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Anthocyanin content and physical characteristics of kernels from 300 pigmented maize accessions

Contenido de antocianinas y características físicas de granos de 300 accesiones de maíz pigmentado

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ABSTRACT

Pigmented corn boasts more antioxidants and superior nutritional quality than white or yellow corn. Identifying varieties with high anthocyanin content is crucial due to their positive impact on human health. This research aimed to characterize 300 accessions of pigmented maize from Mexico based on the physical characteristics of the grain and total anthocyanin content, targeting to employ them in genetic improvement programs. The averages of total anthocyanin content, percentage of floating seeds (indicating hardness), weight of one thousand seeds, length, width, thickness, dimension, coloration percentage, and coloration location were estimated. Subsequently, a correlation analysis of anthocyanin content, hardness, length, width, thickness, dimension, and weight of one thousand seeds was conducted, followed by a principal component (PC) analysis using these variables. The anthocyanin content ranged from 136.53 (HIDA 247) to 723.9 (HIDA 250) μg g^{-1}. Approximately 47.3% of the accessions exhibited a desirable degree of hardness, and 68% demonstrated acceptable hardness for the nixtamal industry. Moreover, 100% showed an average width greater than 4.76 mm, rendering them suitable for this industry. The weight of one thousand seeds ranged from 239 to 532 g for CHIS 1089 and MICH 86, respectively. Blue coloration predominated (70 %). Pigmentation was mainly in the aleurone (88 %). Anthocyanin content showed no correlation with physical characteristics, but hardness correlated with length and dimension ($p \leq 0.05$). In the first two principal components, the variables with the greatest relevance were thousand-seed weight, length, and thickness (PC1), and anthocyanin content, hardness, and kernel width (PC2). The variability observed in this research may enable the selection of maize accessions with high total anthocyanin content.

KEY WORDS: *Zea mays*, anthocyanin, seed color, kernel hardness, maize accession.

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RESUMEN

Los maíces pigmentados poseen una gran cantidad de antioxidantes y una calidad nutricional mayor que el maíz blanco o amarillo. La importancia de identificar variedades con alto contenido de antocianinas radica en su impacto positivo sobre la salud humana. El objetivo de esta investigación fue caracterizar 300 accesiones de maíz pigmentado provenientes de la república mexicana, en función de las características físicas de grano y el contenido de antocianinas totales, con la finalidad de que puedan utilizarse en programas de mejoramiento genético. Se estimó los promedios del contenido de antocianinas totales, porcentaje de semillas flotantes (dureza), peso de mil semillas, largo, ancho, espesor, dimensión, porcentaje de la coloración y ubicación de la misma; luego, se efectuó un análisis de correlación del contenido de antocianinas, dureza, largo, ancho, espesor, dimensión y peso de mil semillas; con estas variables se realizó un análisis de componentes principales (PC). El contenido de antocianinas fue de 136.53 (HIDA 247) a 723.9 (HIDA 250) µg g^{-1}. El 47.3 % de las accesiones presentaron un grado de dureza deseable y el 68 % dureza aceptable para la industria del nixtamal; el 100 % mostró un ancho promedio superior a 4.76 mm, lo que las hace aptas para dicha industria. El peso de mil semillas fue de 239 a 532 g para CHIS 1089 y MICH 86. Predominó la coloración azul (70 %). La pigmentación se presentó principalmente en la aleurona (88 %). El contenido de antocianinas no tuvo correlación con las características físicas, pero la dureza se correlacionó con longitud y dimensión ($p \leq 0.05$). En los primeros dos componentes principales, las variables con mayor relevancia fueron: peso de mil semillas, el largo y el espesor (PC1), y contenido de antocianinas, dureza y ancho de grano (PC2). La variabilidad encontrada en esta investigación puede contribuir a la selección de accesiones de maíz que proporcionen un alto contenido de antocianinas totales.

PALABRAS CLAVE: *Zea mays*, antocianina, color de semilla, dureza de grano, accesión de maíz.

Introduction

Mexico serves as a significant reservoir of corn diversity, boasting 59 breeds (Sanchez *et al.,* 2000). All these breeds feature pigmented kernel variants, ranging in color from black to pale pink. However, the most prevalent colors include blue/purple, black, and red (Salinas *et al.,* 2012b). Pigmented kernel corn is rich in anthocyanins, flavonoid pigments linked to the prevention or development of chronic degenerative diseases such as cancer (Bello *et al.,* 2016; Alegría *et al.,* 2020), and exhibiting anticancer, anti-neurodegenerative, and anti-inflammatory properties (Kraft, 2008; Bello *et al.,* 2016). Typically, these pigments are found in the pericarp, aleurone, or both kernel structures (González *et al.,* 1999; Agama *et al.,* 2011).

According to Salinas et al. (2013b), the quantity and type of anthocyanins in corn kernels vary based on the kernel color and the concentration of the pigment in different structures; magenta-red kernels concentrate anthocyanins in both the pericarp and aleurone layer and can possess up to 10 times more anthocyanins than blue-purple kernels, which only concentrate anthocyanins in the aleurone layer. Furthermore, if the pigment is predominantly found in the aleurone, the kernel is suitable for nixtamalization, whereas if it accumulates adequately in the pericarp, the kernel could be utilized for pigment extraction. The significance of genetic enhancement for the development and utilization of varieties with high anthocyanin content stems from two primary reasons: first, their impact on the sensory characteristics of foods, which can influence their technological performance during food processing, and second, their implications for human health through several pathways (Aguilera *et al.,* 2011).

The anthocyanin content in pigmented corn ranges, according to one study, between 276.8 and 904.0 µg g^{-1} (Salinas *et al.,* 2012a), while another study found values between 271.01 and 1,989 µg g^{-1} (Hernández *et al.,* 2017), where the highest values were found in samples originating from Tlaxcala. In breeding work carried out by Ballesteros *et al.* (2019) to improve the nutraceutical properties in western corn, the anthocyanin content was reduced with respect to the original populations, which justifies the importance of taking advantage of and characterizing native varieties in their original state, as well as continuing breeding to increase or maintain the highest anthocyanins contents and other nutraceutical compounds, as well as other desirable characteristics.

The corn used for nixtamalization must comply with certain physical characteristics according to Mexican standards NMX-FF-034/1-SCFI-2020 and NMX-FF-034/1-SCFI-2002; for white corn, the maximum percentage of floating grains must be 10 to 50 % (flotation index to measure hardness and cooking time), i.e., hard and very hard grains are preferred (Secretaría de Economía-México 2002). Kernel width should exceed 4.76 mm, (Secretaría de Economía-México 2020). Since pigmented corn undergoes nutraceutical compound extraction processes, the above standards are not necessarily applicable to pigmented corn but could be useful as a standard for quality traits.

The objective of this research was to characterize 300 accessions of pigmented maize from the Mexican Republic, according to the physical characteristics of the grain and the total anthocyanin content, to use them in genetic improvement programs.

Material and Methods

The methodology described below was designed to achieve the general objective by fulfilling the following specific objectives:

- Identify the accession(s) with the highest anthocyanin content.
- Identify the accession(s) with appropriate hardness for nixtamalization.

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- Identify the accession(s) with thousand-kernel weight and width meeting the requirements of the corn standard for nixtamalization.

- Determine the location of coloration within the kernels.

- Identify the physical characteristics that correlate with each other and with anthocyanin content.

- Identify the variables that enable the characterization, differentiation, and grouping of the studied accessions.

Genetic material

Three hundred accessions of maize with pigmented grain, sourced from the germplasm bank of the International Maize and Wheat Improvement Center (CIMMYT), were analyzed. These accessions belong to 31 maize races, namely: Ancho, Arrocillo, Arrocillo Amarillo, Azul, Bofo, Bolita, Cacahuacintle, Celaya, Chalqueño, Cónico, Cónico Norteño, Cristalino de Chihuahua, Elotes Cónicos, Elotes de Sinaloa, Elotes Occidentales, Maíz Dulce, Mushito, Nal-Tel, Negro de Chimaltenango, Negro de Tierra Caliente[1], Olotillo, Olotón, Pepitilla, Perla, Reventador, San Juan[2], San Marceno[1], Tabloncillo, Tabloncillo Perla, Tepecintle, and Tuxpeño.

Physical characteristics of the grain

The physical characteristics of the grain were assessed at the seed quality laboratory of the Universidad Autónoma Agraria Antonio Narro. The weight of one thousand seeds was determined by weighing 100 randomly selected grains from each accession using a Precisa Gravimetrics analytical balance. This process was repeated three times per accession, and the results were averaged. Subsequently, the average weight was multiplied by 10 to obtain the weight of one thousand seeds. The length, width, and thickness were measured using a Truper® digital caliper, with a random sample of 10 grains from each accession. A variable called "dimension" was calculated by multiplying the values of length, width, and thickness, expressing the results in mm^3. The procedure followed the NMXFF-034/1-SCFI-2002 standard (Secretaria de Economía-México, 2020). This standard involves placing 100 grains in a sodium nitrate solution with a density of 1.25 g mL^{-1} (+/- 0.001 g mL^{-1}) at a temperature of 22 to 23 °C. The number of grains that rise to the surface was then quantified (Table 1). This procedure was conducted in duplicate.

1 Negro de Tierra Caliente and San Marceño are recognized as Breeds by Wellhausen, E. J., Alejandro, F. O., & Antonio, H. C. (1957). Razas de maíz en América Central. *National Academy of Sciences-National Research Council*, 136. https://doi.org/10.17226/21166

2 Race not well defined, or it is not clear if it belongs to any of the primary races; however, it is recognized as a primary race by the CIMMyT-Germplasm Bank.

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Determination of total anthocyanin content

The analyses were conducted at the Laboratory of Nutritional Quality of Maize "Evangelina Villegas" at CIMMYT, following the methodology outlined by Palacios (2018). Twenty kernels from each accession were ground, and anthocyanin extraction was performed by weighing 20 mg of flour into 1.5 mL Eppendorf tubes and adding 1.3 mL of trifluoroacetic acid. Subsequently, the absorbance of the samples was measured at 520 nm using a Quant BioTek microplate reader. The results are presented in µg pelargonidin per gram of dry weight sample (µg g^{-1}).

Table 1. Number of floating grains per 100 grains.

Hardness	Floating grains
Very hard	0 - 12
Hard	13 - 37
Intermediate hardness	38 - 62
Soft	63 - 87
Very soft	88 - 100

Source: NMXFF-034/1-SCFI-2002 (Secretaria de Economía-México 2020). Cooking time is related to hardness.

Statistical analysis

To identify the accession(s) with the highest anthocyanin content, the mean anthocyanin content of each accession was calculated. Subsequently, to classify the accessions based on their anthocyanin content, the number of classes (c) was determined using Sturge's Rule. This involved calculating the logarithm base 10 of the average number of accessions for each race (N) and rounding c to the nearest odd number. Then, the class width (a) was estimated from the number of classes and the range (r) of the average anthocyanin per accession, using the following equations:

$$c = 1 + 3.322 \log N$$

$$r = max - min$$

$$a = \frac{r}{c - 1}$$

Similarly, to identify the accession(s) with appropriate hardness for nixtamalization, the mean percentage of floating seeds of each accession was estimated.

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To determine which accession(s) met the requirements of the corn standard for nixtamalization regarding thousand-seed weight (PMS) and dimensions, the mean PMS, length, width, and thickness were estimated. Additionally, the percentage of each color and the location of coloration were assessed. These analyses were conducted using Microsoft Excel (2016).

To identify physical characteristics correlating with each other and with anthocyanin content, a Pearson correlation analysis was performed using SAS® Studio software.

To pinpoint the variables enabling the characterization, differentiation, and grouping of the studied accessions, a multivariate principal component analysis was conducted. This analysis included the variables total anthocyanin content, kernel hardness, thousand-kernel weight, kernel length, kernel width, and kernel thickness. Subsequently, a cluster analysis was performed, generating a dendrogram using the centroid method for the 31 maize races. These analyses were carried out in the statistical package Rstudio version 4.1.2, utilizing the ggplot2, factoextra, readxl, and nortest libraries.

Results and Discussion

Table 2 presents the average values of total anthocyanin content for the 31 breeds of pigmented corn. The breed with the highest total anthocyanin content was Tabloncillo Perla, with a value of 511.74 μg g^{-1}, while the breed with the lowest value was Pepitilla, averaging 248.7 μg g^{-1} of total anthocyanins. Breeds such as Elotes de Sinaloa, Mushito, Reventador, Tabloncillo, and Tepecintle fall within the average values.

In terms of total anthocyanin content, the HIDA 250 accession recorded the highest value at 723.9 μg g^{-1}, while the HIDA 247 accession had the lowest value at 136.53 μg g^{-1} (Table 2). These values fall below those reported by Salinas *et al.* (2012b), with a range from 276.8 to 904.0 μg g^{-1}, and Hernández *et al.* (2017), averaging 281.01 μg g^{-1} of total anthocyanins. Remarkably, variability was observed within the samples analyzed, with high values of 1,989 μg g^{-1} reported in samples from Tlaxcala. Additionally, these values were lower than those reported by Arellano *et al.* (2021), who obtained 774.7 μg g^{-1} of anthocyanins.

The flotation index results indicate that 62 accessions exhibited very hard kernels, including accessions HIDA 250, PUEB 509, and CHIS 1053. Furthermore, 80 accessions had hard kernels, 62 accessions had intermediate hardness, 59 accessions had soft kernels, and 37 accessions had very soft kernels. This implies that 142 of the 300 accessions (47.3%) possess a desirable degree of hardness for the nixtamal industry, while a total of 204 (68%) have an acceptable degree, according to the white corn guidelines required by NMX-FF-034/1-SCFI-2020 (Secretaría de Economía-México, 2020).

The lowest kernel size value was found in accession HIDA 74 and the highest value was obtained in accession OAXA 783 (data not shown). According to Salinas *et al.* (2012b), kernel size affects the concentration of anthocyanins, influenced by a dilution effect of anthocyanins

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in the flour of the kernel used for quantification, where a large kernel will have a greater dilution effect than a small kernel. On the other hand, 100 % of the accessions showed an average width greater than 4.76 mm, conforming to NMX-FF-034/1-SCFI-2020 (Secretaría de Economía-México 2020), the VERA 87 accession showed the lowest width value and DURA 163 the highest, 5.6 and 11.7 mm respectively (data not shown).

Table 2. Total anthocyanin content of the 31 races of pigmented maize, and accessions grouped into five intervals.

Primary Race	AC†	Anthocyanin contents†† (μg g^{-1})	Accessions per interval group of anthocyanin contents§				
			(63.0975, 209.9625]	(209.9625, 356.8275]	(356.8275, 503.6925]	(503.6925, 650.5575]	(650.5575, 797.4225]
PEPITILLA	2	248.7		PUEB GP79			
				PUEB 102			
CACAHUACINTLE	5	272.2	MICH 355	MICH 324			
				MEXI 628			
				MICH 369			
				PUEB 436			
MUSHITO	4	285.7		MICH 360			
				MICH 362			
				MICH 376			
				MICH 414			
SAN MARCENO§§	1	287.1		CHIS 689			
ANCHO	6	301.5	MORE 72	GUER 130	GUER 310		
				MORE 92			
				MORE 52			
				MORE 88			

†Accessions included per Primary Race. ††Results are presented in μg of pelargonidin per g of dry weight sample. §Lower and upper limits per class are shown, classes include values greater than the lower limit and less than or equal to the upper limit. The accessions are ordered from lowest to highest anthocyanin content, from base to tip, within the class columns for each race. §§San Marceño and Negro de Tierra Caliente were recognized as Races by Wellhausen *et al.* (1957). ‡Race not well defined, or it is not clear whether it belongs to any of the primary races; however, it is recognized as Primary Race by the CIMMyT-Banco de Germoplasma.

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ELOTES CONICOS	44	308.6	HIDA 223	JALI 54	TLAX 230		
			HIDA 116	GUAN 97	OAXA 796		
			PUEB 302	JALI 633	TLAX 248		
				GUER 305	SNLP 130		
				PUEB 647	PUEB 699		
				PUEB 711	MEXI 27		
				TLAX 232	PUEB 479		
				PUEB 719	OAXA 121		
				MEXI 282	QUER 94		
				PUEB 210	PUEB 14		
				PUEB 39	PUEB 708		
				PUEB 546			
				QROO 36			
				PUEB 594			
				PUEB 148			
				OAXA 26			
				PUEB 611			
				PUEB 149			
				TLAX 234			
				PUEB 589			
				PUEB 18			
				PUEB 600			
				PUEB 618			
				PUEB 304			
				QUER 84			
				PUEB 668			
				GUAN 139			
				HIDA 150			
				GUAN 373			
				PUEB 561			

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MAIZ DULCE	2	309.7		MICH 412 JALI 188			
ELOTES OCCIDENTALES	22	312.0	GUAN 429	GUER 364 PUEB 510 JALI 77 JALI 89 COLI 6 PUEB 98 QUER GP15 GUAN 9 GUAN 98 GUER 173 JALI 321 ZACA 168 MICH 138 SNLP 23 GUER 223 VERA 73 HIDA 276 NAYA 116 GUAN GP22	NAYA 38		PUEB 509
OLOTILLO	8	313.4		OAXA 243 SNLP 368 SNLP 370 CHIS 444 CHIS 434 PUEB 185	OAXA 249 OAXA 245		
CHALQUENO	7	317.1	MEXI 613	PUEB 81 MEXI 145 PUEB 535	PUEB GP75 PUEB 461 MEXI 33		

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NEGRO DE CHIMALTENANGO	1	317.2		CHIS 1099			
BOFO	18	317.7		ZACA 194	NAYA 280		
				NAYA 224	NAYA 191		
				SINA 58	NAYA 243		
				NAYA 201	NAYA 287		
				DURA 100	ZACA 180		
				SINA 49			
				SINA 52			
				ZACA 188			
				NAYA 222			
				NAYA 242			
				NAYA 281			
				NAYA 196			
				DURA 94			
CONICO NORTENO	18	317.8		CHIH 355	CHIH 378	QUER 3	
				AGUC 27	CHIH 441		
				QUER 58			
				GUAN 158			
				CHIH 353			
				GUAN 164			
				QUER 24			
				QUER 17			
				JALI 307			
				DURA 240			
				GUAN 146			
				PUEB 211			
				GUAN 149			
				DURA 238			
				SNLP 76			

[†]Accessions included per Primary Race. [††]Results are presented in µg of pelargonidin per g of dry weight sample. [§]Lower and upper limits per class are shown, classes include values greater than the lower limit and less than or equal to the upper limit. The accessions are ordered from lowest to highest anthocyanin content, from base to tip, within the class columns for each race. [§§]San Marceño and Negro de Tierra Caliente were recognized as Races by Wellhausen *et al*. (1957). [‡]Race not well defined, or it is not clear whether it belongs to any of the primary races; however, it is recognized as Primary Race by the CIMMyT-Banco de Germoplasma.

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CONICO	45	320.4	PUEB 388	MEXI 532	MEXI 553	MICH 119	
			VERA 358	PUEB 485	MEXI 521		
			VERA 328	MEXI 258	PUEB 332		
			PUEB 271	HIDA 85	PUEB 414		
			MEXI 129	PUEB 547	MEXI 235		
				MEXI 471	PUEB 387		
				PUEB 351	MICH 394		
				OAXA 382	PUEB 394		
				TLAX 250	PUEB 215		
				OAXA 326	MEXI 150		
				PUEB 495	MEXI 543		
				PUEB GP80	VERA 322		
				OAXA 300			
				MEXI 237			
				OAXA 288			
				MICH 319			
				QUER GP16			
				MEXI 244			
				PUEB 507			
				OAXA 374			
				HIDA 74			
				MEXI 250			
				OAXA 269			
				PUEB GP76			
				VERA 340			
				MEXI 275			
				MEXI 542			
ARROCILLO	3	321		PUEB 947	PUEB 746		
				PUEB 917			
PERLA	1	321		NAYA 233			

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TUXPENO	19	321.5	VERA 169	QROO 35	OAXA 272	VERA 161	
				VERA 496	SNLP 121		
				VERA 42	SNLP 75		
				VERA 486			
				VERA 148			
				VERA 214			
				VERA 508			
				HIDA 37			
				NAYA 175			
				GUER GP36			
				VERA 56			
				VERA 173			
				SNLP 67			
				NAYA 221			
CRISTALINO DE CHIHUAHUA	2	324.1		CHIH 218			
				DURA 193			
ARROCILLO AMARILLO	4	324.7		MEXI 53	MEXI 64		
				VERA 359			
				VERA 87			
NEGRO DE TIERRA CALIENTE§§	1	327.4		CHIS 978			
AZUL	19	327.7		CHIH 421	CHIH 427		
				CHIH 365	CHIH 420		
				CHIH 373	CHIH 384		
				CHIH 360	CHIH 133		
				CHIH 403	CHIH 430		
				CHIH 367	CHIH 377		
				CHIH 402			
				CHIH 439			
				CHIH 503			
				CHIH 354			
				CHIH 395			
				JALI 290			
				CHIH 357			

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Table 2. Total anthocyanin content of the 31 races of pigmented maize, and accessions grouped into five intervals.

Primary Race	AC†	Anthocyanin contents†† (µg g-1)	Accessions per interval group of anthocyanin contents§				
			(63.0975, 209.9625]	(209.9625, 356.8275]	(356.8275, 503.6925]	(503.6925, 650.5575]	(650.5575, 797.4225]
NAL TEL	3	328.7		OAXA 165	YUCA 147		
				YUCA 162			
OLOTON	12	332.2	OAXA 305	CHIS 420	CHIS 1098	CHIS 1053	
				CHIS 1089		CHIS 1044	
				CHIS 1022			
				CHIS 1100			
				CHIS 674			
				OAXA 310			
				CHIS 1060			
				OAXA 370			
BOLITA	20	332.5	OAXA 321	OAXA 804	OAXA 685	OAXA 789	
			OAXA 391	OAXA 725	OAXA 810		
				OAXA 929	OAXA 751		
				OAXA 811	OAXA 720		
				OAXA 698	OAXA 320		
				OAXA 707	OAXA 695		
				OAXA 783			
				OAXA 259			
				OAXA 800			
				OAXA 730			
				OAXA 758			
CELAYA	1	348.6		PUEB 233			
TABLONCILLO	6	361.4		DURA 142	SINA 41		
				DURA 141	MICH 86		
				JALI 313	DURA 169		
REVENTADOR	7	361.9		SINA 37	SINA 38		
				SINA 22	SINA 44		
				SINA 17	DURA 109		
					NAYA 292		

†Accessions included per Primary Race. ††Results are presented in µg of pelargonidin per g of dry weight sample. §Lower and upper limits per class are shown, classes include values greater than the lower limit and less than or equal to the upper limit. The accessions are ordered from lowest to highest anthocyanin content, from base to tip, within the class columns for each race. §§San Marceño and Negro de Tierra Caliente were recognized as Races by Wellhausen *et al.* (1957). ‡Race not well defined, or it is not clear whether it belongs to any of the primary races; however, it is recognized as Primary Race by the CIMMyT-Banco de Germoplasma.

Continuation

Table 2. Total anthocyanin content of the 31 races of pigmented maize, and accessions grouped into five intervals.

Primary Race	AC†	Anthocyanin contents†† (µg g^{-1})	Accessions per interval group of anthocyanin contents§				
			(63.0975, 209.9625]	(209.9625, 356.8275]	(356.8275, 503.6925]	(503.6925, 650.5575]	(650.5575, 797.4225]
TEPECINTLE	15	374	HIDA 247	HIDA 296 MORE 6 HIDA 239 VERA 837 HIDA 316 HIDA 297	OAXA 161 HIDA 251 OAXA 266 OAXA 337 SNLP 281 HIDA 312	VERA 801	HIDA 250
ELOTES DE SINALOA	2	375.1			CHIS 1117 MICH 232		
SAN JUAN‡	1	420.3			DURA 123		
TABLONCILLO PERLA	1	511.7				DURA 163	

†Accessions included per Primary Race. ††Results are presented in µg of pelargonidin per g of dry weight sample. §Lower and upper limits per class are shown, classes include values greater than the lower limit and less than or equal to the upper limit. The accessions are ordered from lowest to highest anthocyanin content, from base to tip, within the class columns for each race. §§San Marceño and Negro de Tierra Caliente were recognized as Races by Wellhausen *et al.* (1957). ‡Race not well defined, or it is not clear whether it belongs to any of the primary races; however, it is recognized as Primary Race by the CIMMyT-Banco de Germoplasma.

Accession CHIS 1089 obtained the lowest thousand-seed weight, 239 g, while accession MICH 86 obtained the highest value, 532 g (Data not shown). These values are similar to those obtained by Maldonado *et al.* (2021) who report values between 223 g and 522 g for thousand-seed weight and Cieza *et al.* (2020) who when evaluating 21 maize hybrids obtained results for thousand-seed weight between 297 and 381 g. Standard NMX-FF-034/1-SCFI-2020 (Secretaría de Economía-México 2020), does not specify minimum or optimum values for this variable, but it is useful in the characterization of varieties.

The accessions showed variable coloration, with 70 % of blue kernels, 20 % of red kernels, and 10 % of absent coloration; 88 % of the kernels showed coloration in the aleurone, 11 % in aleurone and pericarp, and 1 % in pericarp. In Chalqueño accessions, coloration in the aleurone layer predominated (78 %) followed by coloration in aleurone and pericarp (19 %), and finally, in pericarp (3 %); Salinas *et al.* (2012b) found that anthocyanins were located only in the aleurone layer in blue-purple grain samples of that race, thus making evident the association of coloration

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localization with the presence of anthocyanins.

There was a negative correlation between the percentage of grains floating (inverse of grain hardness according to NMX-FF-034/1-SCFI-2020), and length and size ($p \leq 0.05$, Table 3), thus it is evident that the larger the size, the greater the hardness (fewer grains floating). Anthocyanin content was not correlated with any of the physical characteristics (Table 3).

Table 3. Pearson correlation coefficient among variables of the physical characteristics of the grain and anthocyanin content.

	Float percentage	Weight of a thousand seeds	Length	Width	Thickness	Dimension
Anthocyanin	0.060 0.75 NS	-0.104 0.58 NS	0.164 0.38 NS	-0.198 0.29 NS	-0.31316 0.09 NS	-0.234 0.21 NS
Float percentage		-0.212 0.25 NS	-0.405 0.02 *	-0.134 0.47 NS	0.014 0.94 NS	-0.359 0.05 *
Weight of a thousand seeds			0.450 0.01 **	-0.272 0.14 NS	0.596 <0.01 **	0.679 <0.01 **
Length				-0.312 0.09 NS	0.053 0.78 NS	0.539 <0.01
Width					-0.390 0.03 *	0.122 0.51 NS
Thickness						0.605 <0.01 **

**, *, NS: Significant at 1 and 5 % probability levels and non-significant, respectively. Grain Hardness is inversely related to Float percentage, according to NMX-FF-034/1-SCFI-2020.

Table 4 shows the characteristics of the accessions with the highest anthocyanin content, those corresponding to the two highest intervals in Table 2. These accessions, in addition, could be considered with potential for the nixtamalization industry, since: the coloration is predominantly located in the aleurone, which is preferable according to Salinas *et al.* (2013a), although if it accumulates in the pericarp in sufficient quantity it could be used for pigment extraction. The flotation rate of 10 to 50 % predominates, which is desirable according to NMXFF-034/1-SCFI-2002 (Secretaría de Economía-México 2020). In nixtamalization, it could be desirable that the coloration is located in the aleurone, because if it is located in the pericarp, it is more likely that the anthocyanins are lost when they are detached during the process together with the pericarp.

Table 4. Physical characteristics of the accessions of groups 4 and 5 for anthocyanin content.

Access	Anthocyanin contents† (μg g^{-1})	Interval group††	Color	Color location	Float percentage, %	Hardness§	Hardness acceptance§§	Primary race
HIDA 250	723.99	(650.5575, 797.4225]	Blue	Aleurone	15	Hard	Yes	Tepecintle
PUE 509	677.71		Red	Aleurone	10	Very hard	Yes	Elotes Occidentales
CHIS 1053	620.12	(503.6925, 650.5575]	Blue	Aleurone	25	Hard	Yes	Oloton
CHIS 1044	616.11		Blue	Aleurone	75	Soft	No	Oloton
MICH 119	606.12		Blue	Aleurone (80 %) Pericarp and Aleurone (20 %)	55	Intermediate hardness	No	Cónico
VERA 161	602.26		Blue	Aleurone	10	Very hard	Yes	Tuxpeño
VERA 801	538.93		Blue	Aleurone	0	Very hard	No	Tepecintle
QUER 3	535.53		Blue	Aleurone	50	Intermediate hardness	Yes	Cónico Norteño
OAXA 789	520.78		Blue	Aleurone	60	Intermediate hardness	No	Bolita
DURA 163	511.74		Blue	Aleurone	90	Very soft	No	Tabloncillo Perla

†Results are presented in μg of pelargonidin per g of dry weight sample. ††Lower and upper limits per class are shown, classes include values greater than the lower limit and less than or equal to the upper limit. §According to NMX-FF-034/1-SCFI-2020 (Secretaría de Economía-México 2020). §§According to NMX-FF-034/1-SCFI-2020 (Secretaría de Economía-México 2020) for white maize, acceptable hardness goes from 10 to 50 % floating grains.

Exclusive coloration in the pericarp was observed in accessions GUER GP36 (95%) [294.64 μg g^{-1}], PUEB 589 (100%) [274.8 μg g^{-1}], SNLP (35%), MEXI 64 (20%), PUEB 461 (20%), and others with percentages equal to or less than 10% (CHIH 403, CHIH 421, GUAN 146, PUEB 233, NAYA 191, MORE 52, MICH 414). These accessions may be suitable solely for pigment extraction, although their total anthocyanin content is lower than the average (323.17 μg g^{-1}) and falls below the median range: (356.8275, 503.6925). GUER GP36 and PUEB 589 accessions

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exhibited blue (100%) and red (100%) coloration, respectively.

The results of the principal component (PC) analysis indicate that the first three components accounted for 66.35% of the proportion of variance (Table 5). The most relevant variables in PC1 were 100-kernel weight, length, and thickness. CP2 highlighted anthocyanin content, hardness, and kernel width as the most relevant variables. Figure 1 depicts the biplot of the first two principal components, illustrating the dispersion of the 300 pigmented maize accessions. Previous studies have also demonstrated the relevance of variables related to kernel and ear dimensions for the characterization of maize varieties, as shown in the study by Rocandio *et al.* (2014).

Table 5. Values and eigenvectors of the first three principal components that describe the variation of physical properties and total anthocyanin content of 300 accessions of pigmented corn.

	PC1	PC2	PC3
Eigenvalue	1.36	0.31	1.01
Variance proportion (%)	30.95	18.27	17.13
Cumulative proportion (%)	30.95	49.22	66.35
Anthocyanins	0.07	-0.60	-0.70
Hardness	0.05	**0.50**	-0.39
Weight of a thousand seeds	**-0.64**	-0.05	-0.13
Grain length	**-0.54**	0.03	-0.31
Grain width	-0.06	**-0.62**	0.39
Grain thickness	**-0.54**	0.07	0.30

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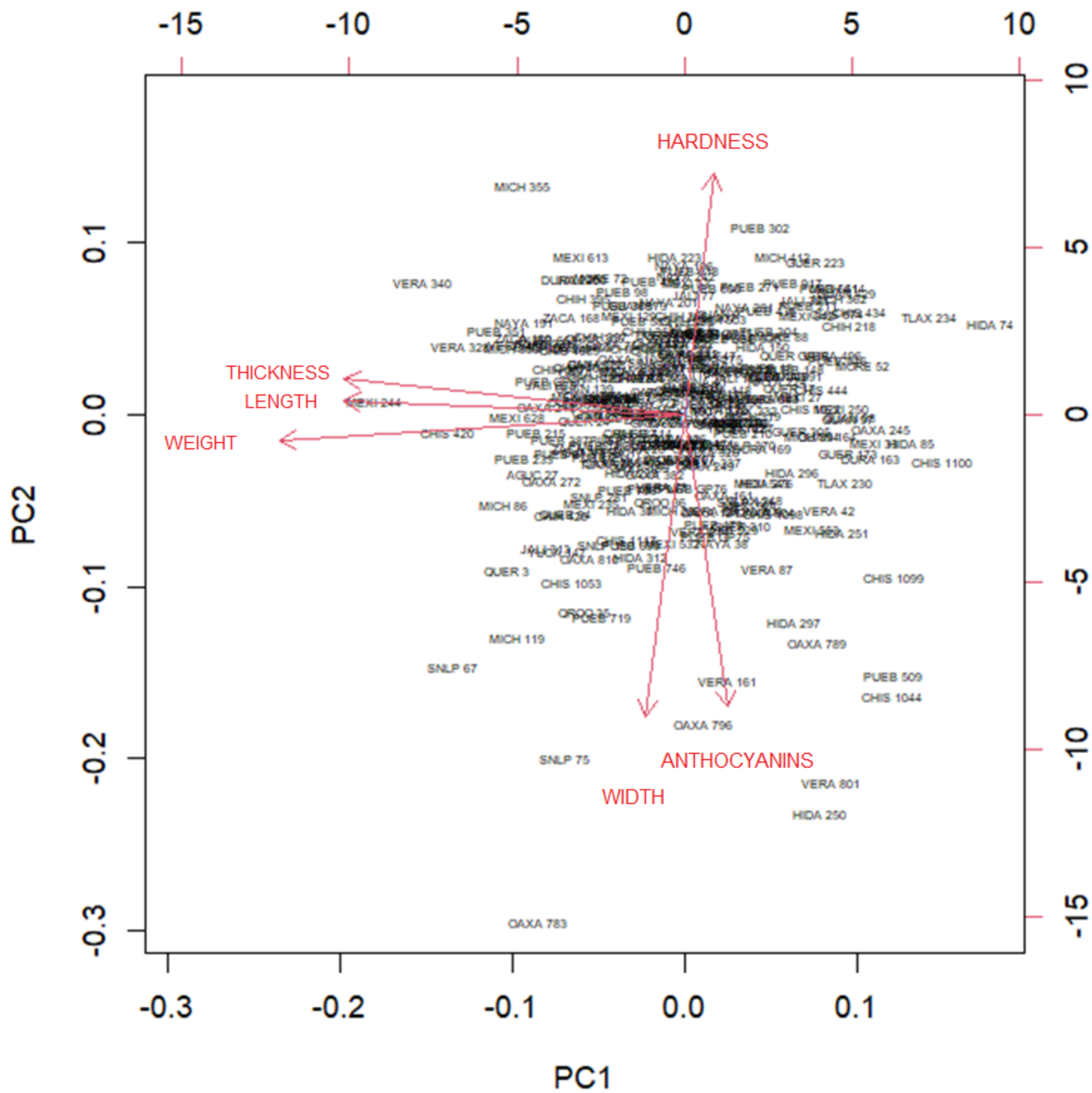

Figure 1. Dispersion of 300 corn accessions from 31 races.

The most relevant variables for PC1 were the weight of a thousand seeds and grain length, and for PC2, were anthocyanin content and grain width.

The dendrogram generated from the physical characteristics and total anthocyanin content of the 31 corn breeds (Figure 2) shows four groups at a Euclidean distance of 5: The first group comprises Dulce, Cristalino de Chihuahua, San Marceno, Negro de Tierra Caliente, Perla, and Pepitilla. The second group includes Olotillo, Arrocillo, Nal-tel, Cónico, Tuxpeño, Bolita, Arrocillo Amarillo, Tepecintle, Elotes de Sinaloa, Cónico Norteño, Chalqueño, Olotón, Elotes Occidentales, Elotes Cónicos, Ancho, Tabloncillo, Mushito, Cacahuacintle, Bofo, Azul, San Juan, Reventador, and Celaya. The third group consists of Negro Chimaltenango, and the fourth group comprises Tabloncillo Perla. A greater affinity was observed between the races Cónico Norteño and Chalqueño, as well as a notable similarity between the races Elotes Occidentales and Elotes Cónicos. Torres *et al.* (2022) analyzed the morphological diversity of seven maize races, identifying four groups in the dendrogram at an Euclidean distance of 7.8 units. Similarly, in the study of genetic diversity of 10 accessions, four groups were delineated from an Euclidean distance of 5.16 (Sanchez *et al.,* 2019).

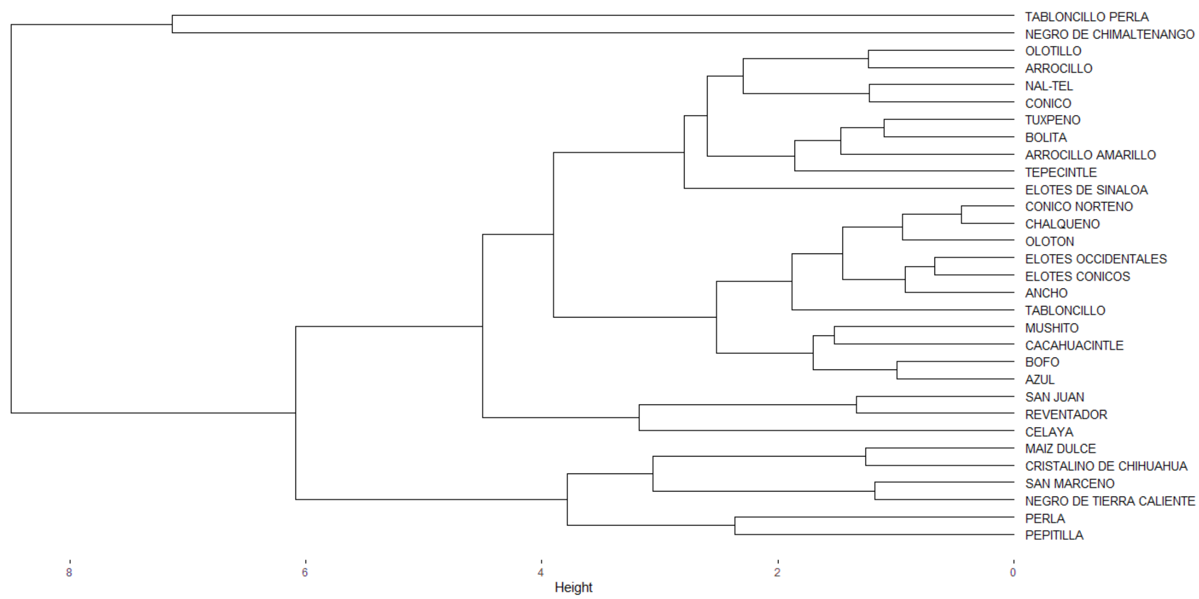

Figure 2. Dendrogram of 31 corn races, considering the variables: total anthocyanins, one hundred grain weight, hardness, length, width, and grain thickness.

Conclusions

The variability uncovered in this research could significantly contribute to the selection of maize accessions offering high total anthocyanin content. Accessions such as HIDA 250 (Tepecintle), PUE 509 (Elotes Occidentales), CHIS 1053 (Olotón), CHIS 1044 (Olotón), MICH 119 (Cónico), and VERA 161 (Tuxpeño) exhibited total anthocyanin content exceeding 600 µg g^{-1}. These accessions demonstrate substantial potential for utilization in breeding programs due to their alignment with the requirements of the nixtamalization industry. Furthermore, most of these

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accessions display coloration predominantly in the aleurone layer, reducing the likelihood of its release during nixtamalization compared to those with coloration in the pericarp. The breeds were grouped into four categories based on the characteristics under study.

Authors' contribution

Conceptualization of the work, HRDM, RHSA. Methodology development, HRDM, RHSA, MRA, PRN. Software management, LBA, GOHT. Experimental validation, LdelRAJ, PRN. Analysis of results, MRA, LBA, LdelRAJ, GOHT. Data management, HRDM, RHSA. Manuscript writing and preparation, HRDM. Writing, revising, and editing, MRA, LBA, PRN, LdelRAJ, GOHT; project manager, RHSA.

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Statement of Informed Consent

Not applicable.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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