Phenotypic variation in grain mineral compositions of pigmented maize conserved in indigenous communities of Mexico

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Abstract

In recent decades, pigmented corn has aroused great interest due to its contributions of bioactive compounds and nutritional elements for improvement of health. To evaluate the mineral grain contents in a pigmented corn collection from Oaxaca, Mexico, 57 accessions from indigenous communities and three commercial varieties were grown in two cultivation locations. A random grain sample was obtained from the crops, and the Cu, Fe, Mn, Zn, P, Mg, K, Ca, Na and S contents were quantified using an optical emission spectrophotometer with inductively coupled plasma (ICP-OES). The cropping localities significantly influenced the mineral contents. Likewise, significant differences were observed between accessions, groups of pigmented grains and accession-locality interactions except for zinc. The groups of pigmented grains interact with Cu, Fe, Ca and Na in the environment. Among the accessions, high variation was observed in all elements except for Zn. The results show that a breeding program can be initiated for the outstanding blue, red and yellow grain accessions.

Introduction

Mexico is the center of the origin, domestication and diversification of corn. High phenotypic and genetic corn diversity is preserved on farms (Kato et al., 2009). During the evolution of teosinte to modern corn, natural and human selection has generated changes in the diversity and genetic structure of the cultivated populations not only agronomically but also in the structure and chemical composition of the grain (Vigouroux et al., 2002; Wright et al., 2005; Olsen and Wendel, 2013). Recently, seed selection and management by farmers have generated strong phenotypic and genetic differences between populations or local varieties (landraces) grown in the same or different communities (Pressoir and Berthaud, 2004). Each community exerts different selection pressures according to the need to adapt the populations to cropping plots (agroecological niches) and local uses (Perales et al., 2005). At the local level, each farmer recognizes and preserves a small number of local varieties of corn that can be differentiated by grain pigmentation, crop cycle, plant traits, ears or grains by their own choice; however, this process is not an indicator of reduced genetic diversity.

The phenotypic and genetic diversity of corn in its centers of origin or diversification has been associated with ethnic social factors, diversity management by farmers and environmental variation, among other factors (Pressoir and Berthaud, 2004; Perales et al., 2005; Orozco et al., 2016). This process generates differences in the grain chemical composition between local varieties –landraces- (Cázares-Sánchez et al., 2015; Vera-Guzmán et al., 2012). In turn, this variety has enormous importance for local farmers and consumers (Fernández-Suárez et al., 2013; Nascimento et al., 2014).

Minerals are important in human health for different specific metabolic functions and are crucial for the chemical structures of different macromolecules (Welch and Graham, 2004; Fraga, 2005). For example, deficient Zn and Fe intakes are associated with malnutrition in pregnant women and children (WHO/FAO, 2004; Spiller, 2001; Mejía-Rodríguez, 2013). The per capita volume of corn consumption in different countries of the Americas and Africa is high (> 250 kg/year), and corn kernels provide carbohydrates, crude fiber, fats, proteins, amino acids, vitamins and minerals to the diet (WHO/FAO, 2004; Utrilla-Coello et al., 2009). The germ contributes greater P, K and Mg contents and smaller proportions of Fe, Zn, Mn, Cu, Na, Ca and Se (O'Dell et al., 1972; Rodríguez-Pichiling, 1999; Benitez-Cadoza and Peiffer, 2006; Waters and Sankaran, 2011); these contents may vary according to genotype, environment, crop management and interactions.

Accumulation of minerals in the corn grain depends on different factors that initiate in absorption, mobilization or remobilization and storage in the grain according to the phenological state of the plant, fertility, addition of nutrients to the plant, biotic or abiotic stress from pre-anthesis and formation, filling and grain maturation (Benke and Stich, 2011; Chen et al., 2016; Zhang et al., 2017a). In different studies on fertilization, changes in the mineral content of the grains were quantified with the addition of foliar and soil applications of zinc (Wang et al., 2012), phosphorus (Zhang et al., 2017a) and nitrogen (Feil et al., 2005; Yu-Kui et al., 2009; Chen et al., 2016), among others. In addition, Prasanthi et al. (2017) noted that the mineral compositions in corn grains differed significantly depending on the use or type of corn (e.g., baby, sweet or dent corn or corn processed as popcorn, corn grits, flour and corn flakes). Suri and Tanumihardjo (2016) emphasize that grain processing for consumption directly affects bioavailability and human assimilation; for example, soaking and cooking strongly reduces bioavailability, but the decrease in minerals is less drastic with fermentation and nixtamalization.

The mineral contents in corn grains are strongly affected by the genotype, environment and genotype-environment interaction. In tropical corns, Oikeh et al. (2003, 2004) and Feil et al. (2005) estimated a greater magnitude of environmental variance than the effects of genotypes and genotype-environment interactions. However, the opposite effect was determined by Menkir (2008) when evaluating inbred tropical corn lines. Genetic analysis and quantitative trait locus (QTL) mapping have identified from 12 to 28 (or more) chromosomal regions associated with high mineral contents in different environments. Nonetheless, the results vary according to the type of genetic material used and the number of environments evaluated (Jin et al., 2013; Gu et al., 2015; Zhang et al., 2017b). Additionally, Chakraborti et al. (2011) identified more defined chromosomal regions for iron and zinc content.

Regarding conservation strategies and on-farm use of native corn with high mineral contents in the grain or for breeding, the function of these gene pools must be evaluated in the responses to micro- and macromineral elements in different environments; however, pigmented corn has received scarce or no attention. Bodi et al. (2008) showed certain improvements when determining that blue corn grains presented higher mineral contents than red grains. Cantaluppi et al. (2017) reported that native yellow grain varieties from South Africa presented higher calcium contents than white grain varieties. Notably, the high mineral contents in pigmented corn and high carotenoid content in yellow and anthocyanins in red or blue grains (Egesel et al., 2003; López-Martínez et al., 2009; Salinas-Moreno et al., 2012) provide a greater number of functional compounds in the same product, which is beneficial for health. In addition, in some cases, evidence for the prevention of certain types of cancer has been found (Serna-Saldívar, 2010; Serna-Saldívar et al., 2013; Guzmán-Gerónimo et al., 2017; Herrera-Sotero et al., 2017). In this context, our objective was to evaluate variation in the mineral contents in the grains of a collection of pigmented corn from two different growing environments.

Materials and Methods

Collection and cultivation of germplasm

From December 2015 to March 2016, pigmented corn preserved by farmers in 39 communities in Oaxaca, Mexico, was collected. In addition, a collection of 57 yellow (30), red (13) and blue (14) local samples was generated (Table 1). To ensure the native origin of the samples, indicating on-farm conservation, the collect was conducted only in communities from the Zapoteco, Mixteco, Mixe and Chinanteco ethno-linguistic groups. At the time of the collection, the name of the donor farmer, visual grain pigmentation, descriptive characteristics of the cropping plot and origin of the seed were recorded. A georeference of the community was included, among other aspects (Table 1).

The collection of 57 populations of pigmented corn crops plus three commercial varieties used as controls (VC-42 blue grain, Basic Tuxpeño and SB4-4032 yellow grain) were planted in San Agustin Amatengo (July 5, 2016) and Santa María Coyotepec (July 21, 2016), Oaxaca, Mexico, under a randomized complete block design with four replicates. The localities present climate variations from semidry to semiwarm with annual precipitation of 500 to 700mm, an average annual temperature of 20°C and alkaline pH soils with moderate organic content and acceptable soil fertility conditions (Table 2). The experiment was performed in rainfed conditions supplemented with irrigation and basic fertilization with 120-100-60 of N, P and K, respectively.

Table 1 Accession lists of pigmented maize collected in 39 communities from Oaxaca, Mexico.

Acc.a	Community of origin	Alt. (masl)^{b}	Lat. (W)	Long (N)	Acc.^{a}	Community of origin	Alt. (masl)^{b}	Lat (W)	Long (N)
AM05^{Z}	Santo Domingo Xagacia	1672	17° 16' 07"	96° 29' 01"	AM7^{Z}	San Agustín Amatengo	1361	16° 30' 37"	96° 47' 21"
AM06^{Z}	San Pablo Yaganiza	1530	17° 09' 07"	96° 14' 01"	AZ02^{Z}	Santa Catarina Lachatao	2111	17° 16' 05"	96° 28' 19"
AM07^{Z}	San Melchor Betaza	1431	17° 15' 30"	96° 09' 09"	AZ03^{Z}	Santa María Yavesia	1977	17° 14' 03"	96° 25' 46"
AM08^{Z}	Santo Domingo Roayaga	1615	17° 14' 30"	96° 22' 09"	AZ06^{Z}	Santo Domingo Roayaga	1615	17° 14' 30"	96° 22' 09"
AM10^{Z}	San Francisco Cajonos	1650	17° 10' 15"	96° 15' 00"	AZ09^{M}	Santo Domingo Tonalá	1653	17° 40' 40"	98° 01' 19"
AM21^{Z}	Talea de Castro	1642	17° 22' 45"	96° 14' 56"	AZ10^{M}	Silacayoapan	1847	16° 33' 68"	96° 72' 74"
AM22^{Z}	Santa Ana, Miahuatlán	1584	17° 14' 30"	96° 22' 09"	AZ13^{M}	San Pedro Topiltepec	2168	17° 31' 40"	97° 20' 42"
AM23^{M}	Santo Domingo Tonalá	1653	17° 40' 40"	98° 01' 19"	AZ16^{M}	San Juan Ñumi	2065	17° 21' 14"	97° 42' 27"
AM24^{M}	Ixpantepec Nieves	1718	17° 31' 68"	96° 03' 74"	AZ18^{M}	Tlaxiaco	1907	17° 31' 11"	97° 36' 50"
AM26^{M}	Silacayoapan	1847	16° 33' 68"	96° 72' 74"	AZ21^{M}	San Miguel Achiutla	1988	17° 18' 48"	97° 28' 34"
AM27^{M}	Santiago Tamazola	1662	17° 59' 59"	98° 19' 69"	AZ23^{Z}	Tlacolula de Matamoros	1624	16° 57' 15"	96° 28' 45"
AM29^{M}	Santiago Ayuquililla	1616	17° 57' 28"	98° 00' 44"	AZ24^{Z}	Santo Domingo Teojomulco	1060	16° 30' 57"	97° 13' 16"
AM30^{M}	Santiago Cacaloxtepec	1640	17° 41' 17"	97° 56' 31"	AZ25^{Mi}	Totontepec Villa de Morelos	1776	17° 12' 55"	96° 03' 28"
AM35^{M}	San Juan Mixtepec	1776	17° 28' 63"	97° 71' 36"	AZ27^{M}	Santa Catarina Tayata	2055	17° 08' 28"	97° 23' 30"
AM37^{M}	Tezoatlán de Segura y Luna	1473	17° 43' 20"	97° 44' 33"	AZ28^{M}	Silacayoapan	1506	17° 51' 24"	98° 05' 38"
AM40^{M}	Magdalena Peñasco	2062	17° 14' 11"	97° 33' 50"	RJ01^{Z}	Santa María Yavesia	1977	17° 14' 03"	96° 25' 46"
AM41^{M}	Tlaxiaco	1907	17° 31' 11"	97° 36' 50"	RJ02^{M}	San Mateo Peñasco	1940	17° 09' 20"	97° 32' 00"
AM42^{M}	San Mateo Peñasco	1940	17° 09' 20"	97° 32' 00"	RJ03^{M}	San Miguel Achiutla	1859	17° 15' 22"	97° 30' 18"
AM45^{M}	San Miguel Achiutla	1988	17° 18' 48"	97° 28' 34"	RJ04^{M}	San Bartolomé Yucuañe	1830	17° 14' 29"	97° 27' 00"
AM49^{M}	Santa María Tataltepec	1629	17° 05' 55"	97° 24' 50"	RJ05^{M}	San Bartolomé Yucuañe	1830	17° 14' 29"	97° 27' 00"
AM50^{Z}	Tlacolula de Matamoros	1624	16° 57' 15"	96° 28' 45"	RJ06^{M}	San Juan Mixtepec	1776	17° 28' 63"	97° 71' 36"
AM51^{Z}	San Andrés Zabache	1438	16° 36' 25"	96° 51' 27"	RJ07^{M}	Santiago Cacaloxtepec	1640	17° 41' 17"	97° 56' 31"
AM52^{Z}	Santa María Sola	1468	16° 34' 11"	97° 00' 50"	RJ08^{M}	San Antonino Monte Verde	2213	17° 31' 53"	97° 43' 13"
AM53^{Z}	Santo Domingo Teojomulco	1257	16° 34' 11"	97° 00' 50"	RJ09^{M}	Santiago Tamazola	1662	17° 59' 59"	98° 19' 69"
AM55^{Z}	San Jacinto Tlacotepec	1062	16° 31' 11"	97° 23' 15"	RJ10^{M}	Silacayoapan	1847	16° 33' 68"	96° 72' 74"
AM58^{Mi}	Santa María Tlahuitoltepec	2087	17° 05' 43"	96° 03' 17"	RJ11^{M}	Silacayoapan	1847	16° 33' 68"	96° 72' 74"
AM59^{Mi}	Totontepec Villa de Morelos	1776	17° 12' 55"	96° 03' 28"	RJ12^{M}	Ixpantepec Nieves	1718	17° 31' 68"	96° 03' 74"
AM60^{Z}	San Juan Comaltepec	707	17° 20' 06"	95° 58' 30"	RJ13^{C}	Santiago Jocotepec	278	17° 37' 49"	96° 01' 01"
AM62^{Z}	Santiago Choapan	904	17° 14' 06"	96° 00' 30"					

^{a}Prefix means grain color, AM = yellow; AZ = blue and RJ = red; Superscript indicate ethnic groups; ^{Z} = Zapotec, ^{M} = Mixtec, ^{Mi} = Mixe, and ^{C} = Chinantec; ^{b} masl = meters above sea level

Grain sampling and sample preparation

In each locality, a sample of 10 ears per population was taken based on the experimental design of the field distribution. The ears were shelled and mixed manually. A sample consisting of 500 g of grain was obtained, and a subsequent subsample of 100 g was finely ground in an Apex mill (Apex Construction®, Ltd.) and later in a grain mill (Krups®, Mexico). Finally, the flour was sieved on a 500-µm mesh screen and stored in amber vials at -20°C prior to analysis.

Determination of mineral contents

A 1.5 g sample of corn flour was brought to constant weight in an oven (Barnstead/Thermolyne Oven series 9000, USA) at 105°C (AACC 44-15) and then incinerated at 570°C in a flask (Barnstead/Thermolyne 1400, USA) to a constant weight (AACC 08-01.01), (AACC 1976). Then, 3 mL of concentrated hydrochloric acid (JT Baker®)) was added to the ash residue and gauged at 50 mL with deionized water. Finally, the solution was filtered with fine pore filtering material and stored under refrigeration prior to analysis. In addition, a blank was prepared without sample following the same procedure

Table 2. Description of two evaluation locations in Oaxaca, Mexico.

Site and soil descriptors	Locations	
	San Agustín Amatengo	Santa María Coyotepec
Site descriptors:		
Latitude (N)	16°30'37 "	16°57'58”
Longitude (W)	96°47'21"	96°42'23”
Altitude (masl)	1361	1518
Annual average temperature (°C)	20.9	20.0
Annual average precipitation (mm)	693.8	526.5
Climate	*semi-dry to semi-warm*	*semi-warm to semi-warm*
Soil descriptors:		
pH (in H_2O)	8.30	7.80
CE (dSm^{-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 analysis under current Mexican norm NOM-021-RECNAT 2000

to rule out possible contamination by reagents.

Quantification of micro- and macronutrients (Cu, Fe, Zn, Mn, P, Ca, Mg, K, Na and S) was performed by optical emission spectrometry with inductively coupled plasma (ICP-OES Thermo Scientific iCAP 6500 DUO, UK) with radial and axial configurations. Argon was used as the auxiliary gas and autosampler (CETAC ASX-520, USA). The analysis was performed with an auxiliary gas flow of 0.4 L min^{-1}, 1200 W RF power and 50 rpm analysis speed with 10 s in peristaltic pump stabilization. The quantification was based on multielement reference standards (High Purity® Standards, USA) in a range from 1 to 100 mg L^{-1} for P, Mg, K, Ca, Fe and Na and 0.2 to 5 µg mL^{-1} for Cu, S, Mn and Zn. The lower limits of detection for Mn, Cu, Zn, Mg, Na, S and Mg were 0.0001, 0.0002, 0.002, 0.003, 0.005, 0.009 and 0.00006 mg L^{-1}, respectively, and 0.01 g L^{-1} for P, K and Ca. All tests were performed in triplicate, and the results were expressed in mg 100 g^{-1} dry base.

Statistical analysis

Combined variance analysis was conducted to evaluate the effects of locality, grain pigment group, accession and the locality-pigment group and locality-accession interactions. In this analysis model, the effect of accessions nested in the grain pigment groups was considered. In addition, multiple mean comparisons were performed using Tukey's method ($p \leq 0.05$). All tests were conducted in the SAS statistical package (SAS Institute, 2002).

Results

In the analysis of variance, significant differences were determined ($p \leq 0.05, 0.01$) between the evaluation localities, between the grain pigment groups (red, blue and yellow) and between the accessions for all minerals evaluated except for zinc in the pigment groups and accessions. The locality-grain color interaction (L x C) showed differences in Cu, Fe, Ca and Na. In the locality-accession interaction (L x A), significant differences were found in Cu, Fe, Mn, Ca, Na and S; Na, Fe and S presented the highest coefficients of variation at 34.4, 33.0 and 28.7, respectively (Table 3).

In the mean comparisons between evaluation locations, the Cu, Fe, Mn and S contents were significantly higher in San Agustín Amatengo than in Santa María Coyotepec but presented an opposite trend for Zn, Mg,

Table 3. Significance in the variance analysis of the mineral content in a collection of fifty-seven accessions and three commercial varieties of pigmented maize.

	Sources of variation							
Mineral contents in grain	Location (L)	Color (C)	L x C	Accessions (A)/C[1]	L x A	Rep./L[1]	Error	C.V. (%)
Cu	0.26**	0.08**	0.03*	0.03**	0.02**	0.004^{ns}	0.01	16.6
Fe	22.40**	0.60**	1.20**	0.30**	0.20*	0.20^{ns}	0.10	33.0
Mn	0.30**	0.05**	0.01^{ns}	0.02**	0.01*	0.03**	0.01	15.0
Zn	0.54**	0.04^{ns}	0.16^{ns}	0.07^{ns}	0.04^{ns}	0.09^{ns}	0.05	12.9
P	893230**	19303**	931^{ns}	3583**	2666^{ns}	4258*	1948	13.0
Mg	42648**	2041**	132^{ns}	375**	291^{ns}	475^{ns}	225	12.0
K	552377**	76551**	3026^{ns}	4426**	2050^{ns}	2621^{ns}	1957	11.0
Ca	105.4**	21.1**	23.3**	1.5**	1.0**	0.6^{ns}	0.5	21.8
Na	125.1**	32.6**	14.9**	0.8**	1.4**	1.1**	0.4	34.4
S	35.9**	22.6**	0.7^{ns}	0.9**	0.8**	0.4^{ns}	0.2	28.7

[1] Indicates accessions nested in groups of grain pigments and replications nested in locations; ns = Not significant ($p > 0.05$); * significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; CV = coefficient of variation

K, P, Ca and Na. This result shows that environmental edapho-climatic characteristics have significant effects on pigmented corn grain mineral contents. In relation to the comparison of grain pigment groups, the red grain accessions showed a higher mineral content except for Fe, which was higher in the yellow grains, whereas S was highest in the blue grains (Table 4)

In the locality-pigment group interaction, the differences were not significant ($p < 0.05$) for the Zn, Mn, P, Mg, K and S contents (Table 5). Namely, for these minerals, independence existed in the selection of grain pigments with these mineral contents by farmers. The Cu content was higher in the red grains in both locations and in the blue grains from San Agustín Amatengo. Specifically, in the town of Santa María, a lower Fe content was determined compared to that in San Agustín, which indicated that the agroecological conditions of Santa María had a negative influence on the accumulation of Fe in the grains of all evaluated materials (Table 5).

Among the three groups of grain color, significant differences ($p < 0.05$) were observed in all evaluated minerals, including the commercial controls, except for Zn. Regarding the micronutrients, different accessions of yellow, blue or red grains significantly surpassed ($p < 0.05$) the controls in Cu, Fe and Mn (Table 6). The variations in the Cu, Fe and Mn contents ranged from 0.10 to 0.32, 0.70 to 2.12 and 0.45 to 60 in the yellow

Table 4. Comparison of mean micro- and macronutrient contents in pigmented corn grains between localities and pigment groups.

Mineral contents in grain (mg 100 g^{-1})	Locations		Grain color group (n = samples)		
	San Agustín Amatengo	Santa María Coyotepec	Blue (16)	Red (13)	Yellow (32)
		Micronutrients:			
Cu	0.27 a[†]	0.21 b	0.24 b[†]	0.26 a	0.22 b
Fe	1.55 a	0.79 b	1.07 b	1.08 b	1.24 a
Mn	0.55 a	0.50 b	0.53 ab	0.54 a	0.52 b
Zn	3.2 b	3.5 a	3.5 a	3.3 a	3.2 a
		Macronutrients:			
P	292.2 b	395.4 a	332.6 b	350.4 a	347.5 a
Mg	107.7 b	129.8 a	116.2 b	122.2 a	118.9 ba
K	336.6 b	414.3 a	385.8 b	401.2 a	362.4 c
Ca	8.3 b	14.4 a	11.3 b	14.6 a	10.2 b
Na	2.4 b	6.6 a	5.4 b	6.5 a	3.4 c
S	5.0 a	2.7 b	5.5 a	4.6 b	2.9 c

[†] Between locations or among grain pigments, means with the same letter are not significantly different ($p < 0.05$)

Table 5. Mineral contents in the interaction between localities and groups of accessions for the yellow, blue and red grain pigments

Minerals (mg 100 g^{-1})	San Agustin Amatengo (n)			Santa Maria Coyotepec (n)		
	Yellow (32)	Blue (16)	Red (13)	Yellow (32)	Blue (16)	Red (13)
Micronutrients:						
Cu	0.24 bc[†]	0.29 a	0.27 ab	0.20 dc	0.19 d	0.25 ab
Fe	1.54 a	1.58 a	1.57 a	0.94 b	0.64 b	0.60 b
Mn^{ns}	0.56	0.54	0.56	0.48	0.51	0.52
Zn^{ns}	2.99	6.62	3.08	3.52	3.40	3.37
Macronutrients:						
P^{ns}	295.2	277.0	300.1	400.2	380.7	400.7
Mg^{ns}	108.9	102.0	110.8	129.0	128.4	133.5
K^{ns}	327.8	342.8	353.7	397.3 a	422.8	448.9
Ca	7.4 d	5.1 e	13.9 bc	13.0 c	16.6 a	15.3 ba
Na	1.0 d	2.6 c	5.7 b	5.8 b	7.8 a	7.3 a
S^{ns}	3.8	7.5	5.8	2.0	3.7	3.5

[†] In a row, means with the same letters do not differ significantly (Tukey's test, $p < 0.05$); nsdifference not significant ($p > 0.05$).

grain collections, from 0.15 to 0.37, 0.52 to 1.64 and 0.46 to 0.60 in the blue grain collections and from 0.22 to 0.32, 0.50 to 1.36 and 0.41 to 0.64 mg 100 g^{-1} in the red grain collections respectively. In terms of their microelement contents, the best accessions were AM40, AM58 and AM59 in Cu and Mn, and AM21 in Fe and Mn for yellow grain; in the group of blue grains were AZ24 and AZ25 in Cu, AZ09 and AZ10 in Fe but in Mn were A13, AZ02 and AZ03; and in the red grain group were RJ01, RJ05 and RJ13 for Cu, RJ03 and RJ06 for Fe, and RJ01, RJ03 and RJ05 for Mn. VC-42 (blue grain) presented high values in all evaluated compounds. All accessions had similar Zn values (Table 6).

The macroelement content was determined for the outstanding accessions in each grain pigment group were determined for two or more elements that differed between pigments. In general, the yellow accessions showed low variation intervals for all macroelements except for P. For P, Mg, K, Ca, Na and S, the yellow grain accessions ranged from 291.7 to 391.5, 100.0 to 130.9, 320.1 to 425.6, 5.5 to 14.5, 1.4 to 6.2 and 1.0 to 5.7 mg 100 g^{-1}, respectively. These same values ranged from 311.3 to 377.5, 108.8 to 131.0, 371.1 to 420.4, 7.3 to 18.8, 3.0 to 9.5 and 3.8 to 7.9 mg 100 g^{-1} in the blue accessions and from 318.4 to 387.9, 107.7 to 143.6, 381.6 to 498.0, 12.4 to 17.8, 2.2 to 7.6 and 0.8 to 6.5 mg 100 g^{-1} in the red accessions, respectively (Table 7). These results show that the microelement contents of the blue and red grain accessions surpassed those of the yellow grains and commercial varieties that were used as controls in this work. The accessions with potential based on their macroelement value was RJ08 (red grain) in P, Mg and K; in the blue grains were AZ03 for Ca and Na, and AZ24 for S; and in the accession of yellow grain, AM58 presented high values in P and Mg. The blue grain variety VC-42, was among the most competitive controls.

In the locality-accession interaction, no significant differences were detected in Zn, P, Mg and K. In contrast, the Cu, Fe, Mn, Ca, Na and S contents in the grains differed significantly among the accessions in their stability according to the evaluation location (L x A). Figure 1 graphically represents the responses of accessions grouped by grain pigment in interactions with two localities and expresses variation in the response. For example, for Fe (Fig. 1a), eight accessions of yellow grain, four of blue grain and four of red grain had higher Fe contents in San Agustín Amatengo than Santa María Coyotepec. In this study, the minerals evaluated across cultivation locations to allowed discriminate accessions by mineral contents, in this case a group of accessions always presented averages above general average of each location (upper right quadrants of the Figure 1). Therefore, we can selection accessions with greater Fe, Cu, Ca, Mn, Na and S and directly to use as food source or to start to a planned breeding program. In this sense, according to our interest, we can select red grain accessions with high content of Ca, Na and S; some yellow grain accessions with high Fe, Cu, Ca and Mn values; and some blue grain accessions for their Fe, Ca, Mn, Na and S contents. In general, the blue accessions showed stronger interaction with their evaluation location in mineral compositions.

Table 6. Mineral microelement contents in the grains of pigmented corn native to Oaxaca, Mexico.

Acc. ID	Cu^1	Fe^1	Mn^1	Zn^1	Acc. ID	Cu^1	Fe^1	Mn^1	Zn^{ns}
AM05	0.24 a-e^2	0.97 ab	0.46 bcd	2.89	AZ02	0.23 a-e	1.27 ab	0.58 a-d	3.50
AM06	0.24 a-e	1.28 ab	0.54 a-d	3.28	AZ03	0.29 a-d	1.12 ab	0.58 a-d	3.28
AM07	0.21 b-e	0.86 ab	0.46 bcd	2.77	AZ06	0.22 b-e	1.37 ab	0.49 a-d	3.23
AM08	0.23 a-e	1.33 ab	0.56 a-d	3.35	AZ09	0.16 cde	1.64 ab	0.50 a-d	3.08
AM10	0.22 b-e	1.40 ab	0.57 a-d	3.29	AZ10	0.19 cde	1.42 ab	0.47 a-d	3.29
AM21	0.23 a-e	1.94 ab	0.60 abc	3.39	AZ13	0.20 cde	0.71 ab	0.60 abc	3.21
AM22	0.25 a-e	1.72 ab	0.59 abc	3.17	AZ16	0.18 cde	1.10 ab	0.46 bcd	3.15
AM23	0.23 a-e	2.12 a	0.49 a-d	3.60	AZ18	0.20 cde	1.22 ab	0.51 a-d	3.09
AM24	0.19 cde	1.91 ab	0.49 a-d	3.31	AZ21	0.17 cde	0.52 b	0.46 bcd	2.90
AM26	0.24 a-e	1.20 ab	0.53 a-d	3.33	AZ23	0.23 a-e	0.61 ab	0.51 a-d	3.09
AM27	0.24 a-e	0.83 ab	0.48 a-d	2.76	AZ24	0.37 ab	0.66 ab	0.51 a-d	5.19
AM29	0.22 b-e	1.05 ab	0.57 a-d	3.68	AZ25	0.30 a-d	0.75 ab	0.56 a-d	3.47
AM30	0.22 b-e	0.98 ab	0.51 a-d	3.28	AZ27	0.26 a-e	1.38 ab	0.54 a-d	3.56
AM35	0.23 a-e	1.08 ab	0.49 a-d	3.06	AZ28	0.15 de	0.62 ab	0.57 a-d	2.90
AM37	0.20 cde	0.99 ab	0.45 bcd	3.08	RJ01	0.28 a-d	1.25 ab	0.59 abc	2.91
AM40	0.32 a-d	1.66 ab	0.54 a-d	3.83	RJ02	0.25 a-e	1.19 ab	0.58 a-d	3.33
AM41	0.26 a-e	1.71 ab	0.52 a-d	3.37	RJ03	0.27 a-d	1.30 ab	0.64 a	3.74
AM42	0.19 cde	1.20 ab	0.55 a-d	3.40	RJ04	0.22 b-e	0.50 b	0.54 a-d	3.06
AM45	0.20 cde	0.70 ab	0.52 a-d	3.29	RJ05	0.28 a-d	1.22 ab	0.59 abc	3.71
AM49	0.23 a-e	1.20 ab	0.51 a-d	3.11	RJ06	0.27 a-d	1.36 ab	0.58 a-d	3.38
AM50	0.22 b-e	0.95 ab	0.52 a-d	3.07	RJ07	0.22 b-e	0.92 ab	0.48 a-d	3.16
AM51	0.22 b-e	1.14 ab	0.51 a-d	3.05	RJ08	0.27 a-d	0.71 ab	0.56 a-d	3.43
AM52	0.21 b-e	1.14 ab	0.55 a-d	3.33	RJ09	0.26 a-e	1.10 ab	0.51 a-d	3.00
AM53	0.23 a-e	0.76 ab	0.45 bcd	3.09	RJ10	0.25 a-e	1.16 ab	0.55 a-d	3.02
AM55	0.21 b-e	1.00 ab	0.49 a-d	2.79	RJ11	0.26 a-e	0.82 ab	0.53 a-d	3.31
AM58	0.30 a-d	1.81 ab	0.59 abc	3.45	RJ12	0.25 a-e	1.15 ab	0.41 d	2.96
AM59	0.32 abc	0.82 ab	0.57 a-d	3.57	RJ13	0.32 a-d	1.11 ab	0.51 a-d	3.06
AM60	0.10 e	0.76 ab	0.56 a-d	2.90	VC-42	0.39 a	1.53 ab	0.62 ab	5.30
AM62	0.16 cde	0.96 ab	0.52 a-d	3.32	Tuxpeño	0.22 b-e	1.45 ab	0.43 cd	3.15
AM70	0.19 cde	1.26 ab	0.54 a-d	3.54	SB4-4032	0.16 cde	1.37 ab	0.48 a-d	3.68

ID prefixes; AM, AZ and RJ indicate yellow, blue and red grains, respectively.^{1} mg 100 g^{-1}; ^{2}in column, means with same letter are not significantly different (Tukey's test, $p \leq 0.05$) and without letters differences not significant (ns).

Discussion

The accumulation of minerals in corn grains depends on several factors associated with the physiology of the plant from the vegetative stage to grain filling and maturation. The environment and/or cultivation practices (e.g., the use of fertilizers), genotype and genotype-environment or genotype-cultivation practice interactions are crucial for the final mineral contents in corn grains (Feil et al., 2005; Yu-Kui et al., 2009; Wang et al., 2012; Gu et al., 2015; Zhang et al., 2017b). Accumulation of minerals in the grain depends on the physiology of the corn plant based on soil absorption, translocation, nutrient demand relationships in different organs (e.g., foliar expansion, flowering, fertilization and cell multiplication in the future ear), drought during grain filling and final grain accumulation (Chen et al., 2016; Zhang et al., 2017a, b). Therefore, the present work provides an estimation of the micro- and macromineral contents accumulated in the grain as a result of genotypic (accessions) and environmental (evaluation localities) effects and genotype-environment interactions.

The results show that significant differences existed in all minerals evaluated between locations. In all cases,

Table 7. Mineral macroelement contents in pigmented corn native to Oaxaca..

Acc. ID	P^1	Mg^1	K^1	Ca^1	Na^1	S^1	Acc. ID	P^1	Mg^1	K^1	Ca^1	Na^1	S^1
AM05	325.3 bc^2	110.7 ab	332.2 bcd	9.6 c-h	3.9 c-h	1.8 b-g	AZ02	368.0 abc	126.1 ab	399.7 bcd	17.2 a-d	7.0 a-f	6.3 a-d
AM06	369.8 abc	127.6 ab	354.3 bcd	12.2 a-h	3.7 c-h	1.1 efg	AZ03	376.9 abc	131.0 ab	415.9 a-d	18.8 a	9.5 a	6.4 abc
AM07	324.0 bc	108.0 b	320.1 d	12.7 a-h	5.3 a-h	1.7 c-g	AZ06	340.2 bc	123.3 ab	377.5 bcd	6.8 gh	2.6 e-h	3.7 a-g
AM08	370.1 abc	126.2 ab	354.7 bcd	13.4 a-h	2.8 d-h	1.6 c-g	AZ09	318.8 bc	114.2 ab	391.3 bcd	8.5 e-h	3.0 c-h	6.5 ab
AM10	365.0 abc	124.7 ab	369.6 bcd	10.0 b-h	2.9 d-h	2.1 b-g	AZ10	322.9 bc	108.8 b	387.1 bcd	7.3 fgh	3.3 c-h	4.0 a-g
AM21	373.8 abc	130.9 ab	364.3 bcd	13.7 a-g	4.2 b-h	3.0 b-g	AZ13	377.5 abc	125.7 ab	420.4 abc	12.0 a-h	4.6 a-h	3.8 a-g
AM22	354.7 abc	117.5 ab	352.7 bcd	9.1 e-h	3.3 c-h	1.6 c-g	AZ16	311.3 bc	112.0 ab	366.7 bcd	8.4 e-h	4.2 b-h	5.1 a-g
AM23	332.2 bc	111.8 ab	369.5 bcd	10.4 b-h	4.0 b-h	4.3 a-g	AZ18	341.2 bc	113.7 ab	420.0 abc	8.4 e-h	4.4 a-h	5.1 a-g
AM24	327.5 bc	112.4 ab	341.8 bcd	7.2 fgh	2.6 e-h	1.8 b-g	AZ21	311.3 bc	110.1 b	354.4 bcd	9.9 b-h	4.7 a-h	3.9 a-g
AM26	354.6 abc	119.6 ab	372.1 bcd	8.2 e-h	2.9 d-h	1.8 b-g	AZ23	324.2 bc	115.4 ab	374.2 bcd	9.9 b-h	4.4 a-h	4.5 a-g
AM27	291.7 c	100.0 b	335.7 bcd	9.5 d-h	2.7 e-h	4.5 a-g	AZ24	318.7 bc	112.1 ab	394.4 bcd	12.0 a-h	6.8 a-g	8.0 a
AM29	344.8 bc	119.0 ab	353.6 bcd	10.8 b-h	3.2 c-h	2.3 b-g	AZ25	336.7 bc	113.7 ab	379.9 bcd	12.9 a-h	7.9 a-d	5.1 a-g
AM30	340.2 bc	116.7 ab	373.3 bcd	13.0 a-h	4.3 b-h	4.2 a-g	AZ27	329.5 bc	116.2 ab	380.2 bcd	13.6 a-g	5.8 a-h	5.8 a-e
AM35	352.4 abc	117.9 ab	410.9 a-d	12.1 a-h	4.5 a-h	4.3 a-g	AZ28	319.7 bc	111.2 ab	371.1 bcd	17.5 abc	9.1 ab	5.2 a-g
AM37	320.3 bc	110.6 ab	343.6 bcd	10.3 b-h	3.6 c-h	2.1 b-g	RJ01	387.9 abc	130.9 ab	420.4 abc	11.8 a-h	5.4 a-h	3.5 a-g
AM40	358.4 abc	120.6 ab	397.3 bcd	10.0 b-h	6.2 a-h	5.7 a-f	RJ02	361.4 abc	129.1 ab	384.7 bcd	14.4 a-g	6.9 a-g	3.6 a-g
AM41	337.0 bc	120.2 ab	386.7 bcd	5.6 h	3.1 c-h	5.5 a-g	RJ03	377.1 abc	132.9 ab	428.0 ab	17.8 ab	7.6 a-e	6.5 ab
AM42	362.2 abc	124.1 ab	373.8 bcd	14.5 a-g	3.8 c-h	1.9 b-g	RJ04	358.5 abc	121.1 ab	400.5 a-d	14.5 a-g	6.3 a-h	2.9 b-g
AM45	360.7 abc	123.7 ab	379.7 bcd	11.4 a-h	3.0 c-h	1.0 fg	RJ05	355.0 abc	123.3 ab	411.4 a-d	14.8 a-g	5.9 a-h	4.7 a-g
AM49	363.6 abc	122.9 ab	365.2 bcd	8.9 e-h	3.5 c-h	1.5 efg	RJ06	352.0 abc	122.7 ab	425.5 ab	15.4 a-e	7.1 a-f	6.3 a-d
AM50	323.5 bc	113.3 ab	336.0 bcd	12.7 a-h	4.2 b-h	2.1 b-g	RJ07	328.1 bc	115.1 ab	389.7 bcd	13.6 a-h	5.4 a-h	4.4 a-g
AM51	336.6 bc	114.5 ab	334.2 bcd	8.0 e-h	2.3 fgh	1.7 c-g	RJ08	444.1 a	143.6 a	498.0 a	12.4 a-h	2.3 fgh	0.8 g
AM52	362.2 abc	121.6 ab	361.6 bcd	10.7 b-h	3.0 c-h	2.3 b-g	RJ09	329.9 bc	118.8 ab	394.5 bcd	15.0 a-f	7.0 a-f	5.5 a-g
AM53	338.1 bc	117.0 ab	375.9 bcd	7.8 e-h	3.7 c-h	5.5 a-g	RJ10	331.4 bc	118.5 ab	396.0 bcd	15.3 a-e	6.5 a-h	4.9 a-g
AM55	338.2 bc	120.7 ab	327.2 cd	9.1 e-h	4.2 b-h	2.4 b-g	RJ11	333.2 bc	120.4 ab	394.2 bcd	13.7 a-g	6.0 a-h	4.8 a-g
AM58	387.2 abc	127.6 ab	425.6 ab	10.4 b-h	4.0 b-h	3.9 a-g	RJ12	318.4 bc	107.7 b	381.6 bcd	14.9 a-f	6.9 a-g	5.0 a-g
AM59	363.1 abc	126.3 ab	391.3 bcd	11.1 a-h	2.9 d-h	3.9 a-g	RJ13	350.8 abc	121.0 ab	362.2 bcd	15.0 a-f	8.2 abc	4.3 a-g
AM60	356.8 abc	119.2 ab	366.6 bcd	8.8 e-h	1.8 gh	2.6 b-g	VC-42	342.9 bc	121.2 ab	388.8 bcd	10.4 b-h	6.3 a-h	7.8 a
AM62	391.5 ab	129.7 ab	401.5 a-d	9.4 d-h	1.4 h	2.4 b-g	Tuxpeño	314.0 bc	117.2 ab	332.8 bcd	7.8 e-h	2.4 fgh	4.5 a-g
AM70	340.7 bc	116.5 ab	343.1 bcd	8.9 e-h	2.1 fgh	4.0 a-g	SB4-4032	341.7 bc	117.9 ab	359.5 bcd	10.1 b-h	2.7 e-h	4.2 a-g

ID prefixes; AM, AZ and RJ indicate yellow, blue and red grains, respectively. ^{1}mg 100 g^{-1}; ^{2} in column, means with same letter are not significantly different (Tukey's test, p ≤ 0.05).

the variance attributed to the effect of localities was greater than that of the accessions (genotypes) and the location-accession interactions. Santa Maria Coyotepec presented higher values for six of the ten elements (Table 3). This result shows that the environment exerts a strong influence on the accumulation of minerals in the grains due to an ecological influence, physical characteristics, soil fertility (Table 2), differential agronomic management practices or a combination of all of these factors, even though the same fertilization formula has been applied. These findings agreed with the reports of de Oikeh et al. (2003) and Feil et al. (2005) in tropical corn, which showed a strong environmental effect on grain minerals. This finding suggests that evaluation of the mineral contents of grains from a single crop cycle and/or a few environments is insufficient to determine the stability of genotypes through environments.

Grain pigment is a simple trait that is used by farmers to differentiate and manage corn types independently and also to select seed lots for each crop cycle (Perales et al., 2005). This documented fact underlying independent management of grain populations with different pigmentation was also reflected in differences in the average mineral composition. For example,

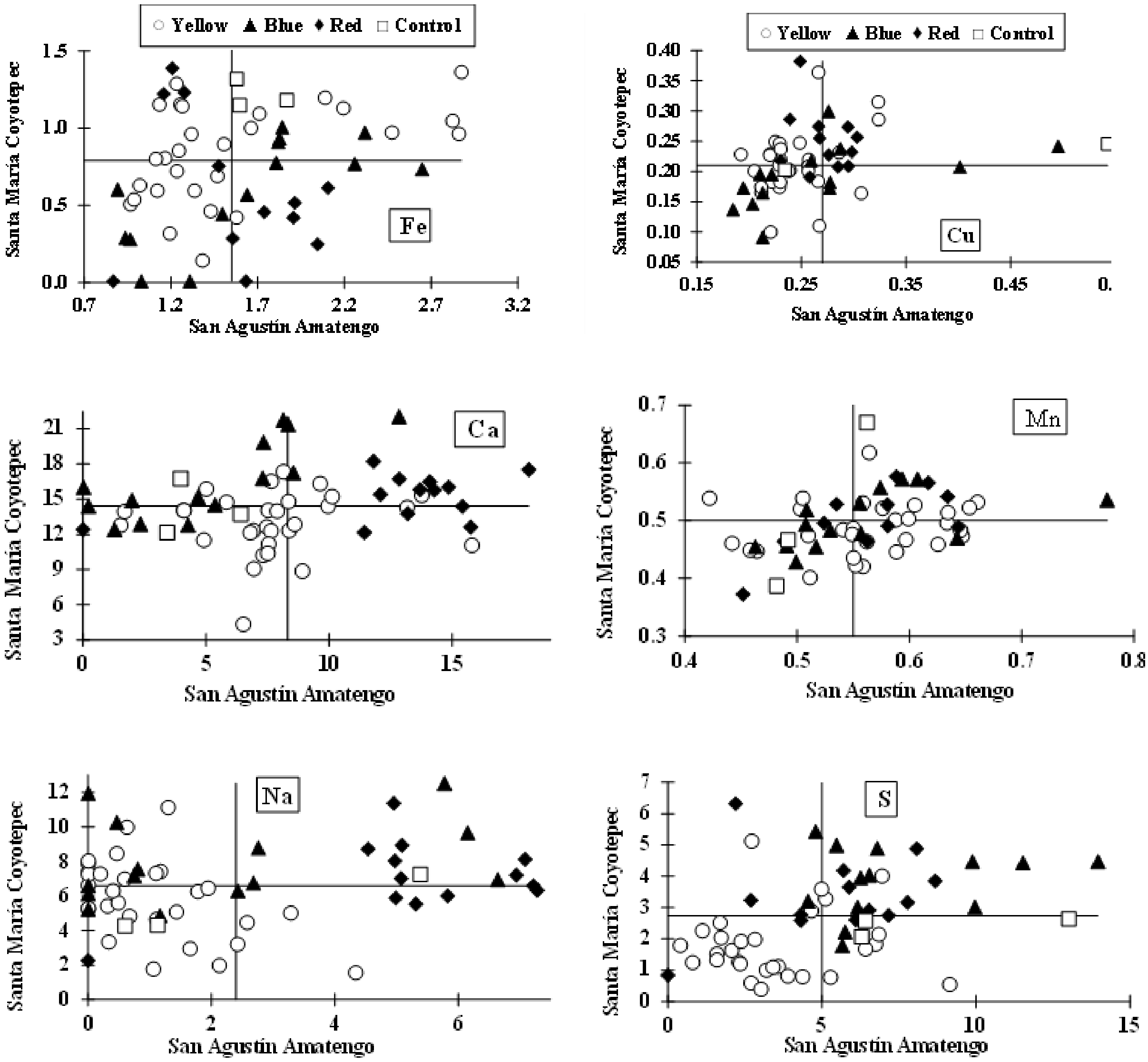

Fig. 1. Dispersion of pigmented corn accessions according to the mineral contents (mg 100 g^{-1}) in the grains and crop products at the two evaluation locations

the Fe content of the group of yellow accessions was significantly superior to those of the blue and red accessions, which was similar to the reports by Oikeh et al. (2003, 2004). Regarding the S content, the blue accessions were superior to the red and yellow accessions. On average, the red accessions were high in seven elements and showed signs of greater isolation or species management among farmers (Table 4). These results differed from the report by Bodi et al. (2008), who determined higher P, K, S, Mg, Fe, Cu and Zn contents in blue grain corn than in other pigmentations. The group of red grain populations in the current study or the blue grain populations in the case de Bodi et al. (2008) suggest that there is greater translocation and probably greater demand and accumulation of minerals in black or red grain corn than in corn with light pigmentations, which may also suggest potential for greater use and consumption to improve the health conditions of rural communities. In addition, pigmented grains contribute bioactive compounds, such as carotenoids, anthocyanins, phenols and flavonoids (Urias-Lugo et al., 2015; Herrera-Sotero et al., 2017).

In the locality-pigment group interaction, significant differences in the Cu, Fe, Ca and Na contents were determined. This result indicates that a differential response exists between grain pigment groups depending on the environment. San Agustin Amatengo was a representative example, in which the three pigment groups (yellow, red and blue) presented higher Fe values than Santa María Coyotepec, Mexico. On the other hand, Ca and Na presented the opposite effects in the blue and red grain groups. In the red grain group, the Cu content was high in both locations. These differences between grain pigment groups based on locality are partially due to differences in soil fertility; for example, in Santa María Coyotepec, the organic matter P, Mg, Fe and Cu contents were slightly higher than those in San Agustín Amatengo (Table 2). Namely, the chemical composition or soil fertility usually affects the mineral content similar to the effect of adding more zinc, nitrogen or phosphorus to the soil during fertilization of the crop (Feil et al., 2005; Yu-Kui et al., 2009; Wang et al., 2012; Chen et al., 2016; Zhang et al., 2017a, b).

The results show no evidence of a significant interaction between grain pigment groups and evaluation localities in the grain Mn, Zn, P, Mg, K and S contents. This result suggests that classification of grain by visual color of the pericarp and/or aleurone is not always associated with differences in the grain mineral composition. A similar finding was documented by Menkir (2008) when evaluating different groupings of 278 inbred lines across locations in eight evaluation studies in Nigeria. However, Menkir (2008) evaluated homogeneous lines, whereas the present study evaluated accessions or collections of the producer with high ear and grain heterogeneity traits. Likewise, the variation in the evaluated minerals within each grain pigment group was high, as confirmed in Tables 6 and 7.

The results show high variation between collections in the grain mineral contents except for Zn and partially reflect the genetic diversity between different genetic stocks of corn grown by farmers in Oaxaca, Mexico. Among accessions with different grain pigments, different degrees of absorption, translocation and accumulation of Cu, Fe and Mn in the grain were determined (Table 6). However, Zhang et al. (2017a) noted that the partition and accumulation of microelements in corn differed depending on the type of organ or structure; for example, Cu, Mn and Fe accumulated in greater proportions in the leaves and bracts than in the grains. However, the demand in grain is increased due to the flowering and fecundation of ovules in the female flower (Chen et al., 2016).

The microelement results present significant differential patterns that can be used to initiate a genetic breeding program. The variation among accessions in Fe, Zn, Cu and Mn was similar to those reported by Feil et al. (2005) in tropical corn. Likewise, Bänzinger and Long (2000), among other authors, estimated similar Fe and Zn contents in corn germplasms in Mexico and Zimbabwe. For the purpose of genotype selection, direct use or undertaking a genetic breeding strategy, Chakraborti et al. (2011) and Zhang et al. (2017b) considered potential genotypes with 25 g kg^{-1} or more of Fe and Zn. Therefore, we should note that estimations of microelements differ according to the analysis method [inductively coupled plasma mass spectrometer (ICP-MS), ICP-OES or atomic absorption spectrometer (AAS)] as reported by Chakraborti et al. (2011) and Nascimento et al. (2014). Unlike white corn, pigmented corn provides bioactive compounds with importance for health in addition to microelements (Herrera-Sotero et al., 2017).

The accessions differed significantly for macronutrients. The variation in Ca, Mg, K, Na, P and S was useful to discriminate and select a group of promising accessions. In blue corn, the values determined in this work for Ca, P, K, Na and Mg were similar to those reported by Urías-Lugo et al. (2015) and Nascimento et al. (2014) in both hybrids and local varieties of blue corn in Mexico and Argentina, respectively. This finding agrees with the report of de Qamar et al. (2017) in a variety of yellow corn from Pakistan. Namely, despite heterogeneity in the native populations (accessions) evaluated in the present study, the average macroelement contents were similar to those of the improved varieties and inbred lines evaluated in other works (Menkir, 2008; Urías-Lugo et al., 2015). In addition, the differences between accessions are partially due to the translocation and grain accumulation capacity.

In the interaction between evaluation locations (environment effect) and accessions, significant differences were determined for Fe, Cu, Ca, Mn, Na and S, with differential patterns depending on the grain pigment (Fig. 1). Between accessions, differences in the response stability were differentiated by the grain pigment. Regarding the Fe content, different accessions of yellow grain and some of blue stood out in both localities as indicators of greater stability. Qamar et al. (2017) reported a similar finding when comparing yellow and white grain corn varieties. However, the yellow grain varieties did not stand out in terms of Ca, Na and S. Accessions of red and blue grains excelled in Ca, Na and S concentrations, whereas a combination of blue, red and yellow corn expressed Mn. These values were similar to those reported by Menkir (2008) for inbred lines and white grain hybrids, which indicated that a breeding program could be initiated with each group of pigmented corn from outstanding accessions. Additionally, the differential management of corn by grain color conducted by farmers has generated isolations and phenotypic differentiation with genetic loads of interest to the breeder.

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