489	-1.407	-1186.25	-932.500	-932.874	-191.557
14-18-1-1	-2.844	-8.322	-0.062	-1764.02	-1588.05	-506.490	-132.752
18-2-1-1	<b>0.600**</b>	<b>6.344**</b>	-0.523	-27.222	<b>173.056**</b>	<b>54.606**</b>	-78.385
K.CollierA	<b>2.322**</b>	<b>4.289**</b>	<b>1.132**</b>	<b>1033.194**</b>	<b>1209.861**</b>	<b>1070.953**</b>	<b>213.569**</b>
Potranca	-0.956	-0.822	<b>0.860**</b>	1944.306**	<b>1137.639**</b>	<b>313.805**</b>	<b>189.126**</b>
Testers							
2-1-2	-0.85	<b>1.522**</b>	-0.01	<b>1706.389**</b>	<b>1310.278**</b>	<b>309.595**</b>	<b>107.904**</b>
3-1-2	0.144	<b>2.122**</b>	-2.05	-3046.94	-2281.38	-1044.2	-354.12
3-2-1	<b>0.611**</b>	<b>12.789**</b>	<b>1.091**</b>	<b>1633.05**</b>	<b>1404.444**</b>	<b>327.637**</b>	<b>186.775**</b>
4-1-1	-0.92	-5.21	<b>1.651**</b>	<b>1017.22**</b>	<b>713.611**</b>	<b>114.023**</b>	<b>222.164**</b>
Keller	<b>1.011**</b>	-13.21	-0.60	-1263.61	-545.556	<b>98.868**</b>	-90.290
TS-3-2-1	0.011	<b>1.989**</b>	-0.07	-46.111	-601.389	<b>194.128**</b>	-72.434

DF: days to flowering; PH: plant height; °Brix: juice sugar content; PFW: total plant weight; SFW: stem weight; JW: juice weight; BIO: bioethanol production. \*, \*\*: Significant at 0.05 and 0.01 % probability respectively.

According to Elangovan *et al.* (2014) sweet sorghums are those that present values of sugar content in the juice between 12.4 to 24 °Brix; thus, except for the hybrid Potranca\*3-2-1 that presented a value of 12.3 °Brix, the rest of the genotypes are within this classification (Table 4). Sorghum with the lowest bioethanol production value was the 14-18-1-1B tester, which was significantly lower than the hybrids Potranca\*2-1-2, K.CollierA\*3-2-1, K.CollierA\*4-1-1, Potranca\*4-1-1, and the 4-1-1 tester. The explanation for the difference is due to the lower plant height of this last material (120 cm), about other genotypes, which have a greater average plant height (213 to 260 cm). This coincides with that reported by Anami *et al.* (2015) and Williams *et al.* (2017); who indicate that sweet sorghums with lower plant height present lower bioethanol production.

**Table 3. Estimated line x tester Specific Combining Ability (SCA) effects on the agronomic variables evaluated.**

Crosses	DF (days)	PH (cm)	Brix	PFW (kg/ha) <sup>-1</sup>	SFW (kg/ha) <sup>-1</sup>	JW (kg/ha) <sup>-1</sup>	BIO (l/ha) <sup>-1</sup>
11-1-1-1*2-1-2	0.189	<b>2.089*</b>	-1.480	-2377.917	-2120.000	<b>860.708**</b>	-301.247
11-1-1-1*3-1-2	-0.478	<b>7.156**</b>	0.800	<b>3633.750**</b>	<b>2092.500**</b>	<b>338.221**</b>	<b>206.479**</b>
11-1-1-1*3-2-1	1.056	<b>8.156**</b>	1.553	-625.417	<b>90.000**</b>	-838.817	<b>170.076**</b>
11-1-1-1*4-1-1	0.589	-2.178	0.960	<b>540.417**</b>	-269.167	-693.762	<b>76.004**</b>
11-1-1-1*Keller	-1.344	-14.178	-1.453	-70.417	<b>406.667**</b>	-162.616	-126.237
11-1-1-1*TS-3-2-1	-0.011	-1.044	-0.380	-1100.417	-200.000	<b>496.266**</b>	-25.075
14-18-1-1*2-1-2	0.911	-5.411	0.376	-2800.139	-2181.111	-756.427	-105.269
14-18-1-1*3-1-2	0.911	<b>3.989**</b>	0.256	<b>1694.861**</b>	<b>1893.889**</b>	<b>770.620**</b>	<b>109.735**</b>
14-18-1-1*3-2-1	-1.889	-3.344	0.209	-560.139	-1425.278	-2167.285	-45.360
14-18-1-1*4-1-1	-1.022	-8.678	-0.384	-777.639	-576.111	348.445**	-77.882
14-18-1-1*Keller	<b>2.044*</b>	<b>15.989**</b>	1.502	<b>3782.361**</b>	<b>2783.056**</b>	<b>1301.766**</b>	<b>351.422**</b>
14-18-1-1*TS-3-2-1	-0.956	-2.544	-1.958	-1339.306	-494.444	<b>502.881**</b>	-232.646
18-2-1-1*2-1-2	0.800	<b>16.256**</b>	-2.063	<b>1800.556**</b>	<b>1778.611**</b>	<b>529.944**</b>	-113.553
18-2-1-1*3-1-2	-0.533	-12.344	-0.350	-2721.111	-2346.389	-537.401	-106.924
18-2-1-1*3-2-1	0.000	<b>8.656**</b>	-1.397	<b>498.889**</b>	<b>476.111**</b>	<b>118.594**</b>	-221.227
18-2-1-1*4-1-1	1.200	-5.011	0.310	-2793.611	-1691.389	-1150.818	-120.303
18-2-1-1*Keller	-1.733	1.322	1.497	<b>583.056**</b>	<b>259.444**</b>	<b>1469.554**</b>	<b>174.328**</b>
18-2-1-1*TS-3-2-1	0.267	-8.878	2.003*	<b>2632.222**</b>	<b>1523.611**</b>	-429.873	<b>387.680**</b>
K.CollierA*2-1-2	-1.922	-10.022	0.714	-2151.528	-1541.528	-1558.078	-85.126
K.CollierA*3-1-2	-0.922	-0.289	0.294	-344.028	-774.861	-875.382	-5.561



## Continuation

**Table 3. Estimated line x tester Specific Combining Ability (SCA) effects on the agronomic variables evaluated.**

Crosses	DF (days)	PH (cm)	°Brix	PFW (kg/ha) <sup>-1</sup>	SFW (kg/ha) <sup>-1</sup>	JW (kg/ha) <sup>-1</sup>	BIO (l/ha) <sup>-1</sup>
K.CollierA*3-2-1	1.611	-19.289	<b>1.814*</b>	<b>1005.139**</b>	<b>826.806**</b>	<b>1815.713**</b>	<b>318.852**</b>
K.CollierA*4-1-1	-0.856	<b>28.711**</b>	-1.279	<b>2225.139**</b>	<b>2059.306**</b>	<b>853.010**</b>	<b>49.828**</b>
K.CollierA*Keller	1.211	<b>3.378**</b>	-1.026	<b>830.972**</b>	-277.361	-697.852	-148.891
K.CollierA*TS-3-2-1	0.878	-2.489	-0.519	-1565.694	-292.361	<b>462.588**</b>	-129.103
Potranca*2-1-2	0.022	-2.911	<b>2.453**</b>	<b>5529.028**</b>	<b>4064.028**</b>	<b>923.854**</b>	<b>605.195**</b>
Potranca*3-1-2	1.022	1.489	-1.000	-2263.472	-865.139	<b>303.942**</b>	-203.729
Potranca*3-2-1	-0.778	<b>5.822**</b>	-2.180	-318.472	<b>32.361**</b>	<b>1071.795**</b>	-222.340
Potranca*4-1-1	0.089	-12.844	0.393	<b>805.694**</b>	<b>477.361**</b>	<b>643.125**</b>	<b>72.352**</b>
Potranca*Keller	-0.178	-6.511	-0.520	-5125.972	-3171.806	-1910.853	-250.622
Potranca*TS-3-2-1	-0.178	<b>14.956**</b>	0.853	<b>1373.194**</b>	-536.806	-1031.863	-0.856

DF: days to flowering; PW: plant height; °Brix: juice sugar content; PFW: total plant weight; SFW: stem weight; JW: juice weight; BIO: bioethanol production. \*, \*\*: Significant at 0.05 and 0.01 % probability respectively.

**Table 4. Results of the best sweet sorghum genotypes, evaluated at Estación Cuauhtémoc Tam, (hybrids, lines, and testers) spring-summer 2017 cycle.**

Genealogy	DF	PH (cm)	°Brix	PFW (kg/ha) <sup>-1</sup>	SFW (kg/ha) <sup>-1</sup>	JW (kg/ha) <sup>-1</sup>	BIO (kg/ha) <sup>-1</sup>
Potranca*2-1-2	68.0 d-h	230 a-c	15.9 a-b	35 467 a	24 996 a	6 740 a-b	2 188 a
K.CollierA*3-2-1	74.3 a-b	230 a-c	16.6 a-b	29 958 a-b	21 925 a-b	8 407 a	2 005 a-b
K.CollierA*4-1-1	70.3 b-h	260 a	14.1 a-b	30 563 a-b	22 467 a-b	7 231 a-b	1 771 a-c
Potranca*4-1-1	68.0 d-h	213 a-c	15.5 a-b	30 054 a-b	20 813 a-b	6 264 a-b	1769 b-c
4-1-1	72.0 b-e	232 a-b	15.8 a-b	25 188 a-d	19 129 a-c	4 904 a-b	1713 b-c
2-1-2	68.7 c-h	220 a-c	15.1 a-b	25 921 a-d	19 188 a-c	4 279 a-b	1593 a-d
18-2-1-1*TS-3-2-1	70.7 b-g	232 a-b	14.0 a-b	28 846 a-b	19 579 a-b	5 012 a-b	1523 a-d

## Continuation

**Table 4. Results of the best sweet sorghum genotypes, evaluated at Estación Cuauhtémoc Tam, (hybrids, lines, and testers) spring-summer 2017 cycle.**

Genealogy	DF	PH (cm)	°Brix	PFW (kg/ha) <sup>-1</sup>	SFW (kg/ha) <sup>-1</sup>	JW (kg/ha) <sup>-1</sup>	BIO (kg/ha) <sup>-1</sup>
K.CollierA*2-1-2	69.3 b-h	228 a-c	14.4 a-b	26 875 a-c	19 463 a-b	5 016 a-b	1522 a-d
11-1-1-1*3-2-1	72.3 b-e	252 a	13.8 a-b	26 108 a-d	19 046 a-c	3 749 a-b	1451 a-d
Potranca*3-2-1	68.7 c-h	250 a	12.3 a-b	29 546 a-b	21 058 a-b	6 906 a-b	1439 a-d
14-18-1-1*Keller	70.0 b-h	227 a-c	13.4 a-b	27 042 a-c	19 133 a-c	6 087 a-b	1414 a-d
Potranca*TS-3-2-1	68.7 c-h	248 a-b	14.2 a-b	29 558 a-b	18 483 a-c	4 669 a-b	1402 a-d
11-1-1-1*4-1-1	70.3 b-h	223 a-c	13.8 a-b	26 658 a-c	17 996 a-c	3 681 a-b	1392 a-d
TS-3-2-1	73.0 a-d	247 a-b	15.5 a-b	21 729 a-d	15 688 a-d	3 933 a-b	1383 a-d
Keller (T)	74.7 a-b	228 a-c	14.9 a-b	21 563 a-d	16 975 a-c	5 763 a-b	1366 a-d
3-2-1	74.0 a-c	245 a-b	19.7 a	15 529 b-d	12 021 b-d	3 901 a-b	1310 a-d
18-2-1-1*4-1-1	70.7 b-g	228 a-c	14.0 a-b	24 483 a-d	17 679 a-c	4 211 a-b	1309 a-d
11-1-1-1B	73.3 a-d	165 c-d	14.3 a-b	16 692 b-d	12 025 b-d	2 627 b	928 b-d
18-2-1-1B	78.0 a	184 b-d	16.3 a-b	12 304 c-d	8 646 c-d	2 068 b	776 b-d
14-18-1-1B	68.0 d-h	120 d	13.2 a-b	10 600 d	6 021 d	3 273 a-b	435 d

DF: days to flowering; PH: plant height; °Brix: juice sugar content; PFW: total plant weight; SFW: stem weight; JW: juice weight; BIO: bioethanol production. Different literals (a, b, c) in each variable and within the same group denote statistical significance (Tukey;  $p = 0.05$ ).

## Conclusions

The hybrids Potranca\*2-1-2, K.CollierA\*3-2-1, K.CollierA\*4-1-1, and Potranca\*4-1-1, were the best candidates for possible use in bioethanol production since they presented highly significant values in Specific Combining Ability (SCA). The best hybrids were formed with two commercial lines (Potranca and K.CollierA) and three experimental testers (2-1-2, 3-2-1, and 4-1-1). In addition, the lines and testers involved in their formation were highly significant ( $P \leq 0.01$ ) for the General Combining Ability.

## Authors' contribution

Formation of the genetic material; Williams-Alanís, H., Aranda-Lara, U. Arcos-Cavazos, G. Conceptualization of the work; Williams-Alanís, H., Aranda-Lara, U. Zavala-García, F. Development of the methodology; Williams-Alanís, H., Aranda-Lara, U. Zavala-García, F. Development of the methodology; Williams-Alanís, H., Aranda-Lara, U. Hernández Martínez, R. Galicia-Juárez, M. Software management; Aranda-Lara, U. Hernández-Martínez, R. Experimental validation and analysis of results; Aranda-Lara, U. Hernández Martínez, R. Elizondo-Barrón, J. Williams-Alanís, H. Writing and preparation of the manuscript; Williams-Alanís, H., Aranda-Lara, U. Elizondo-Barrón, J, López-Guzmán J.A. Writing, revising and editing; Williams-Alanís, H., Aranda-Lara, Elizondo-Barrón, J, López-Guzmán J.A.

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## Conflict of interest

The authors declare that they have no conflict of interest.

## References

- Ali, M. L., Rajewski, J. F., Baenziger, P. S., Gill, K. S., Eskridge, K. M., & Dweikat, I. (2008). Assessment of genetic diversity and relationship among a collection of US sweet sorghum germplasm by SSR markers. *Molecular Breeding*, 21, 497-509. <https://doi.org/10.1007/s11032-007-9149-z>
- Anami, S. E., Zhang, L., Xia, Y., Zhang, Y., Liu, Z., & Jing, H. (2015). Sweet sorghum ideotypes: improvement of the biofuel syndrome. *Food and Energy Security*, 4(3), 159-177. <https://doi.org/10.1002/fes3.63>
- Arzu, K. (2017). Gene action and combining ability in line x tester population of safflower (*Carthamus tinctorius* L.). *Turkish Journal of Field Crops*, 22(2), 197-203. <https://doi.org/10.1007/s11032-007-9149-z>

- [org/10.17557/tjfc.356216](https://doi.org/10.17557/tjfc.356216)
- Aslam, R., Munawar, M., & Salam, A. (2014). Genetic architecture of yield components accessed through line × tester analysis in wheat (*Triticum Aestivum* L.). *Universal Journal of Plant Science*, 2(5), 93-96. <https://doi.org/10.13189/ujps.2014.020501>
- Bunphan, D., Jaisil, P., Sanitchon, J., Knoll, J. E., & Anderson, W. F. (2015). Heterosis and Combining Ability of F1 Hybrid Sweet Sorghum in Thailand. *Crop Science*, 55(1), 178-187. <https://doi.org/10.2135/cropsci2014.05.0363>
- Dávila-Gómez, F. J., Chuck-Hernández, C., Pérez-Carrillo, E., Rooney, W. L., & Serna-Saldivar, S. O. (2011). Evaluation of bioethanol production from five different varieties of sweet and forage sorghums (*Sorghum bicolor* (L) Moench). *Industrial Crops Production*, 33(3), 611-616. <https://doi.org/10.1016/j.indcrop.2010.12.022>
- De la Cruz-Lázaro, E., Castañón-Najera, G., Brito-Manzano, N. P., Gómez-Vázquez, A., Robledo-Torres, V., & Lozano del Río, A. J. (2010). Heterosis y aptitud combinatoria de poblaciones de maíz tropical. *Phyton*, 79(1), 11-17. [http://www.scielo.org.ar/scielo.php?pid=S1851-56572010000100003&script=sci\\_arttext&lng=pt](http://www.scielo.org.ar/scielo.php?pid=S1851-56572010000100003&script=sci_arttext&lng=pt)
- Ekefre, D. E., Mahpatra, A. K., Latimore, M. J., Belmer, D. D., Jena, U., Withehead, D. J., & Williams, A. L. (2017). Evaluation of three cultivars of sweet sorghum as feedstocks for ethanol production in the Southeast United States. *Heliyon*, 3(12). <https://doi.org/10.1016/j.heliyon.2017.e00490>
- Elangovan, M., Kiran, B. P., Seetharama, N., & Patil, J. V. (2014). Genetic diversity and heritability characters associated in sweet sorghum (*Sorghum bicolor* (L) Moench). *Sugar Tech*, 16, 200-210. <https://doi.org/10.1007/s12355-013-0262-5>
- Falconer, D. C. (1981). Introduction to quantitative genetics. 2nd edition Longman Group Ltd. New York. 133 p.
- García, E. (2004). Modificaciones al Sistema de clasificación climática de Köppen. Universidad nacional Autónoma de México. Quinta edición. México, D.F. 91 p.
- Hussien, E. H. (2015). Line x tester analysis and heterosis in grain sorghum hybrids under Arab ElAwamer conditions. *Assiut Journal of Agricultural Sciences*, 46(4), 1-11. <https://doi.org/10.21608/AJAS.2015.537>
- Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias [INIFAP]. (2015). Campo Experimental Huastecas. Estación Cuauhtémoc, Tamaulipas, México. <http://www.inifapcirne.gob.mx/celashuastecas.php>
- Kanbar, A., Shakeri, E., Alhajturki, D., Riemann, M., Bunzel, M., Morgano, M. T., Staff, D., & Nick, P. (2021). Sweet versus grain sorghum: Differential sugar transport and accumulation are linked with vascular bundle architecture. *Industrial Crops and Products*, 167, 113550. <https://doi.org/10.1016/j.indcrop.2021.113550>
- Kemphorne, O. (1957). An introduction to genetic statistics. John Wiley & Sons, Inc., N.Y. 545 p.
- Khawaja, C., Janssen, R., Rutz, D., Luquet, D., Trouche, G., Oriol, P., Reddy, B., Srinivasa, R. P., Basavaraj, G., Schaffert, R. E., Damasceno, C. M. B., Parella, R., Zacharias, A., Bushmann, R., Rettenmaier, N., Reinhardt, G., Monti, A., Zegada, L. W., Amaducci, S., & Braconnier, S. (2014). Manual del sorgo dulce: Cultivo con potencial energético. München: WIP, 67 p. ISBN 978-3-936338-31-7. <https://agritrop.cirad.fr/574768/>
- Kutlu, I., & Sirel, Z. (2019). Using line x tester method and heterotic grouping to select high yielding genotypes of bread wheat (*Triticum aestivum* L.). *Turkish Journal of Field Crops*,

- 24(2), 185-194. <https://doi.org/10.17557/tjfc.643546>
- López-Sandin, I., Zavala-García, F., Levin, L. N., Ruiz, H. A., Hernández-Luna, C. E., & Gutiérrez-Soto, G. (2021). Evaluation of bioethanol production from sweet sorghum variety Roger under different tillage and fertilizer treatments. *BioEnergy. Research*, 14(4), 1058-1069. <https://doi.org/10.1007/s12155-020-10215-7>
- Meena, B. L. & Ranwah, B. R. (2020). General and specific combining ability in dual purpose sorghum [*Sorghum bicolor* (L.) Moench]. *Journal of Pharmacognosy and Phytochemistry*, 9(5), 384-391. <https://doi.org/10.22271/phyto.2020.v9.i5f.12254>
- Mohammed, M. I. (2009). Line x tester analysis across locations and years in Sudanese x exotic lines of forage sorghum. *Journal of Plant Breeding and Crop Science*, 1(9), 311-319. <http://www.academicjournals.org/jpbcs>
- Montes-García, N., Vargas-Valero, E., Salinas-García, J. R., Espinoza-Ramírez, M., & Loredopérez, R. (2013). Tecnología de producción de sorgo dulce para la elaboración de bioetanol para Tamaulipas. <http://www.inifapcirne.gob.mx/Biblioteca/Publicaciones/957.pdf>
- Pabendon, M. B., Efendi, R., Santoso, S. B., & Prastowo, B. (2017). Varieties of sweet sorghum Super-1 and Super-2 and its equipment for bioethanol in Indonesia. In IOP Conference Series. *Earth and Environmental Science*, 65(1), 012054. <https://doi.org/10.1088/1755-1315/65/1/012054>
- R. (2020). R: A Language and environment for statistical computing. R foundation for statistical computing. Vienna, Austria. Retrieved from. <http://www.r-project.org/>
- Rachman, F., Tikoesoemaningtyas, T., Wirnas, D., & Reflinur, R. (2022). Estimation of genetic parameters and heterosis through line x tester crosses of national sorghum varieties and local Indonesian cultivars. *Biodiversitas*, 23(3), 1588-1597. <https://doi.org/10.13057/biodiv/d230349>
- Rakhmetova, S. O., Vergun, O. M., Blume, R. Y., Bondarchuck, O. M., Shymamanska, O. V., Tsyganskov, S. P., Yemets, A. I., Blume, Y. V., & Rakhmetov, D. B. (2020). Ethanol production potential of sweet sorghum in north and central ukraine. *The Open Agriculture Journal*, 14(1), 321-338. <https://doi.org/10.2174/1874331502014010321>
- Ratnavathi, C. V., Suresh, K., Kumar, B., Vijay-Pallavi, M., Komala, V., & Seetharama, N. (2010). Study on genotypic variation for ethanol production from sweet sorghum juice. *Biomass and Bioenergy*, 34(7), 947-952. <https://doi.org/10.1016/j.biombioe.2010.02.002>
- Rooney, W. L., Miller, F. R., & Rooney, L. W. (2003). Registration of RTx437 sorghum parental line. *Crop Science*, 43(1), 445-447. <https://doi.org/10.2135/cropsci2003.4450>
- Secretaría de energía [SENER]. Balance Nacional de Energía, (2021). México. [http://www.gob.mx/cms/uploads/attachment/file/44353/Balance\\_Nacional\\_de\\_Energ\\_a\\_2021](http://www.gob.mx/cms/uploads/attachment/file/44353/Balance_Nacional_de_Energ_a_2021)
- Servicio de Información Agroalimentaria y Pesquera [SIAP]. (2020). Panorama Agroalimentario - Sorgo. <https://pubhtml5.com/vqdk/osny/Panorama-Agroalimentario-2020/>
- Takare, D. P., Ghorade, R. B., & Bagade, A. B., (2014). Combining ability studies in grain sorghum using line x tester analysis. *International Journal of Current Microbiology and Applied Sciences*, 3(10), 594-603. <https://www.cabdirect.org/cabdirect/abstract/20143374377>
- Vanderlip, R. L. (1993). How a sorghum plant develop. Kansas Agricultural Experiment Station. Contribution No. 1203, Manhattan, Kansas, USA. 19 p.
- Williams-Alanís, H. & Arcos-Cavazos, G. (2015). Comportamiento agronómico de híbridos y progenitores de sorgo para grano en las Huastecas. *Agronomía Mesoamericana*, 26(1), 87-

97. <https://doi.org/10.15517/am.v26i1.16926>
- Williams-Alanís, H., Aranda-Lara, U., Arcos-Cavazos, G., Zavala-García, F., Galicia-Juárez, M., Rodríguez-Vázquez, M del C., & Elizondo-Barrón, J. (2022). Line x tester analysis to estimate combining ability in grain sorghum (*Sorghum bicolor* L.). *Revista de la Facultad de Ciencias Agrícolas UNCuyo*, 54(2): 12-21. <https://doi.org/10.48162/rev.39.078>
- Williams-Alanís, H., Montes-García, N., & Pecina-Quintero, V. (2006). Sorgo. En: Rodríguez-del-Bosque, L. A. editor, 50 años de investigación agropecuaria en el norte de Tamaulipas, historia, logros y retos. Libro técnico No. 1. INIFAP, Río Bravo, Tamaulipas, MEX. p. 33-54.
- Williams-Alanís, H., Pecina-Quintero, V., Zavala-García, F., & Montes-García, N. (2004). RB-Patrón, nuevo híbrido de sorgo para grano en el noreste de México. *Revista Fitotecnia Mexicana*, 27(3), 291-293. <https://doi.org/10.35196/rfm.2004.3.291>
- Williams-Alanís, H., Zavala, G. F., Arcos-Cavazos, G., Rodríguez-Vázquez, M. C., & Olivares-Sáenz, E. (2017). Características agronómicas asociadas a la producción de bioetanol en genotipos de sorgo dulce. *Agronomía Mesoamericana*, 28(3), 549-563. <https://doi.org/10.15517/ma.v28i3.26690>
- Williams-Alanís, H., Zavala-García, F., Arcos-Cavazos, G., Rodríguez Vázquez, M. C., Olivares-Sáenz, E. & Treviño-Ramírez, E. (2020). Potrillo, nuevo híbrido de sorgo dulce para la región central de nuevo león y sur de Tamaulipas. *Revista Fitotecnia Mexicana*, 43(3), 355-356. <https://doi.org/10.35196/rfm.2020.3.355>
- Yücel, C., Yücel, D., Hatipoğlu, R. & Dweikat, I. (2022). Research on the potential of some sweet sorghum genotypes as bioethanol source under Mediterranean conditions. *Turkish Journal of Agriculture and Forestry*, 46(2), 141-151. <https://doi.org/10.55730/1300-011X.2966>
- Zhang, B., & Wang, Q. (2015). MicroRNA-based biotechnology for plant improvement. *Journal of Cellular Physiology*, 230(1), 1-15. <https://doi.org/10.1002/jcp.24685>