BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITIES IN PIGMENTED MAIZE LANDRACES

Raquel Martínez-Martínez, Araceli Minerva Vera-Guzmán, José Luis Chávez-Servia, Elia Nora Aquino-Bolaños, José Cruz Carrillo-Rodríguez and Aleyda Pérez-Herrera

SUMMARY

In Mexico, Central and South America, maize exhibits high diversity that is recognized phenotypically by color, grain shape and size, and cob and plant characteristics. The objective was to evaluate the effect of cropping location, accessions, and location-accession interaction on the phenolic compound contents and antioxidant activities in a collection of pigmented native maize from Oaxaca, Mexico. To accomplish this goal, 57 native maize accessions and three commercial varieties were planted and cultivated in the towns of San Agustín Amatengo and Santa María Coyotepec, Oaxaca, using a randomized complete block design with four replicates. The total anthocyanin, phenolic, and flavonoid contents were evaluated in samples of dry and ground grain, and DPPH and FRAP antioxidant activities were spectrophotometrically evaluated. The results showed significant differences (P≤0.05) between cropping locations, grain color groups, accessions within groups and locality-group and genotype-location interactions. The cropping location affected the total anthocyanins and phenolic contents and the antioxidant activities. The anthocyanin content in blue maize was higher than that in red maize. Significantly, decreasing differences were observed among the different grain colors (i.e., blue red>yellow in total phenolic, total flavonoids, and antioxidant activity), and significant interactions were observed between the localities and grain colors. High variability was observed between the accessions for bioactive compounds and antioxidant activities, and significant interactions were observed between locations and accessions of pigmented grains. Some accessions with remarkable composition and antioxidant activity were RJ02, RJ03, RJ04, RJ06, AZ10, AZ13, AZ16, and AZ18.

Introduction

Maize is a cereal with high commercial value and of global consumption; out of the 1060×10^6t million tons produced, 40% is used for human consumption (FAOSTAT, 2013, 2016). Consumption varies between regions and countries. As examples, the countries with the highest consumption volume in Africa are Lesotho, Malawi and Zambia, ranging from 325.15 to 433.97 g/person/day, while the countries with the highest consumption volume in the Americas are Mexico, Guatemala and Honduras, ranging from 213.60 to 318.74 g/person/day, and the countries with the highest consumption in Europe are Moldova, Bosnia-Herzegovina, and Romania, ranging from 110.88 to 251.42 g/person/day. This consumption suggests that the calorie consumption in carbohydrates, protein, minerals, and bioactive compounds from corn is also high and influences individual health.

Mexico is the center of origin, domestication, and diversification of maize, and it preserved a high diversity of colorations and characteristics of grain, precocity, plant and ear height, plant physiology, grain chemical composition, nutritional value and use (Kato *et al.*, 2009; Vera-Guzmán *et al.*, 2012; Fernández-Suárez *et al.*, 2013). As a consequence, maize diversity is essentially distributed among indigenous communities or ethnolinguistic groups (Perales and Golicher, 2014). There is bias exists in maize research due to the importance of its trade, and most studies on corn grain composition focused on yellow and white grain; research on blue corn has only begun to focus in the last decade.

Pigmented grain maize contains phenolic compounds, carotenoid and anthocyanin, compounds associated with nutraceutical properties and health promotion benefits. Therefore, such grain is considered to be a functional food and features high antioxidant activities and preventive functions against cancer, diabetes, obesity and neurodegenerative disorders (Bañuelos-Pineda *et al.*, 2018; Navarro *et al.*, 2018). The most evaluated carotenoids in yellow maize are lutein, zeaxanthin, and α and β cryptoxanthin (Prasanthi *et al.*, 2017), while the major anthocyanins in blue, red, and purple grains are cyanidin-3-glucoside

KEYWORDS / Anthocyanins / Bioactive Compounds / Genotype-Environment Interaction / Spectrophotometry / *Zea mays* L. /

Received: 03/21/2018. Modified: 09/18/2019. Accepted: 09/21/2019.

Raquel Martínez-Martínez. M.Sc. in Conservation and Use of Natural Resources, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR)-Oaxaca, Instituto Politécnico Nacional (IPN). Independent researcher.

Araceli Minerva Vera-Guzmán. Doctor in Science and Technology of Foods, Centro de Investigación en Alimentación y Desarrollo, Hermosillo, México. Professor Researcher, CIIDIR-Oaxaca, IPN, México.

José Luis Chávez-Servia (Corresponding author). Doctor in Genetics, Colegio de Posgraduados, México. Professor Researcher, CIIDIR-Oaxaca, Instituto Politécnico Nacional. Address: Hornos 1003, Santa Cruz Xoxocotlán, 71230, Oaxaca, México. e-mail: jchavezs@ipn.mx.

Elia Nora Aquino Bolaños. Doctor in Food Science, Universidad Autónoma de Querétaro, México. Professor Researcher, Universidad Veracruzana, México.

José Cruz Carrillo-Rodríguez. Doctor in Regional Development, Instituto Tecnológico de Oaxaca, México. Professor Researcher, Instituto Tecnológico del Valle de Oaxaca, México.

Aleyda Pérez-Herrera. Ph.D. in Nutrigenomics, Universidad de Córdoba, Spain. Professor Researcher, CIIDIR-Oaxaca, IPN, México.

0378-1844/14/07/468-08 $ 3.00/0

COMPUESTOS BIOACTIVOS Y ACTIVIDAD ANTIOXIDANTE EN POBLACIONES NATIVAS DE MAÍZ PIGMENTADO

Raquel Martínez-Martínez, Araceli Minerva Vera-Guzmán, José Luis Chávez-Servia, Elia Nora Aquino-Bolaños,
José Cruz Carrillo-Rodríguez y Aleyda Pérez-Herrera

RESUMEN

En México, Centro y Sudamérica, el maíz presenta alta diversidad, reconocida fenotípicamente por color, formas y dimensiones de grano, mazorca y características de planta. El objetivo fue evaluar el efecto de localidades de cultivo, accesiones e interacción localidades-accesiones en el contenido de compuestos fenólicos y actividad antioxidante de una colección de maíces pigmentados nativos de Oaxaca, México. Se sembró y cultivó a 57 accesiones de maíz nativo y tres variedades comerciales en San Agustín Amatengo y Santa María Coyotepec, Oaxaca, bajo un diseño de bloques al azar con cuatro repeticiones. En una muestra de grano seco y molido, se evaluó, por espectrofotometría, el contenido de antocianinas totales, fenólicos totales, flavonoides totales y actividad antioxidante por DPPH y FRAP. Hubo diferencias significativas (P≤0,05) entre localidades de cultivo, grupos de color de grano, accesiones dentro de grupos, interacciones localidades-grupos y localidades-accesiones. La localidad de cultivo tuvo efectos significativos en el contenido de antocianinas totales, fenólicos totales y actividad antioxidante. El contenido de antocianinas en maíz azul fue mayor que en granos rojos. Entre grupos de color de grano se determinaron diferencias significativas decrecientes (azul>rojo>amarillo) en fenoles, flavonoides y actividad antioxidante, e interacciones significativas entre localidades y grupos de color de grano. Se determinó una alta variabilidad entre accesiones en compuestos bioactivos y actividad antioxidante, e interacciones significativas entre localidades y accesiones de granos pigmentado. Algunas de las accesiones sobresalientes en composición y actividad antioxidante fueron: RJ02, RJ03, RJ04, RJ06, AZ10, AZ13, AZ16 y AZ18.

COMPOSTOS BIOATIVOS E ATIVIDADE ANTIOXIDANTE EM POPULAÇÕES NATIVAS DE MILHO PIGMENTADO

Raquel Martínez-Martínez, Araceli Minerva Vera-Guzmán, José Luis Chávez-Servia, Elia Nora Aquino-Bolaños,
José Cruz Carrillo-Rodríguez e Aleyda Pérez-Herrera

RESUMO

No México, Centro e América do Sul, o milho apresenta alta diversidade, reconhecida fenotipicamente pela cor, formas e dimensões de grão, espiga e características de planta. O objetivo foi avaliar o efeito de localidades de cultivo, acessões e interação localidades-acessões no conteúdo de compostos fenólicos e atividade antioxidante de uma coleção de milhos pigmentados nativos de Oaxaca, México. Plantou-se e cultivou-se 57 acessões de milho nativo e três variedades comerciais em San Agustin Amatengo e Santa Maria Coyotepec, Oaxaca, sob um desenho de blocos aleatórios com quatro repetições. Em uma amostra de grão seco e moído, foi avaliado, por espectrofotometria, o conteúdo de antocianinas totais, fenólicos totais, flavonoides totais e atividade antioxidante por DPPH e FRAP. Houve diferenças significativas (P≤0,05) entre localidades de cultivo, grupos de cor de grão, acessões dentro de grupos, interações localidades-grupos e localidades-acessões. A localidade de cultivo teve efeitos significativos no conteúdo de antocianinas totais, fenólicos totais e atividade antioxidante. O conteúdo de antocianinas em milho azul foi maior que em grãos vermelhos. Entre grupos de cor de grão foram determinadas diferenças significativas decrescentes (azul>vermelho>amarelo) em fenóis, flavonoides e atividade antioxidante, e interações significativas entre localidades e grupos de cor de grão. Determinou-se uma alta variabilidade entre acessões em compostos bioativos e atividade antioxidante, e interações significativas entre localidades e acessões de grãos pigmentados. Algumas das acessões sobressalentes em composição e atividade antioxidante foram: RJ02, RJ03, RJ04, RJ06, AZ10, AZ13, AZ16 e AZ18.

(C3G), cyanidin-3,5-diglucoside, pelargonidin, and peonidin-3-glucoside and its malonyl derivatives (Aoki *et al*., 2002; Pedreschi and Cisneros-Zevallos, 2007; Guzmán-Gerónimo *et al*., 2017; Herrera-Sotero *et al*., 2017). Flavonoids are the least studied compounds and require more attention (Navarro *et al*., 2018).

Anthocyanin in maize grains is found in the aleurone layer, pericarp, or in both grain structures, and differ in concentration and structural distribution in the grain (González-Manzano *et al*., 2008; Espinosa-Trujillo *et al*., 2009; Salinas-Moreno *et al*., 2013). The genotype, environment, cultivation practices, genotype-environment interactions and pigment combinations in the grain influence the quantity and variety of anthocyanins or anthocyanidins (Giordano *et al*., 2018). Salinas-Moreno *et al*. (2012) evaluated different Mexican maize populations from the races Chalqueño and Bolite, and determined the anthocyanin contents to be 304.1 to 1332mg of C3G/kg of sample. Similarly, in populations of native maize from Colombia, Velásquez-Landino *et al*. (2016) reported values of 1.88 to 70mg C3G/100g and variations in total phenolic compounds of 538 to 1633mg gallic acid equivalents (GAE)/100g. Zilic *et al*. (2012) reported a content of total phenolic compounds of 5393.2mg·kg^{-1} in yellow kernels. These results indicate that the genotype is relevant and interacts with the environment or cultivation practices. However, little has been documented in Mexican landraces, and this information is required to promote consumption and design conservation strategies that take advantage of this diversity.

In corn phenology, the filling phase and grain ripening are crucial for the synthesis of pigments and can be divided into four subphases. For example, yellow grains in the filling stage begin as a white-cream grain, then become yellow-cream, then yellow, and finally ripen to a yellow-golden or bright yellow. Xu *et al*. (2010) reported that moisture, sugar content, total phenolic compounds and carotenoids decrease, but the corncob subphase or yellow grain state showed the highest values of lutein, zeaxanthin and total carotenoids. A homologous event occurs with anthocyanin biosynthesis of the blue color in grain. These results indicate that some biotic and abiotic stress conditions in this phase influence the final bioactive compound content and may vary among genotypes due to the intrinsic variability in the time required by each genotype to complete this phase and how each genotype interacts with the growing environment. Kapcum and Uriyapongson (2018) reported that the temperature and storage time of blue-pigmented grain produced decreased anthocyanins (cyanidian-3-glucoside, pelargonidin-3-glucoside, and peonidin-3-glucoside) but showed no decrease in flavonoids or antioxidant activity, and consumption in postharvest days is recommended.

Biosynthesis, transport and/or accumulation of bioactive compounds such as anthocyanins, total phenolic and flavonoids in corn grain depend on multiple factors, such as environmental and cultivation practices, soil fertility, fertilizations, genotype, epigenetic factors and genotype-environment interactions (Harrigan *et al*., 2007; Jing *et al*., 2007; Khampas *et al*., 2015; Giordano *et al*., 2018). Other environmental factors affect the accumulation of anthocyanins, total phenolic compounds and antioxidant activity, including the amount and intensity of solar radiation, low temperatures and water stress (Chalker-Scott, 1999; Ali *et al*., 2010; Kishore *et al*., 2010). In this context, the objective of the present work was to evaluate the effects of cropping location, genotype (accession) and location-genotype interaction on the content of total phenolic compounds and antioxidant activities in a collection of pigmented native maize from Oaxaca, Mexico.

Materials and Methods

Collection and cultivation of germplasm

From December 2015 to March 2016, samples from 58 populations of yellow (30), red (13) and blue (15) native maize were collected in 39 municipalities of Valles Centrales, Sierra Norte, Sierra Sur, Mixteca and Papaloapan, Oaxaca, Mexico. During collection, the community, municipality, and region of origin, visual color of the grain, and the georeferenced locations of each community, from 278 to 2213masl, and within 17°59'59" to 16°30'37"W, and 98°19'69" to 96°00'30"N, were recorded. Three commercial varieties were added as controls: blue grain (VC-42 and SBA-4032) and yellow grain (Tuxpeño Basica). The collection and controls were planted in San Agustin Amatengo (July 5, 2016) and in Santa Maria Coyotepec (July 21, 2016), Oaxaca, under a randomized complete block design with four replicates. The traditional rainfed planting method was used at both locations, with irrigation support during grain filling to avoid water stress, and a fertilization formula of 120N-100P-60K was applied. Soil samples were extracted from the experimental locations for a general edaphic analysis using NOM-021-RECNAT (NOM, 2000) to describe the soil physicochemical properties (Table I).

Sampling and sample preparation for analysis

At harvest, a random sample of 10 healthy ears per accession or maize population was taken at each experimental location. The samples were manually threshed. A subsample of 100g of grain per population was then ground and crushed (Apex Construction®, LTD and Krups®, Mexico). The final flour was sieved through a 500µm mesh and stored in amber vials at -20°C until analysis.

Anthocyanin content in blue and red grain maize

The extraction of monomeric anthocyanins was performed from 3g flour samples with ethanol acidified at 85%. The monomeric anthocyanin content was determined by the differential pH method described by Wrolstad (1976). The absorbance of the reaction was measured with KCl pH 1 and CH_3COONa pH 4.5 in a spectrophotometer (Shimadzu UV-1800, Japan) at a wavelength range of 470-720nm, with measurements performed in triplicate. The monomeric anthocyanin (MA) concentration was calculated using the Wrolstad (1976) equation as follows: $MA= (A×MW×DF×1000)/(ε×I)$, where $A= (A_{510}-A_{700})$ pH 1.0 - $(A_{510}-A_{700})$ pH 4.5, MW= 449.2g·mol^{-1} cyanidin-3-glucoside (C3G), ε= 26900 l·mol^{-1}·cm^{-1} C3G molar extinction coefficient, DF= the dilution factor, and I= the cell length (1cm). The results are expressed as mg equivalent of C3G per 100g of dry sample (mg C3G/100g dw).

Total phenolic and flavonoid contents and antioxidant activities

The evaluation of the total phenolic and total flavonoid contents and the antioxidant activity was performed by extraction with 80% methanol from 3g of corn flour. The total phenolic content was determined by the method described by Singleton and Rossi (1965). We added deionized water and Folin-Ciocalteu reagent to 400µl of the diluted extract and left it to rest for 5min. Subsequently, 7% Na_2CO_3 was added, and the sample was incubated for 1h at room temperature (23 ±3ºC). Finally, absorbance readings were performed in triplicate in the spectrophotometer using distilled water as the blank. The total phenolic content was estimated as mg of gallic acid equivalent per 100g

TABLE I
DESCRIPTION OF TWO LOCATIONS FOR THE EVALUATION OF PIGMENTED MAIZE IN OAXACA, MEXICO

Location and soil descriptors	Experimental locations of cultivation	
	San Agustin Amatengo	Santa Maria Coyotepec
Location descriptors:		
Latitude (N)	16°30'37"	16°57'58"
Longitude (W)	96°47'2"	96°42'23"
Altitude (masl)	1361	1518
Annual average temperature (°C)	20.9	20.0
Annual average precipitation (mm)	693.8	526.5
Climate	semidry to semi-warm	semidry to semi-warm
Soil descriptors		
pH (in H_2O)	8.30	7.80
CE (dS·m^{-1})	0.15	0.22
Organic matter (%)	3.0	3.3
P-Olsen (mg·kg^{-1})	4.8	6.4
K (cmol·kg^{-1})	0.9	1.0
Ca (cmol·kg^{-1})	27.8	26.4
Mg (cmol·kg^{-1})	2.5	4.0
Fe (mg·kg^{-1})	13.4	36.8
Zn (mg·kg^{-1})	0.7	0.9
Mn (mg·kg^{-1})	11.9	10.9
Cu (mg·kg^{-1})	2.8	5.5
Inorganic N (mg·kg^{-1})	38.5	29.8

Sources: CNA (2016) and soil analyses under current Mexican norm (NOM, 2000).

in dry basis (mg GAE/100g dw), compared with a calibration curve of gallic acid in concentrations from 0.02 to 0.125mg·ml^{-1} (r^2= 0.9982).

The flavonoid content was determined by the method of Zhishen *et al.* (1999). $NaNO_2$ 5% was added to the methanol extract, and it was stirred and left to rest for 5min. $AlCl_3·6H_2O$ at 10% plus 1M NaOH and deionized water were then added. The absorbance was read in triplicate at 510nm and compared with a reactive blank. The flavonoid content was calculated as mg of catechin equivalent per g of dry sample (mg CE/g dw), based on a (+)-catechin calibration curve in concentrations from 0.0122 to 0.122µg·ml^{-1} (r^2= 0.9976).

The antioxidant activity of DPPH (2,2-diephenyl-1-picrylhydrazyl) was analyzed using the method of Brand-Williams *et al.* (1995). The DPPH radical was added to 100µl of the methanol extract. The solution was vortexed and allowed to stand for 30min in darkness. Subsequent readings were performed in triplicate at 517nm using a spectrophotometer and 80% methanol as a reference. Antioxidant activity was recorded based on a Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) calibration curve from 0.13 to 0.79µmol·ml^{-1} (r^2= 0.9984) and expressed in µmol Etrolox/g dw.

Antioxidant activity was also determined by the FRAP method. The antioxidant capacity, expressed as iron reduction, was determined by the method described by Benzie and Strain (1996). A total of 3ml of FRAP reagent (sodium acetate buffer pH 3.6, 10mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) and 10mM $FeCl_3$ $6H_2O$) were added to 100µl of the extract. This solution was incubated for 30min at 37°C. The absorbance was recorded at 593nm in the spectrophotometer. The quantification of antioxidant activity was performed based on a Trolox calibration curve from 100 to 1000µmol·l^{-1} (r^2= 0.9994), and the results are expressed as µmol equivalent of Trolox per g of dry weight (µmol ET/g dw).

Statistical analysis

To evaluate differences between cultivation locations, grain color groups, accessions nested in color groups, and location-color groups and location-accession interactions, analysis of variance was performed for each response variable based on the lineal model of combined analysis of randomized complete block design. Multiple means comparisons were performed using the Tukey method ($p \leq 0.05$). The location-accession interactions are represented in a graphical-descriptive manner, where each point represents the accessions as a function of the evaluation locations (= axes). All statistical analyses were performed using the SAS statistical package (SAS, 2000).

Results and Discussion

The anthocyanin content was evaluated in the red and blue grain maize populations. The analysis of variance detected significant differences ($p<0.05$) in anthocyanin content between locations, groups of color grains, accessions within groups, and locations-color groups and locations-accessions interactions. Regarding the total phenolic and flavonoid contents and the antioxidant activity (DPPH and FRAP), significant differences were found for all variables evaluated between grain color groups, cropping locations (except in flavonoids), accessions, and locations-groups and locations-accessions interactions. Notably, the anthocyanin content was evaluated only in samples of red and blue grains and, as previously reported, was absent in yellow grains where carotenoids were predominant (Espinosa *et al.*, 2009; Zilic *et al.*, 2012).

The cropping location influenced the average anthocyanin content in grain. In Santa Maria Coyotepec, blue and red maize had a significantly higher concentration of anthocyanins than San Agustin Amatengo. In addition, blue grain maize showed higher values than red grain maize. The significant interaction between the planting locations and grain color groups indicates that the blue grain group had higher anthocyanin concentrations in Santa Maria and San Agustin than red grain in both locations. Conversely, the lowest anthocyanins content was recorded in red maize cultivated in San Agustin. The significant interaction indicates that blue maize cultivation in Santa Maria had higher anthocyanin content and was favorably affected by the cultivation location (Table II). Therefore, the biosynthesis of secondary metabolites occurs via the synthesis of phenylalanine ammonia lyase, which is the enzyme responsible for catalyzing the transformation of L-phenylalanine to trans-cinnamic acid, and the enzymatic activity was influenced by environmental factors and water stress, high temperature, and incidence of UV radiation (Efeoğlu *et al.*, 2009; Vogt, 2010; Chaves-Barrantes and Gutiérrez-Soto, 2017). Additionally, the synthesis of anthocyanins in maize occurs during the last grain-filling phase, and any environmental or physiological alteration in this phase influences the final anthocyanin content.

A high variation in the content of monomeric anthocyanins was identified between the red and blue grain accessions. The response patterns of the accessions follow the patterns observed previously in grain color groups. For example, in the group with the highest anthocyanin content, four accessions of blue grain and three of red grain with a content >30mg C3G equivalent in 100g dw were found. A variation between 22.7 and 29.5mg C3G/100g was observed in a group of 11 accessions of blue grain. Furthermore, three accessions of red corn varied between 20.7 and 25.2, five accessions exhibited values lower than 17.3, and practically no anthocyanins were detected in two accessions, with values <1mg C3G/100g. These results indicate that the visual coloration of faint or pale red was also indicative of low anthocyanin content in the grain, which was the case for accessions RJ01, RJ02, and RJ04 (Table III). On-farm, the preservation of blue, red, or yellow color in maize grain implies the planting of lots that require isolation in space or time, to avoid crossing and consequently the presence of different colors of grain in same cob. This independent management explains part of the difference in anthocyanin content between groups of red and blue grains.

The significant differences in anthocyanins among accessions or genotypes mean that the biosynthesis and storage capacity of anthocyanins in the grains differ from genotype to genotype, and the cropping location influenced these differences. Similar results were obtained by Salinas-Moreno *et al.* (2012) between races and maize populations of Chiapas, by Espinosa-Trujillo *et al.* (2009) in maize from central Mexico, by López-Martínez *et al.* (2009) in maize from different regions of

TABLE II
AVERAGE CONTENT OF MONOMERIC ANTHOCYANINS IN PIGMENTED MAIZE GROUPS, CROP LOCATIONS, AND LOCATION-COLOR GRAIN GROUP INTERACTION

Grain color groups	Santa Maria Coyotepec	San Agustin Amatengo	Average by color
Blue	29.90 a	25.84 b	28.04 a
Red	20.70 c	17.95 d	19.30 b
Average by location	25.93 a	21.98 b	

Among groups of grain color, locations and location-grain color groups interaction, means with different letters indicate significant differences (Tukey's test, $P \leq 0.05$) in anthocyanin content (mg C3G/100g dw).

TABLE III

ANTHOCYANIN CONTENTS OF MAIZE ACCESSIONS OF BLUE (AZ) AND RED (RJ) GRAINS FROM OAXACA, MEXICO

Accessions with blue grain	Anthocyanins (mg C3G/100g)	Accessions with red grain	Anthocyanins (mg C3G/100g)
AZ02	30.88	RJ01	4.59
AZ03	25.88	RJ02	0.73
AZ06	28.96	RJ03	34.68
AZ09	22.74	RJ04	0.85
AZ10	29.46	RJ05	17.93
AZ13	28.30	RJ06	36.12
AZ16	32.93	RJ07	17.22
AZ18	26.53	RJ08	20.71
AZ21	37.50	RJ09	13.32
AZ23	28.88	RJ10	22.37
AZ24	26.72	RJ11	25.15
AZ25	25.49	RJ12	31.70
AZ27	26.85	RJ13	15.77
AZ28	22.82		
VC-42	31.81	HSD-Tukey*	6.66

* Equal or higher differences between the means according to Tukey's honestly significant difference test (Tukey's test, P≤0.05), indicate a significant difference between the means.

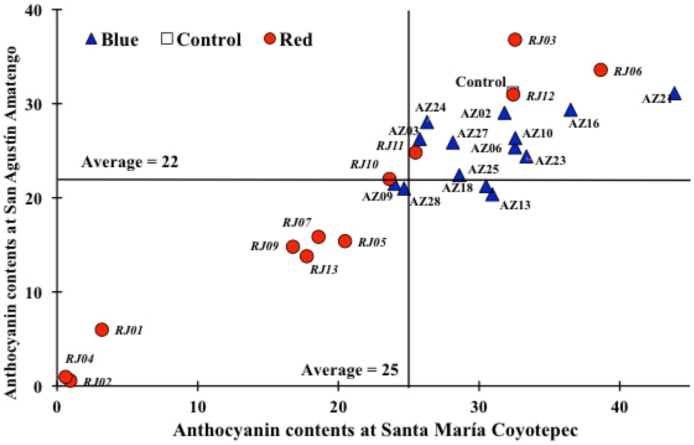

Figure 1. Scatterplot of accessions-locations based on anthocyanin contents (mg C3G/100g dw) in two cropping locations from Oaxaca, Mexico.

Mexico, and by Guzmán-Gerónimo *et al.* (2017) in maize from the Oaxaca Mixteca region (21.4 to 66.9mg C3G/100g). These examples show that, despite the differences in laboratory protocols to obtain estimators of grain composition, the genetic diversity preserved in Mexico has been underutilized by rural communities and it not a priority in the maize breeding programs.

The interaction between cropping locations and groups of grain color affecting anthocyanin content distinguishes divergent patterns by grain color. For example, the red grains showed a downward and stable trend in both cropping locations. In contrast, ten accessions of blue grain exceeded the averages at both locations, over 25 and 22mg C3G/100g dw, in Santa Maria and San Agustin, respectively. As a function of the trend toward stability of anthocyanin content via cropping locations and in relation to the commercial variety (VC-42), two red and two blue grain samples stand out and surpassed the control. These four grains have a potential for continuous evaluations in other environments to demonstrate their stability across environments (Figure 1).

The content of total phenolic compounds, flavonoids and antioxidant activities in pigmented grains showed that the location and growing environment had significant effects on the concentration of the total phenolic content and antioxidant activity but not on flavonoids. In these assays, differentiation patterns among the grain color groups were in descending order of blue>red>yellow in content of total phenolic compounds, flavonoids, and antioxidant activities of DPPH and FRAP and were distinct (Table IV). Therefore, the higher content of bioactive compounds and antioxidant activity in the blue grain maize than in the other groups means that farmers exhibit independent management and seed selection in their lots of seeds as a function of grain color (Badstue *et al.*, 2007), and these practices generated differences in the composition of total phenolic compounds, flavonoids and antioxidant activities.

The cropping locations had a significant effect on monomeric anthocyanin, total phenolic contents, and antioxidant activities (Tables II and IV), and the Santa Maria location exhibited higher values than San Agustin. Location effects were considered climatic, and edaphic conditions grouped as an environmental effect. In this study, the Santa Maria location exhibited high values in soil electric conductivity (CE), organic matter, P, Mg, and Cu but a low pH compared to San Agustin Amatengo (Table I). These soil physical and chemical characteristics of Santa Maria favored the major absorption and mobilization of nutrients, biosynthesis of anthocyanins and phenolic compounds, and the high antioxidant activity, similar to the findings reported by Harrigan *et al.* (2007), Jing *et al.* (2007) and Khampas *et al.* (2015). For example, a pH close to 7.0 and sufficient organic matter in the soil enhance nutrient absorption (Farina *et al.* 1980).

A high variation in total phenolic content and flavonoids among the accessions allows the choice of the best

TABLE IV

TOTAL PHENOLIC AND FLAVONOID CONTENTS, AND ANTIOXIDANT ACTIVITY AS A FUNCTION OF CROPPING LOCATIONS AND GRAIN COLOR GROUPS IN PIGMENTED MAIZE

Location / grain color groups	Total phenolic (mg GAE/100g)	Flavonoids (mg CE/g)	Antioxidant activity (µmol ET/g)	
			DPPH	FRAP
Cropping location				
Santa Maria Coyotepec	96.58 a	0.075 a	4.315 a	4.763 a
San Agustin Amatengo	94.98 b	0.075 a	4.141 b	4.535 b
Grain color groups				
Blue	123.550 a^{1}	0.129 a	4.842 a	5.585 a
Red	113.458 b	0.123 b	4.776 b	5.510 b
Yellow	77.34 c	0.034 c	3.767 c	3.931 c

Between locations or grain color groups, means with same letter are not significantly different (Tukey's test, P≤0.05).

TABLE V
VARIATION IN TOTAL PHENOLIC CONTENTS AND ANTIOXIDANT ACTIVITIES AMONG MAIZE ACCESSIONS OF BLUE (AZ), RED (RJ), AND YELLOW (AM) GRAIN

Acc.	Total phenolic[1]	Flavonoids[2]	Antioxidant act.[3]		Acc.	Total phenolic[1]	Flavonoids[2]	Antioxidant act.[3]	
			DPPH	FRAP				DPPH	FRAP
AZ02[4]	125.12	0.10	4.43	5.42	AM07[4]	71.20	0.02	3.69	3.66
AZ03	121.69	0.14	4.55	4.62	AM08	69.46	0.03	3.98	3.91
AZ06	117.89	0.14	4.56	5.12	AM10	71.57	0.04	3.59	3.70
AZ09	125.46	0.16	4.85	5.31	AM21	74.44	0.04	3.32	3.68
AZ10	122.29	0.13	5.15	5.94	AM22	72.86	0.03	3.84	3.84
AZ13	122.85	0.18	4.71	5.79	AM23	78.81	0.03	3.81	3.96
AZ16	130.05	0.13	5.22	6.29	AM24	78.09	0.04	4.20	4.16
AZ18	137.39	0.12	4.46	6.69	AM26	81.98	0.03	4.51	4.11
AZ21	119.80	0.11	4.36	5.76	AM27	77.01	0.03	4.20	3.68
AZ23	117.06	0.15	4.49	4.99	AM29	74.45	0.03	3.92	3.78
AZ24	122.15	0.10	4.74	5.13	AM30	77.73	0.03	3.97	3.73
AZ25	126.12	0.13	5.04	5.49	AM35	84.06	0.03	3.58	4.04
AZ27	119.82	0.14	4.39	5.64	AM37	81.09	0.04	3.65	4.00
AZ28	116.84	0.13	4.65	5.17	AM40	78.94	0.03	3.17	3.69
VC-42	130.46	0.11	5.70	6.17	AM41	81.54	0.04	3.24	3.97
RJ01	117.21	0.14	4.88	5.67	AM42	73.88	0.04	3.27	3.75
RJ02	113.69	0.16	4.67	5.52	AM45	75.81	0.04	3.17	3.52
RJ03	166.03	0.19	6.75	8.12	AM49	74.18	0.05	3.34	3.82
RJ04	123.20	0.15	5.56	6.50	AM50	72.31	0.05	3.35	3.64
RJ05	108.10	0.09	4.25	5.02	AM51	69.99	0.03	3.44	3.82
RJ06	127.93	0.10	5.45	5.93	AM52	68.19	0.03	3.51	3.68
RJ07	109.83	0.13	4.51	4.94	AM53	81.47	0.05	3.90	4.21
RJ08	120.81	0.09	5.12	5.26	AM55	73.55	0.04	3.39	4.01
RJ09	102.14	0.10	4.26	3.97	AM58	84.40	0.05	4.03	4.31
RJ10	101.63	0.08	4.76	4.59	AM59	85.83	0.03	4.10	4.20
RJ11	93.23	0.11	4.33	5.43	AM60	79.46	0.03	4.02	4.16
RJ12	97.43	0.11	4.66	5.43	AM62	86.66	0.03	4.44	4.20
RJ13	103.75	0.14	4.23	5.41	AM70	79.99	0.03	3.79	3.92
AM05	76.27	0.02	3.73	3.93	Tuxpeño	82.18	0.03	3.92	4.35
AM06	69.94	0.03	3.81	3.83	SBA-4032	88.96	0.04	4.58	4.51
					HSD-T*	9.534	0.027	0.51	0.58

[1]mg GAE/100g, [2]mg CE/g, [3]µmol Etrolox/g, *equal or higher differences between means according to Tukey's honestly significant difference test (HSD-Tukey's test, P≤0.05) indicate a significant difference between means.

accessions for direct utilization. The variation in total phenolic content ranged from 116.8 to 137.4mg GAE/100g in the blue grain accessions, from 93.2 to 166.4mg GAE/100g in red kernels and from 69.9 to 86.74 mg GAE/100g in yellow kernels. Flavonoids varied from 0.10 to 0.18mg CE/g in the blue grain accessions, from 0.08 to 0.19mg CE/g in red and from 0.02 to 0.05mg CE/g in yellow ones. The yellow grain accessions had the lowest contents of total phenolic compounds and flavonoids (Table V). These values are within the estimates of Salinas-Moreno *et al.* (2017) for the total phenolic content of maize accessions from different regions of Mexico, which ranged from 59.3 to 82.78mg GAE/100g, and for the values reported by Guzmán-Gerónimo *et al.* (2017) in Oaxacan Mixteca blue maize of 142.8 to 203.2mg GAE/100g. Our values surpass the values determined by Prasanthi *et al.* (2017) in different types of commercial maize in the USA, of 1.26 to 14.53mg GAE/100g. In all cases, high variability existed among the genotypes that can be used directly in food or to start a breeding program. However, the processing of the grain to its consumables (tortilla, cereal, totopo and other local foods) decreases the estimated contents of grain but retains up to 40% of the total (Herrera-Sotero *et al.*, 2017; Prasanthi *et al.*, 2017). These patterns are somewhat similar to the antioxidant activities of DPPH and FRAP as observed, for example, in the antioxidant activities evaluated by DPPH and FRAP in the accessions of blue grain. These results indicate that the accessions with higher total phenolic and total flavonoid content also have higher antioxidant activity.

Significant interactions between cropping locations and grain color groups were observed. In blue maize, a greater response was found in Santa Maria compared to San Agustin in the total phenolic compounds, flavonoids and antioxidant activities. A similar pattern of total phenolic and total flavonoid composition was also observed in red maize, and in particular, the antioxidant activity of DPPH in red grains was similar in both locations. The response for yellow maize was low with similar behavior in both cropping locations (Table VI). The agroecological conditions of the growing location affected the bioactive compounds and antioxidant activity of experimentally raised and developed grains.

For the cropping location-accessions interaction, only some accessions interacted with the environment in total phenolic and flavonoids content. Yellow grain accessions showed low phenolic and flavonoid contents at both growing locations. Conversely, some accessions of red grain strongly interacted with the cropping location (RJ10, RJ11, and RJ12),

TABLE VI
INTERACTION BETWEEN CROPPING LOCATIONS AND GROUPS OF GRAIN COLOR IN RELATION TO TOTAL PHENOLIC AND FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITIES IN PIGMENTED MAIZE

Total phenolic and antioxidant activity	Santa Maria Coyotepec			San Agustin Amatengo		
	Blue	Red	Yellow	Blue	Red	Yellow
Total phenolic (mg GAE/100g)	127.3 a	115.0 c	75.6 f	119.1 b	112.0 d	79.1 e
Flavonoids (mg CE/g)	0.133 a	0.120 c	0.034 d	0.127 ba	0.126 b	0.036 d
Antioxidant activity by DPPH *	4.84 a	4.79 ab	3.90 c	4.69 b	4.89 a	3.63 d
Antioxidant activity by FRAP *	5.87 a	5.64 b	3.93 e	5.24 d	5.38 c	3.93 e

In the same row, means with same letter are not significantly different (Tukey's test, P≤0.05). *µmol Etrolox/g dw.

and others had high values (RJ01, RJ02, RJ04, and RJ06) and were stable. Blue grain accessions were stable from one location to another in phenol content but did not show a specific pattern in flavonoid content. Yellow grain and control accessions showed low flavonoid content. Two red grain accessions (RJ02 and RJ03) and two blue (AZ13 and AZ06) are remarkable, with flavonoid content >0.15mg CE/g (Figure 2). These data indicate that accessions of blue and red grains include a germplasm of high interest to initiate a process of maize breeding.

The antioxidant activity contributes to estimates of the potential of free radicals or the reducing capacities of the set of bioactive compounds stored in maize grains. Positive relationships between the composition of anthocyanins, total phenolic compounds, and flavonoids with their antioxidant activities were determined. Differences were observed between accessions, based on the antioxidant activities evaluated by DPPH together with FRAP. Nine blue grain and eight red grain accessions are noteworthy and showed higher antioxidant activity. For the total phenolic and total flavonoid contents, RJ02, RJ03, RJ04, RJ06, AZ10, AZ13, AZ16, and AZ18 stand out. Yellow grain accessions showed lower antioxidant activity and lower total phenolic and flavonoids levels (Figure 3). Relative to the FRAP-reducing capacity, the degree of hydroxylation and conjugation of phenolic compounds was confirmed (Pulido *et al*., 2000).

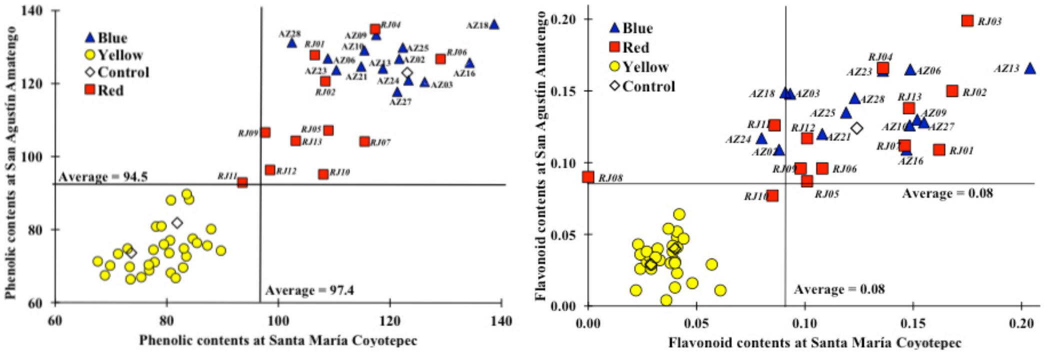

Figure 2. Scatterplot from accession-location interactions based on phenolic (GAE/100g) and flavonoid (mg CE/g) contents at two cropping locations in Oaxaca, Mexico.

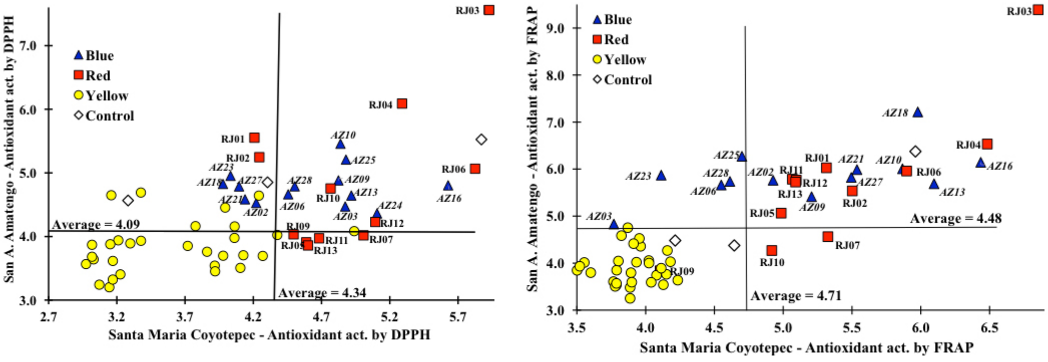

Figure 3. Distribution of maize accessions as a function of the antioxidant activity evaluated using DPPH and FRAP (µmol Etrolox/g dw), in two cropping locations from Oaxaca, Mexico.

The evaluation of the interaction of locations and accessions revealed high variability and differential patterns among accessions in anthocyanin, total phenolic and flavonoid contents and antioxidant activities, which followed the patterns associated with the grain color group (Figures 1, 2 and 3). In bioactive compounds, the accessions of the blue grain always showed a pattern of higher content in Santa Maria and San Agustin, in contrast with the accessions of yellow grain, which showed low values, with the red grain falling between these two groups. In these cases, it possible to differentiate the accessions of blue and red corn with higher contents of anthocyanins, total phenolic compounds and flavonoids compared to yellow accessions, but their use in an integrated breeding program is possible in all cases. In terms of total phenolic compounds, some outstanding red accessions were RJ02, RJ03, RJ04 and RJ06, and some outstanding blue accessions were AZ10, AZ13, AZ16 and AZ18, among others.

Conclusions

The experimental results indicate that the environment and growing location affected anthocyanin and total phenolic content and antioxidant activity, and the effect differed as a function of the grain color group. The content of anthocyanin in blue grain maize was significantly higher compared to that of red grain maize. Among the groups of grain color, significant decreasing differences were determined as blue>red>yellow in total phenolic compounds, flavonoids, and antioxidant activities, and significant interactions between locations and grain color groups were observed. The highest values of anthocyanins, total phenolic compounds, flavonoids and antioxidant activities were found in Santa Maria Coyotepec. Additionally, a high variability between accessions was determined for all evaluated bioactive compounds and antioxidant activities, and significant interactions between locations and accessions of pigmented grains were observed. Some outstanding accessions in composition and antioxidant activity were RJ02, RJ03, RJ04, RJ06, AZ10, AZ13, AZ16 and AZ18, among others.

ACKNOWLEDGMENTS

The authors are grateful for the financial support provided by CONACYT-Problemas Nacionales (Project no. 2015-1-1119) and the Instituto Politécnico Nacional (Project no. 20196672).

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