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Environmental effects on chemical composition and physical properties of polyembryonic maize grain

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Abstract

Polyembryony has been reported in maize (*Zea mays* L.) since the 20th century. This trait is defined as the appearance of two or more seedlings from a germinated seed and has been associated with improvements in the nutritional content of the grain. Maize kernel traits are affected by different factors among them the growing location. Thus, it is important to determine the environmental (location) effect on polyembryonic kernel traits. The purpose of this study was to determine the environmental effects on the physical and chemical properties of polyembryonic maize kernels. Thirty-two maize genotypes with different levels of polyembryony (high, low and non-polyembryonic) were planted in two different locations with distinct climate (Buenavista, Coahuila and Río Bravo, Tamaulipas, Mexico). Results showed that at the Buenavista location, maize lines had a greater weight of one thousand grains (272 - 367 g), the non-polyembryonic lines showed the highest weight. Besides, the low polyembryonic maize lines promoted the largest grain dimensions. The polyembryonic maize had the biggest embryos, which occupied 11.37 -11.59% of the grain. At the Río Bravo, in comparison to Buenavista, the polyembryonic maize lines had the highest protein (4.56%) and fat (5%) contents, while the raw fiber (1.2%) was highest in non-polyembryonic maize, meanwhile at, the Buenavista location, the starch content (59.2%) was higher in non-polyembryonic maize, while ashes (1.4%) and moisture (13.7%) were higher in polyembryonic maize. Growing polyembryonic maize genotypes in different environments, it was observed that the bromatological profile is affected by environmental conditions. In this study, a better nutritional content was observed in maize genotypes grown at Río Bravo in comparison to those maize genotypes grown at Buenavista. So, this study suggests that it is possible to produce polyembryonic maize genotypes in different environments which will have a different bromatological profile and can be employed for specific industrial uses.
Keywords: *Zea mays* L, grain, polyembryony, nutritional quality.

Efecto del medio ambiente sobre la composición química y propiedades físicas del grano de maíz poliembriónico

Resumen

La poliembrionía, ha sido reportada en el maíz (*Zea mays* L.) desde el siglo XX. Este rasgo se define como la aparición de dos o más plántulas de una semilla germinada, y se ha asociado con mejoras en el contenido nutricional del grano. Las características del grano de maíz son afectadas por diferentes factores, entre ellos la localidad de cultivo**.** Por lo tanto, es importante determinar el efecto del ambiente (localidad) en las características de los granos poliembriónicos. El propósito de este estudio fue determinar los efectos ambientales sobre las propiedades físicas y químicas de los granos de maíz poliembriónicos. Se sembraron 32 genotipos de maíz con diferentes niveles de poliembrionía (alta, baja y sin poliembrionía) en dos localidades diferentes (Buenavista, Coahuila y Río Bravo, Tamaulipas, México). Los resultados mostraron que, en la ubicación de Buenavista, las líneas de maíz tenían un peso mayor de mil granos (272 - 367 g) las líneas no poliembriónicas mostraron el mayor peso. En las líneas de maíz de baja poliembrionía el tamaño del grano fue mayor con embriones más grandes que ocupaban un espacio entre 11.37-11.59%. En Río Bravo, en comparación con Buenavista, las líneas de maíz poliembriónico tuvieron el mayor contenido de proteína (4.56%), grasa (5%) y fibra cruda con (1.2%) más alta en el maíz no poliembriónico. En Buenavista, el contenido de almidón fue de (59.2%) mayor en el maíz no poliembriónico, con (1.4%) de cenizas y la humedad de (13.7%) que fueron mayores en el maíz poliembriónico. Al cultivar genotipos de maíz poliembriónico en diferentes ambientes, se observó que el perfil bromatológico se ve afectado por las condiciones ambientales. En este estudio, se observó un mejor contenido nutricional en los genotipos de maíz cultivados en Río Bravo, en comparación con los genotipos de maíz cultivados en Buenavista. Por lo tanto, este estudio sugiere que es posible producir genotipos de maíz poliembriónico en diferentes ambientes y aunque tuvieron un perfil bromatológico diferente pueden emplearse para usos industriales específicos.
Palabras clave: maíz, grano, poliembrionía, calidad nutricional.

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Introduction

Maize has high worldwide economic importance, because, it is used for both, human consumption and animal fodder. It is the major food crop grown in myriad countries, where, it is cultivated mainly by small holder farmers (Methu, Owen, Abate & Tanner, 2001; Qamar, Aslam & Javed, 2016). Maize is also the most produced agricultural commodity around the world, with more than 1 billion tons produced yearly, therefore, maize stover is the most abundant by-product (Rocha-Villarreal, Hoffmann, Levien Vanier, Serna-Saldivar & García-Lara, 2018). México is the center of origin of maize (*Zea mays* L.), where it forms the main livelihood of indigenous cultures (González-Amaro, Figueroa-Cárdenas, Perales & Santiago-Ramos, 2015).

In some maize genotypes, the polyembryony (PE) phenomenonhas been reported, which is defined as the simultaneous emergence of two or more seedlings from a germinated seed (Espinoza, Vega, Navarro & Burciaga, 1998; Rebolloza Hernández, Espinoza Velázquez, Sámano Garduño & Zamora Villa, 2011; Michel *et al*., 2018). The polyembryony was discovered in seeds of Citrus by Van Leeuwenhoek in 1719, where he found two embryos developing from the same seed. This trait has an adaptive sense and improves species survival under normal and stress conditions (Batygina & Vinogradova, 2007). This phenomenon in maize has been reported by different scientists (Pešev, Petrović, Zečević & Milošević, 1976) and it is very important in the study of plant embryogenesis and genetics. Besides, this trait is useful to design new maize varieties with high productive potential since two or more embryos per seed may yield a higher content of protein (Espinoza *et al*., 1998; González Vázquez, Espinoza Velázquez, Mendoza Villareal, De León Castillo & Torres Tapia, 2011).

It has been suggested that this characteristic could have a preponderant effect on the physical-chemical quality of maize compared to that of normal maize grain (Musito Ramírez, Espinoza Velázquez, González Vázquez, Gallegos Solórzano & León Castillo, 2008). In the last four decades, the Mexican Institute of Maize-the Universidad Autónoma Agraria Antonio Narro (IMM-UAAAN), different maize populations have been improved in order to increase and fix high polyembryonic frequency, along with high grain production and nutritional potential (Espinoza *et al*., 1998).

Maize kernel is composed of endosperm (82-83%), embryo (10-11%), pericarp (5-6%), and the tip cap (0.8-1.0 %) (Singh, Kaur & Shevkani, 2013). Its chemical components, such as carbohydrates, proteins, fats, fiber, minerals, vitamins, and antioxidants have great importance on daily human diet (Pasjanant, Kerdchoechuen & Laohakunjit, 2016). The physical grain quality is represented by size, weight, and moisture. While, the major chemical component in the maize kernel is starch, which makes up 73% of the grain, followed by protein which varies up to 11% of the grain and is mostly represented by proteins in the endosperm (75%), the proteins with best amino acid profiles are found in the embryo (Rocha-Villarreal *et al*., 2018). After starch and protein, fat in the form of oil is the third (3.5% to 6%) most important component in maize kernel (embryo contains about 80% while the endosperm 1%). It has also been mentioned that soil quality has an important impact on the grain mineral content (Nuss & Tanumihardjo, 2010).

The pericarp is a semipermeable barrier that covers the maize kernel with high fiber content (Rocha-Villarreal *et al.*, 2018), fiber constitutes until 7% of total kernel composition (Nuss & Tanumihardjo, 2010). For polyembryonic maize, like for many other kinds of cereals, the proximal analysis and physics characteristics are scarcely studied and poorly reported. Some kernel traits have been determined using genotypes grown only in a single location (Musito Ramírez *et al*., 2008). However, maize kernel traits are affected by different factors among them the growing location. Thus, it is important to determine the environmental effect on polyembryonic kernel traits. In this study were tested two very different environments, which are very contrasting, one is dry (Buenavista) and the other sub-tropical (Río Bravo), and the difference in the elevation above the sea level between these two locations is more than 1,700 meters. Therefore, the objective of this study was to evaluate the environmental effects on the physical and chemical traits of maize kernels from genotypes with different levels of polyembryony.

Materials and Methods

Environments and maize genotypes

In this study thirty-two maize genotypes (Table I) were planted in two locations; these genotypes had a different level of polyembryony. Fifteen of these genotypes had more than 30% of polyembryony under field conditions and were called "NAP" (Plant with normal height and high polyembryony), other, fifteen genotypes had less than 4% of polyembryony under field conditions and were called "NBP" (Plant with normal height and low polyembryony). In addition, two genotypes were used as controls "T" (non-polyembryonic), one of them was developed at the UAAAN which was called T-1 Hernán Cortez (IMM-UAAAN) and another one, a commercial cultivar called T-2 Garañon (Asgrow), both of them with normal grain. These genotypes were planted on April 2017, under irrigation conditions at the UAAAN experimental station located at Buenavista, Coahuila, with geographic situation: 25° 22" north latitude, 101° 02" west longitude and at an altitude of 1,756 masl, where the weather is dry, semi-arid, the soil has a clay loam texture with low organic matter content or at the Río Bravo experimental station located at Río Bravo, Tamaulipas, with geographic situation: 25º 57" north latitude, 98º 01" west longitude and at an altitude of 26 masl, where

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Table I. Maize genotypes used in this study.

Polyembryony level	Genotypes sown at Río Bravo (RB)	Genotypes sown at Buenavista (BUV)
High (30 to 60%)	NAP-1, NAP-2, NAP-3, NAP-4, NAP-5, NAP-6, NAP-7, NAP-8, NAP-9, NAP-10, NAP-11, NAP-12, NAP-13, NAP-14, NAP-15	NAP-1, NAP-2, NAP-3, NAP-4, NAP-5, NAP-6, NAP-7, NAP-8, NAP-9, NAP-10, NAP-11, NAP-12, NAP-13, NAP-14, NAP-15
Low (< 4%)	NBP-1, NBP-2, NBP-3, NBP-4, NBP-5, NBP-6, NBP-7, NBP-8, NBP-9, NBP-10, NBP-11, NBP-12, NBP-13, NBP-14, NBP-15	NBP-1, NBP-2, NBP-3, NBP-4, NBP-5, NBP-6, NBP-7, NBP-8, NBP-9, NBP-10, NBP-11, NBP-12, NBP-13, NBP-14, NBP-15
None (controls)	T-1, T-2	T-1, T-2

NAP means: N (normal height of the maize plant), A (high), P (polyembryony). NBP means: N (normal height of the maize plant), B (low) and P (polyembryony). Río Bravo, Tamaulipas (RB), Buenavista, Coahuila (BUV). Each genotype number corresponds to a different genotype.

the climate is semi-arid, subtropical warm with vertisol soil with clay texture. Both experiments were established under a randomized complete block design with three replications. Standard agricultural practices for maize production under irrigation were employed. The experimental plot size was defined as a row for each genotype. The rows were 5 m in length and 0.80 m of row spacing. Cobs were collected 30 days after grain physiological maturity when kernel reached moisture of 15%, maize was shelling by hand.

Physical properties

Thousand grains weight

This trait was determined according to the methodology mentioned by Bonifacio *et al*., 2005. First, 100 kernel grain weight was determined by randomly weighing 100 grains, using a semi-analytical balance, after that, the resulting weight was multiplied by 10.

Grain size

Measurement of kernel dimensions (length, width, and thickness) was performed using a Mitutoyo Digital Vernier. For this test, 10 grains were randomly selected from each maize genotype. Each of the three kernel dimensions was measured in mm.

Percentage composition of maize grains

Percentage composition was performed using five grains of each genotype (10 repetitions), these grains were weighed and soaked with distilled water in a 50 mL beaker for 24 hours. After this time, with the aid of a scalpel, the grain was divided into pericarp, endosperm, and embryo, each fraction being weighed separately, and the percentage obtained concerning the weight of the whole grain (AACC, 1995).

Grain density maize

Grain density was determined by the mass of the grain per unit volume. Briefly, 5 grains chosen at random, were weighed using an analytical balance, then those 5 grains were poured into a 10 mL sample of water. The volume of water displaced by the grains was measured.

Floating rate

The flotation index was determined by placing 50 clean kernels in a beaker containing 150 mL of sodium nitrate ($NaNO_3$) adjusted to a density of 1,250 g/mL. The sample was stirred to separate the kernels and left standing for 1 min. The number of floating kernels on the solution was counted, which indicated the flotation index (AACC, 1995).

Determination of Proximate Compositions of Maize

Moisture content

The moisture content was determined in the maize samples as follows: an empty and covered glass dish was left to dry into the oven at 105 °C for 3 h, then, the glass was transferred to a desiccator to cool, the plate and lid were weighed. Subsequently, 3g of powder from each maize genotype was weighed in a single plate, and the sample was evenly distributed. After, the plate was placed into the oven and left to dry for 3 hours at 105 ºC. Then, the plate was transferred to the desiccator to cool. Finally, the plate was weighed with the dry sample.

Calculations

$$\text{Moisture (\%)} = \frac{(W1\text{-}W2)}{W1} \times 100$$

Where: W1 = weight (g) of a sample before dry and W2 = weight (g) of a sample after dry.

Protein

The protein content was measured by the Kjeldahl method as mentioned by Qamar, Aslam & Javed, 2016. Protein content were estimated by using the next equation:

$$\text{Crude Protein (\%)} = \text{nitrogen content} \times 6.25$$

Raw fat

A 12 mL glass bottle with cap was used. It was placed overnight in the incubator at 105 °C to assure the constant weight. Then, 250 mg of the maize sample was weighed, and 10 mL of hexane was added to the bottle. The sample was placed on an orbiter at constant agitation for 4 hours. The bottles were centrifuged, and the supernatant were poured into bottles that were kept previously at constant weight. The bottles were incubated for 8 -10 hours at 70 ° until the solvent was evaporated completely, and the bottle was completely dry. Later, the bottle with the partially covered lid was transferred to the desiccator. The bottle and its dry contents were re-weighed. The fat content in every genotype was calculated as follow:

$$\text{Fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \text{ x } 100$$

Crude fiber

The crude fiber content was determined by utilizing the AOAC (1990) method. Two grams of degreased maize sample were used. The sample was placed in a Berzelius glass, then 100 mL of 0.255 N sulfuric acid solution was added. Berzelius glass was connected to the reflux apparatus for 30 min, counted from when it starts to boil. Once the time finished, it was dried and filtered through the linen cloth and washed with 3 volumes of 100 mL of hot distilled water. The litmus paper test was carried out to verify the presence of acid. The fiber (residue remaining in the linen fabric) was passed to the Berzelius glass with 100 mL of 0.313 N sodium hydroxide solution and was connected to the reflux apparatus for 30 min. Then, the sample was filtered through linen and washed with 3 volumes of hot distilled water. The litmus test was performed to verify if there is an alkaline reaction. The excess of water was drained by pressing the linen fabric. The fiber was removed with a spatula and deposited in a porcelain crucible. The crucible was placed in the muffle at 550-600 °C for 2 h, sacked and left to cool (to constant weight in a desiccator) and weighed. The calculations were made with the following formula.

$$\text{Fat (\%)} = \frac{\text{weight crucible with dry fiber-} \\ \text{weight crucible with fiber ashes}}{\text{g of sample}} \text{ x } 100$$

Ash content

Ash content was determined using a muffle according to the AOAC 925.10 method, based on the weight loss the sample suffers by heating until a constant weight is obtained. It was weighed 5 g of sample in a tared melt pot. The sample was burned using a Bunsen burner. When vapors were no longer produced, the melt pot were placed on a muffle and heated to 550 °C during 5 h. Then, the melt pot with ashes was left to cool in the desiccator and finally, the ash was weighed. Content of ashes was calculated according to the next equation:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \text{ x } 100$$

Starch

The starch content was measured in an INSTALAB equipment container which uses NIR technology (near-infrared). The technology is based on the absorption of light energy by starch at specific wavelengths. One gram of sample was placed in a plastic container and introduced to the equipment previously stabilized at 50 ºC. The samples were read at 2,180 nm and the results were compared to a standard curve. The results were shown on the screen.

Statistical analysis

The experiments were established using the same number of grains for each genotype and under a randomized complete design with three replications. The obtained data were analyzed by the ANOVA. When needed, the treatment means were compared using the Tukey multiple range test. Analyses were performed using the SAS software 9.0 version.

Results and Disscusion

Physical properties

Thousand grains weight

The weight of one thousand grains was obtained in the range of 231 g and 367 g (Figure 1), showing a significant difference between the maize types (an average of the 15 genotypes for each level of polyembryony). In the present work, the polyembryony maize grains (NAP and NBP) were less heavy than the control (T), this may be due to the energy invested to produce more embryos resulting in the production of more than one ear per plant. The non-polyembryonic maize (T-1, T-2) had the highest weight in both environments (Río Bravo

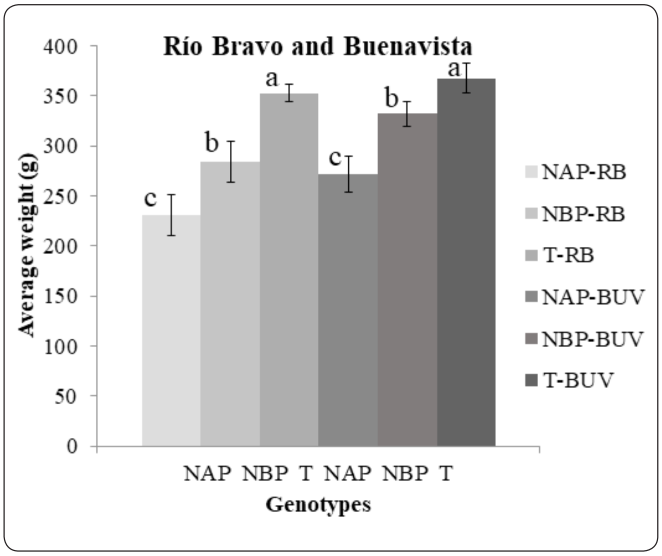

Figure 1. The average weight of one thousand grains for high (NAP), low (NBP) and non-polyembryonic (T) maize groups, planted at Río Bravo (RB) and Buenavista (BUV) environments. Means with in a row with a common superscript are not significantly different (P < 0.05).

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and Buenavista), followed by those maize genotypes with low polyembryony (NBP), being the less heavy grains those from high polyembryony (NAP) genotypes. These differences for the weight of one thousand grains among the types of polyembryony were statistically significant ($p < 0.05$).

The polyembryonic maize has low and medium weights, which makes it favorable for use in the nixtamalization process to obtain soft tortillas. Weight is an indirect variable to predict the size of grains (Roque Maciel, Arámbula Villa, López Espíndola, Ortiz Laurel, Carballo Carballo & Herrera Corredor, 2016). It has been mentioned that the weight of one thousand grains of commercial maize grown at the North of the Mexican Bajío ranges from 220 g to 400 g predominating small grains with large embryo (Guzmán-Maldonado, Vázquez-Carrillo, Aguirre-Gómez & Serrano-Fujarte, 2015). While, in landrace maize isolates there are reports that grain weight ranges from 186.6 g to 343.7 g / 1,000 grains (Figueroa Cárdenas *et al*., 2013). The polyembryonic and the non-polyembryonic maize genotypes evaluated in this study showed a grain weight (231 ± 20.1 to 367 ± 14.7g/1,000 grains) similar to that reported for commercial and landrace maize isolates in Mexico (small-sized grains tend to have large embryos) (Guzmán-Maldonado *et al*., 2015).

Grain weight was affected by the genotype and the maize growing environment. According to the Multiple Range Tukey Test ($\alpha = 0.05$) test, there was a significant difference between the environment and also among the level of polyembryony (Figure 1). The maize genotypes evaluated at Río Bravo had a grain weight as follow: NAP231 g (± 20.1), NBP 284 g (± 20.6), and T 352 g (± 8.6). The evaluated maize at the Buenavista environment had a grain weight of 272 g (± 17.8) for NAP, 332 g (± 12.3) for NBP, and 367 g (± 14.7) for the control.

Particularly, the genotypes with higher weights were NAP-5 with 287.3 g (± 15.9), NBP-1 with 313.3 g (± 5.9), and T-2 (Garañon) with 357.4 (± 10.8) (for high, low and no polyembryonic group, respectively) all harvested in Río Bravo. In contrast, at Buenavista the maize genotypes with the highest grain weight were NAP-3 with 341g (± 5.2), of NBP-2 with 370. 7 g (± 2.8) and T-1 (variety Hernán Cortez) with 382.1 g (± 3.7) (for high, low and no polyembryonic group, respectively). Buenavista and Río Bravo were chosen to evaluate the polyembryonic and non-polyembryonic maize genotypes because they have very contrasting temperatures and relative humidity during grain filling, and very different geographical conditions.

There are no reports related to the polyembrionic maize weight. Bonifacio, Salinas, Ramos & Carrillo, 2005, reported that the cacahuazintle (a non-polyembryonic genotype) maize presented weight values in the range 600 to 700 g / 1000 grains. The weight of a thousand grains is an important factor in the nixtamalization process because it impacts directly on the water absorption capacity of the grains and also on the cooking process (Salinas-Moreno *et al*., 2013).

Grain size

Growing location affected the grain size. At the Río Bravo environment, there were no significant differences among the high, low polyembryony and non-polyembryonic groups of maize genotypes (Figure 2). While, in the Buenavista environment (Figure 2), there were significant differences between grain length, width, and thickness for the groups with different levels of polyembryony. The grains with low polyembryony (NBP) were longer, wider and thicker according to the multiple ranges Tukey test ($\alpha = 0.05$). At, the environment of Buenavista, the kernels showed high values in the parameters, long, width and thickness, due to cooler temperatures and low relative humidity at this location in comparison to Río Bravo.

Small grains tend to have larger embryos. The presence of larger embryos increases the oil yield useful in the industry and reduces the use of this ingredient in balanced foods (Figueroa Cárdenas *et al*., 2013). High-content of oil in polyembryonic maize is useful for the elaboration of tortillas with flours with less retrogradation (Cruz-Requena *et al*., 2011).

The percentage of endosperm was major in the non-polyembryonic (T) genotypes. The size of endosperm affects the hardness of the grain and the amount of starch. In hard maize, the starch granules are polygonal and highly packaged (Figueroa Cárdenas *et al*., 2013).

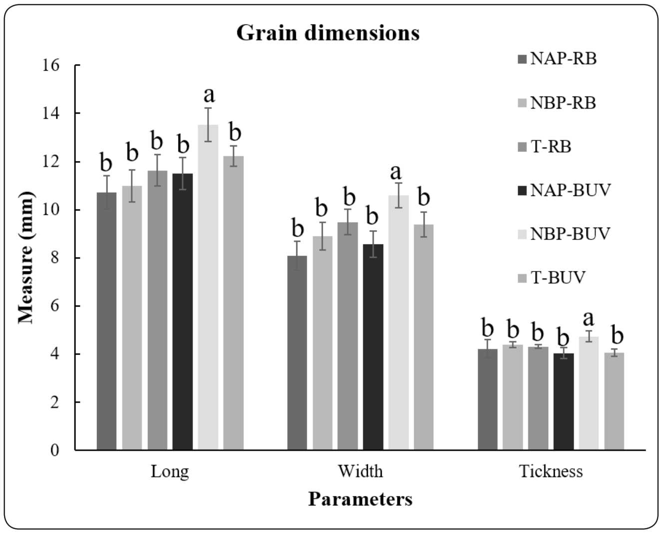

Figure 2. Río Bravo (RB) and Buenavista (BUV) grain dimensions of high (NAP), low (NBP) polyembryonic and non-polyembryonic (T) maize genotypes. Means with in a row with a common superscript are no significantly different (P < 0.05).

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Table II. Percentage of the kernel parts in maize genotypes with different levels of polyembryony.

	NAP	NBP	T
% Pericarp	4.80 ± 0.71*	6 ± 0.78	4.35 ± 0.65
% Embryo	11.59 ± 2	11.37 ± 2.12	9.95 ± 0.36
% Endosperm	84 ± 4.59	80.7 ± 3.81	85.5 ± 0.97

*= Standard deviation.

The percentage of pericarp was higher in the low polyembryonic maize (Table II). The percentage of pericarp reported in Cacahuacintle maize ranges from 4.7 to 6.1% of the grain (Bonifacio *et al*., 2005). The pericarp is a layer covering the grain that is characterized by high crude fiber content. Pericarp percentage and thickness varies according to maize varieties, the endosperm is mainly composed of starch granules, and the embryo is characterized by fat (Singh *et al*., 2013). There are reports for maize collections in northern of El Bajío, México, where the pericarp percentage ranges between 5.2–6.9% and the embryo between 10 – 12.9% (Guzmán-Maldonado *et al*., 2015). For the nixtamalization process, the pericarp is removed, so the less of its percentage in the grain is better for this procedure. Therefore, high polyembryony maize can be used for the nixtamalization process, because it contains the least amount of pericarp (Bonifacio *et al*., 2005).

Grain density

The density of the polyembryonic and non-polyembryonic maize grain was from 1.32 to 1.4 g/mL^{-1}, this trait is important for storage and transport (Table III). The density of the kernel maize genotypes did not show differences between environments and between levels of polyembryony. Bonifacio *et al*., (2005) reports lower densities of 1.07 g/mL $^{-1}$ of Cacahuacintle maize. These authors suggested that grain density is associated with the cooking time for busting maize grain. The lower density of the grain shows a greater porous space, which can allow better penetration and distribution of the water during the grain cooking.

Table III. Density of the grain in different levels of polyembryony and environments.

Density g /mL $^{-1}$	NAP	NBP	T
Río Bravo	1.32 ± 0.04*	1.38 ± 0.04	1.35 ± 0.05
Buenavista	1.36 ± 0.05	1.39 ± 0.05	1.4 ± 0.08

*P < 0.001, * = Standard deviation.

Grain floating rate

The grain flotation index was 2.25 to 8.37 % (Table IV) with values suggesting a predominance of maize genotypes with hard endosperm, which are more resistant to pests. The floating rate of the maize grain did not present differences significantly between environments and levels of polyembryony. Low grain flotation index is representative features of hard grain maize (Roque Maciel *et al*., 2016). Some authors have reported maize genotypes with a flotation index between 46 to 94%, which is indicative of very soft maize (Guzmán-Maldonado *et al*., 2015). The grains hardness is related to the food application, so it is essential to define the industrial quality of the grain concerning the product that is desired to be elaborated. For example, small and hard grains are used for palomero maize, the long and hard grains are used for snacks. Therefore high polyembryony maize could be used as a palomero and low polyembryony maize for snacks (Figueroa Cárdenas *et al*., 2013). As was mentioned, the maize kernels appropriate for the nixtamalized products industry must have a maximum grain floating rate of 40% (Salinas-Moreno *et al*., 2013). Therefore, these polyembryonic maize genotypes are suitable for later use in nixtamalization. There is a positive relationship between grain hardness and protein content in maize, which is attributed to a greater presence of protein bodies (prolamines) that surround the starch granules in the endosperm (Salinas-Moreno *et al*., 2013).

Table IV. Floating rate of the grain in different levels of polyembryony and environments.

Floating rate %	NAP	NBP	T
Río Bravo	8.37 ± 3*	4.53 ± 3.05	2.25 ± 1.21
Buenavista	6.53 ± 2.3	4.6 ± 2.6	2.75 ± 1.70

*P > 0.001, * = Standard deviation.

Proximate chemical composition

The proximate compositions of maize grain varied due to genetics, level of polyembryony, and growing environment (some significantly) (Table V). The maize grains harvested at Río Bravo contained slightly more protein and fat than the maize grains harvested at Buenavista. Protein content varied substantially among the level of polyembryony according to the growing environments. Polyembryonic maize grain from Río Bravo was characterized by higher protein content. It was obvious that the control (non-polyembryonic) grain had lower protein content than the polyembryony grains. This is because two or more embryos per seed could yield higher protein content (Espinoza *et al*., 1998; González Vázquez *et al*., 2011). Storage proteins in maize kernels are usually short of some of the amino acids that are essential for the human diet.

The fat content of maize in this study was found to be about 5%, and there was no significant difference between high, low, and non-polyembryonic maize genotypes. Rocha-Villarreal *et al*., (2018) mentioned that oil in maize grain is mainly in the embryo with an estimate of 3 to 18%. In the present work, for

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polyembryonic maize genotypes were found values between 4.5 to 5%, which are similar to those reported for normal maize (Rocha-Villarreal *et al*., 2018). Salinas-Moreno *et al*., 2013, reported oil values of 4.2 to 4.8 %. Although the polyembryony maize grain produces more than one plant per seed, it would be thought that the level of nutrients would decrease. However, it can be observed that they were maintained, and in some genotypes were higher.

In the polyembryonic maize grain, there was an increase in the size of the embryo and decrease in endosperm. This is a prominent physical characteristic of these genotypes, and this results because these maize genotypes require more energy to produce fat (embryo), decreasing the starch accumulation (endosperm) (Preciado-Ortíz *et al*., 2018). Singh *et al*., (2013) reported that the lipid content of the endosperm is relatively low (1%), compared to that in the embryo.

The fiber content was higher in the maize genotypes grown at Río Bravo, with an average of 1.1 % for the high polyembryony and 1.2 % for the non-polyembryonic, the ranges between genotypes were very variable. Maize intake is the only source of fiber in some regions of Mexico consumed as tortillas. The daily average consumption of tortillas in Mexico is 325 g per person. Maize with high fiber could contribute with a part of the daily recommended fiber, which may help to improve the health status of the Mexican population, since fiber participates in the regulation of intestinal transit, glycemia, and lipemia, and also helps to reduce the risk of chronic diseases (Ramírez-Moreno, Córdoba-Díaz, Sánchez-Mata, Díez Marqués & Goñi, 2015).

On the other hand, the ash content was higher in the maize genotypes grown at the Buenavista environment for polyembryonic maize where the average was 1.4 % and 1.3 % for high and low polyembryony, respectively. The content reported of ashes in commercial special white maize is 0.94% and the whole white grain is 1.73%, the polyembryonic maize is an intermediate-range between these types of maize (Nkhabutlane, du Rand & de Kock, 2014).

The starch content was higher in the maize genotypes grown at Buenavista, and the highest content was registered in the non-polyembryonic maize with 59.2% there was a significant difference from the other types of maize. The differences in the content of starch and protein for maize are attributable to the respective climatic conditions during the grain-filling period (the harvest time of the Río Bravo environment was different from that of Buenavista). Besides, the accumulation of starch is promoted by moderate rains and colder temperatures, which was the Buenavista climate. However, the lack of rain and higher average temperatures result in lower starch and higher protein content (Ramchandran, Hojilla-Evangelista, Moose & Rausch, 2016), as occurs in Río Bravo during grain filling.

The moisture content was higher in the Buenavista environment for high polyembryony maize (13.7%), instead of Río Bravo it was found for 10.5%. The results shown that maize (polyembryony and non-polyembryony) grains moisture content were in the range of 10.5 to 13.7%. It was is in agreement with the reported information, that there is a small variation that may be due to the different variety of maize (in this case polyembryonic) used in the current experiment, environmental

Table V. Proximate composition of polyembryonic and non-polyembryonic.

Component	Río Bravo			Buenavista		
Maize PE	NAP-RB	NBP-RB	T-RB	NAP-BUV	NBP-BUV	T-BUV
Protein %	3.9	4.56	3.4	1.5	2.1	1.6
Range	1.5 – 6.6	1.6 – 6.2	0.4 – 6.1	0.1 – 3.5	0.4 – 3.5	0.1 – 3.4
Fat %	5	4.7	4.8	4.9	4.7	4.5
Range	3.4 – 6.8	4 – 5.3	4.2 – 5.5	3.7 – 6.1	3.8 – 5.5	4.1- 4.8
Crude Fiber %	1.1	1	1.2	0.9	0.82	0.63
Range	0.3 – 1.9	0.1 – 3.5	0.9 – 1.6	0.2 – 1.5	0.2 – 1.8	0.1 – 1
Ash %	1	1	0.8	1.4	1.3	0.8
Range	0.6 – 1.2	0.6 – 1.3	0.6 – 1	1.2 – 1.6	0.8 – 1.6	0.6 – 0.9
Starch %	53.2	56.8	57.5	57.9	57.8	59.2
Range	54 – 59	54.6 – 58.7	55 – 59.8	56 – 59	56 – 59	58 – 60.4
Moisture	10.64	10.53	10.50	13.70	13.60	13.20
Range	10.3 – 11.3	10.3 – 10.8	10.3 – 10.8	12.8 – 14.4	12 – 14.7	12.4 – 13.9

factors and agronomic practices (Qamar, Aslam & Javed, 2016). The moisture differences between the genotypes and environments studied were not significant, indicating that the maize kernels used were homogeneous for this variable.

The polyembryonic maize, in addition to its agronomic advantage, had a higher content of nutrients such as protein, fat, and ash. Also, being a hard-maize type makes this maize genotype an interesting source of grains resistance to pests.

Conclusions

This study provided information on the grain chemical and physical properties of maize genotypes with different levels of polyembryony, which were grown in two different environments (locations). The polyembryony level demonstrated positive effects on the physical and chemical properties of maize grain. The polyembryonic maize had higher embryo content, low weight, and size when it was grown at Río Bravo. Nevertheless, at Buenavista, the low polyembryonic maize grain was larger and showed higher flotation rates which make it less hard. In the proximate composition analysis, it was found that polyembryony maize affects the grain composition by increasing protein, fat, fiber, ash in both environments.

Conflict of interest

The authors declare that they have no conflict of interest.

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