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GRAIN YIELD STABILITY AND BIOCHEMICAL NUTRIENT CONTENT IN WHITE CORN EXPERIMENTAL CROSSES

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ABSTRACT

In Mexico, about half of white corn production is used for human consumption. In a breeding program, in addition to increasing the yield and genotypes stability, the aim is to improve grain quality. Assuming that there is genetic variation among the elite genotypes evaluated in this research, at least one genotype was considered to be outstanding for its high productive potential and desirable characteristics for grain quality. The objective of this work was to determine the genetic variation present in experimental hybrids in terms of yield potential and grain quality, as well as to identify the most outstanding genotypes.

In 2016, twenty white corn experimental crosses were evaluated under a randomized block design with three replications in two locations of the ecological area of the High Valleys of Mexico and one location in a transitional area. Yield stability was determined using the additive main effects and multiplicative interaction model. Grain quality was evaluated through starch, protein and oil content. Most of the crosses had higher yield and stability than the controls (commercial hybrids). Likewise, nutrient content varied among them for each biochemical compound; oil concentration ranged from 3.5 to 6.4 g 100 g^{-1} dry matter (ms); protein, from 5.5 to 9.8 g 100 g^{-1} ms; and starch, from 72.6 to 96.6 g 100 g^{-1} ms.

Based on their phenotypic stability, yield and grain quality, we observed that crosses 1213, 1217, 1218, 1314, 1418, and 1812 were outstanding, which can be used to produce grain with promising starch and oil content. In general, we infer that the crosses showed potential for starch utilization and tortilla production.

Keywords: *Zea mays* L., phenotypic stability, grain quality.

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INTRODUCTION

Because of its high carbohydrate content, corn is one of the three main sources of energy for the world population (Serna-Saldivar and Perez, 2019). In Mexico, it is the most produced (24.2 million tons) and consumed (25.0 million tons) cereal (SIAP, 2019).

It is reported that 49 % of the national white corn production is destined for human consumption (SIAP, 2020), particularly as tortillas, the main element of the Mexican diet (Vázquez-Carrillo *et al*., 2014). Corn kernels are mainly composed of carbohydrates, proteins and lipids (Arendt and Zannini, 2013). Among the carbohydrates present in this grain, starch represents about 85 % of the total weight of the endosperm and 72 % of the total weight of the grain.

Worldwide, corn is the largest commercial source of starch and is the basis for the production of a large number of chemically, enzymatically, and physically modified functional products, as well as hydrolyzed maltodextrins and syrups. Recently, non-gelatinized native corn starch has been shown to be a source of slowly digested starch, which stands out for its glycemic index (Hamaker *et al*., 2019). In addition, it has gained importance for its use in the preparation of gluten-free foods (Serna-Saldivar and Perez, 2019).

The endosperm is also made up of protein (between 8 and 15%), and is the second most abundant biomolecule in the kernel. Zein constitutes 60 to 70 % of endosperm protein. It is not an ideal nutrient for humans, but due to its abundance, hydrophobicity and price, it is valuable for its wide range of industrial applications, such as the production of plasticizers, coatings, fibers, inks and moldings. Nutritionally, QPM (quality protein maize) corns have improved protein quality with respect to essential amino acids, such as lysine and tryptophan, which has increased their nutritional value (Arendt and Zannini, 2013). Although corn is not considered an oilseed, as it has only 3-6% oil, it is important as a by-product of the wet milling process (Wang and White, 2019). In the case of tortilla making, there is evidence that oil can confer desirable characteristics such as softness, flexibility and chewiness (Vázquez-Carrillo *et al*., 2014).

In Mexico, the demand for white corn is covered by domestic production, which represents 86 % of the total grain produced (SIAP, 2019). This sufficiency of white corn has been achieved through the optimization of agronomic practices and genetic improvement. However, although there are precedents in the country on grain quality improvement (CIMMYT, 2017), until a few years ago breeding was mainly focused on increasing grain yield and less work was done to improve grain quality. Recently, Serna-Saldivar and Perez (2019) have reported that native maize populations represent a valuable genetic resource to increase the grain quality and nutritional content.

The incorporation of native maize in the genetic improvement programs of national public research institutions has also been evidenced, generating genotypes with outstanding nutritional characteristics such as starch, oil and protein content (Vázquez-Carrillo *et al*., 2014; Vázquez-Carrillo *et al*., 2018).

Based on the above, it is considered of utmost necessity to characterize the biochemical nutritional content of the genetic material of the current breeding programs in order to know its variation, potential for utilization and to define breeding strategies.

In Mexico, 94 % of the improved seed used for maize crop establishment is obtained from private transnational companies (Luna-Mena *et al*., 2012). Although production can supply the demand for grain, genetic improvement is still required to supply seeds at a lower cost, with competitive yields and grain quality for tortilla production (one of its main uses). Therefore, this research evaluated germplasm obtained from a maize breeding program by hybridization for the High Valleys of Mexico, whose main objectives were: increase grain yield, generate rustic genotypes (low fertilization doses, scarce humidity and minimal cultivation work), and improve grain quality, the latter carried out in a non-systematized sensory way.

The evaluation was carried out to test the existence of genetic variation among the elite genotypes studied, and consequently identify at least one outstanding genotype for its high productive potential and desirable characteristics for grain quality. Thus, the potential of the outstanding experimental crosses for grain yield was determined in different locations and compared to commercial hybrids. Their quality was characterized through the quantification of starch, protein, and oil in order to detect suitable crosses for the production of food for human consumption.

MATERIALS AND METHODS

Germplasm

At the Plant Genetic Resources Program from Colegio de Postgraduados, Mexico, we evaluated ten inbred lines (S_6) that were donated by Dr. Moisés Mendoza Rodríguez† and derived from a maize breeding program developed for the High Valleys.

These lines were formed from broad-based populations that were obtained from variants of the Chalqueño and Cónico landraces; they were selected under rustic production conditions and were characterized by their outstanding attributes (ear weight, 100 kernel weight, ear diameter and ear length) for grain yield. From these lines, all the possible direct and reciprocal crosses were formed, which were evaluated agronomically in 2014. From them, in 2016, twenty outstanding crosses were selected and evaluated for grain yield, incorporating as controls the commercial hybrids: H-San Josecito®, H-San José®, Caimán®, Águila®, and Jabalí®. In the same cycle, in Montecillo, Mexico, controlled pollinations were performed in each cross to obtain the required grain samples to perform the quality analysis.

The grain obtained came from a conventional agronomic management suitable for production, where a 120N-60P-60K fertilization rate was used (60N-60P-60K before planting, and 60N-00P-00K at the time of hilling), and six irrigations were made according to the needs of the crop. The biological cycle was allowed to develop without agronomic restrictions.

Experimental design

The trial was established under a randomized complete block design with three replications. The experimental unit consisted of a 5 m long furrow and a distance between furrows of 0.80 m; and a density of 65,000 plants ha^{-1} was used.

The experiment was established in three locations: Montecillo (20° 17′ N, 98° 36′ W), and Tecámac (19° 42′ N, 98° 96′ W), State of Mexico, and Mixquiahuala, Hidalgo (20°

09′ N, 99° 06′ W). The first two showed the environmental conditions of the High Valleys: altitude of 2200 masl, mean annual temperature (Temp) of 16.2 and 15.6 °C, annual precipitation (PPA) of 590 and 440 mm, respectively.

In Mixquiahuala, located in the Valle del Mezquital, the ecological conditions corresponded to a transition area: altitude of 1850 m, semi-dry temperate climate, Temp 18.3 °C and 400 mm PPA.

Agronomic management

In Montecillo, the assay establishment was done on May 2, 2016, using the fertilization rate 120N-60P-60K, and the crop cycle under irrigated conditions. In Tecámac, the trial was established on June 6, and the same fertilization rate was applied; irrigation was provided after planting and the rest of the cycle was conducted under rainfed conditions. In Mixquiahuala, planting took place on May 5; fertilization was not applied due to the use of sewage water, in accordance with the agronomic practices of the region. Three irrigations were applied during the growing cycle: before planting, twenty days after planting and during flowering.

The agronomic conditions of each site correspond to the local production system, and from these, the yield of each experimental cross was differentiated.

Evaluated characteristics

Grain yield

Grain yield per experimental unit (Rto_1) was determined as:

$$Rto1 = (PMz * Id * (100 - \% Hum) / 100) / 0.86$$

where PMz was the weight of the harvested ears per experimental unit; Id, the shelling index; % Hum, the humidity of the field sample; and 0.86, a correction factor to adjust the yield to 14 % humidity. With this result and the area of the useful plot, the grain yield (Rto) was estimated in Megagrams (tons) per hectare (Mg ha^{-1}).

Genotype-by-environment interaction for grain yield

The genotype-by-environment interaction was assessed using the additive main effects and multiplicative interaction model (AMMI) (Zobel *et al*., 1988) for grain yield. Both data analysis and two-dimensional plot (biplot) were performed using GEA-R software (Pacheco *et al*., 2015).

Biochemical and nutritional composition of the grain

The sample for the study of each cross consisted of 72 g of grain obtained from the central part of five cobs from controlled pollination from Montecillo, State of Mexico; the grain was pulverized with a cyclone mill (UDY Corporation®, Fort Dollins, CO, USA), from which homogenized flour with a particle size of 0.5 mm was obtained.

Samples, reagents and other materials were weighed on an analytical balance (A&D®, Model GR 202, San Jose, CA, USA).

The methods used for biochemical analysis were as follows: method 30-25.01 for oil content, method 46-11.02 for protein (AACC, 2009), the methodology of Brunt *et al*. (1998), and method 76-13.01 of AACC (2009) to starch determination.

The determination of biochemical content was carried out in three replicates per cross. The concentration obtained was expressed in g 100 g^{-1} of dry matter (ms).

With the information obtained from the nutritional biochemical components, the analysis of variance was carried out under the model of a completely randomized experimental design complemented with Tukey's test of means ($p \leq 0.05$). Statistical analysis was performed with the Statistical Analysis System software (SAS Institute, 2003).

RESULTS AND DISCUSSION

The analysis of variance obtained using the AMMI model showed statistical differences ($p \leq 0.01$) in the genetic component and locations (Table 1); it also indicated significant interaction ($p \leq 0.01$) between both components.

Zobel *et al*. (1988) and Gauch (2013) mentioned in their studies that these results are a generalized response showing variation among genotypes, differences in environmental potential, and variation in the ordering of genotypes in each location; which allows differentiating genotypes by yield and stability.

According to the sum of squares of treatments (SCT), the genotypes x locations interaction (GenxLoc) concentrated the greatest variation (51.1 %), which highlighted the importance of this phenomenon, both in the evaluation and selection of genotypes and, therefore, in genetic improvement. The full AMMI model represented 85.7 % of the sum of squares of the treatments and the residual was 14.2 %. Under this

Table 1. Combined analysis of variance across locations for grain yield of experimental crosses and commercial hybrids of white maize.

SV	DF	SS	MS	SSPercentage
Treatments	74	1169.75	15.8	
Genotypes	24	159.76	6.65†	13.66¶
Locations	2	411.90	205.9†	35.21¶
GenxLoc	48	597.85	12.45†	51.11¶
CP1	25	431.67	17.26†	72.20§
Residual	23	166.18	7.22	14.2¶
Error	225	284.76	1.26	
Total	299	1454.51		

†$p \leq 0.01$; SV: Source of variation; DF: Degrees of freedom; SS: Sum of squares; MS: Mean squares; CP1: Principal component one; ¶: Proportion with respect to the sum of squares of the treatment design; §: Proportion with respect to the sum of squares of the GenxLoc interaction.

consideration and according to Zobel *et al*. (1988), the model adequately represented the data. For the explanation of the interaction, only principal component one (CP1) was significant ($p \leq 0.01$), comprising 77.2 % of the interaction variance. This allows its representation in a biplot figure (AMMI1) through CP1 and grain yield.

Genotype-by-location interaction (GenxLoc)

The biplot figure showed the differences in environmental potential between locations (Figure 1), particularly between Montecillo and the rest, since the former had a higher grain yield (> 9.0 Mg ha^{-1}).

The high productivity of the locality was attributed to the environmental potential basically defined by the possibility of irrigation, as well as the adaptation of the genotypes to this environment. It was also found that, given the vector length, Montecillo had a greater interaction effect, which indicated that there was more variation in grain yield among genotypes and facilitated both discrimination and selection of genotypes.

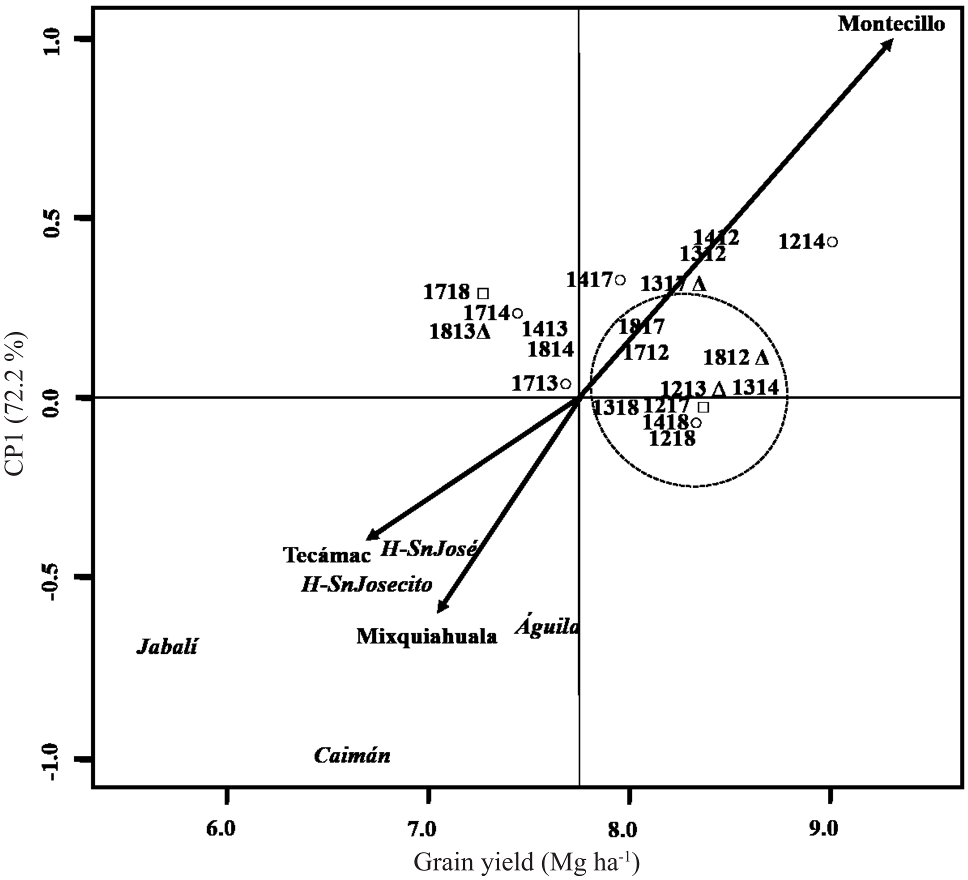

Figure 1. AMMI. Grain yield and interaction of genotypes (experimental crosses and commercial hybrids) by location. ○, □, Δ are crosses with outstanding starch, oil and protein content, respectively. The oval surrounds the outstanding crosses for their stability and grain yield.

In contrast, in Mixquiahuala and Tecámac the yield was lower than the average of the localities (7.8 Mg ha^{-1}); this was mainly due to the lower availability of water, since moisture shortage was considered to be the most limiting environmental factor for production; however, such a restriction represents the production conditions of those locations.

In the case of the interaction effect, the angle between the vectors and the mean yield value was similar in both locations, so that an analogous classification of the genotypes' behavior could be made. This implied that there was a lower discrimination capacity among the crosses evaluated, since the variation in the expressed yield was reduced.

According to Zobel *et al*. (1988), genotypes that are located in the same direction as the locations show positive interaction with these; that is, they have adaptation and higher production. Although in this study, in general, the crosses showed positive interaction with Montecillo, it was also observed that their genetic constitution had an important effect on the expression of superior yield compared to commercial hybrids. Among genotypes, 14 crosses were observed that exceeded the average yield (close to 8 Mg ha^{-1}) (Figure 1); the average yield of commercial hybrids (< 7.0 Mg ha^{-1}) was considered outstanding, which demonstrated the hardiness of the crosses under adverse production conditions. Likewise, ten of them were found to have superior performance in Montecillo (Figure 1, quadrant I). It was also identified that crosses 1214, 1412, and 1312 were adapted to this location and had yields ≥ 8.5 Mg ha^{-1}. Although their performance was considered adequate, given the high GenxLoc interaction, these crosses were defined as suitable to be used in Montecillo for grain production.

Based on the criteria of Zobel *et al*. (1988), crosses 1213, 1314, 1812, 1218, 1418, 1217, 1318, 1712, and 1817 presented interaction values close to zero; consequently, they were identified as stable, with an average yield of 8.5 Mg ha^{-1}, and were defined as outstanding crosses for their yield and stability. Within this group, 4 crosses were also observed with contrasting behavior between direct and reciprocal cross for grain yield and environment interaction (1718-1817, 1813-1318 and 1317-1713, 1213-1312, respectively).

Despite the differences, common parents were identified in crosses 1713, 1318, 1213, and 1314, where parent 13 participated; and crosses 1812, 1213, 1217, and 1218, where parent 12 participated. It was deduced that, depending on their interaction with another line, parents 13 and 12 possess stability characteristics that could be inherited to their offspring, which shows the possibility of obtaining stable crosses from the identification of parents with this attribute

The grain yield of the commercial hybrids was lower than the overall mean, and they exhibited the largest interaction effects. These genotypes were associated with the Mixquiahuala and Tecamac locations. Although their phenotypic expression was lower than expected (because they are hybrids marketed in the region), it corresponds to their areas of adaptation: Águila®, Jabalí® and Caimán® are hybrids recommended for the Mezquital Valley, while H-San José® and H-San Josecito® are recommended for production conditions with water deficit. Caimán® had the highest GenxLoc interaction, and Jabalí® had the lowest grain yield. It was also suggested that the limited expression of the commercial hybrids, especially Águila®, Caimán® and Jabalí®, was due to the low water availability present in both locations; however, these restrictions helped to easily identify the potential of the experimental crosses and to differentiate them from the commercial hybrids.

Nine outstanding crosses were identified (Figure 1) that showed stability across locations and had acceptable yields superior to those of commercial materials. Their use is feasible for grain production in the evaluation localities and other similar ones.

Biochemical nutritional content of the grain

Analysis of variance showed that there were statistical differences ($p \leq 0.01$) in the three biochemical components evaluated in the grain of the twenty experimental crosses (Table 2).

The range of the biochemical nutrient content of the crosses (Table 3) showed a higher variation than that reported in maize grains by Arendt and Zannini (2013). It was also greater than the variation found in improved varieties (Méndez-Montealvo *et al*., 2005), hybrids from the High Valleys of Mexico (Vázquez-Carrillo *et al*., 2018), and in populations with high oil content (Vázquez-Carrillo *et al*., 2014).

In the present study, lower and higher contents were observed for the defined elements, and higher extremes were desirable.

Oil content fluctuated between 3.5 and 6.4 g 100 g^{-1} ms, with a mean value of 5.2 g 100 g^{-1}. Crosses 1718 and 1217 stood out, with oil concentrations greater than 6.0 g 100 g^{-1} ms, which exceeded the concentration found by Méndez-Montealvo *et al*. (2005), and were similar to the maximum value (6.7 %) found by Vázquez-Carrillo *et al*. (2014). According to the same authors, an average loss of 30 % of oil during the nixtamalization and tortilla manufacturing process was considered. Based on the results, we inferred that crosses with concentrations ≥ 5.0 g 100 g^{-1} can form soft, firm tortillas, with remarkable chewiness and an acceptable shelf life.

Regarding protein content, it was observed that, although there was variation (Table 3) and there were crosses with more than 9.0 g 100 g^{-1} of ms (1813, 1317, 1213, and 1812),

Table 2. Mean squares of analysis of variance for biochemical composition of grain in single crosses of white maize.

SV	DF	Oil	Protein	Starch
Genotypes	19	1.72[†]	4.46[†]	78.50[†]
Repetitions	2	0.004	0.27	14.07
Error	38	0.051	0.61	32.76
CV (%)		4.46	10.75	6.51

[†] $p \leq 0.01$; SV: Source of variation; DF: Degrees of freedom; CV: Coefficient of variation.

Table 3. Mean values of biochemical nutrient content of grain from single crosses of high yielding white maize.

Cross	Starch g 100 g^{-1}	Protein g 100 g^{-1}	Oil g 100 g^{-1}
1813	90.1 ab	9.8 a	3.5 k
1317	84.9 ab	9.2 ab	5.2 cdef
1213	72.6 b	9.1 abc	5.6 bc
1812	87.1 ab	9 abcd	5.4 cd
1318	86.1 ab	8.2 abcde	4.7 efghi
1413	85.6 ab	7.8 abcdef	4.5 fghi
1214	92.4 a	7.6 abcdef	3.7 jk
1412	85.9 ab	7.6 abcdef	5.5 cd
1417	95.5 a	7.2 bcdef	4.2 ijk
1314	85.3 ab	7.2 bcdef	4.5 ghi
1714	93.8 a	6.9 bcdef	5.3 cde
1712	87.1 ab	6.9 bcdef	4.7 efghi
1713	92.1 a	6.7 bcdef	5.3 cde
1718	87.7 ab	6.6 cdef	6.4 a
1217	87.2 ab	6.6 cdef	6.3 ab
1817	87.6 ab	6.4 ef	5.6 c
1218	84.6 ab	6.3 ef	5.1 cdefg
1814	89 ab	5.9 ef	4.9 efgh
1418	96.6 a	5.7 f	4.4 hij
1312	85 ab	5.5 f	5.6 cd
HSD	17.8	2.4	0.7

Concentrations expressed in g 100 g^{-1} of dry matter (dm). HSD: Honest Significant Difference. Means with different letters indicate statistical difference (Tukey, $p \leq 0.05$).

the grain of the twenty crosses had an average protein content in accordance with that reported by Arendt and Zannini (2013).

Concentrations were lower than those reported in hybrids for High Valleys (Vázquez-Carrillo *et al*., 2014), and also with respect to protein quality genotypes (Coutiño-Estrada and Vázquez-Carrillo, 2018).

The limited protein content of most genotypes, determined from grain produced under irrigated conditions, could be attributed to that found by Thomison *et al*. (2014). They reported that conventional hybrids exposed to irrigation conditions reduced their protein content compared to those exposed to drought during grain filling.

The variation in protein content associated with genotypes coincided with that reported by Aguilar-Carpio *et al*. (2016), who also mentioned that genotypes respond differently to fertilization rates. Thomison *et al*. (2014) found a consistent response of protein increase associated with nitrogen increase.

Aguilar-Carpio *et al*. (2016), in soils similar to those of the present study, found a positive response with doses of 160 kg ha^{-1} of N, similar to those used in the evaluation (120N-60P-00K).

Based on the above arguments, we considered that although the fertilization dose was adequate, protein expression and variation among crosses were defined by the genotype.

The starch concentration found in the crosses ranged from 72.6 to 96.6 g 100 g^{-1} of dm (Table 3); these values were higher than the ranges defined for previously studied maize (Arendt and Zannini, 2013), with different origin and level of improvement (Vázquez-Carrillo *et al*., 2018; Vázquez-Carrillo *et al*., 2014; Agama-Acevedo *et al*., 2011). Crosses 1214, 1417, 1418, 1713, 1714, and 1813 had more than 92 g 100 g^{-1} of dm and were rated as outstanding in this trait. Grain from these crosses is considered suitable for use in the food industry, where starch is used in a variety of processes to thicken, gelatinize, retain moisture, and improve the texture of a wide range of foods (Hamaker *et al*., 2019).

It is important to take into account that the rheological and functional characteristics of starch are determined by the amylose:amylopectin ratio and the structure of its molecules (Salinas-Moreno *et al*., 2003), which is why studies on the starch characteristics of these corns are required.

Commonly, starch in a corn kernel is represented by 75 to 80 % amylopectin, and 20 to 25 % is amylose (Hamaker *et al*., 2019).

A modification of the proportions promotes changes in the hardness, color and moisture conservation of the tortillas (Salinas-Moreno *et al*., 2003); therefore, although the starch content of the crosses was outstanding, they also require complementary studies to know with greater certainty the final destination of the grain produced.

Grain yield stability and biochemical nutrient content

When grain yield stability and nutrient content were analyzed together, it was found that the stable crosses with acceptable yield (≥ 8.0 Mg ha^{-1}) were: 1213, 1217, 1712, 1218, 1812, 1314, 1318, 1418, and 1817 (Figure 1); these also had outstanding starch content (> 85 g 100 g^{-1} of dm), with the exception of 1213 (72.6 g 100 g^{-1} of dm).

Cross 1418 showed the highest value (96.6 g 100 g^{-1} of dm). Since corn starch represents one of the main sources of this compound, it is considered that these crosses could be directed towards its production and benefit. However, it is still necessary to identify the type and quality of starch in each for optimal utilization. The high starch content of the crosses was associated with racial origin, although the variation exceeded the content found by Agama-Acevedo *et al*. (2011) in populations of the Chalqueño landrace.

Cross 1217 showed acceptable yield and stability, as well as high starch (≥ 85 g 100 g^{-1} of dm) and oil (6.3 g 100 g^{-1} of dm) content, which are attributes that can improve the rheological characteristics of tortillas made with this grain, according to Vázquez-Carillo *et al*. (2014).

Although cross 1812 was found to have acceptable yield, stability and starch content, and ranked among the crosses named as outstanding for protein concentration (9.0 g 100 g^{-1} of ms), its protein content was considered to be in the normal range of variation, in agreement with the values reported by Vázquez-Carrillo *et al*. (2014) and Vázquez-Carrillo *et al*. (2018).

In general, it was observed that the higher the protein content, the lower the starch concentration. This behavior had already been described by Vázquez-Carrillo *et al*. (2014), who found significant correlations of oil and protein (r = 0.33), oil and starch (r = -0.39) and starch and protein (r = -0.84). Lambert *et al*. (1998) explained that oil and starch accumulation are mutually exclusive.

Based on the results obtained, despite finding crosses with high grain yield and starch content, no relationship was observed between grain yield and biochemical content, which was anticipated, given that no direct selection had been made for the specific quality components evaluated, but basically for yield. However, it is possible to commercially exploit outstanding genotypes and initiate selection for biochemical components based on the characterization of nutrient content and yield expression.

With respect to stability, grain yield and nutrient content, crosses 1213, 1217, 1218, 1314, 1418, and 1812 were outstanding, with significant starch content. This is deduced by taking as a reference the values of Vázquez-Carrillo *et al*. (2014) and Vázquez-Carrillo *et al*. (2018), from which the grain can be destined to the production of quality tortillas. Because of the oil content, they could also be improved in softness, firmness and chewiness (Vázquez-Carrillo *et al*., 2014); however, studies of the nixtamalization and industrialization processes are required.

CONCLUSIONS

Of all the experimental crosses evaluated, six (1812, 1314, 1213, 1217, 1418, and 1218) showed outstanding characteristics of stability, grain yield and starch and oil content; therefore, in the breeding program defined, there is genetic material with outstanding characteristics that could be used commercially. There was also sufficient genetic diversity to continue improving nutrient content, particularly starch and oil. Protein content, although with variations, was considered basic and in accordance with a normal corn grain.

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