

# Effect of crop location and genotype on phenolic compounds, mineral contents, and antioxidant activity in yellow maize landraces

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## Abstract

The Mesoamerican region is center of origin, domestication, and diversification of maize. In this ecogeographic context, the objective was to evaluate the variation in the phenolic compounds, antioxidant activity and concentration of minerals in the grain of a population collection of yellow maize landraces from southeastern Mexico. During 2016, samples of landraces of yellow maize were collected and integrated into 32 populations and experimental varieties, which were planted in two locations in Oaxaca, Mexico, under a random block design. At harvest, a sample of grain was taken, which was grounded to evaluate the polyphenolic compound contents and antioxidant activity by UV-visible spectrophotometry, and macro- and microelement contents were determined using inductively coupled plasma-optical emission spectrometry. The effect of crop location was significantly greater than the effects of populations and location-population interaction on polyphenol contents and concentration of Ca, P, Mg, K, Na, S, Cu, Fe, Mn and Zn. In the Amatengo locality, a higher macroelement contents were recorded, and in the second locality, the concentration of microelements, polyphenols, and flavonoids contents were higher. The populations showed high variability, with significant interactions with crop location in bioactive compounds, antioxidant activity and Ca, Cu, Na, Mn and S contents.

**Keywords:** Bioactive compounds, spectrophotometry, optical emission spectrometry (OES), interactions genotype-environment, communitarian food systems.

## 1. Introduction

Maize (*Zea mays* L.) is one of the most important crops in the world, and its grain provides carbohydrates, protein, lipids, vitamins, fiber, minerals, and a high diversity of bioactive compounds with high antioxidant activity. Pigmented grains are considered functional foods. However, genetic factors inherent to grain and environmental and genetic-environmental interactions affect the variation in quantity and quality of its nutritional-nutraceutical constituents (Palacios-Rojas et al., 2020). Pigmented grain maize provides secondary metabolites of high nutraceutical value, such as carotenoids, anthocyanins and phenolic

compound complexes (Domínguez-Hernández et al., 2022). Experimentally, the extracts of products or subproducts of pigmented grains have shown antimutagenic and anticarcinogenic activity (Loarca-Piña et al., 2019; Herrera-Soto et al., 2020) and have shown potential to counteract the increase in chronic diseases related to diet (e.g., diabetes, cancer, and degenerative diseases).

The greatest genetic diversity of the species is concentrated in the centers of the origin and diversification of maize, and this specie continues to evolve under domestication, commonly classified phenotypically in native populations, landraces, or races. These landraces and races are highly heterogeneous and phenotypically share common morpho-agronomic biochemical characteristics and are adapted to certain geographic regions (Vielle-Calzada and Padilla, 2009; Newton et al., 2010). The biochemical composition of the grain is the product of selection by farmers in their cultivation plots and storage places (Hoogendoorn et al., 2018) to satisfy their family nutritional needs and of adaptation to agroecological crop conditions and is a research hotspot for the landraces of blue, red, yellow, purple and variegated grains as sources of secondary metabolites, minerals, protein and starch and their interaction with abiotic factors and crop conditions (Domínguez-Hernández et al., 2022).

Phenolic compounds are biosynthesized in all plants and are subject to different regulatory mechanisms, both genetic and biotic and abiotic interactions where plants develop (Cheynier et al., 2013). The main phenolic compounds in maize kernels are simple phenols and polyphenols, phenolic acids, flavonoids, coumarins, stilbenes, carotenes, anthocyanins, lignans and lignins, tocopherols and others, and their concentrations vary among populations, landraces, races and varieties. Their composition is affected both by crop agroecology and during postharvest processing (Salinas-Moreno et al., 2017; Gálvez-Ranilla, 2020). Yellow grain maize, with a high content of phenolic compounds, including carotenoids and anthocyanins, has been associated with greater antioxidant activity and nutritional-nutraceutical potential in the prevention of diseases associated with food (Žilić et al., 2012; Bae et al., 2021).

Feil et al. (2005) indicated that the mineral composition of corn grains is affected by ecological-environmental factors such as pre-anthesis drought and the rate of assimilation of nitrogen added through fertilization. In this regard, Seebauer et al. (2010) indicated that grain

composition is a product of the source-demand relationships after anthesis and that during grain maturation, the phenolic compound content, starch composition and antioxidant activity are affected (Borrás et al., 2002; Xu et al., 2010; Martínez et al., 2019). In addition to environmental effects, a complex of genes and genetic-environmental interactions also regulate phenolic compound biosynthesis and mineral concentration (Chakraborti et al., 2011; Zhang et al., 2020). The proposed objective of this study was to evaluate the variation in total polyphenol and flavonoid content, antioxidant activity and mineral concentration in the grain of a population collection of yellow maize landraces from southeastern Mexico.

## **2. Materials and Methods**

### *2.1 Sampling of yellow maize landraces and crop locations*

During the first months of 2016, maize with yellow grain was collected from farmers in Oaxaca, Mexico (16° 30' 37" at 17° 59' 59" W latitude, 95° 58' 30" at 98° 19' 69" N longitude; from 700 to 2087 masl), generating a collection of 30 population samples plus two experimental varieties as controls: YTB (yellow grain) and BSBA-4032 (blue grain). The collection was planted in San Agustín Amatengo and Santa María Coyotepec, Oaxaca, under a random block design; both locations have a semidry to semiwarm climate, an average temperature of 20 °C, average rainfall of 526.5 to 693.8 mm, a soil pH of 7.8. to 8.3 and excellent organic matter availability. San Agustín is located at 1361 masl and Santa María at 1518 masl. Fertilization (120N-100P-60K) and management were constant for both evaluation sites, and both sites had full exposure to rainy conditions (rainfall).

### *2.2 Sample preparation and evaluation of total polyphenols, flavonoids, and antioxidant activity*

*Sample preparation.* At harvest, a random sample of ten healthy ears per maize population was taken at each experimental location. The ears sampled were manually threshed to generate a sample of approximately 600 g. Later, a subsample of 100 g grain per population was ground and crushed (Apex Construction®, LTD and Krups®, Mexico), and the final flour was sieved through a 500-µm mesh and stored in amber vials at -20 °C until analysis. A sample of flour

(3 g) was extracted with 80% methanol, and the total polyphenol and flavonoid contents and antioxidant activity were determined.

*Total polyphenols.* The total polyphenol contents were determined using the method described by Singleton and Rossi (1965); deionized water and Folin-Ciocalteu reagent were added to 400  $\mu\text{L}$  of the diluted extract and left to rest for 5 minutes. Subsequently, 7%  $\text{Na}_2\text{CO}_3$  was added, and the sample was incubated for 1 h at room temperature ( $23 \pm 3$  °C). Absorbance readings were conducted in triplicate in a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) using distilled water as the blank. The total polyphenol content was estimated as milligrams of gallic acid equivalents per 100 g in dry weight ( $\text{mg GAE } 100 \text{ g}^{-1} \text{ dw}$ ) using a gallic acid calibration curve with concentrations ranging from 0.02 to 0.125  $\text{mg mL}^{-1}$ .

*Flavonoid content.* The flavonoid content was determined using the method described by Zhishen et al. (1999). First, 75  $\mu\text{L}$  of  $\text{NaNO}_2$  was added to 400  $\mu\text{L}$  of the methanolic extract and left to rest for 5 minutes; then,  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  at 10% plus 1 M NaOH and deionized water were added. The absorbance was read in triplicate at 510 nm. The flavonoid concentrations were calculated as milligrams of catechin equivalents per gram of dry sample ( $\text{mg CE g}^{-1} \text{ dw}$ ) using a (+)(-)-catechin calibration curve with concentrations ranging from 0.0122 to 0.122  $\text{mg mL}^{-1}$ .

*Determination of antioxidant activity by DPPH and FRAP.* Antioxidant activity was analyzed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method described by Brand-Williams et al. (1995). DPPH radical was added to 100  $\mu\text{L}$  of the extract. The solution was vortexed and allowed to rest for 30 minutes in darkness. Subsequently, readings were performed at 517 nm using a spectrophotometer and 80% methanol as a reference. Antioxidant activity was recorded using a Trolox calibration curve (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) with concentrations ranging from 0.13 to 0.79  $\mu\text{mol mL}^{-1}$  and expressed in  $\mu\text{mol TE g}^{-1} \text{ dw}$ . Antioxidant activity was determined using the FRAP method described by Benzie and Strain (1996). A total of 3 mL of FRAP reagent (sodium acetate buffer pH 3.6, 10 mM TPTZ, and 10 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) was added to 100  $\mu\text{L}$  of the extract. This solution was incubated for 30 minutes at 37 °C, and its absorbance was recorded at 593 nm in a spectrophotometer. The quantification of antioxidant activity was performed using a Trolox calibration curve with concentrations ranging from 100 to 1000  $\mu\text{mol L}^{-1}$ , and

the results were expressed as  $\mu\text{mol}$  equivalent of Trolox per gram of dry weight ( $\mu\text{mol TE g}^{-1}$  dw).

### *2.3 Determination of mineral contents*

Two grams of corn flour was dried in an oven at  $105\text{ }^{\circ}\text{C}$  (Barnstead/Thermolyne Oven series 9000, USA) (AACC 44-15). Then, ash was obtained using a muffle furnace at  $570\text{ }^{\circ}\text{C}$  (Barnstead/Thermolyne. 1400, USA) (AACC 08-01.01), (AACC 1976). A total of 4 mL of HCl (JT Baker®) was added to the ash, which was further dissolved with 50 mL of deionized water. Finally, the solution was filtered and stored under refrigeration until analysis. The quantification of micro- and macronutrients (Fe, Zn, Mn, P, Ca, Mg, K, Na and S) was performed by inductively coupled plasma–optical emission spectrometry (ICP–OES Thermo Scientific iCAP 6500 DUO, United Kingdom) using the methodology proposed by Martínez-Martínez et al. (2019). The quantification was based on multielement standards (High Purity® Standards, USA). All tests were performed in triplicate, and the results were expressed in mg of the element  $100\text{ g}^{-1}$  dry weight ( $\text{mg } 100\text{ g}^{-1}$  dw).

### *2.4 Statistical analysis*

A database was compiled to evaluate the differences among populations, crop locations and the location-population interaction (genotype-environment) using analysis of combined variance and a linear model with a randomized complete block design for which blocks, or repetitions were nested in locations. In addition, multiple comparisons of means were performed by Tukey's test ( $p \leq 0.05$ ), and only significant comparisons of the location-population interaction were visualized in scatter plots (SAS Institute, 2002).

## **3. Results and Discussion**

In the analysis of variance, significant differences ( $p \leq 0.05, 0.01$ ) were identified between evaluation locations (environments), between populations (genotypes) and in the location-population interaction (genotype-environment) for all the variables evaluated, with the exceptions of antioxidant activity, as evaluated by the FRAP method, between locations, Mg content between populations and P, Mg, K, Fe and Zn concentrations for the locality-population interaction. The variance or mean square of polyphenols was higher in localities than in

populations and in the locality-population interaction, a pattern that was repeated for antioxidant activity, as evaluated by DPPH, and mineral content, indicating that the effect of the environment is significant for these compounds. For flavonoids, the variance was greatest for the locality-population interaction, followed by the variance among populations and finally between localities (Table 1).

**Table 1.** Significance of square means in the analysis of variance in the total polyphenol, flavonoid and mineral contents and antioxidant activity in grains of yellow maize landraces.

Evaluated compounds	Sources of variation						CV (%)
	Locations (L)	Populations (P)	L x P	Rep./L	Lab. Replicates/R	Error	
Total polyphenols	2084.90**	709.40**	373.70**	316.1**	1.20*	26.50	6.6
Flavonoids	9.76**	15.04**	16.58**	1.36*	0.09 <sup>ns</sup>	0.44	19.2
Antiox. act. by DPPH	13.06**	3.51**	2.00**	0.21 <sup>ns</sup>	0.03 <sup>ns</sup>	0.11	8.8
Antiox. act. by FRAP	<0.01 <sup>ns</sup>	1.37**	0.95**	0.61**	<0.01 <sup>ns</sup>	0.10	8.2
Ca	54.92**	1.51**	1.35**	0.69 <sup>ns</sup>	-	0.59	25.3
P	697969**	3789*	2874 <sup>ns</sup>	4914*	-	2225.7	13.6
Mg	26027**	360 <sup>ns</sup>	316 <sup>ns</sup>	528 <sup>ns</sup>	-	252.2	13.3
K	307623**	5111**	2225 <sup>ns</sup>	1419 <sup>ns</sup>	-	2181.9	12.9
Na	25.35**	0.12**	0.29**	0.10 <sup>ns</sup>	-	0.068	7.2
S	9.45**	0.62**	0.51**	0.16 <sup>ns</sup>	-	0.11	15.5
Cu	0.066**	0.01**	0.008**	<0.01 <sup>ns</sup>	-	<0.01	23.8
Fe	1.737**	0.071**	0.044 <sup>ns</sup>	0.081*	-	0.035	10.5
Mn	0.345**	0.016**	0.010*	0.013*	-	<0.01	14.6
Zn	18.12**	0.59*	0.44 <sup>ns</sup>	0.73 <sup>ns</sup>	-	0.38	18.8

<sup>ns</sup>Not significant ( $p > 0.05$ ) \* Significant at  $p \leq 0.05$ , \*\* Significant at  $p \leq 0.01$ ; CV = coefficient of variation.

The landraces of yellow maize were strongly influenced in grain composition by crop location, but the effect of the environment was different based on the type of compound. For total polyphenols, flavonoids and microelements such as Cu, Mn and Fe, higher values were recorded in San Agustín Amatengo, but in Santa María Coyotepec, greater antioxidant activity, as determined by the DPPH method, and macronutrient contents, such as Ca, P, Mg, K and Na, were observed. It means that crop locations have significant effects on grain composition, as supported by Nankar et al. (2016), who studied amino acid, protein, anthocyanins, starch and ash content. Menkir (2008) and Gu et al. (2015) also reported that mineral content is affected not only by the location but also by the year of cultivation; that is, the composition of maize grain changes or is influenced by the location and year of cultivation, two conditions that refer to the effect of the environment on composition.

**Table 2.** Average total polyphenol, flavonoid and mineral contents and antioxidant activity of yellow maize landraces cultivated in two locations of Oaxaca, Mexico.

Compound evaluated	Crop locations in Oaxaca, Mexico	
	San Agustín Amatengo	Santa Maria Coyotepec
Total polyphenols (mg GAE 100 g <sup>-1</sup> )	79.1 ± 7.80 a <sup>1</sup>	75.6 ± 8.85 b
Flavonoids (mg EC g <sup>-1</sup> )	0.036 ± 0.01 a	0.033 ± 0.01 b
Antioxidant act. by DPPH (μmol TE g <sup>-1</sup> )	3.6 ± 0.60 b	3.9 ± 0.54 a
Antioxidant act. by FRAP (μmol TE g <sup>-1</sup> )	3.9 ± 0.39 a	3.9 ± 0.50 a
<i>Mineral content (mg 100 g<sup>-1</sup>)</i>		
Ca	7.5 ± 4.6 b	13.0 ± 4.1 a
P	295.2 ± 51.5 b	400.2 ± 49.7 a
Mg	108.8 ± 17.6 b	129.0 ± 15.8 a
K	327.8 ± 47.3 b	397.3 ± 53.3 a
Na	1.04 ± 1.5 b	5.77 ± 3.1 a
S	3.79 ± 2.6 a	2.01 ± 1.9 b
Cu	0.24 ± 0.1 a	0.20 ± 0.1 b
Mn	0.56 ± 0.1 a	0.48 ± 0.1 b
Fe	1.54 ± 0.9 a	0.93 ± 0.8 b
Zn	2.99 ± 0.5 b	3.52 ± 0.8 a

<sup>1</sup>In the rows, means with the same letter are not significantly different (Tukey's test,  $p \leq 0.05$ ).

The variation in total polyphenol content among populations collected from yellow corn ranged from 68.2 to 88.9 mg GAE 100 g<sup>-1</sup> (Table 3), values that are within the range reported by Mora-Rochin et al. (2010) and Loarca-Piña et al. (2019) for white, yellow, red and blue corn from Mexico and within the values reported by Syedd-León et al. (2020) for white, yellow and red grain from Costa Rica; although the upper range in the reference reports is greater than 140 mg GAE 100 g<sup>-1</sup>, in all cases, the values were lower than the values reported by Bae et al. (2021) and suggest differences in laboratory methodology, i.e., not only in the evaluated genotypes. The references also suggest that there are no differential patterns in total polyphenols between populations with similar or different grain color because there are populations with high and low total polyphenol content between and within each color group. Loarca-Piña et al. (2019) estimated a variation in flavonoid content of <0.001 to 0.12 mg EC g<sup>-1</sup> in blue and red grain from Querétaro, Mexico, and in this study, the estimated variation ranged from 0.016 to 0.053 mg EC g<sup>-1</sup> (Table 3). However, both estimates differ from the higher values recorded by Bae et al. (2021), i.e., 0.074 to 0.591 mg EC g<sup>-1</sup> in yellow maize from Asia, and in a collection of pigmented maize (0.248 to 0.337 mg EC g<sup>-1</sup>) evaluated by Žilić et al. (2012). That is, in this work, the variation observed between landraces of Oaxaca, Mexico, was lower than that estimated by other authors despite possible differences in specific laboratory methods. In this

sense, Zhang et al. (2020) indicated that phenolic compound content decreases as grain maturity advances or as grains lose moisture.

**Table 3.** Variation in total polyphenol, flavonoid, and mineral content and antioxidant activity in the kernel of yellow maize landraces.

Pop. ID	Poly. <sup>1</sup>	Flav. <sup>2</sup>	Antiox. Activ. <sup>3</sup>		Macroelements (mg 100 g <sup>-1</sup> )					Microelements (mg 100 g <sup>-1</sup> )				
			DPPH	FRAP	Ca	P	Mg	K	Na	S	Cu	Fe	Mn	Zn
Y05	76.3	<u>0.020</u>	3.73	3.93	9.6	325.3	110.7	332.2	3.93	1.78	0.24	0.97	0.46	2.89
Y06	69.9	0.032	3.80	3.83	12.2	369.8	127.6	354.3	3.69	1.09	0.24	1.28	0.54	3.28
Y07	71.2	0.016	3.68	3.66	12.7	324.0	108.0	320.1	5.30	1.68	0.20	0.86	0.46	2.77
Y08	69.5	0.027	3.98	3.91	13.4	370.1	126.2	354.7	2.82	1.65	0.23	1.33	0.57	3.35
Y10	71.6	0.036	3.59	3.70	10.0	365.0	124.7	369.6	2.88	2.09	0.22	1.40	0.58	3.29
Y21	74.4	0.043	3.31	3.68	13.7	373.8	<b>130.9</b>	364.3	4.15	3.02	0.23	1.94	<b>0.60</b>	3.39
Y22	72.8	0.025	3.84	3.84	9.1	354.7	117.5	352.7	3.31	1.55	0.25	1.72	0.59	3.17
Y23	78.8	0.033	3.81	3.95	10.4	332.2	111.8	369.5	4.02	4.29	0.23	<b>2.12</b>	0.49	3.60
Y24	78.1	0.044	4.20	4.16	7.2	327.5	112.4	341.8	2.63	1.85	0.19	1.91	0.49	3.31
Y26	82.0	0.034	4.51	4.10	8.2	354.6	119.6	372.1	2.87	1.77	0.24	1.20	0.53	3.34
Y27	77.0	0.031	4.20	3.67	9.5	<u>291.7</u>	<u>100.0</u>	335.7	2.75	4.49	0.24	0.83	0.48	<u>2.76</u>
Y29	74.4	0.029	3.91	3.78	10.8	344.8	119.0	353.6	3.25	2.35	0.23	1.05	0.57	3.68
Y30	77.7	0.033	3.97	3.73	13.0	340.2	116.7	373.3	4.30	4.19	0.22	0.98	0.51	3.28
Y35	84.0	0.032	3.57	4.04	12.1	352.4	117.9	410.9	4.46	4.28	0.23	1.08	0.50	3.06
Y37	81.1	0.039	3.65	4.00	10.3	320.3	110.6	343.6	3.63	2.10	0.20	0.99	0.46	3.08
Y40	78.9	0.031	<u>3.17</u>	3.69	10.0	358.4	120.6	397.3	<b>6.21</b>	<b>5.67</b>	0.32	1.66	0.55	<b>3.83</b>
Y41	81.5	0.036	3.23	3.97	<u>5.6</u>	337.0	120.2	386.7	3.15	5.46	0.26	1.71	0.52	3.37
Y42	73.9	0.041	3.27	3.75	<b>14.5</b>	362.2	124.1	373.8	3.78	1.88	0.19	1.20	0.55	3.40
Y45	75.8	0.035	<u>3.17</u>	<u>3.52</u>	11.4	360.7	123.7	379.7	3.02	<u>1.05</u>	0.20	<u>0.70</u>	0.53	3.29
Y49	74.2	0.046	3.33	3.81	8.9	363.6	122.9	365.2	3.52	1.46	0.23	1.20	0.51	3.11
Y50	72.3	0.045	3.35	3.64	12.7	323.5	113.3	336.0	4.20	2.15	0.23	0.95	0.52	3.07
Y51	70.0	0.028	3.44	3.82	8.0	336.6	114.5	334.2	2.30	1.71	0.22	1.14	0.52	3.06
Y52	<u>68.2</u>	0.032	3.51	3.68	10.7	362.2	121.6	361.6	3.04	2.25	0.21	1.14	0.55	3.33
Y53	81.4	0.045	3.90	4.21	7.8	338.1	117.0	375.9	3.74	5.48	0.23	0.76	0.45	3.09
Y55	73.5	0.041	3.39	4.01	9.1	338.2	120.7	<u>327.2</u>	4.20	2.40	0.21	1.00	0.49	2.79
Y58	84.4	<b>0.053</b>	4.03	4.31	10.4	387.2	127.6	<b>425.6</b>	4.01	3.92	0.31	1.81	0.59	3.45
Y59	85.8	0.032	4.10	4.20	11.1	363.1	126.3	391.3	2.92	3.89	<b>0.32</b>	0.82	0.57	3.57
Y60	79.5	0.029	4.02	4.16	8.8	356.8	119.2	366.6	1.84	2.58	<u>0.10</u>	0.76	0.57	2.90
Y62	86.7	0.032	4.44	4.20	9.4	<b>391.5</b>	129.7	401.5	<u>1.42</u>	2.35	0.16	0.96	0.52	3.32
Y70	80.0	0.035	3.78	3.92	9.0	340.7	116.5	343.1	2.05	4.04	0.19	1.26	0.54	3.54
YTB	82.2	0.033	3.92	4.35	7.8	314.0	117.2	332.8	2.43	4.49	0.22	1.45	<u>0.43</u>	3.15
BSB	<b>88.9</b>	0.043	<b>4.58</b>	<b>4.51</b>	10.1	341.7	117.9	359.5	2.72	4.17	0.16	1.37	0.48	3.68
DMS <sup>1</sup>	<u>5.68</u>	<u>0.007</u>	<u>0.36</u>	<u>0.36</u>	<u>7.5</u>	<u>91.26</u>	<i>ns</i>	<u>90.35</u>	<u>3.87</u>	<u>3.07</u>	<u>0.10</u>	<u>0.36</u>	<u>0.15</u>	<u>1.19</u>

<sup>1</sup> Total polyphenols (mg GAE 100 g<sup>-1</sup>); <sup>2</sup> Flavonoids (mg EC g<sup>-1</sup>); <sup>3</sup> μmol ET g<sup>-1</sup>, difference minimal significant (Tukey's test  $p \leq 0.05$ ); ns = not significantly different.

Regarding antioxidant activity, as determined by the DPPH and FRAP methods, similar interpopulation variation was observed, from 3.17 to 4.58 μmol ET g<sup>-1</sup> and from 3.52 to 4.51 μmol ET g<sup>-1</sup>, respectively (Table 3), activity levels that were significantly different from those recorded by Bae et al. (2021) using the DPPH method (104.1 to 313.4 μmol ET g<sup>-1</sup>) and by



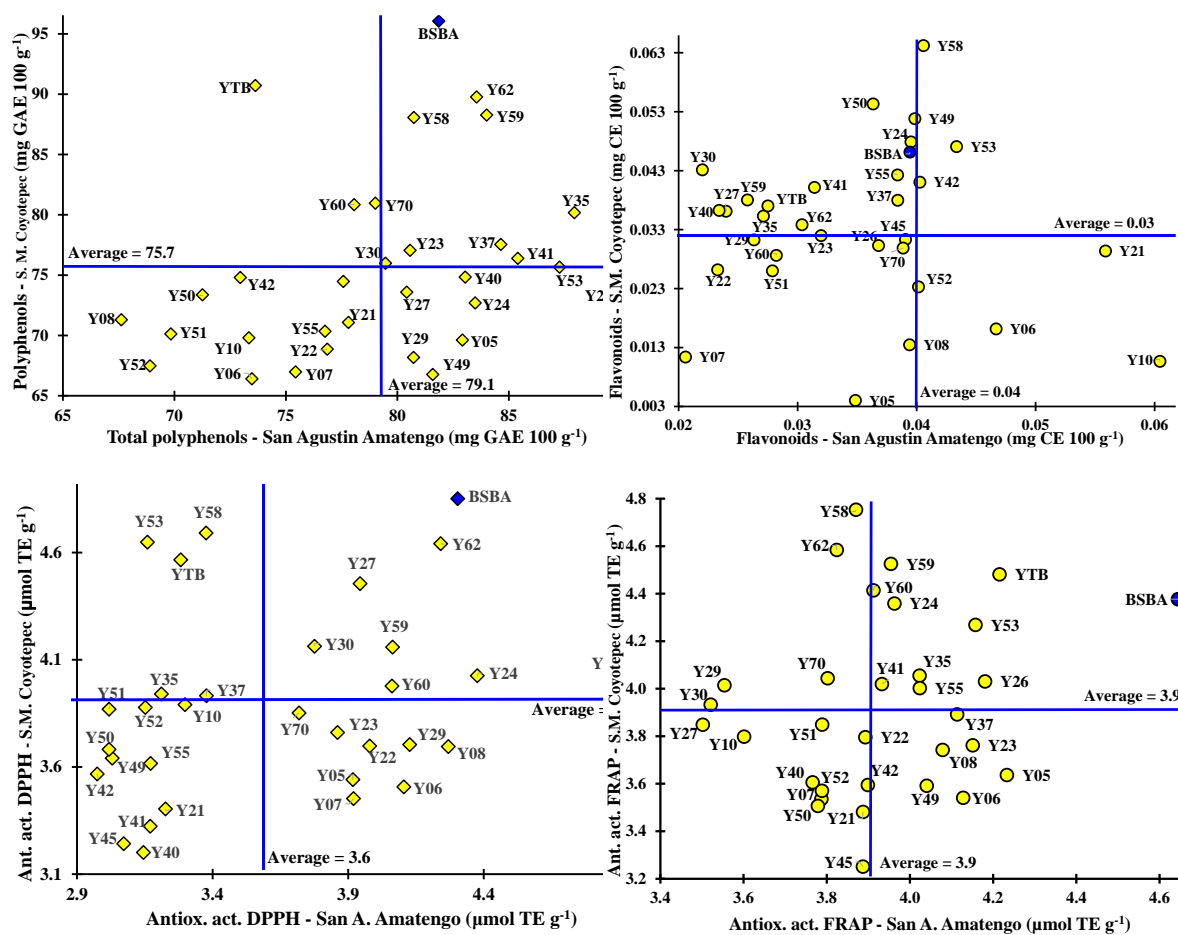
Zhang et al. (2020) using the FRAP method (12.5 to 15.0  $\mu\text{mol ET g}^{-1}$ ). However, the differences in the laboratory methods and antioxidant activity values are indicators of the reducing capacity of free radicals in the sample under study due to the amount of bioactive compounds with these reducing functions. In addition, Zhang et al. (2020) noted that this reducing capacity does not necessarily decrease as the grain matures.

In terms of mineral macroelements, the variation between populations of yellow corn ranged from 5.6 to 14.5 mg 100 g<sup>-1</sup>, from 291.7 to 391.5 mg 100 g<sup>-1</sup>, from 100 to 130.9 mg 100 g<sup>-1</sup>, from 327.2 to 425.6 mg 100 g<sup>-1</sup>, from 1.42 to 6.21 mg 100 g<sup>-1</sup> and from 1.05 to 5.67 mg 100 g<sup>-1</sup> for Ca, P, Mg, K, Na and S, respectively (Table 3). This variation is significantly higher than the reference values reported by Gu et al. (2015) and by Feil et al. (2005), except for Ca for the latter case; in all cases, the same PCI-OES methodology was used. However, the results are consistent with the values reported by Menkir et al. (2008), who used the same methodology in advanced lines of tropical maize. Together, these findings indicate that comparisons between results for maize with different genotypes and results obtained using different laboratory methods are challenging; however, such results contribute with successive approximations to evaluate the genetic and phenotypic diversity of landraces, advanced lines, or cultivated varieties of maize, which may be feasible to apply some genotypic selection methodology.

Regarding mineral microelements, Cu, Fe, Mn and Zn contents varied from 0.1 to 0.32 mg 100 g<sup>-1</sup>, from 0.7 to 2.12 mg 100 g<sup>-1</sup>, from 0.43 to 0.60 mg 100 g<sup>-1</sup> and from 2.76 to 3.83 mg 100 g<sup>-1</sup>, respectively (Table 3). Zn and Fe play essential roles against anemia in vulnerable populations. The values recorded here for Zn were slightly higher than those recorded among the varieties evaluated (2.34 to 2.65 mg 100 g<sup>-1</sup>) by Feil et al. (2005) and those estimated (1.64 to 2.46 mg 100 g<sup>-1</sup>) by Oikeh et al. (2003) but are consistent with the estimated values reported by Demeke (2018) and Menkir (2008) and indicate a certain genetic potential in the evaluated germplasm. However, when the Fe content was evaluated, the pattern was similar and, in some cases, slightly lower than that reported in the studies referred to.

The interaction between populations and evaluation locations was not significant for all the evaluated composition parameters. For example, the Zn, Fe, K, Mg and P interaction was not significant, indicating independence between the effect of localities and germplasm or the native varieties evaluated (Table 1). The polyphenol and flavonoid contents and antioxidant

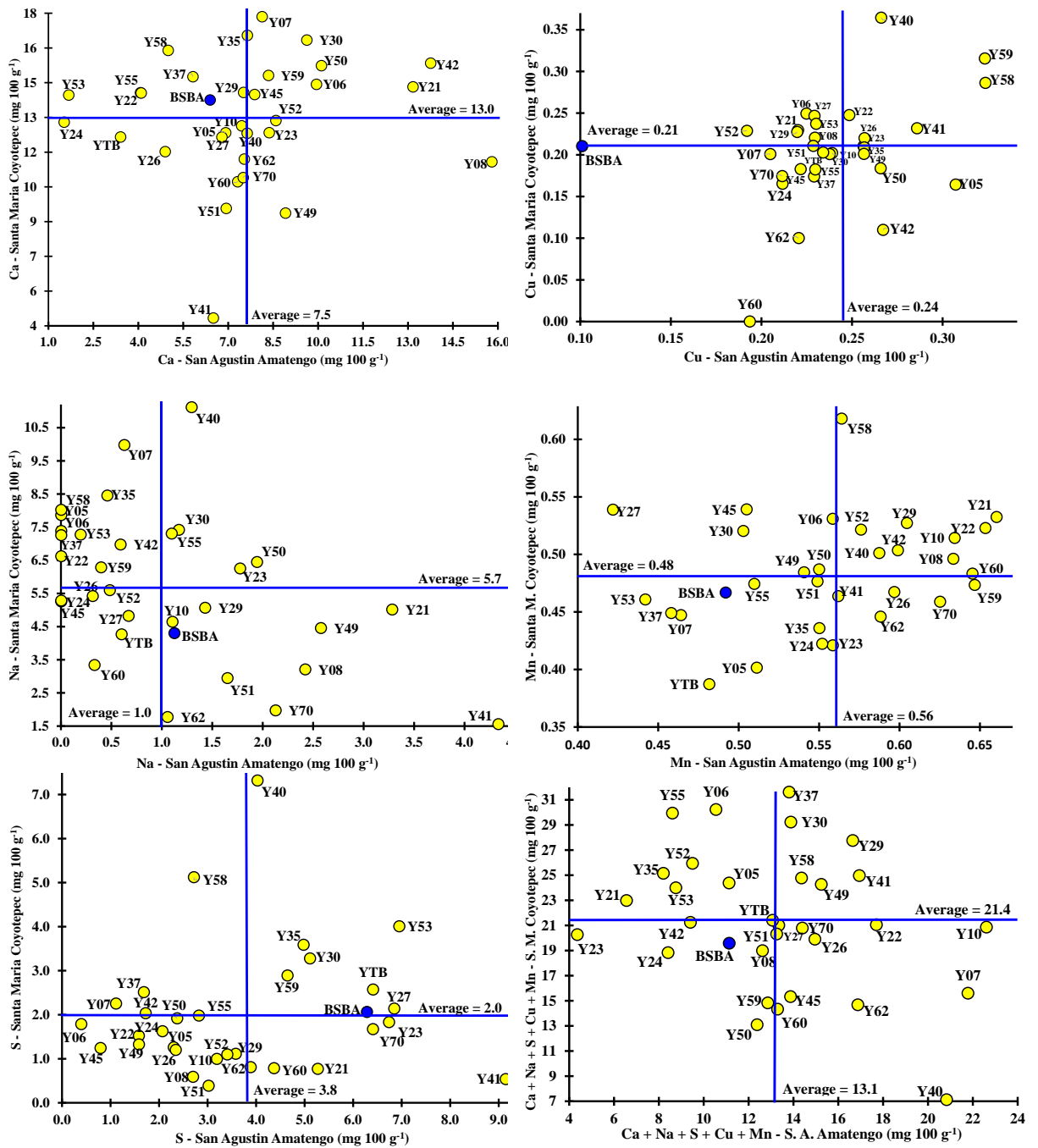
activity were affected by the location-population interaction, and at least four response patterns were differentiated as a function of the average by locality (Figure 1), among which three are of special interest to farmers and consumers. If interested in the response in both environments, the quadrant of interest is the upper right quadrant in each scatter plot (Figure 1), but if the interest is the response in one of the environments, the upper left or lower right quadrant would be of greatest interest. For example, the most stable populations with regard to polyphenols were Y62, Y59, Y58, Y37, Y35, Y41 and Y23 of yellow corn and BSBA of blue corn, those with regard to flavonoids were Y58, Y53 and Y42, and those with regard to antioxidant activity, as determined by the DPPH and FRAP methods, were Y60, Y59, Y26 and Y24. These findings indicate that it is possible to select stable materials, based on the bioactive compound content and antioxidant activity, among the evaluated populations of yellow corn.



**Figure 1.** Scatter plots for crop locations and populations with regard to total polyphenols, flavonoids, and antioxidant activity in yellow maize landraces.

Menkir (2008), Chakraborti et al. (2011) and Gu et al. (2015) reported that maize genotypes interact significantly with the environment or agroecological conditions and years of cultivation with respect to the concentration of mineral microelements and macroelements in grain, either inbred lines or genotypes with a heterogeneous genetic structure. In this study, there was a significant interaction effect between Ca, Na, S, Cu and Mn content and the environment for the populations studied (Table 1, Figure 2). For these mineral elements, populations in the upper right quadrant of each scatter plot (Figure 2) contain consistently high values for more than one mineral element; these populations include Y59, Y58, Y52, Y42, Y40, Y30, Y23, Y22 and Y21. For example, for Ca, Cu, Na, Mn and S, the most established populations had values greater than 7.5, 0.2, 1.0, 0.48 and 2.0 mg 100 g<sup>-1</sup>, respectively; these values are similar to those estimated by Menkir (2008) and Gu et al. (2015). Together, Y58, Y49, Y41, Y37, Y30 and Y29 stood out as the populations with the highest Ca, Cu, Na, Mn and S contents.

Borrás et al. (2002), Feil et al. (2005), Xu et al. (2010) and Zhang et al. (2020) reported that the composition of maize grains depends on source-demand relationships during post-flowering and during grain maturation, the agroecological conditions of cultivation (e.g., drought or soil fertility) and/or mineral fertilizers or management practices. That is, the genetic conditions of a population or variety reflect certain gene expression profiles to generate basic composition levels, but the effects of the cultivation environment and genetic-environmental interactions are significant. In this study, the genetic-population effects or population variations (genotypic variance, Table 1) were lower than the environmental effects of the location of cultivation for most of the parameters evaluated, but the results indicate the possibility of selecting outstanding populations or directly using these populations for feeding the families of farmers who preserve this diversity.



**Figure 2.** Scatter plots for crop locations and populations with regard to Ca, Na, S, Cu, and Mn in yellow maize landraces.

#### 4. Conclusions

In terms of grain composition, the evaluated population sample of yellow maize landraces in two agroecological farming locations showed different response patterns. First, the environmental effect (location) was significantly greater than the effects of population (genotypic) and the location-population interaction on the total polyphenol, Ca, P, Mg, K, Na, S, Cu, Fe, Mn and Zn content. For flavonoid content and antioxidant activity, as evaluated by FRAP, the effect of location and location-population interaction caused greater variation in the populations studied. In this work, the populations evaluated showed high variation in grain composition, and the average or combined effect was that, at one location, the macroelement content was higher and, at the other location, the microelement concentration was higher, combined with a higher polyphenol and flavonoid content. There was a significant interaction between cultivation location and bioactive compound content, antioxidant activity and Ca, Cu, Na, Mn, and S content in yellow corn conserved *in situ* by the indigenous communities of Oaxaca, Mexico.

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#### References

- AACC. (1976). *Approved methods of the AACC*. American Association of Cereal Chemists. St. Paul MN.
- Bae, H-H., Yi, G., Go, Y. S., Ha, J. Y., Choi, Y., Son, J-H., Shin, S., Jung T-W., & Lee, S. (2021). Measuring antioxidant activity in yellow corn (*Zea mays* L.) inbreds from three different geographic regions. *Appl. Biol. Chem.*, 64, 56. <https://doi.org/10.1186/s13765-021-00629-y>.
- Benzie, F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power. The FRAP assay. *Analyt. Biochem.*, 239, 70-76.
- Borrás, L., Curá, J. A., & Otegui, M. E. (2002). Maiz kernel composition and post-flowering source-sink ratio. *Crop. Sci.*, 42, 781-790.
- Brand-Williams, W., Cuvelier M., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.*, 28, 25-30.
- Chakraborti, M., Prasanna, B. M., Hossain, F., Mazumdar, S., Singh, A. M., Guleria, S., & Gupta, H. S. (2011). Identification of kernel iron-and zinc-rich maize inbreds and analysis of genetic diversity using microsatellite markers. *J. Plant Biochem. Biotechnol.*, 20, 224-233.

- Cheyrier, V., Comte, G., Davies, K. M., Latanzio, V., & Martens, S. (2013). Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiol. Biochem.*, 72, 1-20.
- Demeke, K. H. (2018). Nutritional quality evaluation of seven maize varieties grown in Ethiopia. *Biochem. Mol. Biol.*, 32, 45-48.
- Domínguez-Hernández, E., Gaytán-Martínez, M., Gutiérrez-Urbe, J. J., & Domínguez-Hernández, M. E. (2022). The nutraceutical value of maize (*Zea mays* L.) landraces and the determinants of its variability: a review. *J. Cereal Sci.*, 103, 103399. <https://doi.org/10.1016/j.jcs.2021.103399>.
- Feil, B., Moser, S. B., Jampatong, S., & Stamp, P. (2005). Mineral composition of the grain of tropical maize varieties as affected by pre-anthesis drought and rate of nitrogen fertilization. *Crop Sci.*, 45, 516-523.
- Gálvez-Ranilla, L. (2020). The application of metabolomics for the study of cereal corn (*Zea mays* L.). *Metabolites*, 10, 300. <https://doi.org/10.3390/metabo10080300>.
- Gu, R., Chen, F., Liu, B., Wang, X., Liu, J., Li, P., Pan, Q., Pace, J., Soomro, A-A., Lübberstedt, T., Mi, G., & Yuan, L. (2015). Comprehensive phenotypic analysis and quantitative trait locus identification for grain mineral concentration, content, and yield in maize (*Zea mays* L.). *Theor. Appl. Genet.*, 128, 1777-1789.
- Herrera-Sotero, M. Y., Cruz-Hernández, C. D., Oliart-Ros, R. M., Chávez-Servia, J. L., Guzmán-Gerónimo, R. I., González-Covarrubias, V., Cruz-Burgos, M., & Rodríguez-Dorantes, M. (2020). Anthocyanins of blue corn and tortilla arrest cell cycle and induce apoptosis on breast and prostate cancer cells. *Nutr. Cancer*, 72, 768-777.
- Hoogendoorn, J. C., Audet-Bélanger, G., Böber, C., Donnet, M. L., Lweya, K. B., Malik, R. K., & Gildemacher, P. R. (2018). Maize seed systems in different agro-ecosystems; what works and what does not work for smallholders farmers. *Food Security*, 10, 1089-1103.
- Loarca-Piña, G., Neri, M., Figueroa, J. D., Castaño-Tostado, E., Ramos-Gómez, M., Reynoso, R., & Mendoza, S. (2019). Chemical characterization, antioxidant and antimutagenic evaluations of pigmented corn. *J. Food Sci. Technol.*, 56, 3177-3184.
- Martínez, R. D., Cirilo, A. G., Cerrudo, A. A., Andrade, F. H., & Izquierdo, N. G. (2019). Discriminating post-silking environmental effects on starch composition in maize kernels. *J. Cereal Sci.*, 87, 150-156.
- Martínez-Martínez, R., Chávez-Servia, J. L., Vera-Guzmán, A. M., Aquino-Bolaños, E. N., Carrillo-Rodríguez, J. C., & Pérez-Herrera, A. (2019). Phenotypic variation in grain mineral compositions of pigmented maize conserved in indigenous communities of Mexico. *Maydica*, 64(1), eM2. <https://journals-crea.4science.it/index.php/maydica/article/view/1853/1199>.
- Menkir, A. (2008). Genetic variation for grain mineral content in tropical-adapted maize inbred lines. *Food Chem.*, 110, 454-464.
- Mora-Rochin, S., Gutiérrez-Urbe, J. A., Serna-Saldivar, S. O., Sánchez-Peña, P., Reyes-Moreno, C., & Milán-Carrillo, J. (2010). Phenolic content and antioxidant activity of tortillas produced from pigmented maize processed by conventional nixtamalization or extrusion cooking. *J. Cereal Sci.*, 52, 502-508.
- Nankar, A., Grant, L., Scott, P., & Pratt, R. C. (2016). Agronomic and kernel compositional traits of blue maize landraces from the Southwestern United States. *Crop Sci.*, 56, 2663-2674.
- Newton, A. C., Akar, T., Baresel, J. P., Bebeli, P. J., Bettencourt, E., Bladenopoulos, K. V., Czembor, J. H., Fasoula, D. A., Katsiotis, A., Koutis, K., Koutsika-Sotiriou, M., Kovacs, G., Larsson, L., Pinheiro de Carvalho, M. A. A., Rubiales, R., Rissell, J., dos Santos, T. M. M., & Vaz Patto, M. C. (2010). Cereal landraces for sustainable agriculture. A review. *Agron. Sustain. Dev.*, 30, 237-269.
- Oikeh, S. O., Menkir, A., Maziya-Dixo, B., Welch, R., & Glahn, R. P. (2003). Genotypic differences in

concentration and bioavailability of kernel-iron in tropical maize varieties grown under field conditions. *J. Plant Nutr.*, 26, 2307-2319.

Palacios-Rojas, N., McCulley, L., Kaeppler, M., Titcomb, T. J., Gunaratna, N. S., Lopez-Ridaura, S., & Tanumihardjo, S. A. (2020). Mining maize diversity and improving its nutritional aspects within agro-food systems. *Comp. Rev. Food Sci. Food Saf.*, 19, 1809-1834.

Salinas-Moreno, Y., García-Salinas, C., Ramírez-Díaz, J. L., & Alemán-de la Torre, I. (2017). Phenolic compounds in maize grains and its nixtamalized products. In: M. Soto-Hernández, M. Palma-Tenango and R. García-Mateos (eds.), *Phenolic compounds – natural sources, importance, and applications*. Rijeka, Croatia, InTech, pp: 215-232.

Seebauer, J. R., Singletary, G. W., Krumpelman, P. M., Ruffo, M. L., & Below, F. E. (2010). Relationship of source and sink in determining kernel composition of maize. *J. Exp. Bot.*, 61, 511-519.

Syed-León, R., Orozco, R., Álvarez, V., Carvajal, Y., & Rodríguez, G. (2020). Chemical and antioxidants characterization of native corn germplasm from two regions of Costa Rica: a conservation approach. *Int. J. Food Sci.*, 2020, Article ID 2439541. <https://doi.org/10.1155/202/2433541>.

System Analysis Statistical (SAS Institute) (2002) *SAS® Procedures Guide*, ver. 8. SAS Institute Inc. Cary, NC, USA.

Singleton, V. L. & J. A. Jr Rossi. (1965). Colorimetry of total phenolics with phosphor molybdic-phospho tungstic acid reagents. *Amer. J. Enol. Vitic.*, 16(3), 144-158.

Vielle-Calzada, J-P. & Padilla, J. (2009). The Mexican landraces: Description, classification, and diversity. In: J.L. Bennetzen and S.C. Hake (eds.), *Handbook of maize: Its biology*. New York, USA, Springer Science, pp: 543-561.

Xu, J-G., Hu, Q-P., Wang, Z-D., Luo, J-Y., Liu, Y., & Tian, C-R. (2010). Changes in the main nutrients, phytochemicals, and antioxidant activity in yellow corn grain during maturation. *J. Agric. Food Chem.*, 58, 5751-5756.

Zhang, S., Ji, J., Zhang, S., Xiao, W., Guan, C., Wang, G., & Wang, Y. (2020). Changes in the phenolic compound content and antioxidant activity in developmental maize-kernels and expression profiles of phenolic biosynthesis-related genes. *J. Cereal Sci.*, 96, 103113. <https://doi.org/101016/j.jcs.2020.103113>.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.*, 64, 555-559.

Žilić, S., Serpen, A., Akıllıoğlu, G., Gökmen, V., & Vančetović, J. (2012). Phenolic compounds, carotenoids, anthocyanins, and antioxidant capacity of colored maize (*Zea mays* L.) kernels. *J. Agric. Food Chem.*, 60: 1224-1231.