Protein content and quality of seeds in central mexican maize (*Zea mays*) accessions

Contenido de proteína y calidad de semilla en accesiones de maíz (*Zea mays*) del centro de México

César Leobardo Aguirre-Mancilla [1 *], Rosario Yarandín Godínez-Galán [1], Juan Carlos Raya-Pérez [1], Glenda Margarita Gutiérrez-Benicio [1, 2], Juan Gabriel Ramírez-Pimentel [1], Jorge Covarrubias-Prieto [1], J. Guadalupe García-Rodríguez [1]

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Abstract

Mexico is the center of origin, domestication and diversity of maize. This cereal is the main constituent of the Mexican diet, especially for low-income families. In this research, 10 maize accessions derived from a large landrace collection, with the 'INIFAP-QPM' accession and a 'regional landrace' as controls, were studied to identify the accessions with the best biochemical and physiological characteristics showing good adaptation to 'El Bajío' (regional center in Mexico) conditions. The accessions were statistically superior to the two controls in the germination and accelerated aging tests. In the assessment of variable plumule length, variability was observed among the accessions, but the controls showed the lowest values. Protein contents in different fractions (albumins, globulins, prolamins and glutelins) showed variability as did oil and fiber contents. The 'HRH2015' accession showed high contents of albumins and globulins and low contents of prolamins and glutelins. The 'regional landrace' accession exhibited the highest contents of glutelins and prolamins but the lowests content of globulins and albumins. The total percentage of proteins showed variability among the accessions, but the values were within those reported in the literature. The 'RQ2015' accession presented the highest oil content (5.25%). The electrophoretic patterns of prolamins were obtained, and some differences were observed between them. The 'regional landrace' presented the lowest protein content, which was significantly different from those of the other evaluated accessions. This research demonstrates biochemical, germination and vigor variability among the studied maize accessions.

Keywords
Zea mays • corn • protein fractions • vigor • quality protein

1 Tecnológico Nacional de México/I. T. Roque. km 8 Carretera Celaya-Juventino Rosas. CP. 38110. Roque. Celaya. Guanajuato. México. * ceaguirre@itroque.edu.mx
2 Universidad de Guanajuato. Campus Celaya-Salvatierra. Sede Mutualismo. Celaya. Guanajuato. México.

Resumen

México es el centro de origen, domesticación y diversidad del maíz. Este cereal ocupa el primer lugar como constituyente de la dieta de los mexicanos, sobre todo de las familias de bajos recursos. En esta investigación se estudiaron 10 accesiones de maíz derivadas de una colecta amplia de Criollos y como testigos INIFAP-QPM y un criollo de la región, con el objetivo de identificar las accesiones con mejores características bioquímicas y fisiológicas con buena adaptación a las condiciones de El Bajío. Las accesiones fueron estadísticamente superiores a los dos testigos en las pruebas de germinación y envejecimiento acelerado; en la variable longitud de plúmula se observó variabilidad entre las accesiones, pero los testigos siguieron mostrando los valores más bajos. El contenido de proteína en sus diferentes fracciones (albúminas, globulinas, prolaminas y glutelinas) mostró variabilidad, así como el contenido de aceite y fibra. Se encontró una accesión (HRH2015) con alto contenido de albúminas y globulinas y bajo contenido de prolaminas y glutelinas; el 'genotipo criollo' presentó el mayor contenido de glutelinas y prolaminas pero el menor en globulinas y albúminas. El porcentaje total de proteínas presentó variabilidad entre las accesiones, pero los valores están dentro de los reportados en la literatura. El genotipo 'RQ2015' presentó el más alto contenido de aceite (5,25%). Se obtuvieron los patrones electroforéticos de las prolaminas y se observaron algunas diferencias entre ellas. El criollo de la región, tomado como referencia, presentó el menor contenido de proteína estadísticamente diferente al resto de las accesiones evaluadas. Esta investigación demuestra la variabilidad existente a nivel bioquímico, germinación y vigor entre las accesiones de maíz estudiadas.

Palabras clave

Zea mays • maíz • fracciones proteicas • vigor • calidad proteica

Introduction

Maize is the main grain cultivated in the "El Bajío" region of Mexico and is the principal ingredient in the Mexican population's diet (15, 30). Information on the chemical composition of corn grain is abundant and clearly states that the variability of each component is broad as a result of both genetic and environmental factors (20, 28). These factors can influence the chemical composition of different parts of the grain (11).

The prolamin fraction constitutes the highest proportion of the protein present in cereals, followed by glutelins and in smaller amounts albumins and globulins. The nutritional quality of the protein as well as its physicochemical and functional characteristics, depend on the proportion of each protein fraction in the grains (37). Cereal proteins present low biological or nutritional value because they are deficient in some of the essential amino acids, such as methionine and lysine (19, 38). These proteins include wheat gliadins, barley hordeins, corn zeins (prolamins), wheat glutelins, and rice orizein.

High-quality-protein materials contain higher amounts of the albumin and globulin fractions and lower amounts of prolamins, as indicated by Vivas-Rodriguez *et al.* (1990) and Yang *et al.* (2018). The expression of the physicochemical characteristics of maize is influenced by the environment

and depends on genotype-environment interaction (2). Agricultural practices have allowed the characteristics of the grain to be improved according to Zepeda-Bautista *et al.* (2009). Prolamins are stored in the starchy endosperm, and albumins and globulins are concentrated in the embryo and aleurone layer (19). The distribution of these proteins could alter some properties of the grain, depending on their relative abundance. Nitrogen application affects grain protein content. Its effect is greater in the prolamin fraction because approximately 60% of the grain protein consists of prolamins (zeins). The albumin and globulin fractions represent 22% of the protein content and are concentrated in the embryo. Glutelins are found in both the germ and the endosperm and account for up to 25% of the grain protein (26, 38).

Ten gene families encode prolamin proteins in maize, with 3-10 genes in each family (19). Corn contains 7-13% protein, which can be differentiated into three types: 1) storage proteins, which are the most abundant type, constituting a reserve of amino acids deposited during the development of the seeds; 2) enzymes involved in metabolism; and 3) structural proteins (23, 39).

Although studies have been conducted on this topic, more research on the protein quality of maize landraces cultivated in Mexico is required (27). Evaluation of the performance of different accessions in different environments and the influence of agronomic practices on the composition of essential amino acids, which determine the quality of grain proteins, is also required (37). Finally, it is vital to keep in mind that, for breeding purposes, to consider seed vigor is also necessary. This characteristic is usually tested with the accelerated aging test (17). With this objective, maize accessions were evaluated to characterize them biochemically and physiologically. They were integrated from a broad genetic base population (3). The 'INIFAP-QPM' accession and a maize landrace were used as controls.

The objective of this research was to identify accessions with good protein and physiological quality among accessions that are well adapted to the environmental conditions of El Bajío, Mexico, under the hypothesis that the accessions show good seed quality.

Materials and methods

Ten maize accessions derived from the Celaya, Tuxpeño, Cónico Norteño and Bofo races were used (14): '12015', '22015', 'A2015', 'ROQUE2', 'POL2015', 'FVR12015', 'ERH2015', 'MRH2015', 'RQ2015' and 'HRH2015' (12, 27), along with 'INIFAP-QPM' (accession with high protein quality from the "Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias") and a 'regional landrace' harvested in the SS 2015 growing season. The analyzed accessions were derived from collections from the Mexican states of Michoacan, Hidalgo, Estado de México and Puebla. Each collection was developed from 200 plants. Standard germination (18) was evaluated; a sample of 200 seeds from each accession was obtained, and each sample was divided into four replicates of 50 seeds (12) and incubated in a germination chamber (hel-La HC30R®) at 25°C with 80% relative humidity. Two sets of records were obtained: the first was obtained on the fourth day and the second on the seventh day. Germination was registered as the percentage of seeds with emerged radicles (18, 21). The plumule length test (21) was carried out. In the accelerated aging tests, a sample of 40 seeds collected at random

were disinfected with 1% hypochlorite in water (v/v) and then placed in a beaker with a grid. Water was poured into the beaker to a height that did not reach the grid, and the beaker was covered with aluminum foil and placed at 42°C with 80% relative humidity for 72 h. Thereafter, the seeds were removed, and the standard germination test was performed (17).

Protein and oil extraction was performed by grinding the seeds (20 g each accession) in a mill (Techmark ® model A 10), followed by sieving through a No. 60 mesh. The samples were placed in paper bags and stored at 4°C until use. Oil extraction was performed in Soxtec® equipment; the flour was placed in filter paper cartridges at a ratio of 4:1 solvent - sample (hexane) (V/W) in the cup which was then stored in a desiccator for 24 h. Thereafter, the cup was mounted in the equipment. Protein extraction was carried out according to a previously described methodology (12, 25) using 1 g of flour and 4 mL of distilled water. After incubation for 1 h with shaking, the extraction solution was centrifuged for 1 h at 13,200 rpm (in a microfuge). The supernatant was then stored, and the pellet was re-extracted with a solution of 0.3 M NaCl in 50 mM Tris-HCl, pH 8, followed by centrifugation under the conditions described above, this second supernatant was stored. Supernatant was stored. The pellet was extracted two more times: once with 55% isopropanol and then with 50 mM sodium borate solution and 0.1% SDS (sodium dodecyl sulfate), pH 9. Protein quantification was carried out via the Bradford method (4). Electrophoretic patterns were obtained under denaturing conditions in 10% polyacrylamide gels (PAGE-SDS) following the technique of Schagger and von Jagow (1987).

Statistical analysis of physical and physiological quality and protein content traits was performed with the statistical package SAS version 9.0. Statistical tests of the homogeneity of variance assumptions and normality of the data distribution were carried out. Comparisons of means were conducted with Tukey's test ($\alpha = 0.05$).

Results and discussion

The analysis of variance (data not shown) showed significant differences between the accessions for germination percentage, with a coefficient of variation of 1.77%. The experiment was conducted properly, and the degree of precision in the comparison of the accessions was good (13). The germination percentage trait was statistically equal for all accessions (table 1, page 18) but the 'INIFAP-QPM' and 'regional landrace' controls, showed germination percentages of 82 and 87.3%, respectively. Similar results were obtained in the accelerated aging test. The results showed that the evaluated accessions exhibited good adaptation to the environmental conditions of El Bajío, while the controls were statistically inferior in the mentioned tests. This result is in agreement with the findings of Gutiérrez-Hernández *et al.* (2011), who observed differences in tolerance to artificial aging among accessions of blue corn in different maize accessions. Another previous study (12) of 22 accessions, showed 4 accessions with 99% germination after being subjected to accelerated aging. Artificial aging allows low-vigor seeds with a high germination percentage that lose germinative ability when subjected to this treatment, to be distinguished. These previous results are in agreement with those obtained in the present investigation, in which the evaluated accessions showed higher tolerance to accelerated aging, indicating higher seed vigor (8).

Table 1. Means comparison by the Tukey test for the germination percentage, accelerated aging test and plumule length traits in the evaluation of 10 maize accessions, Roque, Guanajuato. Spring-Summer 2015.

Tabla 1. Comparación de medias mediante la prueba de Tukey para las variables porcentaje de germinación, envejecimiento acelerado y longitud de plúmula en la evaluación de 10 accesiones de maíz, Roque, Guanajuato. Primavera-Verano 2015.

Accession	GER[1] (%)	AA[2] (%)	PL[3] (cm)
12015	96.6 a	94.0 a	5.6 f
22015	98.6 a	97.3 a	6.9 ef
A2015	99.3 a	98.0 a	12.6 a
ROQUE2	98.0 a	96.6 a	10.4 c
POL2015	99.3 a	99.3 a	11.3 abc
FVR12015	98.6 a	97.3 a	11.9 ab
ERH2015	98.6 a	98.6 a	10.9 bc
HRH2015	95.3 a	94.0 a	8.4 d
MRH2015	97.3 a	96.6 a	9.1 d
RQ2015	98.6 a	97.3 a	7.9 de
INIFAP-QPM	82.0 c	40.0 c	4.3 f
Regional landrace	87.3 b	83.3 b	6.6 f
Mean	95.83	91.05	8.85

Means with the same letter for each trait are statistically equal, Tukey's test, P≤0.05.
[1] Germination percentage; [2] Accelerated aging; [3]Plumule length.

Medias con la misma letra dentro de cada variable son estadísticamente iguales, Tukey, P≤ 0,05.
[1] Germinación; [2] Envejecimiento acelerado; [3]Longitud de plúmula.

Many factors participate in seed physiological behavior, such as oligosaccharides, which play an important role in seed longevity (16). Methionine sulfoxide reductase activity, tocopherol content, late embryogenesis abundant (LEA) protein accumulation and heat shock proteins (HSPs) (6) as well as changes in membrane permeability (34) are important factors affecting seed vigor.

Plumule length showed higher variation than the two previous traits. Accessions A2015 and FRV12015 exhibited the highest, however statistically similar, seed vigor. This variable best indicates physiological quality. Once more, INIFAP-QPM and Regional landrace, both controls, presented the lowest values together with 12015 and 22015 accessions. This result indicates that these accessions exhibited little vigor, since they obtained the lowest values in these three traits. In this research, plumule length was more effective in evaluating seed vigor, given that it allowed discrimination among them.

Vigor traits and accelerated aging

Analysis of variance (not shown) of the vigor traits indicated significant differences between the accessions with a coefficient of variation of 2.43%, which indicates reliable results. In the means comparisons (table 1), the 'POL2015', 'A2015' and 'FVR12015' accessions showed the highest values for the three traits, indicating that they are vigorous accessions, whereas the 'INIFAP-QPM' accession showed the lowest vigor value. The storage of seeds under

adverse conditions causes aging, which results in a variety of symptoms ranging from a reduction in viability or a decreased ability to germinate, to poor seedling development (24). Stress causing aging due to conditions to which seeds are subjected, provokes higher reserve depletion, reducing seed vigor. The accumulation of proteins such as late embryogenesis-abundant (LEA) proteins, proteases and amylases in the embryo, may confer higher tolerance in breeding (19).

Analysis of variance (not shown) of the protein fraction content showed highly significant differences between the accessions, indicating that the accessions have contrasting characteristics. The coefficient of variation for total protein was good (CV 2.98) (13). Tandag-Silvas *et al.* (2011) noted that the classification of extracted proteins is a convenient approach for studying seed storage proteins due to the relative ease of protein extraction.

The results of the means comparisons (table 2) showed that accession 'HRH2015' presented the highest total soluble protein content of 3.48 g per 100 g flour, which was significantly different from that of 'FVR12015' (3.05 g). The accession with the lowest soluble protein content was the 'regional landrace' (1.75 g of protein) (9).

The QPM accession showed the highest protein quality because its endosperm content includes high levels of albumins and globulins (rich in lysine and tryptophan) with a decreased zein content. These proteins constitute the major fraction of storage proteins, accounting for 50 to 70% of the proteins (33).

Table 2. Means comparison by the Tukey test for the soluble protein contents of maize accessions. Roque, Guanajuato Spring-Summer 2015.

Tabla 2. Comparación de medias mediante la prueba de Tukey para contenido de proteína soluble de los genotipos de maíz. Roque, Guanajuato Primavera-Verano 2015.

Accession	ALB[1]	GBL[2]	PRL[3]	GLT[4]	TOTAL[5]
12015	1.28def	1.11bc	0.26bcd	0.03e	2.69cde
22015	1.31cde	1.19ab	0.26bcd	0.16c	2.93bc
A2015	1.36bc	1.12bc	0.35ab	0.05de	2.90bcd
ROQUE2	1.18f	1.32ab	0.17d	0.04de	2.71cde
POL2015	1.22ef	1.16bc	0.19cd	0.05de	2.63e
FVR12015	1.42bc	1.28ab	0.26bcd	0.083d	3.05b
ERH2015	1.44b	0.95c	0.21cd	0.05de	2.67de
HRH2015	1.61a	1.41a	0.38a	0.07de	3.48ª
MRH2015	1.22ef	1.21ab	0.39ª	0.06de	2.88bcd
INIFAP-QPM	1.22ef	1.21ab	0.39ª	0.06de	2.88bcd
Regional landrace	0.37h	0.24e	0.37ab	0.75ª	1.75g
RQ2015	0.95g	0.57d	0.29abc	0.39b	2.20f
MEAN	0.76	0.512	0.945	0.329	2.55
LSD	0.0706	0.0388	0.0883	0.0412	0.16

Means with the same letter for each trait are statistically equal, Tukey's test, P≤0.05.
[1] Albumins; [2] Globulins; [3] Prolamins; [4] Glutelins; [5] Total soluble protein content (g P / 100 g of flour).

Medias con la misma letra dentro de cada variable son estadísticamente iguales, Tukey, P≤ 0,05.
[1] Albúminas; [2] Globulinas; [3] Prolaminas; [4] Glutelinas; [5] Contenido total de proteína soluble (g P/100 g de harina).

For the albumin fraction, the accessions with the highest content were 'HRH2015', with 1.61 g of protein / 100 g of flour, followed by 'ERH2015', with 1.44 g, and 'FVR12015', with 1.42 g. The accessions with the lowest contents were the 'regional landrace', with 0.37 g, followed by 'RQ2015', with 0.95 g. Similar values were found in a previous study (12).

The accessions with the highest globulin fraction contents were 'HRH2015' (1.41 g), 'ROQUE2' (1.32 g), 'FRV2015' (1.28 g) and '22015' (1.19 g); the accession with the lowest globulin protein content was the 'regional landrace' (control; 0.24 g).

The 'HRH2015' accession contained high percentages of the albumin, globulin and prolamin fractions, but a lower percentage of the glutelin fraction, making it suitable material for breeding. The two first fractions were of better protein quality (32). The 'MRH2015' accession exhibited high percentages of globulins and prolamins. The 'HRH2015', 'INIFAP-QPM', 'MRH2015', 'ROQUE2', '22015' and 'FVR2015' accessions contained high percentages of the globulin fraction. Literature indicates that high percentages of albumins and globulins and a low percentage of prolamins are indicators of the protein quality of accessions, since the first two fractions are rich in essential amino acids such as lysine and tryptophan.

In OPACO-2, maize genes have been introduced to restore grain hardness and even low levels of α. Introduced quantitative trait loci increase the expression of γ-zeins, which appears to restore grain hardness (37).

Figure 1 shows the electrophoretic pattern of the prolamin fraction (zein), which is of interest for determining the possible genetic differences between the accessions. This fraction presented a similar pattern among the obtained bands.

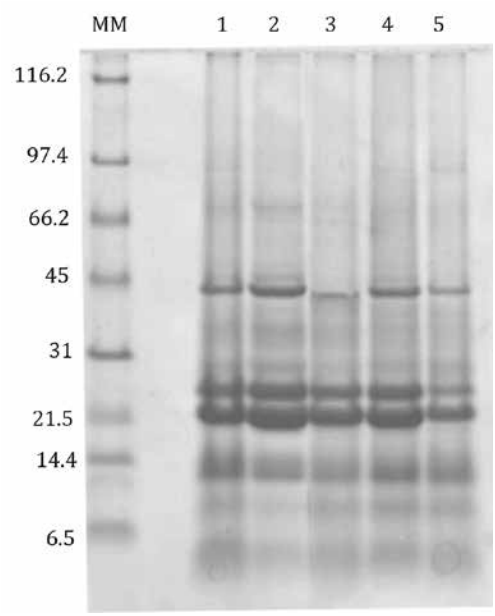

Figure 1. Electrophoretic pattern of the prolamin fraction. MM: Molecular weight marker (kDa); 1: '12013', 2: 2. '22013', 3. 'A2013', 4. 'ROQUE2', 5. 'POL2015'. At the 21 kDa marker position, two prominent bands characteristic of this fraction are shown.

Figura 1. Patrón electroforético de la fracción de prolaminas. MM: Marcador molecular (kDa) 1. Genotipo '12013', 2. '22013', 3. 'A2013', 4. 'ROQUE 2', 5. 'POL2015'. A la altura del marcador de 21 kDa se muestra dos bandas prominentes características de esta fracción.

The 'POL2015' accession showed a band at 95.6 kDa, and the '12013' and '22013' accessions showed a band at 77.8 kDa. All accessions presented bands at 43.6, 39.2, 29.1, 23.7, 19.8 and 13.2 kDa, which are characteristic of maize germplasms.

Prolamins are small globular proteins with a high cysteine content. High percentage accumulation of zeins confers a poor protein quality (37).

Figure 1 (page 20) shows some of the differences between the accessions, such as a missing prominent band in the middle of the gel (45 kDa) in the '12013' accession (third lane). Apparently, the molecular weight of this band changes. This characteristic could help to differentiate the accessions.

Figure 2 shows the electrophoretic pattern of the prolamin fraction; a similar pattern is observed among the bands of accessions 'FVR2015', 'ERH2015', 'HRH2015' and "MRH2015". The 'INIFAP-QPM' accession presents a band at 33.1 kDa that the other materials do not present. Accessions 'FVR12015', 'ERH2015' and 'HRH2015' present a very intense band at 20.1 kDa. In general, the pattern includes bands at 67, 45, 25, 20, 16 and 13.2 kDa. According to Wu *et al.* (2012), the 22 and 19 kDa zein bands are the most prominent storage proteins. Additionally, high accumulation of zeins results in a poor protein quality according to these researchers.

The analysis of variance (not shown) of the bromatological data (moisture, ash, proteins, oil, fiber and carbohydrates) showed significant differences for all traits, indicating that at least one accession presented different characteristics for some of the evaluated traits. These results indicate that the accessions are not related. The coefficients of variation were good for the evaluated traits (13).

The average moisture trait value was 12.40%; the highest values were obtained for accessions 'INIFAP-QPM' and '12015' (13.11%), while the values for '22015' (12.86%) and 'HRH2015' (12.45%) were statistically equal.

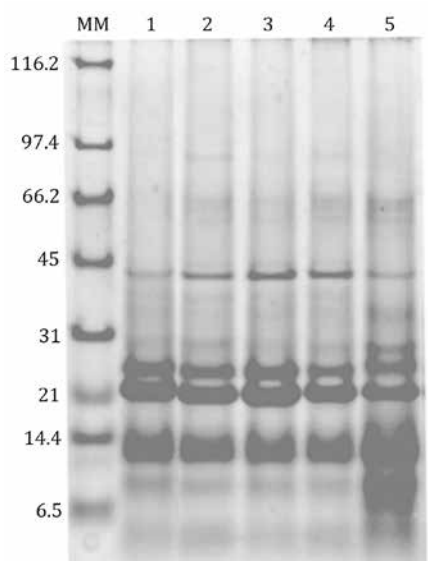

Figure 2. Electrophoretic pattern of the prolamin fraction of 5 maize accessions. MM: Molecular marker 1.-FVR12015, 2.-ERH22015, 3.-HRH2015 4.- MRH2015, 5.- INIFAP-QPM. At the 21.5 kDa marker position, prominent bands of prolamins are visible.

Figura 2. Patrón electroforético de la fracción de prolaminas de 5 genotipos de maíz. Donde: MM: Marcador molecular 1.-FVR12015 ,2.-ERH22015, 3.-HRH2015 4.- MRH2015, 5.- INIFAP-QPM. A la altura del marcador de 21.5 kDa se ven las bandas prominentes de las prolaminas.

The average ash trait value was 1.27%; the accessions with the highest values (statistically equal) were '12015' (1.42%), 'ERH2015' (1.36%), 'RQ2015' (1.33%), 'INIFAP-QPM' (1.32%), 'ROQUE2' (1.3%), the 'regional landrace' (1.29%) and 'HRH2015' (1.26%). For the protein trait, the average value was 8.55%; the highest value was obtained for the '12015' accession (10.44%), which exhibited an excellent protein percentage that was

20.55% higher than that of 'INIFAP-QPM' and 35.8% higher than that of the 'regional landrace'. In addition, it also showed high ash and carbohydrate contents, constituiting good material for breeding.

Accessions 'ROQUE2', 'PLO2015', '22015', 'ERH2015' and the 'regional landrace' showed a low protein content (7.64%), similar to those of non-breeding maize varieties (21). The mean oil percentage trait value was 2.7%; accessions 'RQ2015' (5.2%), 'ERH2015' (4.2%) and 'ROQUE2' (4.28%), showed high oil content, while the accession with the lowest oil content was '12015' (0.66%). The mean fiber percentage trait value was 2.22%; the 'INIFAP-QPM' variety showed the highest value for this trait (3.96%), followed by '22015' (3.21%).

The observed ash contents were consistent with the findings of Cázares-Sánchez *et al.* (2015), who evaluated 41 native maize accessions from central-northern Yucatán, México, and found values of 1.12 to 1.83%. These findings are consistent with those of Agama-Acevedo *et al.* (2005), who evaluated pigmented maize and found ash values of 1.1 to 1.6%. These results are within the range established by the Codex Alimentarius Commission, with a maximum of 3% (10).

Protein content ranged from 7.23 to 10.44%; this range has been reported for different genotypes by others, such as Narváez-González *et al.* (2006), who observed contents between 6.8 and 14.2%, and Díaz-Coronel *et al.* (2009), who found protein values ranging from 10.6 to 12.2% in five corn hybrids. These results indicate that the protein content has a genetic basis. Vidal-Martínez *et al.* (2008) evaluated 45 collections of maize landraces from "Sierra de Nayarit", Mexico, where the highest protein value was 12%. Vázquez-Carrillo *et al.* (2010) observed 12.5% protein as a maximum value in 26 maize landraces from the Mezquital Valley, Hidalgo, Mexico, similar to the percentage found in this research. A high protein quality in maize is caused by the synthesis of larger albumin and globulin fractions, which consist of lysine- and tryptophan-rich proteins, while the zein content is decreased. This means that the total protein content does not vary quantitatively (33). Zeins are the main fraction (up to 60%) of the total endosperm protein (22). These results show that some of the evaluated accessions presented a good protein quality and seed quality, supporting the hypothesis.

Conclusions

The results of the germination test were superior to 80% for all accessions. Accession "INIFAP-QPM" showed susceptibility to the accelerated aging test. The 'HRH2015' accession exhibited the highest amount of soluble protein, and the 'regional landrace' presented the lowest. Accession 'HRH2015' showed high albumin, globulin and prolamin contents; the 'regional landrace' showed the highest content of glutelins plus prolamins. The '12015' accession showed the highest total protein content, followed by 'HRH2015'; the lowest total protein content was presented by 'RQ2015' and the 'regional landrace'. Accession 'RQ2015', on the other hand, presented the highest oil content and '12015' the lowest. In terms of

fiber content, the 'INIFAP-QPM' accession presented the highest value. The average ash content was 1.27%. The electrophoretic patterns of prolamins showed some differences between accessions. These maize accessions could be used for breeding purposes. The 'HRH2015' accession showed high performance in most of the evaluations conducted in this study, except for glutelin content, and this fraction does not provide essential amino acids. Accessions 'HRH2015' and 'FVR12015' were superior in protein quality, and accessions 'A2015', 'POL2015' and 'FVR12015' were superior in seed quality; therefore, these accessions could be used in a future breeding program.

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