

Evaluation of single-cross and trilinear hybrids with *su1* gene from mexican landraces

Abstract

Sweet corn is a vegetable eaten fresh. This research evaluates hybrid sweet maize carrying *su1* gene. In the first stage 30 single-cross and 47 trilinear hybrids were evaluated in separate experiments using Alpha Lattice design. Results showed competence data on 11 hybrids, outstanding in yield and degrees Brix. Those were evaluated in a second stage in three separate experiments, using two environments with a randomized blocks design and eight repetitions. Significant differences were found ($P \leq 0.01$) between experiments, repetitions and treatments with higher yield and degrees Brix over reference items. By crossing native sweet maize with elite lines of non-sweet maize sweet hybrids with same sweetness and significant improvement of plant and corn quality for Mexican landraces were obtained.

Keywords: Maíz dulce, Dulcillo del Noroeste, sweet mexican hybrids, genetic improvement, gene *su1*

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José Alberto Sánchez-Nuño,¹ Lino De la Cruz-Larios,¹ Abraham Guerrero-Corona,² Moisés Martín Morales-Rivera,¹ Eduardo Rodríguez-Guzmán,¹ Fernando Santacruz-Ruvalcaba¹

¹Universidad de Guadalajara, Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA), México

²Instituto Tecnológico José Mario Molina Pasquel y Henríquez, Unidad Académica Cocula, Mexico

Correspondence: Dr. Lino De la Cruz Larios, Universidad de Guadalajara, Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA). Camino Ramón Padilla Sánchez 2100, Nextipac, 44600 Zapopan, Jalisco, México, Tel 3313274355, Email linocucba@hotmail.com

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Introduction

Landraces Maíz Dulcillo del Noroeste and Maíz Dulce are considered special due to their antioxidants, vitamins, proteins, minerals, and sugar contents. "A total of 18 amino acids, crucial for human nutrition have been identified in the corn cob of sweet maize, including serine, glutamine, alanine, proline, and aspartic and glutamic acids",¹ and "mineral components including potassium, calcium, sodium, magnesium, phosphorus, iodine and iron".² Sweet corn also has a long tradition and multifunctionality in nutrition and customs; the great majority of it is marketed through improvised sites in highways, farmers markets, food markets, direct delivery to wholesales sites, and in supermarkets. It is also used to extract high fructose syrups. Sweet corn cobs "are one of the main products obtained from maize and are used to make corn flakes",³ and for making candies. Due to the importance of sweet corn it is necessary to work with it in genetic improvement programs.

Sweet corn has in its genome recessive alleles *su1su1* provoked by a "spontaneous mutation in *su1* gene (*sugary1*) controlling sugar conversion into starch inside the endosperm of the maize grain".⁴ With an alteration of the nucleotide sequence in a "fixed position called *su1*, located in chromosome 4 Bin 4.05".⁵ "The historically most important sweet mutant *su1* produces grains with high phytoglycogen content".⁶ This recessive gene causes polysaccharides soluble in water to raise increasing sweetness. Improved hybrid maize from genetically pure lines with allele *su1su1* present high soluble solids in general, such as phytoglycogen, increasing sugar levels and percentages of (°Bx) degrees Brix, which is the indicator measure used in experimental tests to determine food sweetness in the food industry and to detect sweet grain genotypes for producing seeds and hybrids. A problem existing in Mexico regarding improved seeds and hybrids of sweet maize offer is that it is restricted to hybrids with very limited adaptation to seasonal rain and to certain areas of Mexico. Up to now private and public institutions in Mexico have no knowledge of sweet maize hybrid seeds adapted to seasonal rain which can function

as forage. For this reason, it is important to generate quality hybrids of sweet maize which can adapt to temperate and semi-warm subhumid climates. The present research work has the aim to evaluate single-cross and trilinear hybrids with *su1* gene having good agronomic characteristics to benefit consumer, such as yield, length, diameter, sweetness and quality of plant and corn.

Materials and methods

The germplasm used as donor progenitor was composed by accessions of landrace Maíz Dulce: Chihuahua (Chih-194 from Balleza and Chih-200 from Guachochi), Guanajuato (Gto-181 from Silao), Jalisco (Jal-300 from Mezquitic, M09476 and M09477 from Huejucar) and Michoacán (M06193 from Tarimbaro and M06173 from Huandacareo); of landrace Dulcillo del Noroeste: Nayarit (Nay-47 from Tuxpan) and Sonora (Son-85 from Rosario), and of elite progenitors of no sweet maize of dented grain LUG282, LUG03, CML78 and CML311 used as recurrent progenitors, which are lines of improvement from (IMAREFI) Instituto de Manejo de Recursos Fitogenéticos at (CUCBA) Centro Universitario de Ciencias Biológicas y Agropecuarias from Universidad de Guadalajara, and (CIMMYT) International Maize and Wheat Improvement Center.

Crossings of the recurrent progenitor (pure lines) with donor progenitor (sweet maize landraces) were carried out. F_1 was planted and at harvest, maize grains with sweet grain type characteristics were selected. These are grains with rough aspect in ear stage. They were planted and self-pollinated for several cycles until pure recombinant sweet lines were obtained which latter generated single-cross and trilinear hybrids.

Crossing evaluation was carried out in two locations in the state of Jalisco: CUCBA, Nextipac, municipality of Zapopan, Jalisco, with coordinates 20°44'42"N and 103°30'54"W, altitude 1658 masl, annual average temperature 18°C, annual precipitation of 850-1000 mm, and subhumid climate; and in El Salitre, municipality of

San Martín Hidalgo, Jalisco, with coordinates: 20°30'33.9"N and 103°51'01.4"W, altitude 1260 masl and annual precipitation of 964 mm. annual average temperature 20.9 °C and semidry.

Field work was carried out in three stages; in stage one 47 crossings with four repetitions and a commercial reference, Golden Sweet were evaluated. In a second experiment 30 single-cross with two repetitions, and Golden Sweet, included as reference were evaluated. These experiments were planted in the summer cycle of 2015 in the experimental field at CUCBA. Evaluated variables were: (FF) days for female flowering, (FM)days for male flowering, (AMZ) ear height, (ALPL) plant height, (Peso25) and (Peso35) ear weight at 25 and 35 days after flowering and respectively, (DiaM) ear diameter, (LonM) ear length, (°Bx25) and (°Bx35) sugar content at 25 and 35 days after flowering respectively, (Hil) number of grain rows, and (Grxhil) number of grains per row. Experimental units were made in two furrows 4.00 m length separated by 0.75 m with 50 total plants. Samples consisted of ten corns for weight and three for sweetness (°Bx), took randomly.

In the second stage parental seeds of the best recombinant lines

were incremented to form trilinear hybrids, based on the results of the first stage. These seeds were planted in greenhouse conditions with drip irrigation systems in the winter 2015-2016. Parental increments and hybrid formations were done through manual pollination. In the third stage, during the spring-summer cycle 2016, the best 11(HT) trilinear hybrids were evaluated, as well as the best (HS) single-cross hybrids regarding best yield and corn sweetness from the first stage. Hybrid Golden Sweet and a native corn (Jayamitla) were used as references. They were evaluated in three independent experiments with different planting dates in two locations: El Salitre municipality of San Martín de Hidalgo, Jalisco, and in Nextipac, municipality of Zapopan, Jalisco. The evaluation was carried out with a complete block design and eight repetitions each. Experimental units were in four furrows 4.00 m length with 0.75 m separation between furrows, with 50 total plants. Measured variables were the same as those in stage one, (°Bx25r30 and °Bx35r30) plus sweetness at 25 and 35 days after flowering and refrigerated for 30 days. Sugar percentages were measured with a digital refractometer Atago PAL-1. Table 1 shows genealogy of the 11 hybrids which participated in the third stage evaluations.

Table 1 Hybrids genealogy. Evaluation in 2016 T

Treatments	Trilinear crosses
Trilinear crosses8	Csdulce-x(LUG-03XNAYA-47_Plant12)F2-2-1-1-M-1
Trilinear crosses10	Csdulce-x(LUG-03XGTO-181_Plant14)F2-4-2-3-M-1
Trilinear crosses12	Csdulce-x(LUG-03XJAL-300_Plant9)F2-2-3-1-1-M
Trilinear crosses16	Csdulce-x(LUG-03XNAYA-47_Plant12)F2-2-1-1-M-2
Trilinear crosses18	Csdulce-x(LUG-03XNAYA-47_Plant12)F2-4-4-4-1-M
Trilinear crosses20	Csdulce-x(LUG-03XM-06193_Plant12)F2-3-1-1-1-1
Trilinear crosses21	Csdulce-x(LUG-03XGTO-181_Plant12)F2-2-1-3-M-1
Trilinear crosses29	Csdulce-x(LUG-03XJAL-300_Plant8)F2-1-1-4-1-M
Trilinear crosses36	Csdulce-x(LUG-03XNAYA-47_Plant12)F2-4-4-5-1-M
	Single crosses
Single crosses11	[LUG-03XM-06193_P12)F2-1-2-1-1-M]xLMdulce
Single crosses19	CS_Dulcex(Commercial hyb x M06030-49-1-1)
GS	Golden sweet
Test	Sweet native maize Jayamitla

Fertilizers were applied in the following way: 200 kg/ha, MAP (18-46-00) at planting, and two applications of urea 200 kg/ha (46-00-00), the first one when plants had five true leaves and the second when plants were in flowering stage. Herbicides Convey (Topramezone 33, 6%) and Atrazina with a dosage of 4 Lha⁻¹ were applied, with manual sprayer and fan nozzle number 4 aiming to weeds. Foliar fertilizer Bayfolan® S 4 Lha⁻¹, containing macronutrients and micronutrients was applied with backpack sprayer and directed cone nozzle pointing to corn plants; biological farming insecticide (Palgus Spinetoram: mixture of Spinosyn J and Spinosyn L) 3 Lha⁻¹ was also applied.

Statistical analysis

With the quantitative data obtained in field and laboratory an ANOVA and means comparison tests by treatment for each variable were carried out. Means were adjusted with a (GLM) generalized linear model adjusted by least squares (LSMEANS) with the program SAS® 9.0 and ANOVA with a combined model of the two evaluation stages simulating different environments.

The model for the experiments was alpha lattice:

$$\text{Where: } Y_{ijl} = \mu + \tau_i + \gamma_j + \rho_l(j) + \varepsilon_{ijl}$$

Y_{ijl} = Value of each variable in each experimental unit

τ_i = Effect of treatments

γ_j = Effect of repetitions

ρ_l(j) = Effect of incomplete blocks

ε_{ijl} = Random error

Results and discussion

Variance analysis (Table 2) for trilinear hybrids HT presented differences highly significant (P ≤ 0.01) among treatments for all variables, except for (°Bx25). Nevertheless, there were important numerical differences in means comparison and percentages obtained in laboratory tests for this variable. This can be attributed partly to the pure lines utilized as base; the genotypes presented the same

sweet *su1* gene but from a different geographic origin. There was also an intrinsic genetic variability among genotypes behaving with a sweetness increment from 25 to 35 days after flowering, in accordance with Xu et al.⁷ In the variance analysis for single-cross HSs there were significant differences ($P \leq 0.05$) in treatments for the great majority of variables indicating variability in the evaluated genotypes, as can be seen in Table 3. Characters in quality of corn from sweet maize are complex as they depend on quantitative characters and the sugar content depends on recessive genes; nevertheless, Luch singer and Camilo⁸ point out that taking as reference a commercial item, sugar contents for the evaluated hybrids would be considered of good

quality. The effect on significance ($P \leq 0.01$) was represented in four variables taken into account in the experiment which indicates that at least one block was different from the rest. This is attributed to the effects of environmental genotypes evaluated in the random incomplete blocks which caused differences in weights and degrees of sweetness on corn per experimental unit. HTs showed significant differences in quality, compared to reference item. These results are in accordance with similar studies made by Michaels and Andrew.⁹ In our work, germplasm lines of Nay-47 (Dulcillo delNoroeste) stands out as can be seen in Table 5.

Table 2 ANOVA: Sweet trilinear hybrids CUCBA 2015

Variable	CME	CMR	CMB	CMT	CV	Mean
Peso25	42963	53265.6	106256**	116185.4**	10.4	1985.2 kg
Peso35	47430.1	56406.6	139757.4**	108216.9**	10.5	2068.6 kg
°Bx25	18.9172	34.2641	13.24	21.8598	19.49	22.32 °Bx
°Bx35	23.0757	3.3417	19.3472	80.2798**	20.21	23.77 °Bx
LonM	1.4177	0.3165	1.9002	6.6599**	6.68	17.82 cm
DiaM	0.027	0.0523	0.0659**	0.1353**	3.43	4.79 cm
Hil	0.755	0.2266	0.5013	6.8279**	6.02	14.44 rows
Grxhil	7.2302	4.9222	7.5096	28.8331**	7.7	34.94 grains
Ff	2.4692	0.4417	3.7008	26.7829**	2.37	66.31 days
Fm	2.8154	0.2063	4.3762	26.3303**	2.55	65.89 days
Alpl	0.0173	0.0364	0.1193**	0.1114**	6.37	2.06 m
Amz	0.0191	0.0739*	0.0373*	0.0877**	12.87	1.07 m
GL	105	3	12	39		

† ANOVA CME, CMR, CMB, CMT, mean squared error; repetitions, blocks in repetitions, and treatments respectively; GL, degrees of freedom; CV, coefficient of variation; Mean, general mean of the experiment; Fm, days for male flowering; Ff, days for female flowering; Bx25 and Bx35, quantity of sugar in degrees Brix at 25 and 35 days after flowering, respectively; peso25 and Peso35, weight in kg of ten fresh ears of corn at 25 and 35 days after flowering, respectively; LonM and DiaM, ear of corn length and diameter in cm, respectively; Hil, number of grain rows, Grxhil, number of grains by row; Alpl, plant height in m; Amz, ear of corn height in m, Controls = * = $P \leq 0.05$, ** = $P \leq 0.01$

Table 3 ANOVA: Sweet single and trilinear hybrid CUCBA 2015 T

Variable	CME	CMR	CMB	CMT	CV	Mean
Peso25	38686.45	168363.38*	49160.1	60534.55	10.58	1858.86 kg
Peso35	37011.86	110081.67	27175.42	75585.64*	10.06	1912.72 kg
°Bx25	7.9891	32.4625	8.7571	16.1096*	13.03	21.69 °bx
°Bx35	12.6378	16.4327	24.091	29.9137*	15.71	22.62 °bx
LonM	1.7197	1.1252	2.342	3.696*	7.55	17.36 cm
DiaM	0.0232	0.0634	0.0122	0.0624*	3.22	4.73 cm
Hil	0.6607	0.15	0.3574	1.4598*	5.84	13.93 rows
Grxhil	4.1655	8.563	8.2625	18.0586**	5.91	34.54 grains
Ff	1.9268	4.2667	3.4125	6.6549*	2.04	68.20 days
Fm	2.4351	1.0667	4.1667	4.8322	2.31	67.50 days
Alpl	0.0752	0.0175	0.0464	0.1653*	13.08	2.10 m
Amz	0.024	0.0001	0.0227	0.0646*	14.85	1.04 m
GL	19	1	10	29		

† ANOVACME, CMR, CMB, CMT, mean squared error; repetitions, blocks in repetitions, and treatments respectively; GL, degrees of freedom; CV, coefficient of variation; Mean, general mean of the experiment; Fm, Days for male flowering; Ff, days for female flowering; Bx25 and Bx35, quantity of sugar in degrees Brix at 25 and 35 days after flowering, respectively; Peso25 and Peso35, weight in kg of ten fresh ears of corn at 25 and 35 days after flowering, respectively; LonM and DiaM, ear of corn length and diameter in cm, respectively; Hil, number of grain rows, Grxhil, number of grains by row; Alpl, Plant height in m; Amz, Ear of corn height in m, Controls = * = $P \leq 0.05$, ** = $P \leq 0.01$

Values for total solid soluble contents quality 32.2 °Bx were obtained, other works as the one from Coutiño et al.¹⁰ presented sugar contents of 13.8°Bx. It was observed that maize genotypes with *su1* gene maintain high sugar concentrations and can have an advantage in yield, crop and commercialization, according to works carried out by Michaels and Andrew,⁹ Marshall et al,¹¹ & Tracy et al.¹² regarding quality associated with sugar concentrations and shelf life.

In means comparative tests, considering weight for 10 cobs higher than 2.300 kg, the significant treatments were 8, 16 and 36, and for diameter, treatments 10 and 21. It is important to point out that genealogies of these treatments were lines with germplasm LUG03 per accessions of landraces Dulcillo del Noroeste from Nayarit and Maíz Dulce from Guanajuato. These treatments, with good

quantitative characteristics besides having values of 23 °Bx are good options regarding corn quality.

Table 4 shows combined variance analysis. For this analysis, all variables were significant and/or highly significant, that is, there was interaction with the environment so individual analyses were taken for each variable and locality. On the other hand, there was no significant interaction in the combined analysis for variables (°Bx25r30) and (°Bx35r30). In this evaluations sugar was increasing in the grains as the days after flowering passed by, but all hybrids lowered their sugar concentration after being refrigerated which was observed in the cobs harvested at 35 days after flowering (Table 5). Sucrose synthesis affects sugar concentrations in tissues due to changes in assimilate distribution as grains mature.⁷

Table 4 ANOVA: Combined analysis of 11 simple and Trilinear hybrids 2016 T

Variable	CME	CME _{Exp}	CMR	CMT	CMI	CV	Mean
Peso25	0.0899	27.2546**	0.0883	0.3641**	0.1833*	18.9	1.59 kg
Peso35	0.0768	31.0785**	0.0786	0.4655**	0.2724**	18.71	1.48 kg
°Bx25	12.3942	458.9086**	14.7428	88.1489**	19.5045*	18.48	19.06 °Bx
°Bx25r30	35.3044	784.0064	36.3561	109.2034*	38.7985	32.54	18.26 °Bx
°Bx35	11.0406	42.7767*	14.9586	219.6678**	33.9827**	13.96	23.80 °Bx
°Bx35r30	24.9862	16.5506	10.6511	130.2333**	39.5412	28.11	17.79 °Bx
LonM	5.1037	238.7827**	2.5036	21.0563**	11.7458*	14.03	16.10 cm
DiaM	0.1177	18.2622**	0.1401	0.4873**	0.2547*	8.19	4.19 cm
Hil	1.1182	27.5927**	1.0015	11.0532**	2.5949*	7.09	14.91 rows
Grxhil	29.4268	1853.69**	23.495	129.7332**	63.6234*	16.45	32.97 grains
GL	257	3	24	11	32		

† ANOVACME, CME_{Exp}, CMR, CMT, CMI, mean squared error, experiments, repetitions, treatments, and experiments per treatment, respectively; GL, degrees of freedom; CV, coefficient of variation; Media, general mean of the experiment; Bx25, Bx35, quantity of sugar in degrees Brix at 25 and 35 days after flowering, respectively; Bx25r30 and Bx35r30, Quantity of sugar in degrees Brix at 25 and 35 days after flowering and refrigerated for 30 days after harvesting, respectively; Peso25 and Peso35, weight in kg of ten fresh ears of corn at 25 and 35 days after flowering respectively; LonM and DiaM, ear of corn length and diameter in cm, respectively; Hil, number of grain rows, Grxhil, number of grains per row; Controls = * = P ≤ 0.05, ** = P ≤ 0.01

Table 5 Adjusted means of 11 hybrids in three individual experiments 2016 T

Trat	Cucba 1	Cucba 2	Salitre	Cucba 1	Cucba 2	Salitre	Cucba 1	Cucba2	Salitre
	Kg 10 ears at 25 daf			°Bx at 25 daf			°Bx at 35 daf		
HT8	1.98	1.7**	0.82	17.9**	19.6**	19.5	24.3**	28.5**	24.3
HT10	2.13	1.67**	0.7	16.5	15.6	18.8	20.4**	23.9**	22.9**
hs11	1.91	1.73	1.09	18.5**	18	19	22.5**	23.4**	24.3
HT12	2.03	1.67*	1.03	18**	18.4*	20.8	22.4	25.3**	25.6**
HT16	2.08	1.53**	0.87	17.7**	20.7**	20.8	23.8**	26.2**	25.6**
HT18	1.91	1.68**	1.09	16.2	22**	19.8	25.6**	25.7**	24.2
hs19	2.08	1.65*	0.42	16.2	18.9*	19.2	22	25.1**	25.7**
HT20	2.19	1.56	0.99	17.2*	20.3**	20.8	23.3**	24.1**	22.5**
HT21	2.28	1.9**	0.73	17.4*	18.5*	19.2	25.5**	26.9**	22.3*
HT29	1.93	1.49	0.66	20.4**	15.5	22.6**	26.5**	27.5**	22.9**
HT36	2.3	1.68**	1.07	17.8**	18.9*	18.6	25.4**	26.5**	22*
TEST	2.06	1.18	0.88	11.8	12.5	16.9	14.1	8.7	17.1

†Means of most representative variables: Trat, Treatments; HT, Trilinear hybrids; hs, Single hybrids; Test, Reference; daf, days after flowering; Controls, * = P ≤ 0.05; ** = P ≤ 0.01

Table 5 shows the adjusted means for hybrids evaluated in two environments but three different planting dates. A remarkable superiority was observed in different variables associated with corn quality for hybrids compared to reference items used in different localities and which were not significant in any environment or any variable studied, compared with trilinear and single hybrids which were significant. For example, HTs were superior and more competitive than HSs in variables ($^{\circ}$ Bx25) and ($^{\circ}$ Bx35). The best results in sweetness expression were from trilinear hybrids and happened in the experiment done in the second planting date in CUCBA2, where significant treatments were 12, 20 and 21, coming from landrace Maíz Dulce, accessions from the states of Jalisco, Michoacán and Guanajuato, and for treatments 16 and 18 for landrace Dulcillo del Noroeste with hybrid accessions from Nayarit. Authors like Ramírez-Díaz et al.¹³ have considered that native corns presents lots of similarities to exotic germplasm coming from other regions of the world; both can be a source of useful allele but they can also

carry non-desirable characteristics, like great height of the plant, and susceptibility to stalk and root lodging, and ear diseases. In location El Salitre, differences highly significant between treatments ($P \leq 0.01$) for variables (Peso25), (AMZ), and (ALPL), and significant differences ($P \leq 0.05$) for variable (DIAM) were obtained, while for variables ($^{\circ}$ Bx25) and ($^{\circ}$ Bx25r30) there was no significance (Table 6). In CUCBA2 corn weighted less but had a higher percentage of $^{\circ}$ Bx than in the other two environments. The reason could be that in the second environment at CUCBA, planting was carried out the latter and environmental conditions and water availability, because it was sown in temporary rain and could have been restrictive for the development of the plant and therefore maize had a high percentage of Bx. In the work of Luchsinger and Camilo,⁸ similar results were obtained due to differences in planting dates, where corns planted at a later date were sweeter and, at the same time, maize weight yields were reduced.

Table 6 (Continuation): Adjusted means of 11 hybrids in three individual experiments 2016 T

Trat	Cucba 1	Cucba 2	Salitre	Cucba 1	Cucba 2	Salitre	Cucba 1	Cucba 2	Salitre
	$^{\circ}$ BX Refrigerated for 25 ddf			Ear length at 25 daf			Ear diameter at 25 daf		
HT8	14.2	23	23.2	16.9	16.8	15.2	4.3**	4	3.4
HT10	12.4	11.9	20.2	16.5	16.7	12.6	4.5**	4.1	3.4
hs11	11.5	20.9**	23.3	16.7	17.1	16.9	4.4**	4.3	3.7
HT12	16.8	21.3**	22.6	15.3	15.2	13.9	4.7	4.2	3.9
HT16	13.2	22.5**	25.2	16.9	16.4	15.1	4.3**	4	3.6
HT18	16.3	22.1**	18.6	15.8	17	15.2	4.4**	4.3	3.7
hs19	15.3*	15.6	19.7	17.3	16.5	9.9*	4.7	4.4	3.1**
HT20	13.5	21.1**	21.7	17.5	16.3	15.8	4.4**	3.7**	3.7
HT21	17	21.5**	26	13.9	16.1	13	4.6**	4.4	3.4
HT29	13.4	14.5	19.5	16.4	17.1	10.8	4.5	4	3.7
HT36	17.3	15.1	21.1	18.1	17.4	15.9	4.7	4.2	3.7
TEST	8.6	9.3	16	16.2	15.5	15	4.9	4.5	4

[†]Means of most representative variables: Trat, treatments; HT, trilinear hybrids; hs, single hybrids; test, Reference; daf, days after flowering; controls, * = $P \leq 0.05$; ** = $P \leq 0.01$

Conclusion

Results obtained in this work confirm the possibility of obtaining varieties and hybrids of sweet corn with high potential in yield and quality of grain for fresh corn consumption due to sweetness *su1* gene obtained from native Mexican corn. Combinations of traditional varieties of landraces Maíz Dulce and Dulcillo del Noroete, along with other elite lines with good combinatorial capacities can be the base germplasm that could establish heterotic patterns. Based in the results of two years, it was possible to demonstrate that modifying native sweet maize through non-sweet but outstanding normal lines is especially useful. Due to the existence of genotype-environment interactions, more evaluations in a wide range of environmental and management conditions will be required.

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Conflicts of interest

Authors declare no conflict of interest exists.

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