

Article

Physical Traits and Phenolic Compound Diversity in Maize Accessions with Blue-Purple Grain (*Zea mays* L.) of Mexican Races

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Abstract: Consumer interest in foods enriched with phytochemical compounds for health benefits has prompted plant breeders to focus on developing new cultivars with an enhanced content of specific compounds. Studies regarding the exploration of germplasms of species of great economic importance, such as maize, could be useful in this task. This study aimed to assess the physical grain traits and phenolic compound variations (including anthocyanins, flavonoids, and proanthocyanidins) in blue-purple maize accessions from various Mexican races. We examined 207 accessions from 21 Mexican maize races, evaluating physical grain traits such as weight of one hundred grains (W100G), endosperm type (ET), pigment location, and grain color. Phenolic composition analysis encompassed total soluble phenolics (TSP), total anthocyanin content (TAC), flavonoids (FLAV), and proanthocyanidins (PAs). The predominant endosperm type was floury, with W100G values indicating a large grain size and the pigment primarily located in the aleurone layer. Among phenolic composition variables, only TSP exhibited a normal distribution, while others skewed towards the left side. A hierarchical analysis of phenolic composition data revealed three distinct groups comprising different numbers of Mexican varieties, with TAC proving the most effective for grouping. Our comprehensive exploration of maize diversity featuring blue-purple grain coloration has led to the identification of novel maize varieties with outstanding phenolic contents.

Keywords: *Zea mays* L.; polyphenols; composition; flavonoids; proanthocyanins; anthocyanins



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1. Introduction

Maize is a cereal highly cultivated around the world, with multiple uses for feeding both human beings and animals. Its origin and domestication have been registered in Mexico [1]. The domestication process implies the selection of material from different agro-ecological regions and its adaptation to different environments. This process is guided by the identification and prioritization of plant traits that are of economic and nutritional interest to humans [2]. In Mexico, the high maize diversity, comprised of 64 maize varieties [3], is explained by the topography and different climates, but also by the utilization of the maize grain in the preparation of a great diversity of foods. Furthermore, a high number of dishes in which the maize grain plays a basic role support Mexican gastronomy [4].

The presence in Mexican maize races of landraces with different grain color due to the existence of colored phenolic compounds like anthocyanins has contributed to this diversity. The most common grain colors in Mexican maize diversity are blue-purple or

blue [5]. Landraces with blue-purple maize grain are used all around the country for the preparation of many different food products. Anthocyanins are the pigments responsible for the blue-purple color of the maize grain. These phenolic compounds are highly valued for their biological activities, among which the antioxidant activity is the most evaluated because of its relationship with the protection of cellular tissues from damage by free radicals, which can cause mutations and predispose to cancer development [6].

In recent times, there has been renewed interest among scientists in examining the phenolic variability present in maize landraces and commercial samples with pigmented grains, particularly in countries like Peru [7], India [8], and Italy [9]. The purpose of the previous studies, in addition to evaluating the genetic diversity, was to contribute to the visibility of these valuable plant resources, potentially leading to the discovery of new uses and applications.

The presence of colored phenolic compounds in pigmented maize grains introduces new flavors and colors to the products prepared with them, the consumption of which can help to preserve health due to the different biological activities associated with some of these compounds, like phenolic acids, anthocyanins and flavonoids [10].

Several published studies have focused on describing the phenolic composition of different landraces or accessions from Mexican maize races with blue-purple grain [11–13]. However, a noticeable gap exists as no study has thoroughly analyzed the majority of maize races that exhibit blue-purple grain variants. We consider that these studies can contribute by allowing breeders of this cereal interested in achieving competitive blue-purple grain varieties to locate sources of diversity with high anthocyanin content.

This study aimed to determine physical grain traits of accessions of maize with blue-purple grain from Mexican races and to evaluate the variation in phenolic compounds (anthocyanins, flavonoids, and proanthocyanidins) in the grains.

2. Materials and Methods

2.1. Plant Material

This study was conducted with 207 maize accessions with blue-purple grain that belonged to 21 Mexican races. In maize, the diversity is grouping in races. A race is understood to be that group of individuals who share enough characteristics (phenotypic and genotypic) with each other to be considered as a group. Within each maize race, there are local varieties of maize or landraces. Figure 1 illustrates the grains of some of the blue-purple maize accessions (BPMA) analyzed.

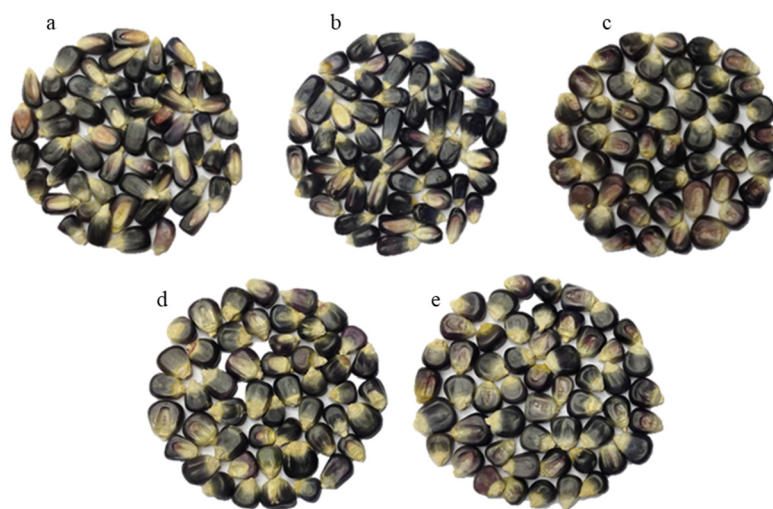


Figure 1. Grain color of some of the blue-purple maize accessions from different varieties analyzed. Varieties are as follows: (a) Olotón, (b) Elotes Cónicos (elongated grain shape), (c) Elotes Occidentales, (d) Azul, and (e) Bolita (rounded grain shape).

The selection of the accessions began with the review of the Mexican CONABIO (National Commission for the Knowledge and Use of Biodiversity) [5] databases, in those classified as blue grain. Subsequently, the available photographs of these accessions were reviewed to corroborate the color of the grain. Accessions were selected based on the uniformity of the grain color (accessions that were a mixture of grains with different colors were not selected), diversity of races, and distribution throughout the Mexican territory. This last condition was achieved by checking the geographical data of the maize grain accession. Samples of 200 to 400 seeds were provided by different germplasm banks located in Mexico. Physical and chemical determinations were made on the grain. Table 1 provides more information regarding the accessions used in the study.

Table 1. Names of Mexican maize varieties, numbers of accessions analyzed per varieties and variations of elevation, meters above sea level (masl), precipitation and temperature in the sites in which the accessions were collected.

Maize Races	Number of Accessions Analyzed per Race	Elevation (masl)	Precipitation (mm)	Temperature (°C)
Arrocillo	4	1900 to 2100	700 to 1200	12 to 20
Amarillo				
Azul	28	2000 to 2600	600 to 1000	10 to 18
Bolita	17	1400 to 1800	800 to 1200	18 to 23
Celaya	2	1600 to 1800	1100 to 2000	16 to 28
Chalqueño	4	1600 to 1800	600 to 1000	19 to 25
Conejo	2	2000 to 2500	1000 to 1200	21 to 28
Cónico	15	2000 to 2500	800 to 1200	12 to 24
Cónico Norteño	2	1600 to 1900	500 to 700	20 to 24
Elotero de Sinaloa	4	600 to 1500	800 to 1300	18 to 26
Elotes Cónicos	51	2000 to 2500	800 to 1200	16 to 22
Elotes Occidentales	33	800 to 1600	700 to 1200	18 to 26
Mushito	8	2200 to 2500	1100 to 1500	10 to 22
Nal-Tel	2	200 to 500	500 to 800	25 to 29
Nal-Tel de Altura	1	1000 to 1200	1000 to 1200	28 to 32
Negro de Tierra Caliente	1	800 to 1000	800 to 1200	24 to 28
Olotillo	6	400 to 1000	600 to 1300	18 to 30
Olotón	4	2000 to 2000	1200 to 1800	14 to 24
Reventador	1	100 to 200	400 to 800	25 to 28
Tabloncillo	1	1000 to 1200	1200 to 1800	15 to 28
Tepecintle	11	400 to 1200	1200 to 2000	20 to 28
Tuxpeño	10	200 to 1500	1200 to 1400	14 to 28

2.2. Reagents and Standards Used

The organic solvents used were methanol, ethanol, methanol HPLC grade, acetone, and petroleum ether, all from JT Baker (Xalostoc, Mexico). Other reagents were acetic acid, trifluoroacetic acid, Folin–Ciocalteu reagent, ferulic acid, catechin, sodium hydroxide, and aluminum chloride bought from Sigma-Aldrich (St. Louis, MO, USA). The anthocyanin standards of cyanidin 3-glucoside, pelargonidin 3-glucoside, peonidin 3-glucoside, and malvidin 3-glucoside were from Extrasynthese (FR).

2.3. Physical Grain Analysis

The traits determined included the weight of one hundred grains (W100G), which involved manually counting 100 seeds of each accession and recording their weight in a semi-analytical balance (Sartorius model BL 610. Gotinga, Germany). The determination of the location of the pigment (PL) was carried out following the procedure described

by Salinas-Moreno et al. [13]; for the type of endosperm (TE), the method of Bedolla and Rooney [14] was used. All determinations were made in duplicate. The grain color was determined with a Hunter Lab colorimeter (MiniScan model 5400L, Reston, WV, USA) on a CIELab scale. The values of “L”, “a”, and “b” were taken on the surface of the crown of the grain mounted on a gray plasticine base, simulating the form in which they are inserted on the cob. Tone angle (hue°) and chroma were calculated [15]. The color determination was performed in quadruplicate.

2.4. Phenolic Composition of Maize Grain

For the preparation of the grain samples before phenolic compound extraction, 30 grains of each accession were triturated with a hammer, taking care to cut the germ into tiny pieces with a cutter. The triturated grain sample was loaded into a cellulose cartridge and extracted for 8 h in a Soxhlet system using petroleum ether as the solvent. The defatted sample was ground in a cyclonic mill (UDY Corporation, Fort Collins, CO, USA) with 0.5 mm mesh. The moisture content of several of the ground samples (10% of the total) was determined using the method 44-19 of AACC [16] to report the content of the variables in the dry base. One gram of ground and defatted sample of grain of each accession was extracted with 20 mL of solvent. Two solvents were used: methanol acidified with trifluoroacetic acid (TFA) at 1% (MOHA) was employed for extracting phenolic compounds like phenolic acids and anthocyanins, while a mixture of acetone/distilled water/acetic acid (75:24.5:0.5 v/v/v) (ACE) was utilized to enhance the recovery of flavonoids and proanthocyanidins. The blend (sample + solvent) was subjected to sonication (Branson 2150 sonication bath, Danbury, CT, USA) for 15 min at room temperature and refrigerated for 105 min. Each sample was centrifuged (Hettich zentrifugen, Mod. Universal 32, Tuttlingen, Germany) at $2200 \times g$ for 10 min and the supernatant was recovered and filtered with Whatman 4 paper and the volume was measured. The MOHA extracts were used to quantify total soluble phenolics (TSP) using the Folin–Ciocalteu method [17], and total anthocyanin content (TAC) using the method of Abdel-Aal and Hucl [18] with the adaptations described by Salinas-Moreno et al. [19]. A standard ferulic acid curve was developed and TSP results were reported as μg equivalents of ferulic acid (FAE)/g of dry weight (DW). TAC results were expressed as μg equivalents of cyanidin 3-glucoside CGE/g of DW. A standard curve of cyanidin 3-glucoside (Extrasynthese, FR) was prepared. The ACE extracts were used for quantifying flavonoids (FLAV) and proanthocyanidins (PAs). FLAVs were quantified according to Sumczynski et al. [20], and reported as μg equivalents of catechin (CE)/g DW. PAs were determined by the method of DMAC (4-(Dimetil amino) cinnamaldehyde), following the method described by Wallace and Giusti [21]. The results were reported in terms of μg equivalents of catechin/g of DW.

2.5. Anthocyanin Profiling of Grain Accessions

Anthocyanin profiling analysis was undertaken only on samples of the two maize races with the highest number of accessions (Elotes Cónicos and Elotes Occidentales), collected at different altitudes. The anthocyanin extraction for HPLC analysis was carried out using 1.0 g of ground defatted grain and 15 mL of aqueous formic acid at 2%. The sample was sonicated for 15 min in the sonication bath and refrigerated for 105 min. Afterward, the sample was centrifuged at a $2660 \times g$ for 10 min, and the supernatant was recovered. The anthocyanin profile was obtained with the use of a Perkin-Elmer HPLC system (series 200, Shelton, CT USA), with a Hypersil ODS-2 (250×46 mm, 5 μm) analytical column. The analytical procedure applied was that described by Fossen et al. [22]. An aliquot of one mL of the extract was filtered through a 0.20 μm Millex-LG[®] membrane (Millex PTFE, 4 mm, Sigma-Aldrich, Toluca, Mexico) prior to injecting into the equipment. The volume injected was 10 μL , the flow rate was 1.2 mL/min, and the running time was 25 min. The temperature of the column was maintained at 25 °C. The detection was performed at 520 nm with a UV-DAD coupled to the HPLC system. One sample of each of the two races was chosen to be analyzed as a raw sample, alkaline-treated sample, and acid-treated

sample, according to the process described by de Pascual-Teresa et al. [23] to identify the esterified and non-esterified anthocyanins, as well as the aglycons. Commercial standards of cyanidin 3-glucoside, perlargonidin 3-glucoside, peonidin 3-glucoside, and malvidin 3-glucoside (Extrasynthese, FR) were used to help in the identification of the compounds. Published studies about analyses of anthocyanins in maize grain were also used [13,24] to complete the identification of some anthocyanins.

2.6. Analysis of the Data

To determine the behavior of the variables related to the physical traits (W100G, ET, and PL) and phenolic composition (TSP, TAC, FLAV, and PAs) of the 207 accessions of blue-purple maize grain, descriptive statistics were used by calculating indicators of central tendency, dispersion, and asymmetry. Additionally, histograms and accumulated frequencies were constructed for the two sets of variables. A cluster analysis was conducted to identify groups among maize races that exhibit similar phenolic properties. For this analysis, the averages of the phenolic composition variables were taken and standardized. The differences between the elements were calculated using the Manhattan method and grouping by the Ward method. In addition to the cluster analysis and to determine the phenolic variables that influenced the formation of the hierarchical groups, a principal component analysis (PCA) was developed for the 21 maize races. In this analysis, the averages by race of the variables TSP, TAC, FLAV, and PAs were considered, and were standardized and ranked by the correlation method [25]. Once the groups had been defined, the statistical differences between them were established for each phenolic variable, using box plots and Tukey's test of means. The software used was R-Studio, version R-4.1.2 [26].

3. Results and Discussion

3.1. Physical Traits of Maize Grain and Phenolic Composition of the Accessions

A first exploratory analysis of the variables of physical characteristics of the grain and the phenolic composition showed that, in general, in each variable, there was not much dispersion between the data; that is, the possibility of finding extreme data that could alter the analyses is ruled out (Table 2). For the physical characteristics, the construction of the histograms allowed us to determine that of the 207 blue-purple maize accessions (BPMA) analyzed, more than 80% were concentrated on the values left of their respective means (Figure 2). The weight of 100 grains (W100G) varied between 16.1 and 70.5 g (Table 1), which illustrates the great variability of the accessions analyzed. However, about 80% presented W100G between 20 and 40 g, a range that is similar to that found by Uriarte-Aceves et al. [27] in 15 landraces with blue-purple maize grain of the Elotero de Sinaloa race.

The predominant endosperm type (TE) in the grain of the accessions was floury, with the majority (~80%) of the samples having 75 to 100% floury endosperm. Nankar et al. [28] reported floury endosperm in the blue maize grain of landraces from the southwestern USA.

BPMA grain presented pigmentation predominantly (>95%) in the aleurone layer, a result that coincides with what has been reported in other studies, like Mahan et al. [29]. The location of the pigment in maize grain is an important trait for adapting the uses of the grain. Most maize landraces destined to prepare nixtamalized maize products have pigments in the aleurone layer, and this layer exerts some protection for the anthocyanins against the alkaline conditions of the nixtamalization process [30].

The color parameters of the accessions under study showed luminosity (L) values between 15.9 and 31.1%, which means that the grain is dark (Figure 3); the variable a^* ranged from -0.63 to 9.22 . Negative values are related to the green tone, while positive values correspond to red tones (Figure 3A). Most of the accessions presented grain with positive values of a^* , and therefore with a reddish-purple tone. In the blue-purple grain accessions examined, anthocyanins were located in the aleurone layer. The pericarp lacked pigments, but had a slightly yellow tone, associated with positive b^* values. The grain of some accessions showed negative values in b^* , indicative of a blue tone, which dominated compared to the yellow tone of the pericarp (Figure 3B).

Table 2. Indicators of central tendency, dispersion, and symmetry of the variables related to the physical characteristics of the grain and phenolic composition, obtained from the analysis of 207 accessions of blue-purple maize grain from Mexico.

		Physical Characteristics of Grain ¹			Phenolic Composition ²			
Type	Name	W100G	TE	PL	TSP	TAC	FLAV	PAs
Central	Mean	34.91	74.52	2.03	1965.93	601.84	562.51	43.11
	Median	33.90	75.00	2.00	1960.99	558.85	529.58	39.59
	Mode	28.05	75.00	2.00	*	*	516.93	39.10
Dispersion	Standard deviation	8.69	16.07	0.18	291.26	237.40	149.88	12.63
	Variance	75.44	258.18	0.03	84,830.17	56,356.60	22,463.99	159.47
	Coefficient of variation	24.88	21.56	8.87	14.82	39.44	26.64	29.29
	Range	54.39	75.00	1.00	1621.75	1375.09	680.56	73.62
	Minimum	16.09	25.00	2.00	1369.15	180.21	319.91	25.63
Symmetric	Maximum	70.47	100.00	3.00 ³	2990.91	1555.30	1000.47	99.26
	Kurtosis	1.73	−0.22	25.53	0.53	2.79	−0.24	3.20
	Asymmetric coefficient	0.94	−0.09	5.22	0.55	1.36	0.69	1.58

W100G: weight of 100 grains (g); TE: type of endosperm (% of floury endosperm); PL: pigment location, 1 = pericarp, 2 = aleurone, 3 = both pericarp and aleurone; TSP: total soluble phenolics (μg FAE/g of DW); TAC: total anthocyanin content (μg CGE/g DW); FLAV: flavonoids (μg CE/g DW); PAs: proanthocyanidins (μg CE/g DW). * Amodal distribution ('none' mode).

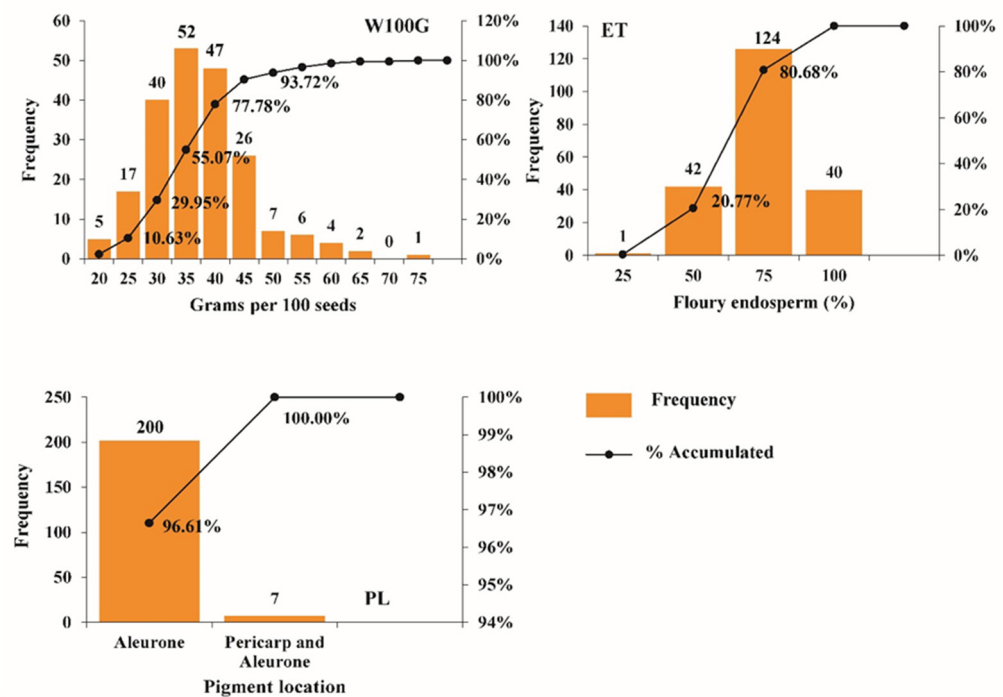


Figure 2. Frequency distribution histograms and accumulated percentage of physical grain characteristics in 207 accessions of blue-purple maize grain of Mexican races.

The hue parameter in the accessions presented a wide variation (1.52 to 358.2°); this variable, together with the luminosity, allowed the separation of the accessions into two groups. The group of accessions with high hue values (197 – 358°), characteristic of a bluish tint, was smaller and more compact than the group of accessions with low values (1.5 – 85.6°), which exhibited a reddish tint (Figure 3C). All accessions with high hue values presented negative b^* values. Chroma values varied between 0.2 and 10.19 (Figure 3D), with most of the accessions having values between 0.2 and 4 , indicative of low color purity and a high presence of gray tones.

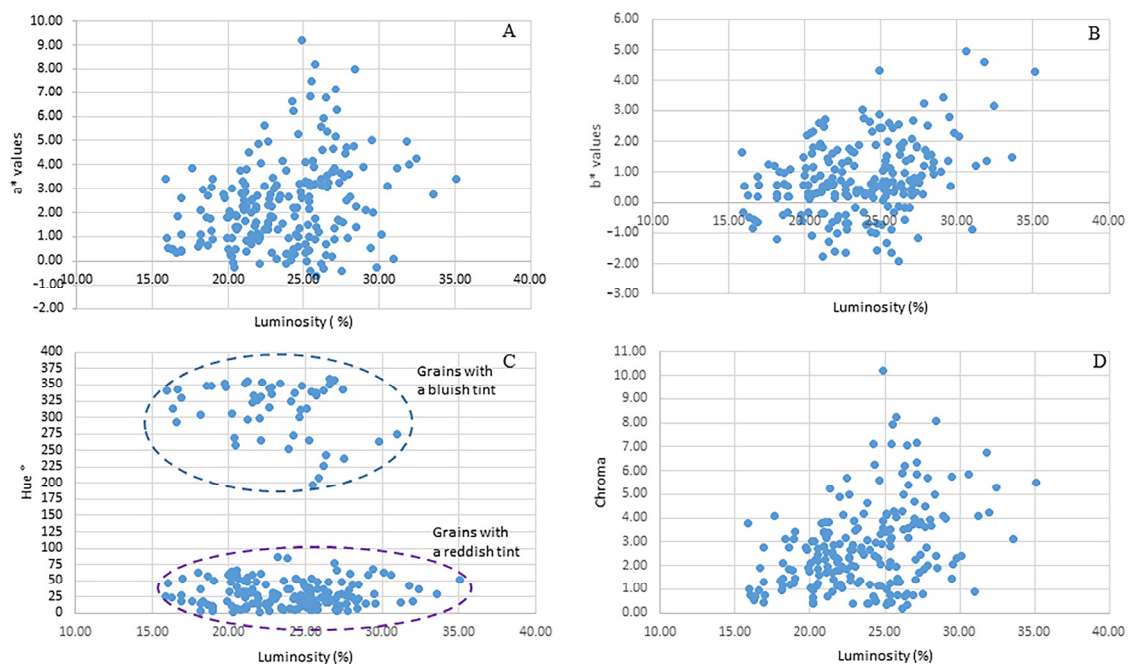


Figure 3. Color of blue- purple maize grain accessions in terms of luminosity, a^* , b^* , hue, and chroma parameters. (A): a^* value, (B): b^* value, (C): Hue°, (D): Chroma.

The surface color of the blue-purple maize grain is not uniform. Areas such as the pedicel and the germ region commonly do not have pigments, in addition to the fact that the pericarp is translucent, so when measuring the color on the surface of a grain sample, the hue values obtained do not correspond to the blue-purple tone characteristic. In this regard, Salinas-Moreno et al. [31] reported an average hue value of 80.3° for the blue-purple grain of 34 maize accessions of seven different races. In addition, Espinosa-Trujillo et al. [32] reported an average value of 108.5° for 18 blue-purple grain accessions from different maize races.

The histograms of the variables related to the phenolic composition of the accession's grains showed an asymmetric distribution slightly shifted to the left end (Figure 4). Of the phenolic variables determined, TSP showed the highest values since this variable includes all phenolic compounds with the capacity to oxidize the key complex reagents of the method [33]. The variability of TSP among the BP maize accessions (BPMA) analyzed was lower than that observed in TAC, FLAV, and PAs (Table 2).

For the variable TSP, there was a variation from 1400 to 3000 µg equivalents of ferulic acid (FAE)/g of the dry sample (DW). The phenolic compounds identified in an acidified methanol extract from a blue-purple maize grain included seven different groups of phenolics, of which the principal ones were phenolic acids and anthocyanins [34].

The values of TSP from blue-purple maize grain can vary in functions of genetics, growing conditions, and the particularities of the quantification method. Regarding the latter, the standard used to express the results has a significant effect. The use of gallic acid as a standard for expressing the TSP of maize grain could lead to lower values than when ferulic acid is used. Urias-Peraldi et al. [35] reported a range of 946 to 1348 mg GAE/kg DW for 25 hybrids of blue maize grain cultivated in two locations. This range is lower than that observed in our study.

The total anthocyanin content (TAC) in the grain of the accessions showed a variation between 200 and 1600 µg equivalents of cyanidin 3-glucoside/g of DW, with the majority (82.8%) of the samples located between 400 and 800 µg CGE/g DW. The range showed in the majority of the accessions is higher than that reported by Mora-Rochín et al. [36] in 15 landraces of blue maize grain from the Elotero de Sinaloa race, which ranged from 141 to 343 µg CGE/g DW.

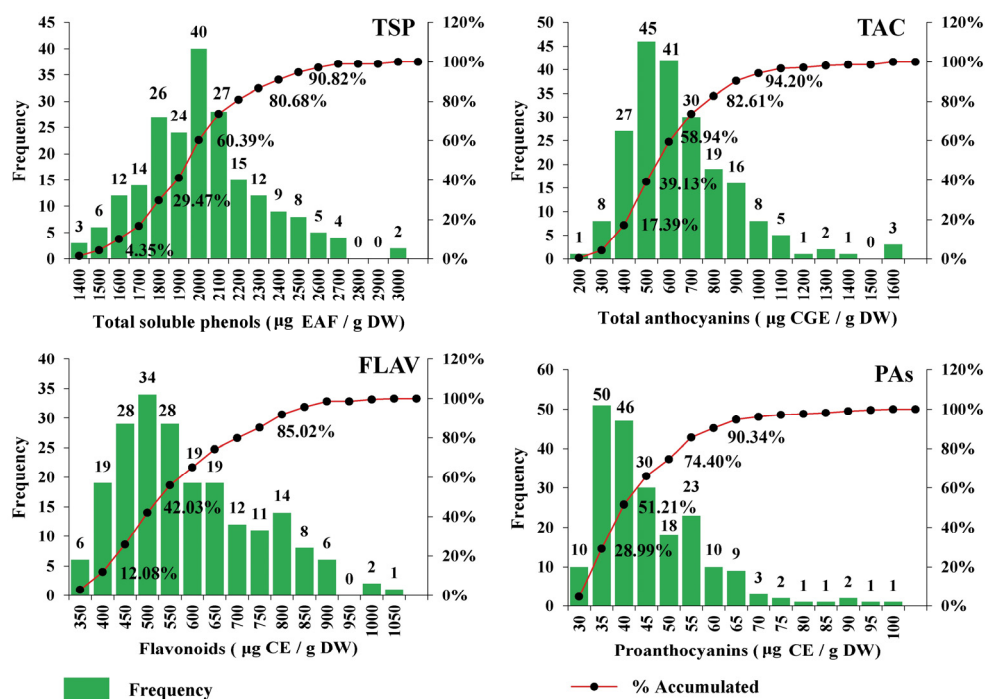


Figure 4. Frequency distribution histograms and accumulated percentages of phenolic compounds in 207 accessions of blue-purple maize grain of Mexican races. TSP: total soluble phenolics, TAC: total anthocyanin content, FLAV: flavonoids, PAs: proanthocyanidins.

The flavonoid content (FLAV) showed values between 350 and 1050 μg catechin equivalents (CE)/g DW, while proanthocyanidins (PAs) were found in low quantities (30 to 100 μg catechin equivalents/g DW). Of the non-anthocyanin flavonoids present in blue-purple maize grains, quercetin derivatives are among the most cited [30]; however, derivatives of kaempferol and myricetin have also been reported in purple maize grains [37]. The low PA content in the blue-purple maize grains analyzed was in agreement with the range reported by Luna-Ramírez [38] in maize landraces with the same grain color from Chalqueño, Cónico, and Bolita races, which was from 24.2 to 59.7 μg CE/g DW using same analytical method. However, with the vanillin method, the PAs content in blue maize was 7100 μg CE/g DW [39]. This high value could be because quantification is carried out at 510 nm, a wavelength at which anthocyanins also absorb. The authors mentioned that catechin and epicatechin are the two most common flavanol monomers present in oligomers of maize grain.

Environmental growing conditions of the site where maize accessions are collected influence the phenolic composition of grain. Therefore, variations in the values of variables used to assess phenolic composition are directly tied to the unique environmental factors of each location. When a maize accession from a germplasm bank (GB) is cultivated in a particular location, the quantity of phenolic compounds may differ. Salinas-Moreno et al. [40] observed variations in the TAC of grains from 48 purple maize accessions cultivated in specific locations compared to grains obtained from the GB. In the majority of the accessions, the content of TAC in grains from the GB was consistent with that of the cultivated grains. However, in other accessions, the values were either higher or lower.

3.2. Anthocyanin Profiling of Maize Grain Accessions in Mexican Varieties

With the protocol of analysis used, 12 peaks were separated, of which the peaks 4 and 9 were the most important, and they corresponded to cyanidin 3-glucoside (C3G) and cyanidin 3-malonilglucoside, respectively (Figure 5, Table 3). The number of anthocyanins reported in blue-purple maize grain varied in function with the particularities of the method of analysis used. It fluctuated from seven to 12 peaks [41,42]. The anthocyanin

profile of the accessions of Elotes Cónicos y Elotes Occidentales races were similar, with slight variations in the proportion of each peak, which probably influenced the maximum absorption exhibited by the extracts of the samples.

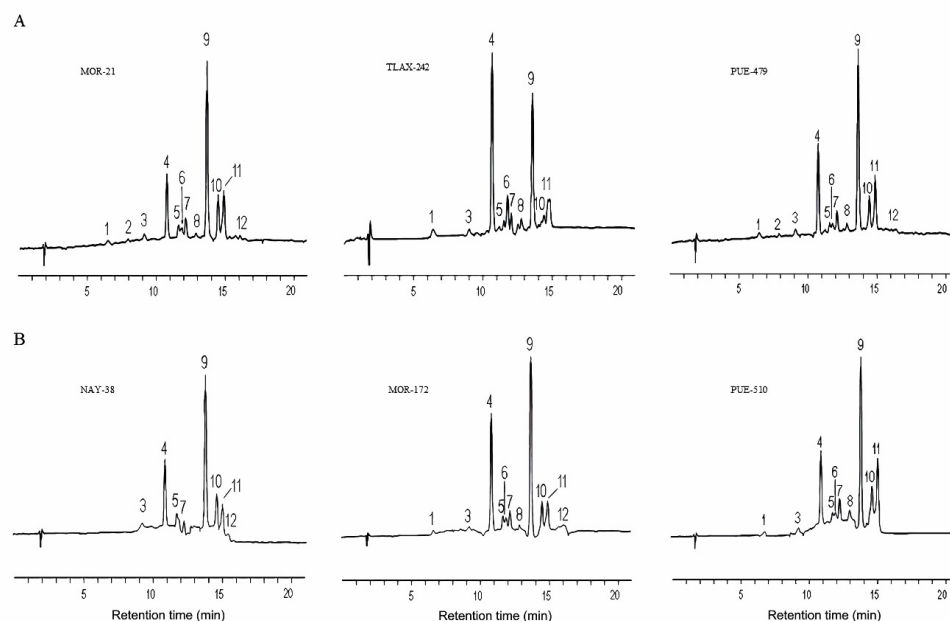


Figure 5. Chromatographic anthocyanin profile of BP maize grain of accessions from the race Elotes Cónicos (**A**) collected at altitudes of 1200 (MOR-21), 2300 (TLAX-242), and 2800 masl (PUE-479), and the race Elotes Occidentales (**B**) collected at altitudes of 100 (NAY-38), 1169 (MOR-172), and 2400 masl (PUE-510). The identity of the peaks is described in Table 3.

Table 3. Chromatographic parameters, identity, type of anthocyanins, and references used to complement the anthocyanins' identification.

Peak	Rt (min)	Identity	Anthocyanin Type	References
1	6.52	NI	No acylated	
2	7.97	NI	No acylated	
3	9.14	NI	Acylated	
4	10.80	Cyanidin 3-glucoside	No acylated	Standard
5	11.65	Acylated derivative	Acylated	Standard
6	11.89	Pelargonidin 3-glucoside	No acylated	
7	12.17	Acylated derivative	Acylated	
8	12.90	Peonidin 3-glucoside	No acylated	Standard
9	13.71	Cyanidin 3-malonyl glucoside	Acylated	Salinas-Moreno et al. [13]; Paulsmeyer et al. [42]
10	14.52	Pelargonidin 3-malonyl-glucoside	Acylated	Salinas-Moreno et al. [13]; Paulsmeyer et al. [42]
11	14.94	Cyanidin 3 dimalonyl-glucoside	Acylated	Salinas-Moreno et al. [13]; Paulsmeyer et al. [42]
12	16.15	NI	Acylated	Standard
13	15.71	NI	Acylated	
14	15.78	Cyanidine	Aglycone	
15	18.00	Pelargonidine	Aglycone	Standard
16	18.67	Peonidine	Aglycone	Standard

Rt: retention time, NI: not identified.

This result shows that the anthocyanin profile in the maize grain is determined by its color, and the race to which the accession belongs is not as important. However, Salinas-Moreno et al. [13] analyzed the anthocyanin profiles in six accessions of the maize races

Chalqueño, Elotes Cónicos, and Bolita and reported a profile lacking peonidin derivatives in accessions from the Bolita race.

The three accessions examined in each race, collected at different altitudes, exhibited similar anthocyanin profiles. Although specific details about the growing conditions of these accessions are unavailable, our results suggest that the altitude of the location (for which we have data) in which they grew did not have a significant impact on the anthocyanin profile. However, it is possible that growing conditions could influence the proportion of each anthocyanin. In this regard, Hu et al. [43] reported that pelargonidin content, in black sweet maize grain, decreased during the autumn compared to the summer season. These changes were associated with variations in photosynthesis rates and gene expression levels between the two seasons.

To complement the identification of anthocyanins, alkaline hydrolysis [23] of the crude extract of the grain of one accession from the Elotes Cónicos race and one from the Elotes Occidentales race was undertaken. Of the twelve peaks separated in the raw sample (Figure 6A), only six peaks were observed in the chromatogram of the hydrolyzed sample (Figure 6B), of which the largest corresponded to C3G, which was observed in a greater proportion than in the raw sample. In the blue-purple maize grain, acylated anthocyanins derived from cyanidin dominate [13,42]. When a raw sample is hydrolyzed with alkali, the ester bond between the sugar and the acyl radical is broken, resulting in a glycosylated anthocyanin, which in this case is C3G. Glucose is the most common sugar glycosylated to the anthocyanidins in maize grain and malonyl is the most common acyl radical [41]. However, Nankar et al. [28] found that succinyl is the acyl radical in the acylated anthocyanins of blue-purple maize grain, and Mora-Rochín et al. [36] reported acylated anthocyanins with both malonyl and succinyl radicals in blue maize grain.

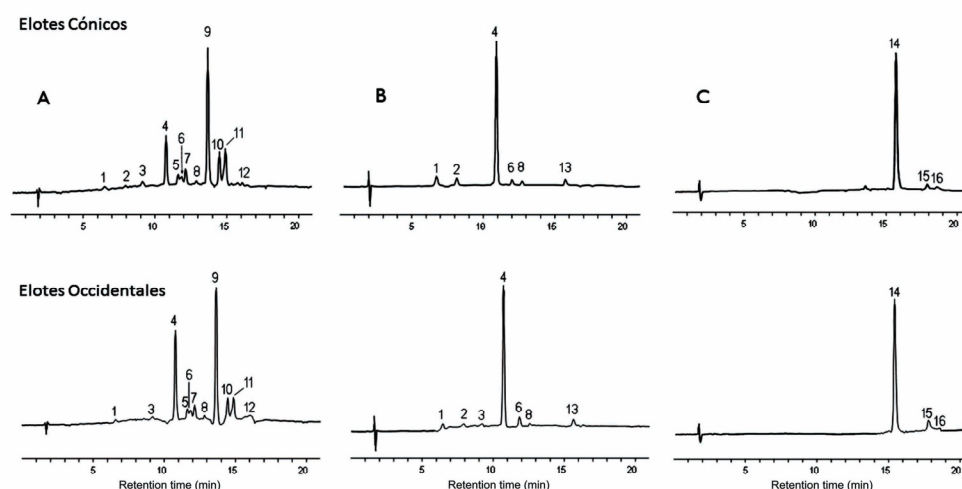


Figure 6. Chromatograms of blue-purple maize accessions from the varieties Elotes Cónicos and Elotes Occidentales. Raw extract (A), alkali hydrolyzed extract (B), and acid hydrolyzed extract (C). The identity of the peaks is described in Table 3.

The non-acylated anthocyanins, pelargonidin 3-glucoside (peak 6) and peonidin 3-glucoside (peak 8), remained after the alkaline hydrolysis. Peaks 1 and 2 resisted the alkaline hydrolysis, meaning that they were not acylated anthocyanins. They are very polarized substances, and it is possible that they are condensed forms of anthocyanins with catechin or epicatechin, as has been reported by González-Manzano et al. [44]. In fact, Paulsmeyer, et al. [42] considered condensed forms of anthocyanins, all peaks that appeared before C3G in the chromatogram. Peak 13, that appears in the alkaline hydrolyzed chromatogram was not identified.

The chromatogram of the sample hydrolyzed with acid and temperature showed only three peaks (Figure 6C). With this treatment, both the acyl radical and the sugar

were eliminated from the structure, leaving only the aglycone (anthocyanidin) preserved. The aglycones found in the blue-purple maize grain are cyanidin, pelargonidin, and peonidin [41]. Among these, cyanidin predominated (89%), while peonidin was found in the lowest proportion (<3%). Cyanidin also predominates in purple maize grain, but the proportion of peonidin is much higher than that of pelargonidin [24].

3.3. Hierarchical Analysis and Principal Component Analysis of Blue-Purple Maize Grain Races in Mexico

The hierarchical analysis allowed us to differentiate three groups in which the 21 races of blue-purple maize grain analyzed, which combine the 207 accessions, were concentrated. Each group was formed according to the similarity of the phenolic variables shared by the races (Figure 7A). The most populous group was group 2, made up of 11 maize races, among which were the two with the highest number of accessions analyzed (Elotes Cónicos and Elotes Occidentales).

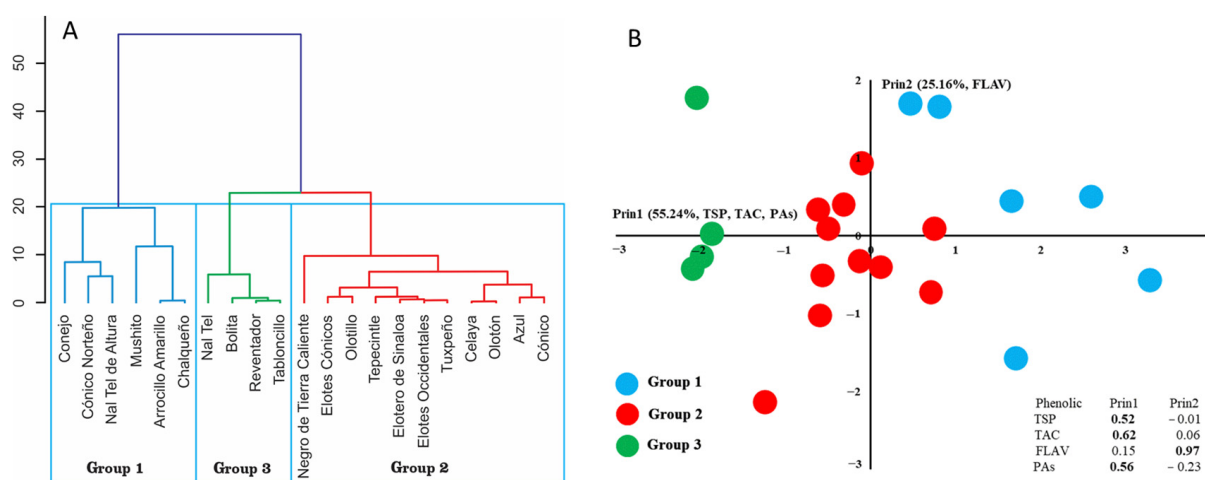


Figure 7. Dendrogram of the 21 maize races, with blue-purple grain reported for Mexico grouped by the similarity of their phenolic properties (A) and the dispersion of the varieties in the function of Prin1 and Prin2 obtained through the principal components analysis (B).

In the PCA, it was found that in Prin1 the variables TSP, TAC, and PAS were grouped and explained 55.24% of the total variation of the data, while in Prin2 the variable FLAV was grouped and explained 25.16% of the total variation. Both components (Prin1 + Prin2) explained 80.40% of the total variation. In Figure 7B, it can be seen that in the races of group 1, the content of TSP, TAC, and PAS tended to be higher than in the races of groups 2 and 3. However, it can also be seen that the FLAV content does not seem to be different among the maize races of the three groups formed. In addition, the contribution of the variable FLAV in Prin2 is lower than the contribution of variables TSP, TAC, and PAS in Prin1; in this last component (Prin1), TAC had the highest contribution.

We did not find published reports about groupings of Mexican maize diversity in the function of phenolic variables in grains. However, several studies aimed at the analysis of maize diversity using morphological and agronomic traits have been conducted in landraces with white [45] or pigmented grains [46]. The use of molecular genetic markers (SSRs or Microsatellites) to measure maize diversity has occurred in studies from other countries [9].

Tukey's test of means, applied to the groups obtained from the hierarchical analysis, revealed differences in the average content of the phenolic variables among groups. Group 1, comprising six maize races, was outstanding in its content of TSP, TAC, and PAS, while group 3 showed lower values in these variables. However, group 1 showed the highest variability in TSP, FLAV, and PAS, which comprised a high diversity of phenolic

compounds. Groups 2 and 3 exhibited similar patterns in TAC and PAs content and no differences among groups were observed for the FLAV variable (Figure 8).

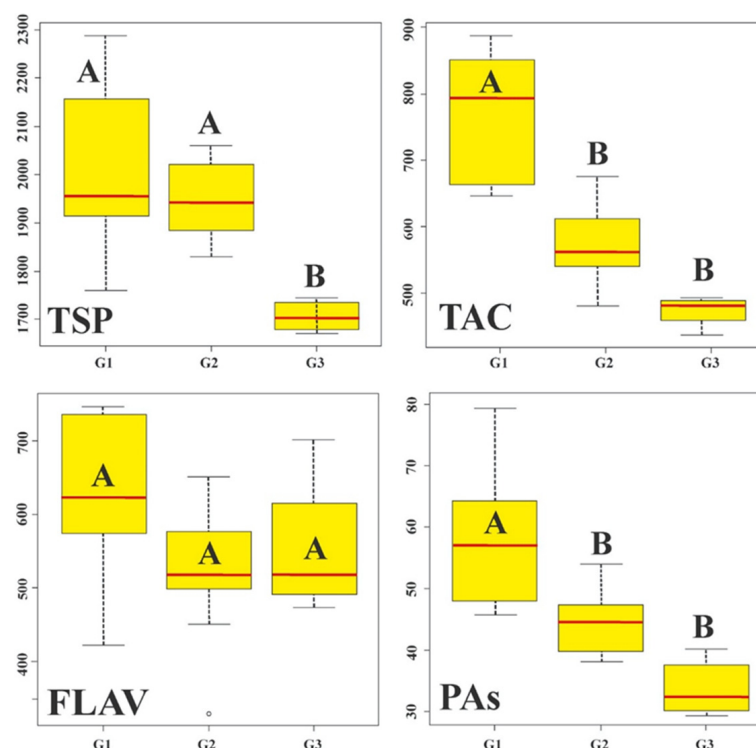


Figure 8. Box plots of the means of the three hierarchical groups by phenolic properties of blue-purple maize grain reported for Mexico. Means with the same letter per diagram are not statistically different (Tukey, $\alpha = 0.05$).

The methodology of environmental units of Gómez-Orea [47] allowed the territorial characterization of the climatic and altitude conditions of the three groups where the 21 races of maize with blue-purple grain are developed in Mexico (Figure 9). These results coincide with those reported by Ruiz-Corral et al. [48], who applied the same hierarchical analysis technique to 42 races of maize with different grain colors and conducted it to differentiate four groups according to the similarity of climatic and altitude variables.

In this way, our analysis for Group 2 differentiated two regions (Centre and North), spatially separated but environmentally similar; in the Center region, ten races are grown, and one in the North region (Blue race). For Group 3 (Centre-South), the races that make it up (4) are distributed from the center of the country to the south, and for Group 1, the six races it includes are distributed from the center of the country to the west. Like Ruiz-Corral et al. [48], our analysis agrees that the temperature regime is a good parameter to differentiate the regions, in such a way that the races of Group 2 are grown in dry–semi-warm climates, those of Group 3 in humid–semi-warm climates, and those of the Group 1 in dry–warm climates.

The environmental characterization of the hierarchical groups and Tukey's mean tests allowed us to assume that the races with the best phenolic properties were distributed from the center to the west of the country (Group 1), in dry–warm climates, with average temperatures less than 22 °C, precipitation less than 1200 mm per year, and altitudes greater than 1500 masl. This fact is explained due to the genotype–environment interaction that gives maize plants unique characteristics depending on the physical environment in which it is grown [49], and even due to social factors in production modes that are unique per region.

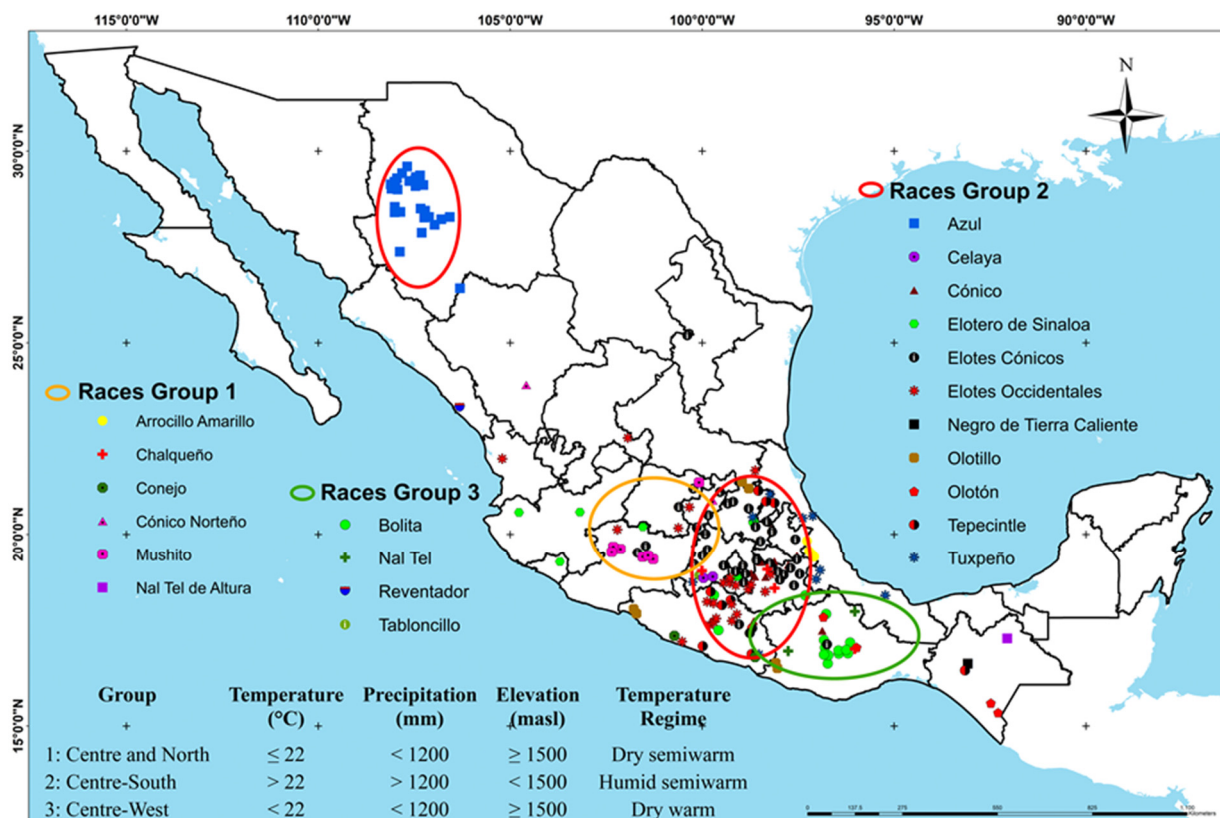


Figure 9. Environmental characterization of the hierarchical groups to define the regions in which the races with the best phenolic properties of blue purple maize grain are distributed in Mexico.

4. Conclusions

In the blue-purple maize grain accessions analyzed, floury endosperm predominated, and a large grain size, and the pigment was mainly located in the aleurone layer.

Of the phenolic composition variables, only the content of soluble phenols showed a normal distribution; the rest of the variables presented an asymmetric distribution oriented towards the left side. Total anthocyanin content was the variable most useful to differentiate and group the maize races. Acylated cyanidin derivatives predominated in the anthocyanin profile.

The extensive exploration of the diversity of maize with a blue-purple grain color in its phenolic composition allowed the identification of outstanding maize races in this characteristic that have not been explored.

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