Emirates Journal of Food and Agriculture. 2016. 28(8): 581-588
doi: 10.9755/ejfa.2016-02-147
http://www.ejfa.me/

REGULAR ARTICLE

Anthocyanin, polyphenol, and flavonoid contents and antioxidant activity in Mexican common bean (*Phaseolus vulgaris* L.) landraces

Elia Nora Aquino-Bolaños[1], Yatzil Denih García-Díaz[1], José Luis Chavez-Servia[2]*, Jose Cruz Carrillo-Rodríguez[3], Araceli Minerva Vera-Guzmán[2], Elena Heredia-García[4]

[1]Instituto de Ciencias Básica-Universidad Veracruzana, Av. Rafael Sánchez Altamirano, Industrial Animas, 91192 Xalapa, Veracruz, México, [2]Instituto Politecnico Nacional, CIIDIR Unidad Oaxaca, Hornos No. 1003, Santa Cruz Xoxocotlan, 71230 Oaxaca, México, [3]Instituto Tecnológico del Valle de Oaxaca, Ex-Hacienda Nazareno, Santa Cruz Xoxocotlan, 71230 Oaxaca, México, [4]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias-Campo Experimental El Bajío, Carr. Celaya-San Miguel de Allende, 38010 Celaya, Guanajuato, México

ABSTRACT

The aim of this work is to assess and describe a collection of Mexican populations of common bean (*Phaseolus vulgaris* L.) based on their contents of monomeric anthocyanins, polyphenols, and flavonoids; antioxidant activity; and grain physical characteristics. A collection of 26 bean populations was assembled at five states and regions in Mexico. The beans were cultivated in Santa Cruz Xoxocotlan, Mexico, using a randomized complete block design with four replications. From a sample of seeds per population, the polyphenol and flavonoid contents were evaluated using UV-visible spectroscopy; the anthocyanin content was evaluated based on differential pH; antioxidant activity was measured using the DPPH method; and a morphologic description of the grain was recorded. Analysis of variance was used to determine significant differences ($P < 0.01$) within and among the population groups for all evaluated variables. Populations from Oaxaca and Puebla presented high anthocyanin contents, 1.6 and 2.1 mg C3GE/g, respectively. Polyphenol content was higher in the seed coat (27.7 to 127.0 mg GAE/g) than in the whole grain (1.3 to 5.4 mg GAE/g). Similar patterns were noted for flavonoids (from 5.9 to 21.5 mg CE/g in the seed coat and from 0.10 to 0.78 mg CE/g in the whole grain) and antioxidant activity (AA; from 132.5 to 1,021.7 μmol ETrolox/g in the seed coat). AA was significantly correlated with anthocyanin and polyphenol contents. Several populations were exceptional regarding the evaluated compounds; these populations were OAX-011-29, OAX-011-30, PUE-011-15, PUE-011-34, EM-01-01 and GRO-10-87. These beans can be used in participatory plant breeding to improve the local beans, and their consumption can be recommended to low income families with poor nutrition.

Keywords: Bean genepools; Phenotypic diversity; Spectrophotometry; Indigenous families; Nutritional value

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) has a high nutritional and nutraceutical value; the grain or seed contains high contents of protein, carbohydrates, vitamins, minerals, fiber, and secondary metabolites, such as polyphenols, flavonoids, anthocyanins, carotenoids, lectins and trypsin inhibitors, among others (Reynoso-Camacho et al., 2006; Golam-Masum-Akond et al., 2011; Suárez-Martínez et al., 2015). Bioavailable secondary metabolites in the grain promote a high antioxidant capacity; thus, the common bean can be considered a functional food (Golam-Masum-Akond et al., 2011; Rocha-Guzman et al., 2013). Bean nutritional value and consumption volume have been important since pre-Columbian times for rural communities in Mexico and Latin America (Singh et al., 1991; Romero-Arenas et al., 2013; Hernández-López et al., 2013).

Mexico is the origin of common beans (*Phaseolus vulgaris* L.), and hundreds of local varieties and genepools of wild populations exist (Singh et al., 1991; Bitocchi et al., 2012; Hernández-López et al., 2013). Bean is a grain legume of worldwide importance in terms of human consumption volume; nevertheless, the precise nutritional and nutraceutical value resulting from this genetic diversity preserved *in situ* are unknown by producers at different

***Corresponding author:**
José Luis Chavez-Servia, Instituto Politecnico Nacional, CIIDIR Unidad Oaxaca, Hornos No. 1003, Santa Cruz Xoxocotlan, 71230 Oaxaca, México. E-mail: jchavezs@ipn.mx

Received: 10 February 2016; **Revised:** 29 April 2016; **Accepted:** 30 April 2016; **Published Online:** 06 May 2016

agroecological production sites and by consumers, despite beans being part of their daily diet. For example, per capita consumption ranged from 0.06 (Argentina) to 20 kg (Nicaragua) during 2011 (Broughton et al., 2003; FAOSTAT, 2011; Beebe et al., 2013; Romero-Arenas et al., 2013); however, bean consumption has decreased considerably in the last decade. Thus, estimating the nutritional content of beans is relevant. Variety, climatological conditions, crop management, and storage conditions affect the nutritional quality of common beans (Santalla et al., 1999; Prolla et al., 2010; Blair et al., 2010); furthermore, common bean landraces and cultivated and wild species differ in their nutritional compound contents (Espinosa-Alonso et al., 2006).

Bean seed also contains non-nutritional components and toxic compounds, such as saponins, lectins, condensed tannins, lectins, trypsin inhibitors, and phytic acid, which interfere with protein digestibility and mineral bioavailability and are associated with other negative nutritional aspects. However, these phytochemicals provide additional health benefits, for example, protection against rotavirus and inhibition of carcinogenesis, and function as chemo-preventive agents (Katyal et al., 2001; de Mejía et al., 2003; Suárez-Martínez et al., 2015).

An increased understanding of the chemical composition of local bean varieties will help farmers and plant breeders to define varieties and genepools with greater nutritional-nutraceutical potential. This knowledge will also help to inform consumers and will enhance the *in situ* conservation of landraces on farms by increasing local, regional and national demand. In this context, the aim of this work was to evaluate and describe a collection of Mexican native common bean populations (*Phaseolus vulgaris* L.) in terms of their contents of monomeric anthocyanins, polyphenols, and flavonoids and antioxidant activity, as well as some physical traits of the seed.

MATERIALS AND METHODS

Germplasm, field experiments and sample preparation

Seed samples of 26 populations of common bean were collected in various rural communities in the states of Oaxaca (5), Guerrero (11), Puebla (6), Tlaxcala (1) and Estado de Mexico (3), Mexico; these populations were considered as origin groups. Seeds from each population were sown (July 9, 2014), and the resulting plants were cultivated following a randomized block design with four replications in the experimental fields of the Instituto Tecnologico del Valle de Oaxaca, Mexico (17º 02' N, 96º 44' W; 1,530 m in altitude; semidry to temperate climate, with 600 to 700 mm of annual precipitation and an average temperature of 18 to 20 °C) (Vidal-Zepeda, 2005). Samples of each population (200 to 500 g of grain per population) were obtained after harvesting and stored at room temperature until processed in the laboratory.

Sample preparation

After manual separation of the seed coat and grain, 3 g of each fraction was weighed. Extracts were prepared based on the method described by Xu et al. (2007). To each 25-mL fraction, a mixture of solvents (acetone/water/acetic acid (70:29.5:0.5, v/v/v)) was added; the mixture was then homogenized (Wisd Laboratory Instruments, Wertheim, Germany) and centrifuged at 4,000 rpm and 4 °C for 20 min (Hettich Zentrifuge, Universal 32R, Tuttlingen, Germany). The supernatant was separated, and the residue was extracted a second time under the same conditions. The supernatant was again separated, and the contents of phenolic compounds, flavonoid compounds, and monomeric anthocyanins were determined. Antioxidant activity was also measured.

Evaluation of polyphenol, anthocyanin, and flavonoid contents, antioxidant activity, and color

Total polyphenol content was quantified using the method described by Singleton and Rossi (1965): 400 µL of extract at an appropriate dilution was mixed with 200 µL of Folin Ciocalteau reagent; 1 mL of distilled water was added, and the reaction was allowed to proceed for 5 to 8 min. Afterward, 2 mL of sodium carbonate (7 g/100 mL) was added, and the solution was diluted to 5 mL with distilled water. After shaking for one hour, the absorbance was measured at 750 nm using a spectrophotometer (JENWAY, 6305, United Kingdom). A gallic acid standard curve (0.02-0.16 mg/mL) was used for the quantification of total polyphenols, and the results are reported as equivalents of gallic acid (GAE) per gram of sample (dry weight; dw).

Flavonoid analysis was carried out following the method of Zhishen et al. (1999). Then, 250 µL of extract at an appropriate dilution was mixed with 75 µL of 5% sodium nitrite ($NaNO_2$) and then shaken using a vortexer. The reaction was allowed to rest for 5 min, after which 150 µL of 10% aluminum chloride ($AlCl_3$) and 500 µL of 1 M NaOH were added; the mixture was then diluted to a final volume of 3 mL with distilled water. The absorbance was measured at 510 nm, and flavonoids were quantified based on a catechin (0.2-0.12 mg/mL) standard curve. The resulting values are expressed as mg equivalents of catechin per gram of sample (mg CE/g) based on dry weight.

Antioxidant activity was measured according to Brand-Williams et al. (1995). One-hundred microliters of extract was added to 2.9 mL of DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent, which was prepared with 3.9 mg/100 mL methanol (80% (v/v)). The mixture was

shaken manually and then incubated for 30 min at room temperature (25 °C). The absorbance was then measured at 517 nm. To quantify the antioxidant activity, a standard Trolox curve (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (0.10 to 0.80 µmol Eq Trolox/mL) was prepared. The results are reported as µmol Eq Trolox/g of sample (dry weight).

To determine the monomeric anthocyanin content, the differential pH method described by Wrolstad (1976) was used. Extract was diluted 1:5 with 0.025 M potassium chloride, pH 1.0, and 0.4 M sodium acetate, pH 4.5, as buffers. An absorption spectrum from 460 nm-710 nm (Shimadzu UV-VIS-1800, Kyoto, Japan) was recorded as a reference for quantifying the maximum absorption wavelength (λ *vis* - max). Monomeric anthocyanin content (AM) was calculated using the formula AM = (A×MW×DF×1000)/(ε × l), where sample absorbance $A=(A_{\lambda\ vis\text{-}max} - A_{700})_{pH1.0} - (A_{\lambda\ vis\text{-}max} - A_{700})_{pH4.5}$, MW is the molecular weight (449.2), ε is the cyanidin-3-glucoside molar absorptivity (26,900), DF is the dilution factor used, and 1 is the path length (1 cm). The results are reported as cyanidin-3-glucoside mg (C3G)/g of sample (dry weight).

Grain color was measured using a Minolta CM-2600d spectrophotometer (Konica Minolta Sensing Inc., Japan) with an illuminant D65 observer angle of 10°. The parameters L*, a* and b* were registered, and chromaticity ($C^*= (a^{*2} + b^{*2})^{1/2}$) and hue angle [$h° = \tan^{-1} (b^*/a^*)$] were calculated according to McGuire (1992).

Morphometry and specific weight of the seeds

In one sample per collection, average grain length, breadth and thickness were recorded using a digital vernier. The weight of 100 grains was quantified by weighing a random sample using an analytic scale (± 0.001 g), and volume was measured based on water displacement; the volume displaced by 100 grains was measured in a 100-mL volumetric test tube, using four replicates. Based on the weight and volume of water displacement of 100 grains, specific weight was calculated as weight/volume and is expressed as g/cm^3.

Statistical analysis

A database was created representing the common bean samples, including morphometric description; specific weight and color of seeds; monomeric anthocyanin, polyphenol and flavonoid contents; and antioxidant activity in the seed coat and whole seed. Later, a variance analysis was carried out to test differences among and within population groups based on a randomized complete block design; accessions were nested in origin groups, and different accessions were numbered by group, considering the analysis models with nested effects proposed by Quinn and Keough (2003). Later, multiple mean comparisons were carried out using the Tukey ($P < 0.05$) test. Pearson analysis was used to determine the correlation between antioxidant activity and polyphenol and anthocyanin contents.

RESULTS AND DISCUSSION

In the variance analysis, significant differences ($P < 0.05$) were determined among common bean origin groups and among populations in each group over all evaluated variables (Table 1). The results showed that phenotypical differences among populations of different geographical origin occur not only in biophysical characteristics and seed color but also in anthocyanin, polyphenol, and flavonoid contents and antioxidant activity. Additionally, within each group, differences were observed among the populations from Estado de Mexico, Guerrero, Oaxaca and Puebla, indicating that genepools, geographic isolation, and bean cultivation management are the reasons why the populations differ from one another.

The bean populations studied here were collected from agricultural fields located in the Mesoamerican region, one of the main origin and diversification centers of the common bean (Singh et al., 1991; Papa and Gepts, 2003; Bitocchi et al., 2012); this core genetic diversity was characterized after 2012. This partially explains the wide variability of seed forms observed in this study. Certain patterns were observed; for instance, populations from Estado de Mexico and Tlaxcala presented greater dimensions and seed densities than those from Puebla, Guerrero and Oaxaca. Nevertheless, within each origin group, seed size varied; for instance, in Guerrero, length varied between 10.2 and 14.9 cm; in Oaxaca, length varied between 10.1 and 13.2 cm; in Puebla, length varied between 9.6 and 14.5 cm; and in Estado de Mexico, length varied between 14.8 and 15.0 cm. Specific weight generally varied between 0.30 and 0.49 g/cm^3, indicating that very small grains and very large grains were present (Table 2). These values are consistent with those reported by Espinosa-Pérez et al. (2015) in a study of the classification of cultivated populations based on seed morphology; the authors state that populations growing in the south of Mexico have smaller seeds than those cultivated in the north.

Morphometric diversity in bean populations and in the specific weight of seeds (Tables 1 and 2) indicate that high genetic bean variability is preserved among farms in the Mesoamerican region (in the center and south of Mexico); this differentiation among populations is also expressed in different contents of anthocyanins and flavonoids and antioxidant activities (Tables 1 and 3).

The monomeric anthocyanin content in grains varied among populations, ranging from 0.04 to 9.07 mg

Table 1: Significance of square means based on an analysis of variance for the physical, chemical and morphometric traits of seeds from a collection of common beans

Composition of seed coat and whole seed								
Sources of variation	DF[a]	An[b]	Seed coat			Whole seeds		
			Po	Fl	AA	Po	Fl	AA
Groups	4	16.05*	6882.48**	94.71**	370872.0**	1.181**	0.066**	227.5**
Accessions/groups	21	13.14**	1858.25**	69.39**	140493.5**	3.248**	0.135**	114.6**
Error	78	<0.001	1.08	0.04	35.0	0.009	<0.01	0.4
C.V. (%)		2.5	1.4	1.7	0.9	4.2	3.9	4.7

Parameters of seed color				
Sources of variation	DF[a]	L*	Chrome index	h° index
Groups	4	105.9**	0.98**	1929.4**
Accessions/groups	21	203.2**	1.72**	698.3**
Error	78	25.4	0.20	57.1
C.V. (%)		9.4	15.8	14.7

Morphometric seed traits							
Sources of variation	DF[a]	Description of seeds (mm)			Weight and volume of 100 seeds		Specific weight (g/cm³)
		Length	Width	Thickness	Weight (g)	Volume (cm³)	
Groups	4	27.15**	1.51**	0.90**	439.5**	353.2**	0.027**
Accessions/groups	21	8.04**	1.33**	0.58**	116.2**	77.4**	0.010**
Error	78	0.59	0.12	0.06	1.7	0.93	<0.001
C.V. (%)		6.3	4.7	4.4	4.2	1.2	3.9

[a]DF: Degrees of freedom, where accessions were nested in groups of origin, [b]An: Monomeric anthocyanins, Po: Polyphenols, AA: Antioxidant activity, Fl: Flavonoids, L*: Lightness, h°: Hue index, C.V.: Coefficient of variation. *P<0.05, **P<0.01

Table 2: Morphometric description and specific weight of grains in a Mexican collection of common beans

Accession[a]	Length (mm)	Width (mm)	Thickness (mm)	100 grains weight (g)	Volume of 100 grains (mL)	Specific weight (g/cm³)
EDOMEX-011-11	15.0	7.1	5.5	41.5	85.8	0.48
EDOMEX-011-7	14.9	8.3	5.9	40.2	84.0	0.48
EM-01-01	14.8	7.6	5.3	41.6	85.5	0.49
Average-Mexico	**14.9ab**	**7.7a**	**5.5b**	**41.1a**	**85.1a**	**0.48a**
GRO-01-103	13.7	7.8	5.6	37.1	81.3	0.46
GRO-011-15	10.9	5.9	5.2	20.5	68.8	0.30
GRO-011-16	11.6	7.0	5.1	30.0	76.3	0.39
GRO-01-118	12.5	7.4	5.5	34.5	79.0	0.44
GRO-011-19	11.3	7.1	4.8	21.6	68.5	0.32
GRO-011-20	10.2	7.1	5.4	25.4	73.0	0.35
GRO-011-23	11.8	8.5	6.3	32.6	79.8	0.41
GRO-10-120	14.9	7.9	5.1	38.6	83.3	0.46
GRO-10-129	10.4	6.8	5.5	25.2	70.8	0.36
GRO-10-87	12.7	7.2	5.0	33.4	79.3	0.42
GRO-10-99	12.2	7.3	5.6	33.8	78.8	0.43
Average-Guerrero	**12.0bc**	**7.2c**	**5.4b**	**30.2c**	**76.2c**	**0.39bc**
OAX-011-07	11.1	7.7	5.6	30.6	75.0	0.41
OAX-011-12	10.1	6.7	5.5	22.9	68.8	0.33
OAX-011-28	13.2	7.3	4.9	30.8	74.8	0.41
OAX-011-29	10.4	6.6	5.7	24.6	71.8	0.34
OAX-011-30	12.3	7.3	5.5	28.6	74.3	0.38
Average-Oaxaca	**11.4c**	**7.1c**	**5.4b**	**27.5d**	**72.9e**	**0.38c**
PUE-011-13	12.5	6.9	5.5	31.1	75.3	0.41
PUE-011-14	11.3	7.4	6.0	32.4	77.3	0.42
PUE-011-15	9.6	6.9	5.5	23.9	69.3	0.35
PUE-011-20	14.5	8.0	5.0	40.3	82.8	0.49
PUE-011-34	12.0	6.7	5.0	26.8	72.0	0.37
PUE-11-33	10.5	6.9	4.9	26.1	73.3	0.36
Average-Puebla	**11.7c**	**7.1c**	**5.3b**	**30.1c**	**74.9d**	**0.40b**
TLA-10-5 (average)	**12.8b**	**8.1a**	**6.3a**	**38.9b**	**83.7b**	**0.46a**
DSH-Tukey	0.76	0.34	0.24	1.30	0.96	0.02

[a]Groups of origin: EDOMEX/EM: Estado de Mexico, GRO: Guerrero, OAX: Oaxaca, PUE: Puebla, TLA: Tlaxcala. [b]Among groups, means with the same letter are not significantly different (Tukey test, P<0.05)

Table 3: Average values of anthocyanin and polyphenol contents, antioxidant activity, and color index in a Mexican collection of common beans

Accession[a]	An[b]	Seed coat			Whole seed			Seed color		
		Po	Fl	AA	Po	Fl	AA	L*	Chrome	h°
EDOMEX-011-11[p]	0.04	82.2	20.9	564.5	2.5	0.70	13.8	59.5	8.0	46.2
EDOMEX-011-7[mc]	0.04	94.6	9.3	751.3	2.5	0.37	14.1	53.5	17.1	58.6
EM-01-01[pr]	1.13	89.5	12.3	667.9	2.7	0.40	11.8	54.4	10.2	50.7
Average-Mexico	**0.4c**[2]	**88.7c**	**14.2b**	**661.2c**	**2.6a**	**0.49a**	**13.2b**	**55.8b**	**11.7ab**	**51.8a**
GRO-01-103[pr]	0.41	67.9	12.8	621.7	2.1	0.33	12.0	51.6	9.4	33.7
GRO-011-15[c]	0.05	61.2	6.5	132.5	2.3	0.30	13.8	68.2	10.7	65.3
GRO-011-16[r]	0.25	55.7	16.8	610.1	2.6	0.78	13.5	50.5	15.5	51.7
GRO-01-118[pr]	0.25	92.5	12.9	724.4	1.3	0.33	10.4	36.9	5.1	21.5
GRO-011-19[pr]	0.34	51.0	12.8	512.9	2.7	0.77	11.0	56.7	8.9	47.0
GRO-011-20[r]	0.07	57.9	14.1	520.7	1.6	0.18	7.1	50.0	9.7	42.5
GRO-011-23[p]	0.22	51.2	19.6	639.4	1.3	0.63	16.6	59.7	9.5	40.8
GRO-10-120[p]	0.53	70.2	15.0	631.4	2.3	0.32	13.1	48.1	11.4	43.6
GRO-10-129[r]	0.42	80.9	8.5	735.9	2.5	0.48	10.9	53.4	7.6	35.2
GRO-10-87[r]	0.59	62.1	21.5	779.2	1.4	0.61	10.2	49.1	12.4	40.3
GRO-10-99[r]	0.24	52.2	11.6	459.7	2.3	0.42	9.1	60.7	6.3	36.4
Average-Guerrero	**0.3d**	**63.9d**	**13.8c**	**578.9d**	**2.0c**	**0.47b**	**11.6c**	**53.2b**	**9.7ab**	**41.6b**
OAX-011-07[pr]	0.38	87.9	15.7	750.8	2.7	0.56	8.3	47.8	11.6	44.9
OAX-011-12[y]	0.37	71.4	10.0	615.7	3.3	0.54	20.3	47.7	13.1	48.7
OAX-011-28[b]	2.14	57.0	5.9	534.7	1.9	0.38	10.5	51.7	5.6	67.7
OAX-011-29[mc]	1.54	108.2	7.3	1021.7	2.3	0.30	15.9	63.6	7.7	61.2
OAX-011-30[b]	3.47	127.0	11.0	973.8	1.9	0.35	12.5	49.1	4.8	81.3
Average-Oaxaca	**1.6b**	**90.3b**	**9.9e**	**779.3b**	**2.4b**	**0.43c**	**13.5b**	**52.0b**	**8.6b**	**60.7a**
PUE-011-13[p]	0.25	104.5	15.7	713.4	2.0	0.27	11.2	48.5	12.1	35.7
PUE-011-14[y]	0.04	39.2	8.9	389.4	1.3	0.10	7.4	62.1	25.4	72.1
PUE-011-15[cp]	9.07	27.7	7.9	321.6	5.4	0.64	32.4	59.5	11.1	62.0
PUE-011-20[b]	1.94	31.3	8.0	240.4	1.3	0.28	23.0	45.5	4.8	79.4
PUE-011-34[mc]	1.32	80.2	11.1	728.0	1.5	0.32	25.3	56.3	9.2	64.1
PUE-11-33	0.05	70.6	9.8	603.6	2.7	0.49	15.9	53.7	10.5	41.4
Average-Puebla	**2.1a**	**58.9e**	**10.2d**	**499.4e**	**2.4b**	**0.35e**	**19.2a**	**54.3b**	**12.2a**	**59.1a**
TLA-10-5cr (average-Tlaxcala)	**0.2e**	**123.4a**	**14.8a**	**985.3a**	**2.6a**	**0.41d**	**13.4b**	**62.2a**	**9.8ab**	**60.6a**
DHS-Tukey	0.02	1.03	0.21	5.9	0.10	0.02	0.65	5.01	3.0	7.52

[a]Groups of origin: EDOMEX/EM: Estado de Mexico, GRO: Guerrero, OAX: Oaxaca, PUE: Puebla, TLA: Tlaxcala. [b]An: Anthocyanins (mg of cynidin-3-glucoside-C3G-/g DW), Po: Polyphenols (mg equivalents of gallic acid –GAE-/g DW), Fl: Flavonoids (mg equivalents of catechin -CE-/g DW), AA: Antioxidant activity (µmol ETrolox/g DW). [c]Among groups, means with the same letter are not significantly different (Tukey test, P<0.05). Visual color of grains: [p]: Pink, [mc]: Mixture of seed colors, [pr]: Pink-red, [cr]: Cream, [cp]: Cream-pink, [r]: Red, [b]: Black, [y]: Yellow

C3G/g (dry weight); among groups, samples from Puebla presented the highest average (2.1 mg C3G/g), followed by samples from Oaxaca (1.6 mg C3G/g) and Tlaxcala (0.2 mg C3G/g). Anthocyanin variation among the studied populations was slightly higher than those described by Gola-Masum-Akond et al. (2011), who studied 29 common bean genotypes of various colors: 0.05 to 0.45 mg C3G/g. Anthocyanin type was not determined in this study; however, it was evident that between groups, accessions of black, intense red, and multicolored (a mixture of grains of different colors) beans presented higher anthocyanin contents (> 1 mg C3G/g); such accessions included EM-01-01, OAX-011-28, OAX-011-29, OAX-011-30, PUE-011-15, PUE-011-20 and PUE-011-34. Tsuda et al. (1994) determined that the anthocyanins delphinidin-3-O-β-D-glucoside, petunidin-O-β-D-glucoside and malvidin-3-O-β-D-glucoside are mostly associated with black beans. In contrast, Xu et al. (2007) found that dephinidin-3-glucoside and petunidin-glucoside were the main compounds in black beans. In red beans, Choung et al. (2003) found that the most abundant anthocyanin was pelargonidin 3-glucoside. Dzomba et al. (2013) found higher anthocyanin contents in brown, spotted black and pinto beans (0.45-0.59 mg C3G/g). This finding indicates that the highest anthocyanin contents are associated with beans having dark seed coats including variations of brown, red and black.

The anthocyanin contents in black bean reported in this study (1.94 to 3.47 mg C3G/g) were superior to those reported by Salinas-Moreno et al. (2005) in 15 black bean varieties (which varied in anthocyanin content from 0.38 to 0.72 mg C3G/g). These variations are partly attributed to the types of laboratory procedure used; nevertheless, differences between the sample genotypes cannot be rejected. This is confirmed by the results of Xu and Chang (2009), who did not find any type of anthocyanins

in the pinto (variegated) variety but found some in a black variety (Turtle Eclipse), including delphinidin-3-glucose, malvidin-3, 5-diglucose, petunidin-3-glucose, malvidin-3-galactoside, and malvidin-3-glucose.

Important differences in polyphenol and flavonoid contents were found among beans with seed coats and without seed coats, favoring the first one in both cases. Among the population groups, polyphenol content varied from 58.9 to 123.4 mg GAE/g and from 2.0 to 2.6 mg GAE/g, and flavonoids varied among groups from 9.9 (Puebla) to 14.8 (Tlaxcala) mg CE/g, and from 0.35 to 0.49 mg CE/g, in the seed coat and seed, respectively. The higher polyphenol and flavonoid concentrations in seed coats were expressed as higher antioxidant activities in seed coats (499.4 to 985.3 μmol ETrolox/g) than in seeds (11.6 to 19.2 μmol ETrolox/g).

These antioxidant activity patterns were repeated among populations within groups. For example, populations PUE-011-15 (27.7 μmol ETrolox/g) and PUE-011-20 (31.3 μmol ETrolox/g) from Puebla presented the lowest polyphenol contents in the seed coat; nevertheless these values were higher than the highest contents in the seeds of accessions OAX-011-12 and PUE-011-15, 20.3 and 32.4 μmol ETrolox/g, respectively.

Flavonoid content varied among populations from 5.9 (OAX-011-28) to 21.5 (GRO-10-87) mg CE/g in the seed coat and from 0.1 (PUE-011-14) to 0.78 (GRO-011-16) mg CE/g in the whole seed (Table 3). Consequently, because the greatest flavonoid content and highest antioxidant activity were found in the seed coat, we think that nutraceutical attention should focus on the grain covering due to its high potential.

The total polyphenol contents found in the grain (from 1.3 to 5.4 mg GAE/g) in this work was slightly less than that reported by Golam-Masum-Akond et al. (2011) in different bean varieties (from 5.9 to 14.1 mg GAE/g) and was slightly less than the contents in the seed coat (from 27.7 to 127.0 mg GAE/g). These last values are similar to those reported by Espinosa-Alonso et al. (2006) in different common bean populations in México (from 49.6 to 131 mg GAE/g). Differences in the laboratory methods used could have influenced the results; nevertheless, populations with nutraceutical potential (high flavonoid content) in the seed coat and in the seed were detected; these populations included OAX-011-29 and TLA-105.

Espinosa-Alonso et al. (2006) reported total flavonoid contents of 0.82 to 10.6 mg CE/g in 62 wild and cultivated bean populations. The lowest values were similar to those determined in the grain in this study; however, in several cases, the values were lower than those found in seed coats in the group with the greatest flavonoid content. Fifteen populations had a higher flavonoid content, as high as 11 mg CE/g. Boateng et al. (2008) estimated flavonoid contents of 0.61 to 0.84 mg CE/g. Despite the differences among the analysis methods used and the estimates that were derived from those results, the populations evaluated here exhibited higher flavonoid contents in the seed coat compared to other genotypes, both cultivated and wild. This leads us to believe that farmers in the evaluated area have a wide knowledge of their bean seeds and cultivate the most valued varieties.

Use of grain color indexes (L*; chromaticity, and h°, tone) were useful for differentiating among populations according to seed coat color or other visually appreciated feature. For instance, the luminosity index (L*) was found useful for distinguishing the visual colors of pink, cream, yellow or red from other visual variants. The chroma index distinguishes red beans from black beans; the tone hue index (h°) was the most precise because it assigns a graded score to each color or variant. In this way, all evaluated bean populations were classified quantitatively. In this case, the lowest values correspond to beans perceived as pink or red, and the highest values correspond to beans perceived as black (Table 3). These indexes can be used as physical parameters to differentiate among local bean varieties of different grain colors.

Antioxidant activity was considerably higher in the seed coat (132.5 to 1021.7 μmol ETrolox/g) than in the seed (7.1 to 32.4 μmol ETrolox/g). The latter was significantly correlated ($r > 0.36$, $P < 0.05$) with total polyphenol content in the seed coat and in the seed and with anthocyanins in the grain. In this sense, differentiation among origin groups and populations was noticeable (Fig. 1). The results confirmed that anthocyanins and polyphenols confer high antioxidant activity to bean grains and seed coats. Similar results were found by Oomah et al. (2005), Golam-Masum-Akond et al. (2011) and Dzomba et al. (2013).

The results presented here show that bean populations exhibiting a wide variety of forms, specific weight and grain colors are cultivated by small farmers in the center-south of México; these beans present significant differences in anthocyanin, polyphenol and flavonoid contents, and these differences are associated (although not in all cases) with the geographical distance from their origin site. The information obtained in this study can be used to select the most appropriate genotypes for participatory plant breeding to improve local beans and can be used to recommend their consumption to low-income families with poor nutrition.

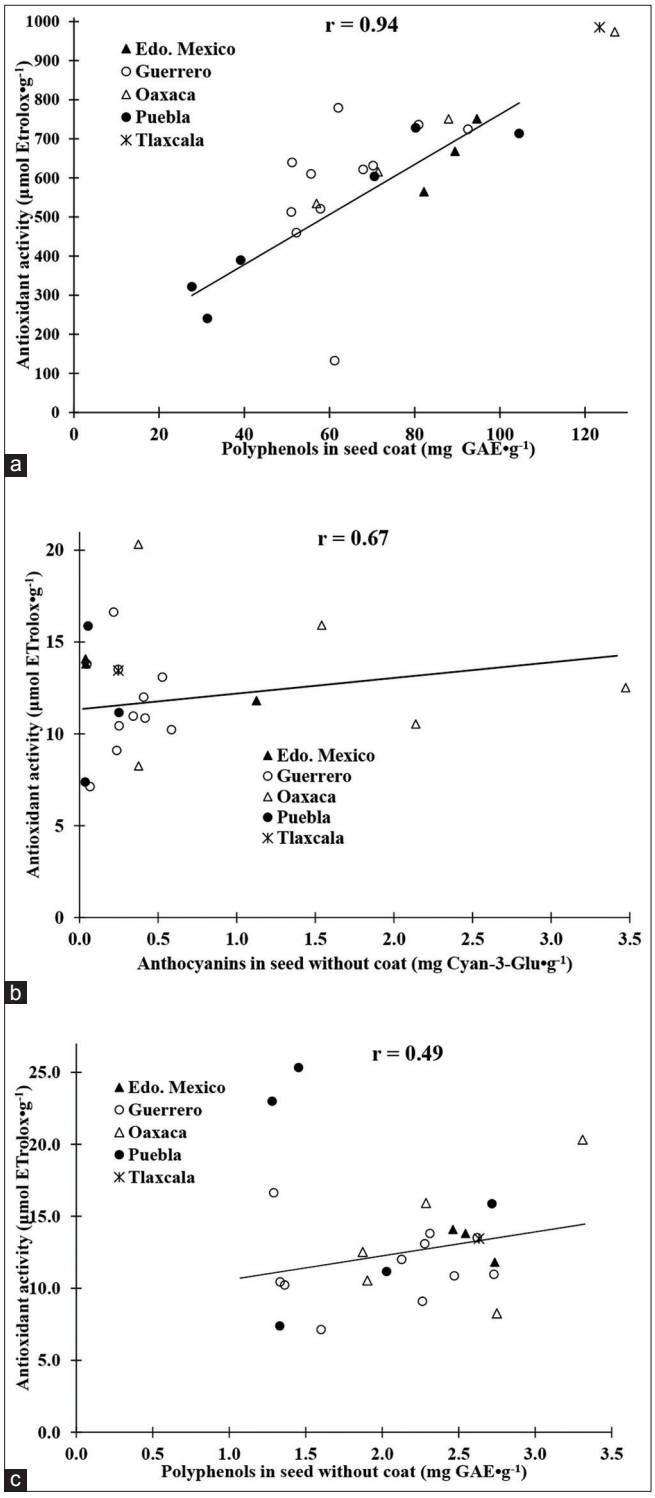

Fig 1. Relationships among polyphenol contents in seed coats (a), total anthocyanin contents (b) and polyphenol contents in seeds (c) and antioxidant activity in 26 populations of Mexican common beans.

CONCLUSIONS

The evaluated bean populations presented significant differences in the chemical composition and physical characteristics of the seed among and within the geographical origin groups. Small seeds with low specific weight are common in populations from Oaxaca and Guerrero. In this work, populations of dark bean (black, red or a mixture of dark-colored grains) presented greater contents of monomeric anthocyanins (> 1 mg C3G/g). Polyphenol and flavonoid contents were always higher in the seed coat than in the grain; this pattern was repeated for antioxidant activity, which was significantly correlated with polyphenol content in the seed coat and grain and with monomeric anthocyanins. The populations with outstanding levels of the evaluated compounds were the following: OAX-011-29, OAX-011-30, PUE-011-15, PUE-011-34, EM-01-01 and GRO-10-87; these findings are based on the total anthocyanin and polyphenol contents and on the antioxidant activity of the whole grain and seed coat.

Author contributions

E.N.A.-B., Y.D.G.-D., J.L.C.-S. and J.C.C.-R. jointly conducted the research, compiled data and conducted the laboratory and statistical analysis; J.L.C.-S., A.M.V.-G. and E. H.-G. wrote the paper.

REFERENCES

Aparicio-Fernández, X., T. García-Gasca, G. G. Yousef, M. A. Lila, E. González-de Mejía and G. Lorca-Piña. 2006. Chemopreventive activity of polyphenolics from black Jamapa bean (*Phaseolus vulgaris* L.) on HeLa and HaCaT Cells. J. Agric. Food Chem. 54: 2116-2122.

Beebe, S. E., M. R. Idupulapati, M. W. Blair, and J. A. Acosta-Gallegos. 2013. Phenotyping common beans for adaptation to drought. Front. Physiol. 4: 1-20.

Bitocchi, E., L. Nanni, E. Bellucci, M. Rossi, A. Giardini, P. S. Zeuli, G. Logozzo, J. Stougaard, P. McClean, G. Attene and R. Papa. 2012. Mesoamerican origin of the common beans (*Phaseolus vulgaris* L.) is revealed by sequence data. Proc. Nat. Acad. Sci. USA 109: E788-E796.

Blair, M. W., L. F. González, P. M. Kimani and L. Butare. 2010. Genetic diversity, inter-gene pool introgression and nutritional quality of common beans (*Phaseolus vulgaris* L.) from Central Africa. Theor. Appl. Genet. 121: 237-248.

Boateng, J., M. Verghese, L. T. Walker, and S. Ogutu. 2008. Effect of processing on antioxidant contents in selected dry beans (*Phaseolus* spp. L.). LWT-Food Sci. Technol. 41: 1541-1547.

Brand-Williams, W., M. Cuvelier and C. Berset. 1995. Use of free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28: 25-30.

Broughton, W. J., G. Hernández, M. Blair, S. Beebe, P. Gepts and J. Vanderleyden. 2003. Beans (*Phaseolus* spp.)- model food legumes. Plant Soil 252: 55-128.

Choung, M., B. Choi, Y. An, Y. Chu and Y. Cho. 2003. Anthocyanin profile of Korean cultivated kidney bean (*Phaseolus vulgaris* L.). J. Agric. Food Chem. 51: 7040-7043.

de Mejía, E. G., S. H. Guzmán-Maldonado, J. A. Acosta-Gallegos, R. Reynosa-Camacho, E. Ramírez-Rodríguez, J. L. Pons-Hernández, M. M. González-Chavira, J. Z. Castellanos and J. D. Kelly. 2003. Effect of cultivar growing location on

the trypsin inhibitors, tannins, and lectins of common beans (*Phaseolus vulgaris* L.) grown in the semiarid highlands of Mexico. J. Agric. Food Chem. 51: 5962-5966.

Dzomba, P., E. Togarepi and M. Mupa. 2013. Anthocyanin content and antioxidant activities of common bean species (*Phaseolus vulgaris* L.) grown in Mashonaland Central, Zimbabwe. Afr. J. Agric. Res. 8: 3330-3333.

Espinosa-Alonso, L. G., A. Lygin, J. M. Widholm, M. E. Valverde and O. Paredes-Lopez. 2006. Polyphenols in wild and weedy Mexican common beans (*Phaseolus vulgaris* L.). J. Agric. Food Chem. 54: 4436-4444.

Espinosa-Pérez, E. N., P. Ramírez-Vallejo, M. M. Crosby-Galván, J. A. Estrada-Gómez, B. Lucas-Florentino and J. L. Chávez-Servia. 2015. Clasificación de poblaciones nativas de frijol común del centro-sur de México por morfología de semilla. Rev. Fitotec. Mex. 29: 29-38.

FAOSTAT. 2011. FAOSTAT Database. Food supply: crop primary equivalent. In: http://faostat3.fao.org/download/FB/CC/S [accessed: 2015-11-28].

Golam-Masum-Akond, A. S. M., L. Khandaker, J. Berthold, L. Gates, K. Peters, H. Delong and K. Hossain. 2011. Anthocyanin, total polyphenols and antioxidant activity of common bean. Amer. J. Food Technol. 6: 385-394.

Hernández-López, V. M., M. L. P. Vargas-Vásquez, J. S. Muruaga-Martínez, S. Hernández-Delgado and N. Mayek-Pérez. 2013. Origen, domesticación y diversificación del frijol común. Avances y perspectivas. Rev. Fitotec. Mex. 36: 95-104.

Hernández-Saavedra, A., M. Mendoza-Sánchez, H. L. Hernández-Montiel, H. S. Guzmán-Maldonado, G. F. Loarca-Piña, L. M. Salgado and R. Reynoso-Camacho. 2013. Cooked common beans (*Phaseolus vulgaris*) protect against β-cell damage in streptozotocin-induced diabetic rats. Plant Foods Hum. Nutr. 68: 207-212.

Ktyal, R., S. V. Rana, S. Ojha, K. Vaiphei, V. Singh and K. Singh. 2001. Soybean trypsin inhibitor confers protection against rotavirus infection in infant mice. Trop. Grastroenterol. 22: 207-210.

Leterme, P. 2002. Recommendations by health organizations for pulse consumption. Br. J. Nutr. 88: S239-S242.

Leterme, P. and L. C. Muñoz. 2002. Factors influencing pulse consumption in Latin America. Br. J. Nutr. 88: S251-S254.

McGuire, R. G. 1992. Reporting of objective color measurements. HortScience 27: 1254-1255.

Oomah, B. D., A. Cardador-Martínez and G. Loarca-Piña. 2005. Phenolics and antioxidative activities in common beans (*Phaseolus vulgaris* L.). J. Sci. Food Agric. 85: 935-942.

Papa, R. and P. Gepts. 2003. Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica. Theor. Appl. Genet. 106: 239-250.

Prolla, I. R. D., R. Garcia-Barbosa, A. P. Lima-Veeck, P. Rossini-August, L. Picolli-da-Silva, N. Dalfollo-Ribeiro and T. Emanuelli. 2010. Cultivar, harvest year and storage conditions affecting nutritional quality of common beans (*Phaseolus vulgaris* L.). Ciênc. Tecnol. Aliment. 30: 96-102.

Quinn, G. P. and M. J. Kough. 2003. Experimental Data and Data Analysis for Biologist. Cambridge University Press, Cambridge, UK.

Reynoso-Camacho, E., M. Ramos-Gomez and G. Loarca-Pina. 2006. Bioactive components in common beans (*Phaseolus vulgaris* L.). In: R.G. Guevara-González and I. Torres-Pacheco, editors. Advances in Agricultural and Food Biotechnology. Research Signpost, Kerala, India, pp. 217-236.

Rocha-Guzmán, N. E., J. A. Gallegos-Infante, R. F. González-Laredo, V. Cardoza-Cervantes, R. Reynoso-Camacho, M. Ramos-Gómez, T. García-Gasca and A. de-Anda-Salazar. 2013. Evaluation of culinary quality and antioxidant capacity for Mexican common beans (*Phaseolus vulgaris* L.) canned in pilot plant. Int. Food Res. J. 20:1087-1093.

Romero-Arenas, O., M. A. Damián-Huato, J. A. Rivera-Tapia, A. Báez-Simón, M. Huerta-Lara and E. Cabrera-Huerta. 2013. The nutritional value of beans (*Phaseolus vulgaris* L.) and its importance for feeding of rural communities in Puebla-Mexico. Int. Res. J. Biol. Sci. 2: 59-65.

Salinas-Moreno, Y., L. Rojas-Herrera, E. Sosa-Montes and P. Pérez-Herrera. 2005. Anthocyanin composition in black bean (*Phaseolus vulgaris* L.) varieties grown in Mexico. Agrociencia 39: 385-394.

Santalla, M., M. A. Fueyo, A. P. Rodino, I. Montero and A. N. de Ron. 1999. Breeding for culinary and nutritional quality of common bean (*Phaseolus vulgaris* L.) in intercropping systems with maize (*Zea mays* L.). Biotechnol. Agron. Soc. Environ. 3: 225-229.

Singh, S. P., P. Gepts and D. G. Debouck. 1991. Races of common bean (*Phaseolus vulgaris*, Fabaceae). Econ. Bot. 45: 379-396.

Singleton, V. L. and J. A. Rossi. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am. J. Enol. Vitic. 16: 144-158.

Suárez-Martínez, S. E., R. A. Ferriz-Martínez, E. Campos-Vega, J. E. Elton-Punete, K. de la Torre-Carbot and T. García-Gasca. 2015. Bean seeds: leading nutraceutical source for human health. CyTA-Journal of Food DOI: 10.1080/19476337.2015.1063548.

Tsuda, T., M. Watanabe, K. Ohshima, S. Norinobu, S. Choi, S. Kawakishi and T. Osawa. 1994. Antioxidative activity of the anthocyanin pigments cyaniding 3-O-β-D-glucoside and cyaniding. J. Agric. Food Chem. 42: 2407-2410.

Vidal-Zepeda, R. 2005. Las Regiones Climáticas de México. Instituto de geografía, Universidad Nacional Autónoma de México, México, D.F.

Wrolstad, R. E. 1976. Color and pigment analyses in fruit products. Oregon State University Agricultural Experiment. Station Bull. 624: 1-17.

Xu, B. J., S. H. Yuan and S. K. C. Chang. 2007. Comparative analyses of phenolic composition, antioxidant capacity, and color of cool season legumes and other selected food legumes. J. Food Sci. 72: S167-S177.

Xu, B. and S. K. C. Chang. 2009. Total phenolic, phenolic acid, anthocyanin, flavan-3-ol, and flavonol profiles and antioxidant properties on pinto and black beans (*Phaseolus vulgaris* L.) as affected by thermal processing. J. Agric. Food Chem. 57: 4754-4764.

Zhishen, I., T. Mengcheng and W. Jianming. 1999. The determination of flavonoids contents in mulberry and their scavenging effects in superoxide radicals. Food Chem. 64: 555-559.