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Biochemical and nutritional characterization of the kernel from whithe maize (*Zea mays* L.) single crosses

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ABSTRACT

Objective: To characterize the nutritional and biochemical content of 25 genotypes: 5 S_4 inbred lines and the 20 possible single crosses formed between them.

Design/Methodology/Approach: The content of oil, starch, protein, ash and phytic acid was determined in kernels of each genotype, using the American Association of Cereal Chemists' methods; the information obtained was analyzed by a complete randomized experimental design and Tukey's means tests.

Results: For the parents and the crosses, correspondingly, the intervals of the substances under study were: a) Oil: from 5.99 to 3.84 and 6.40 to 3.55 $g \cdot 100\ g^{-1}$, b) Protein: from 8.26 to 5.43 and 9.83 to 5.56 $g \cdot 100\ g^{-1}$, c) Starch: from 88.25 to 74.48 and 96.64 to 72.57 $g \cdot 100\ g^{-1}$, d) Ash: from 1.90 to 1.20 and 2.0 to 0.89 $g \cdot 100\ g^{-1}$, e) Phytic acid: from 2.40 to 1.08 and 2.29 to 1.11 $g \cdot 100\ g^{-1}$.

Study Limitations/Implications: The study shows that in comparison to the parents, the crosses were only significantly superior in the content of starch, although there were statistical differences of the contents within each group. The crosses that showed higher contents for a nutritional component were those in which at least one parent had a high composition of the nutrient.

Findings/Conclusions: The variation in the nutritional and biochemical content showed that there is diversity among genotypes, which is linked to the contrasting genetic origin of the inbred lines and is feasible to be used. This research showed the potential of taking advantage of the nutritional components of white maize through crosses (specially its starch content).

Key words: *Zea mays* L., corn breeding, starch, oil, phytic acid

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INTRODUCTION

Food insecurity and malnutrition, in the middle of the 21st century, continue to be a global problem; the figures indicate that in the year 2017, 821 million people (11% of the global population) suffered hunger and some type of poverty, primarily food poverty (FAO, 2019), and in Mexico, 53.4 million inhabitants have this hindrance, of which 46% suffer lack of food (CONEVAL, 2017); this is despite the country being the center of origin


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of a broad variety of plant species of local and even global importance. Maize (*Zea mays* L.), because of its carbohydrate content, is one of the three principal sources of energy of the global population (Serna-Saldivar and Pérez, 2019), and in Mexico it is the main food source, although it is also important for socioeconomic and cultural reasons, because the country is its center of origin, domestication and diversification.

In Mexico, technical genetic breeding has been adapted from North American breeding programs centered primarily in hybridization, regarding traits associated with the production of cobs and grain yield; however, despite the importance and destination of the production, grain quality has received less attention. Authors such as Pollak and Scott (2005) showed that the nutritional content of the plant species through time has declined and they associated this effect to the use of new varieties; however, in recent years, facing the revaluation of genetic diversity, initiatives for improvement have been proposed and developed with the objective of improving the nutritional quality and value.

Currently, demands from society lead to the development of new products with functional properties that provide the nutritional value and other components that improve the standard of living. Among the genetic resources of maize, there are native landraces, which have genetic wealth that can contribute to obtaining or increasing the nutritional value (Serna-Saldívar, 2013; Cázares-Sánchez *et al.*, 2015). Based on this, the biochemical-nutritional content of a group of inbred lines and their respective single crosses, which were evaluated previously for grain yield, was determined and characterized. The hypothesis considered that, given the genetic diversity among the origin of genotypes, the nutritional content of the lines and their respective crosses would be differentiated, and the selection with traits of nutritional and industrial revaluation could be possible.

MATERIALS AND METHODS

The biochemical and nutritional evaluation was carried out with n=25 genotypes, five corresponding to the lines S_4 and the possible single crosses (20) between them. The lines were selected through the *per se* test and from breeds in 2015. In the racial origin, variants and mixtures between the races Cónico and Celaya are distinguished.

In the year 2017, the dialelic cross scheme was developed under the Design I (p^2) by Griffing (1956) and the evaluation of grain yield was conducted in three localities of the area of Valles Altos de Mexico in 2018.

For the biochemical analysis, F_2 seed and from the lines were obtained in an alternate lot through controlled fraternal pollinations. The grain used for the evaluation derived from adequate agronomic conditions for the production such as fertility, irrigation, pest control and diseases.

For the determination of biochemical content, a balanced mixture that included grains from the middle third of five cobs from each genotype was formed, according to the methodology proposed by Galicia *et al.* (2012) for biochemical tests in maize. The grains were put through a cyclone grinder (UDY Corporation®) to obtain flour with particles of 0.5 mm. The weight of the samples, the reagents, and the other materials were recorded in an analytical balance AND® model GR 202.

The methods used for the biochemical analysis were the following: for oil the method used was 30-25.01, protein through Kjeldahl: method 46-11.02, the ashes through the method 08-01.01, all from AACC (2000), and phytic acid through the method by Dragiĉević *et al.* (2011).

The determination of starch was done in two stages. First, that of extraction through the methodology by Brunt (1998) with ethanol washes of the samples to eliminate soluble sugars, and to keep complex sugars that are part of the starch. The second consisted in dissociating the complex to simple sugars through the protocol 76-13.01 of the AACC (2000). The concentration of total starch was determined through the quantification of glucose through the colorimetric method by Antrona. The results were expressed in g 100 g^{-1} of dry matter (dm), based on a standard curve ($y=3.4943x-0.012$, $R^2=0.997$) prepared with glucose (Sigma Aldrich®). The determination of biochemical content was carried out in the Unit of Laboratories of the Puebla Campus from Colegio de Postgraduados.

The analysis of the biochemical information was conducted through an analysis of variance using a completely random design, in addition to conducting Tukey's means test to identify the differences between the parents, the crosses, and between both where they existed.

RESULTS AND DISCUSSION

The analysis of variance showed significant differences between genotypes (lines+crosses) ($P\leq0.01$) for the nutritional content (Table 1); these were considered associated to the groups, that is, lines and crosses. However, between groups (lines and crosses), differences were found only in the content of starch and the yield ($P\leq0.01$).

The lines showed differences ($P\leq0.01$) for the biochemical components, which are associated with their genetic origin. The crosses also showed variation ($P\leq0.01$) indicating the possibility of selecting superior ones and the characterization of the behavior of parents within the crosses.

Genotype comparison (lines+crosses)

Among genotypes, the differences (lines+crosses) were not the ones expected (Table 2); finding an overexpression in the crosses of the lines as commonly happens in the grain yield was considered; this phenomenon happened only for starch, so it was considered that

Table 1. Analysis of variance of the nutritional biochemical components and grain yield of *Zea mays* L. determined in lines S_4 and their respective single crosses.

SV	DF	Yield	Oil	Protein	Starch	Ashes	Phytic Acid
Replications	2	1.08	0.023ns	0.36ns	45.97ns	0.0085ns	0.006ns
Genotypes	24	18.36	1.90**	4.36**	93.35**	0.33**	0.356**
Inbred lines	4	3.75	3.12**	4.30*	93.84**	0.33**	0.69**
Crosses	19	10.67**	1.72**	4.44*	78.5**	0.34**	0.29*
Inbred lines/Crosses	1	207.1**	0.55ns	2.69ns	373.39**	0.156ns	0.088ns
Error	48		0.045	0.74	29.1	0.004	0.051

SV=Source of variation; DF=Degrees of freedom; **=$P\leq0.01$.

Table 2. Nutritional biochemical concentration and grain yield of *Zea mays* L. in the parent lines of single crosses.

Inbred lines	Yield	Oil	Protein	Starch	Ashes	Phytic Acid
P6	6.2	4.67[b]	7.29	79.25[ab]	1.20[c]	1.66[bc]
P7	5.7	3.84[c]	8.26	74.48[b]	1.57[b]	1.85[ab]
P8	7.2	5.80[a]	5.76	82.52[ab]	1.84[a]	2.40[a]
P9	6.4	5.99[a]	5.43	86.68[ab]	1.90[a]	1.97[ab]
P10	6.3	3.90[c]	7.45	88.25[a]	1.21[c]	1.08[c]
Inbred lines	6.36[B]	4.84	6.84	82.2[B]	1.55	1.79
Crosses	8.4[A]	5.05	7.31	87.7[A]	1.43	1.70
$HSD_{Lin\ Cr}$	0.44	0.47	0.80	3.09	0.19	0.22
HSD_{Lines}	1.81	0.42	3.29	12.28	0.14	0.64
C. V.	4.06	3.09	17.08	5.29	3.26	12.81

†The biochemical concentration of each element is shown in g·100 g^{-1} of dry matter; Rto=Grain yield (t ha^{-1}); DMS=Minimum significant difference, C.V.=Coefficient of variation (%). The letters show the grouping according to Tukey's mean test.

the genetic effect that determines the expression of the biochemical characteristics is not the same in every case; in this sense, Núñez-Terrones *et al.* (2019) and Bisen *et al.* (2017) found that the non-additive genetic effects prevailed over the additive effects; in the first case, this behavior is justified through the previous exhaustion of the additive variance through the selection.

The grain's nutritional biochemical content of the lines (Table 2) showed variation for each of the components, except protein; the components, according to Kirk *et al.* (1996) are fundamental for human diet and nutrition, since they are part of the primary metabolism and, therefore, the improvement of this content is widely justified.

Among the lines, the interval of variation was 7.2 to 5.7 t ha^{-1} for Rto; of 5.99 to 3.84 g·100g^{-1} dm for the oil; of 8.26 to 5.43 g 100 g^{-1} dm for the protein; of 88.25 to 74.48 g 100 g^{-1} dm for the starch; of 1.90 to 1.20 g 100 g^{-1} dm for the ash; and of 2.40 to 1.08 g 100 g^{-1} dm for phytic acid (Table 2).

According to Arendt and Zanini (2017), a typical maize grain is composed of 70-75% starch, 8 to 10% protein, 4 to 5% lipids, 1 to 3% sugars, and 1 to 4% ash; therefore, it was determined that the lines studied presented a high starch content and a basic nutritional content of ash and protein, which compared to QPM (Quality Protein) produces 12%.

A similar classification could be done based on the results from Corcueca *et al.* (2016) and Mendez-Montealvo *et al.* (2005). Cázares-Sánchez *et al.* (2015) point out that quantity of protein and oil in the maize grain is not high compared to that of other cereals, although there is variation that should be taken advantage of in plant breeding, since these characteristics confer quality in products such as tortilla.

For the case of oil, the variation was similar to that found by Martínez *et al.* (2009) in their evaluation of 50 maize accessions in Cuba, and they considered this interval as normal, typical of the maize grain. For the case of ash or minerals, the content was also considered similar to the one common in maize grains.

The content of phytic acid, 90 % of what was found in the germ and the main source of phosphorus in the seed, also showed variation; according to Hurrell (2004), the parents showed high concentration of this "anti-nutrient", although according to Serna-Saldivar *et al.* (2013), the lime used during the *nixtamalización* process can reduce at least 25% of the phytic acid, allowing higher bioavailability of iron, primarily.

According to the nutritional concentrations among parents (Table 2), and to the normal concentration for maize grain, according to Arendt and Zanini (2017), the P8 and P9 parents stood out for oil, with 5.88 and 5.90 g 100 g^{-1}, respectively; P7 with more than 8.0 g 100 g^{-1} for protein; P8, P9 and P10, with more than 80 g 100 g^{-1} for starch; P8 and P9 with higher mean values than 1.80 g 100 g^{-1} for ash; P10 with 1.08 g 100 g^{-1} dm of phytic acid. According to the concentrations, the P8 and P9 parents were considered to be prominent and the most limited was P6.

Nutritional biochemical content of the grain in single crosses

The interval of variation for each of the nutritional biochemical components for the crosses was 6.40 to 3.55 g·100 g^{-1} for oil; 9.83 to 5.56 g 100 g^{-1} for protein, 96.64 to 72.57 g 100 g^{-1} for starch, 2 to 0.89 g 100 g^{-1} for ash, and 2.29 to 1.11 g 100 g^{-1} for phytic acid (Table 3). The intervals, compared with those obtained in the parents, showed a higher value in favor of the crosses in the content of oil, protein and starch.

The results showed that the crosses with highest nutritional content were those where at least one parent with a higher content of the desired element participated. Therefore, for the evaluation (Table 3), the highest content of oil was attained through the crosses P9×P10 and P6×P9, with P9 being the line of highest oil content.

For protein, the crosses P6×P7, P7×P9 and P10×P7 stood out, where P7 is involved, both in direct and reciprocal cross, with the highest protein content; for starch, the crosses

Table 3. Yield and nutritional biochemical content of the white grain in single crosses of *Zea mays* L. from Valles Altos of the Mexican Central Plateau.

Cross	**P6×P7**	**P7×P6**	**P6×P8**	**P8×P6**	**P6×P9**	**P9×P6**	**P7×P8**	**P8×P7**	**P7×P9**	**P9×P7**	**P8×P9**
Yield	8.2	8.3	9.1[a]	8.7	8.5	8.8	8.1	8.7	9.7[a]	8.3	10.3[a]
Oil	5.61	5.66	3.75	5.51	6.36[ab]	4.73	4.56	4.55	5.25	5.29	4.22
Protein	9.06	5.56[f]	7.63	7.55	6.58	6.92	7.16	7.83	9.16	6.75	7.16
Starch	72.57[b]	85.04	92.4	85.89	87.2	87.14	85.26	85.64	84.9	92.13	95.51
Ashes	1.98	1.74	1.01	1.35	1.34	1.39	1.74	1.41	1.79	1.29	1.13
Phytic Acid	2.29[a]	1.91	1.27	1.77	1.94	1.9	1.89	1.85	1.93	1.6	1.29
Cross	**P9×P8**	**P6×P10**	**P10×P6**	**P7×P10**	**P10×P7**	**P8×P10**	**P10×P8**	**P9×P10**	**P10×P9**	**Media**	**DMS**
Yield	5.9	7.2	7.8	8.6	8.6	8.2	9.1	7.9	8.8	8.4	1.61
Oil	5.33	5.14	5.46	4.73	3.55[k]	4.41	4.95	6.40a	5.66	5.05	0.7
Protein	6.94	6.28	9.02	8.24	9.83[a]	5.72	5.86	6.63	6.41	7.31	2.44
Starch	93.82	84.6	87.09	86.13	90.07	96.64[a]	88.97	87.74	87.6	87.81	17.77
Ashes	1.09	1.39	0.89[g]	1.68	0.92	1.32	2.00[a]	1.36	1.89	1.43	0.21
Phytic Acid	1.46	2.14	1.11[d]	1.89	1.27	1.51	1.77	1.89	1.52	1.71	0.7

*The biochemical concentration of each element shows in g 100 g^{-1} of dry matter; Yield t ha^{-1}; DMS=Minimum significant difference; C.V.=Coefficient of variation.

P6×P8, P9×P7, P8×P9 and P8×P10 stood out, where P8, P9 and P10, which showed high starch contents, participated.

The results showed the possibility of improving the grain quality through breeding between lines with higher expression for the desired trait.

Among the crosses, the variation of the grain yield was 10.3 t ha^{-1} to 5.9 t ha^{-1}; regarding the diversity, four outstanding crosses were found with yield higher than 9.0 t ha^{-1} (P6×P8, P7×P9, P8×P9 and P10×P8), where the nutritional content in the crosses according to the classification by Arendt and Zanini (2017) showed prominent concentration of starch, exceeding any of the contents reported by Mendez-Montealvo *et al.* (2005), and therefore it was considered that the main use and exploitation of the crosses can be associated to this type of industry and their byproducts.

Based on this, subsequent studies associated with the specific analysis of the quality of starch and its particular uses, as well as nixtamalización for the elaboration of tortillas, are suggested; and in addition, according to Vázquez-Carrillo *et al.* (2014), the content of protein and oil of the outstanding crosses was considered acceptable and therefore it is expected that the tortillas made with this maize have prominent firmness and chewability, as well as good shelf life.

In the crosses, which had less attractive nutritional concentration, trends or behaviors associated to parents of higher concentration were not defined, although they were for the case of lower content, where at least one parent of lower concentration participated; in oil, the cross P10×P7; for protein, the direct and reciprocal cross, P8×P10 and P10×P8; for starch, P6×P7, where an effect of genetic complementarity was found between the parents that could also be associated with limited genetic divergence.

Compared to other studies, the oil concentration was similar to what was reported by Mendez-Montealvo *et al.* (2005) in hybrids and cultivated varieties in Mexico; therefore, it was considered that although the outstanding crosses from the study are not superior, they can fulfill the necessary requirements for quality in the market, in addition to continuing with the genetic improvement of nutritional content together with the grain yield.

For the case of proteins, the content can be unattractive if the expression of the QPM (12% protein) is considered, although the content was considered normal for the maize grain. In the study, the cross of highest protein content was the one of lowest oil concentration; however, Pearson's correlation analysis (data not shown) did not present significant correlations between the biochemical contents, which contrasted with the results by Corcueca *et al.* (2016) and Scrob *et al.* (2014), who mentioned the negative correlation between protein and starch.

In the case of starch, five crosses showed prominent content (P6×P8, P9×P7, P8×P9, P9×P8, P10×P7, P8×P10), of which two were of satisfactory yield (P6×P8 and P8×P9), highlighting the cross between parents P9 and P8, given that both versions stood out, direct and reciprocal; in addition, in each of the outstanding crosses, at least one prominent parent participates based on its starch concentration: P8, P9 or P10.

In the ash content, crosses P6×P7 and P10×P8 stood out, with concentrations of 1.98 and 2.0 g 100 g^{-1}, which were higher than those found by Scrob (2014), although the study showed a greater interval of variation. For the case of phytic acid, P6×P7 and P6×P10 exceeded 2.0 g 100 g^{-1} that were considered high and undesirable given the chelating power of phytates.

In the general data analysis, there are crosses of outstanding yield with prominent content of some nutritional component, which can be used for grain production; others where the yield was not satisfactory in the commercial sense but which present high content of some element and can continue in the breeding process.

The results showed that the particular content of a specific nutritional component is associated to the specific interaction between parents; however, it can be seen that a single cross of outstanding behavior for a nutritional component is associated with the participation of at least one parent with a high composition of the nutrient.

CONCLUSION

The variation in the nutritional biochemical content of the genotypes evaluated showed diversity that is possible to be used; also, the evaluation showed the potential of use of the nutritional components through crosses, with starch being the main usable component.

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