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Polyphenols in Wild and Weedy Mexican Common Beans (*Phaseolus vulgaris* L.)

L. GABRIELA ESPINOSA-ALONSO,[†] ANATOLY LYGIN,[‡] JACK M. WIDHOLM,[‡] MARIA E. VALVERDE,[†] AND OCTAVIO PAREDES-LOPEZ*,[†]

Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Unidad Irapuato, Km 9.6 Libramiento Norte, Carr. Irapuato-León, P.O. Box 629, Irapuato, Gto. 36500, México, and Department of Crop Sciences, 284 ERML, University of Illinois, 1201 West Gregory Drive, Urbana, Illinois 61801

The polyphenolic composition of 62 wild and weedy Mexican bean collections from diverse origins, grouped by their seed coat color, was assessed. According to spectrophotometric analysis, the range of total phenols, condensed tannins, and total anthocyanins presented wide differences. Furthermore, the phenolic acid, flavonoid, and anthocyanin profiles were analyzed using HPLC. Ferulic was the main phenolic acid. Kaempferol and quercetin were the main flavonoids, and the isoflavones daidzein and coumestrol were found in only low levels in few collections. Delphinidin was the main anthocyanidin found, followed by petunidin, cyanidin, malvidin, pelargonidin, and peonidin. The wide variation observed in polyphenolic contents was more related to their genotype than to the color factor. These results show that some wild and weedy beans are good sources of phenolic compounds for use in breeding programs focused on nutrition and health.

KEYWORDS: Common bean; *Phaseolus vulgaris*; wild bean; weedy bean; color seed coats; polyphenols; total phenols; condensed tannins; anthocyanins; phenolic acids; flavonoids

INTRODUCTION

Chronic diseases affect large numbers of people in developed and developing countries, increasing premature death. Lifestyle changes and dietary habits affect diseases that include obesity, diabetes mellitus, cardiovascular disease, hypertension and stroke, and some types of cancer. Epidemiological studies suggest that the consumption of whole fruits, vegetables, and legumes is important in the prevention of chronic diseases. Phytochemicals play an important role in health, because of their antioxidant, antimutagenic, and anticarcinogenic biological activities (*1*). The global intake of fruits and vegetables varies in populations of different countries as well as with socioeconomic status. The mean intake in Mexico and other Latin American countries is one of the lowest levels in the world (72−235 g daily, whereas the recommendation is 600 g to prevent risk of chronic diseases) (*2*). Unfortunately, inappropriate food consumption patterns are taking place in Latin America: total fat, animal product, and sugar intakes increase, whereas cereal, fruit, and vegetable intakes decrease (*3*). Additionally, fruit and vegetable consumption is scarce in some important groups of the population because they are more expensive than diets with high fat and sugar contents (*4*). Therefore, the availability of diverse food choices may help encourage improved intake of phytochemicals.

Common beans are a staple food in many Latin American and African countries. Mexico is the origin of this legume; it has been consumed since prehispanic times, and per capita intake is ≈22 kg per year. Common beans are the second source of protein, carbohydrates, vitamins, and minerals after corn. They are also an excellent source of nutraceutical constituents such as fiber, protease inhibitors, phytic acid, and polyphenols such as tannins (*5*). The bean seed coat color is attributed to the presence and quantity of polyphenols such as flavonol glycosides, condensed tannins, and anthocyanins (*6*−*10*), and their function is to protect the seed against pathogens and predators (*11*). These compounds have antioxidant, antimutagenic, and anticarcinogenic activities and also free radical scavenger properties (*12*−*14*). However, there is little information about the content of phenolic compounds in wild and cultivated common bean seeds, and no possible correlations have been established between phenolic acid content and color of seed coats. Wild common beans, the ancestor of domesticated or cultivated beans, have the most extensive genetic variability in comparison with cultivated beans, and weedy beans are those materials produced by the natural crossbreeding between a wild and a cultivated bean (landrace or improved). They grow in the forest without human manipulation and have more genetic diversity than cultivated beans (*15*). The wild and weedy common beans have not been widely studied; however, it is

* Author to whom correspondence should be addressed [telephone +52 (462) 623-9641 or 623-9674; fax +52 (462) 624-5996; e-mail oparedes@ira.cinvestav.mx].

[†] Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional.

[‡] University of Illinois.

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known that they have high resistance to some pathogens and storage pests because of the presence of arcelin, a protein exclusively found in both types of beans. In addition, higher levels of protein, some essential amino acids, and some minerals (Ca, Fe, and Zn) have been reported in wild and weedy common beans than in cultivated (*16*). Moreover, nutritional and nutraceutical bean characteristics have not been considered in crop-breeding programs. The introduction of novel genetic information on wild germplasm is suggested as an effective tool to increase the genetic variation and nutritional and nutraceutical characteristics of cultivated beans. Consumer's preferences (size, brightness, cooking time, and color) of common beans are also important factors to consider for breeding programs. Color is an attribute that determines the consumption preferences in different Mexican regions, that is, yellow in the northwest, beige with brown mottles and cream in the northeast, black in the south, and various specific colors in the center (*17*). Considering the genetic variation of wild and weedy beans, the presence of polyphenols in beans, and the importance of seed color for consumers, our objective was to determine the main polyphenol classes (condensed tannins, phenolic acids, flavonoids, and anthocyanins) in 62 wild and weedy Mexican bean collections and 2 cultivated beans from several origins and different seed coat colors in order to provide information regarding the levels of polyphenols in these types of beans.

MATERIALS AND METHODS

Wild and Weedy Bean Seeds. The materials were obtained from the germplasm collections of the National Research Institute for Forestry, Agriculture and Livestock (INIFAP, Celaya, Mexico). We used 50 wild and 12 weedy materials. For comparative purposes, two cultivated varieties, 'Jamapa' and 'Pinto', beans were included. Wild and weedy common beans were grown in a greenhouse to obtain new seeds, and after storage at 4 °C for not more than two years the samples were used for analyses. Whole seeds were milled into flour (mesh 60) and protected from light and stored at −20 °C, until analysis.

Chemicals. The Folin−Ciocalteu and vanillin reagents, caffeic acid, (+)-catechin, *p*-coumaric acid, ferulic acid, gallic acid, *p*-hydroxybenzoic acid, sinapic acid, syringic acid, vanillic acid, vanillin aldehyde, and the flavonoids quercetin and kaempferol were purchased as standards from Sigma Chemical Co. (St. Louis, MO). Isoflavonoids, daidzein and genistein, and coumestrol standards were purchased from LC Laboratories (Woburn, MA). The anthocyanin mix, 3-*O*-β-glucopyranosides of pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin, was provided by Polyphenols Laboratories (Hanabrygene Technology Centre, Sandnes, Norway).

Seed Color Evaluation. This was determinated with a Konica Minolta model DP-400 Chromo meter (Konica Minolta Sensing, Inc., Ramsey, NJ), obtaining the Hunter *Lab* values (L, a, and b). L measures the luminosity of the color, with a range of 0 (black) to 100 (white); a has positive (red) or negative (green) values; and b has positive (yellow) or negative (blue) values. The color of beans was expressed as a function of L (luminosity) and C [chroma = $(a^2 + b^2)^{1/2}$]. It was reported as the average from five independent determinations on the whole seeds.

Dendrogram Building. A dendrogram was built using the computational program Statgraphics Plus 5.1 by the nearest-neighbor method (Euclidian distance) comparing each of the average color parameters L and C.

Quantification of Total Phenols. Phenols were determined according to the Folin−Ciocalteu procedure (*18*) by reading the absorbance at 760 nm, and results are expressed as milligrams of gallic acid equivalents (GAE) per gram of bean flour.

Quantification of Condensed Tannins. Tannins were determined according to the vanillin−HCl procedure (*19*) by reading the absorbance at 500 nm, and results are expressed in milligrams of (+)-catechin equivalents (CAE) per gram. To correct for interference of natural pigments in dry beans, a blank sample was prepared with all reagents, without vanillin reagent.

Quantification of Total Anthocyanins. Anthocyanins were determined according to the Abdel-Aal and Hucl method (*20*) by reading the absorbances at 535 nm, and results are expressed as milligrams of cyanidin 3-glycoside per gram of bean flour.

Extraction, Hydrolysis, and Determination of Phenolic Acids and Flavonoids. Phenolic acids and flavonoids from bean flour were determined according to the modified method of Graham (*21*). Briefly, phenolic acids and flavonoids were extracted with 80% methanol and centrifuged; the extract was diluted with 3 mL of 2 N HCl and heated at 95 °C during 2 h to hydrolyze glycosides. Compounds were extracted with 4 mL of ethyl acetate, and the solvent was removed under vacuum. Finally, the residue was resuspended in 80% methanol and centrifuged for HPLC analysis. DAD-HPLC analyses were carried out using a Separation Module 2690 with a PDA detector (Waters Co., Milford, MA) supplied with a 57 × 7 mm i.d. Platinum EPS C-18 Rocket column (Alltech Associates, Inc., Deerfield, IL). The components were identified by their retention time and UV spectra. The solvents were (A) water adjusted to pH 2.8 with acetic acid and (B) acetonitrile. The injection volume was 30 μL and the flow rate 2.5 mL/min. The gradient for phenolic acid elution was linear to 6% B in 8 min, to 12% B in 14 min, to 20% B in 18 min, and to 35% B in 24 min; after that, the column was washed with 95% B for 3 min and equilibrated for 3 min at 100% A. UV absorbance at 295 nm was used to detect vanillic, caffeic, syringic, coumaric, ferulic, and sinapic acids and vanillin aldehyde. Absorbance at 257 nm was used to detect *p*-hydroxybenzoic acid. The gradient for flavonoids elution was linear to 10% B in 2.5 min, to 12% B in 6 min, to 23% B in 18 min, and to 35% B in 24 min; finally, the column was washed with 95% B for 3 min and equilibrated for 3 min at 100% A. UV absorbance at 260 nm was used to detect quercetin, kaempferol, daidzein, and genistein, and absorbance at 342 nm was used to detect *p*-coumestrol. Total running time for each analysis was 30 min.

Extraction, Hydrolysis, and Determination of Anthocyanins. Anthocyanin analysis was carried out according to the method of Romani et al. (*10*) with some modifications. Anthocyanins were extracted from 500 mg of bean flour, using 3 × 15 mL of 70% ethanol, adjusted to pH 2.0 with formic acid. Ethanolic extracts were dried under vacuum and dissolved in $H_2O/CH_3CN/MeOH/HCOOH$ (45:22.5:22.5:10) to a 2 mL final volume. The hydrolysis of the anthocyanin standard and the samples was carried out according to the method of Takeoka et al. (*7*). One milliliter of extract was heated with 1.0 mL of 2 N HCl in a boiling water bath for 60 min and cooled. The anthocyanins were extracted twice with ethyl acetate, then dried, redissolved with 10% formic acid, centrifuged, and used for HPLC analysis. Anthocyanin analysis was done using the HPLC system and column previously described. The solvent was made with (A) 5% HCOOH and (B) 5% HCOOH in acetonitrile. The injection volume was 20 μL and the flow rate 1.0 mL/min. The gradient for anthocyanin elution was linear at 5% B in 0 min, to 10% B in 10 min, and to 18% B in 20 min. UV absorbance at 520 nm was used to detect delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin.

RESULTS AND DISCUSSION

Clusters of Seeds in Based on Grain Color. The first step was to evaluate the color of seeds and build color groups according to their similarity (designated I−IV). **Table 1** shows the origin, type, and color of the analyzed samples and the percentage of seed color. Four main color groups were described: black (I), mottled gray (II), caffeto (III), and pale yellow (IV). Seventeen heterogeneous collections presented seeds with different colors (greenish brown, yellow with black stripes, dark brown and pale brown with dark brown stripes, purple, etc.). The heterogeneous seeds were not incorporated in the seed color groups and were termed a mixture of seed colors (M). Durango had the highest number of caffeto seed coat (38.5%), whereas 50% of the Guerrero collections were black. Oaxaca (70%) and Michoacán (45.5%) were predomi-

Table 1. Common Bean Origin, Type, and Color [a]

no.	collection name	type	color group
	Chiapas		
1	G-19026-C	weedy	mottled gray
	Chihuahua		
2	G-22837	wild	caffeto
	Durango		
	I = 15%, II = 8%, III = 38.5%, IV = 8%, M = 30.5%		
3	G-10999	weedy	black
4	DgoSalt2	wild	black
5	G-11024	wild	caffeto
6	DgoCCamp	wild	caffeto
7	DgoChInd	wild	caffeto
8	DgoPaura	wild	caffeto
9	DgoSBay2	wild	caffeto
10	G-10022	wild	mottled gray
11	G-11025-B	weedy	pale yellow
12	G-11028	wild	mixture
13	G-11029	weedy	mixture
14	G-11034	wild	mixture
15	DgoAgBla	wild	mixture
	Guerrero		
	I = 50%, II = 25%, III = 0%, IV = 0%, M = 25%		
16	G-12878	wild	black
17	G-12879-A	wild	black
18	G-1002-A	wild	mottled gray
19	G-12881-A	wild	mixture
	Jalisco		
	I = 14.25%, II = 21.5%, III = 14.25%, IV = 7%, M = 43%		
20	G12865	wild	black
21	G-12915-A	weedy	black
22	G-12930	wild	caffeto
23	G-12944	weedy	caffeto
24	G-12957	wild	mottled gray
25	G-12966	wild	mottled gray
26	G-9995	wild	mottled gray
27	G-12955	wild	pale yellow
28	G-12934	wild	mixture
29	G-12935	wild	mixture
30	G-12945	wild	mixture
31	G-12952	wild	mixture
32	G-13026	wild	mixture
33	G-13029	wild	mixture
	Michoacán		
	I = 18.2%, II = 45.4%, III = 18.2%, IV = 0%, M = 18.2%		
34	G-12888	wild	black
35	G-12889	wild	black
36	G-12895	weedy	caffeto
37	G-12960	weedy	caffeto
38	G-10019	weedy	mottle gray
39	G-12896	weedy	mottled gray
40	G-12896-B	wild	mottled gray
41	JSG y LOS 151	wild	mottled gray
42	Pátzcuaro	wild	mottled gray
43	G-11050	wild	mixture
44	JSG y LOS 80	wild	mixture
	Morelos		
	I = 20%, II = 40%, III = 0%, IV = 0%, M = 40%		
45	G-12874-B	wild	black
46	G-10010	wild	mottled gray
47	G-10016	wild	mottled gray
48	G-10012	weedy	mixture
49	IB-UNAM	wild	mixture
	Nayarit		
50	JSG y LOS 38	wild	mottled gray
	Oaxaca		
	I = 0%, II = 70%, III = 20%, IV = 0%, M = 10%		
51	OaxSanMi	wild	caffeto
52	OaxNTila	wild	caffeto
53	G-12871	wild	mottled gray
54	G-12876	wild	mottled gray
55	OaxMonAlb	wild	mottled gray
56	OaxNPort	wild	mottled gray
57	OaxSanAnt	wild	mottled gray
58	OaxTeita	wild	mottled gray
59	MaOax	wild	mottle gray
60	G-12875	wild	mixture
	Sinaloa		
61	G-12870-A	wild	mixture
	Zacatecas		
62	G-12987	wild	black
	cultivated		
63	Jamapa	cultivated	black
64	Pinto	cultivated	mottled cream

[a] Color percentage from samples by origin place: (I) black; (II) mottled gray; (III) caffeto; (IV) pale yellow; and (M) mixture of seed colors.

nantly mottled gray. Collections from Morelos presented mottled gray and heterogeneous color mixtures in the same proportion (40%). Finally, the most heterogeneous collections were from Jalisco, with all variations of seed color described before (**Table 1**). Color distribution of wild and weedy collections by regions is not conclusive; however, we can observe a general trend according to the preferences of consumption in Mexico: yellow in the northwest, all colors in the center, and dark or black in the southeast (*17*).

The grain luminosity (L) measured varied from 19.29 to 52.20 and the chrome (C) from 0.34 to 18.20. Black seeds showed the lowest levels of luminosity and chrome, whereas the highest levels were from pale yellow seeds. These data are presented in a supplementary table included in the Supporting Information. Correlation between luminosity and chrome (0.9183, $p = 0.01$) and the dendrogram obtained according to the similarity of seed color are provided in a supplementary figure included in the Supporting Information.

Total Phenol, Condensed Tannin, and Total Anthocyanin Contents. The total phenol and condensed tannin variations in 62 wild and weedy common beans with different color are shown in **Figure 1**. The level of total phenols ranged from 0.90 to 2.11 mg of GAE/g of flour bean seed. The highest value was for Durango G-11025-B (pale yellow no. 11) and the lowest for Jalisco G-12952 (mixture no. 31). The mean total phenol content of all collections was 1.37. We found variation based on origin (i.e., Durango samples exhibited the highest dispersion; Guerrero, Michoacán, Morelos, and Oaxaca were homogeneous in relation to total phenol content; and collections from Jalisco had the lowest levels).

Pinto (cream with brown mottles) and Jamapa (black), cultivated beans, had values of 1.41 ± 0.01 and 1.98 ± 0.06 mg of GAE/g of flour, respectively, in the same range as wild and weedy beans. The total phenol content of beans was similar to that of wild *Vaccinium* berry species (*V. deliciosum*, *V. membranaceum*, *V. oxycoccus*, *V. parvifolium*, and *V. uliginosum*), one of the most important sources of polyphenols in fruits (0.81−1.70 mg of GAE/g) (*22*). This result shows that phenols in beans are comparable with important sources and that they offer a beneficial effect on health. Furthermore, it has been reported that common beans have high contents of phenol antioxidants, comparable with 23 different vegetables mainly consumed in the United States. Kidney and Pinto beans were by far the best sources of these antioxidants (*23*).

In **Figure 1**, the condensed tannins ranged from 9.49 to 35.70 mg of (+)-catechin equivalents (CAE)/g of flour. The highest value was for Durango G-11025-B, the same collection with the highest total phenol content, and the lowest value was for

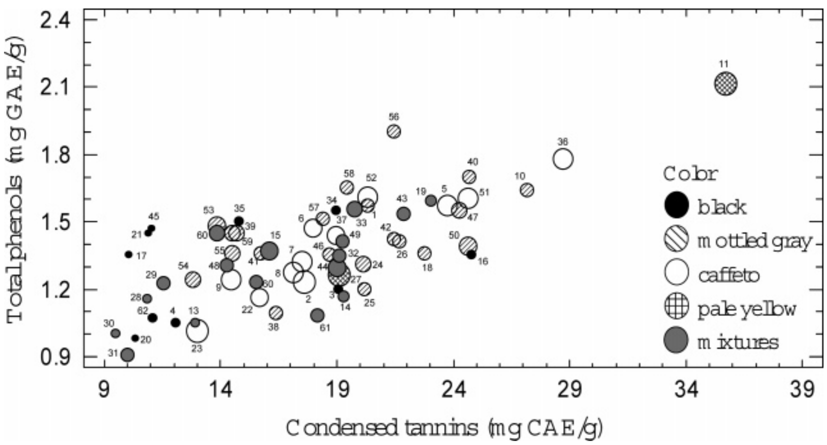

Figure 1. Intra- and intervariability of total phenols and condensed tannins for black, mottled gray, caffeto, pale yellow, and mixture color groups of wild and weedy beans. Circle shadings represent the color of seed coat (expressed as luminosity). Numbers represent collections in the same order as **Table 1**. Total phenols are expressed as milligrams of gallic acid (GAE) per gram of bean flour. Condensed tannins are expressed as milligrams of (+)-catechin equivalents (CAE) per gram of bean flour.

Jalisco G-12945 (mixture 30). The average content was 18.09 mg of CAE/g of flour. These results are in accord with reports for wild and weedy Mexican bean collections from two regions (*16*) and with cultivated beans from Durango and Jalisco races (*24*). However, we found more variability in our analyzed collections because the materials were from much more diverse regions. Pinto and Jamapa showed values of 30.86 ± 2.5 and 21.37 ± 1.37 mg of CAE/g of flour, respectively; these levels are similar to those reported for other cultivars (*24*) but higher than that found for Jamapa (*25*).

Total anthocyanins ranged from 0.01 to 1.85 mg of cyanidin 3-glucoside (C3G)/g of flour. Collections showed important differences, and not all contained anthocyanins. It was not possible to find a profile of total anthocyanin content between collections from the same region. The average content of the analyzed collections was 0.30 mg of C3G/g of flour. The highest and lowest values of total anthocyanins were found in black and mottled gray seed coats, respectively. The highest contents were obtained for the black beans Jamapa, Jalisco G-12915-A, and Guerrero G-12879-A (1.85, 1.74, and 1.52 mg of C3G/g of flour, respectively). Results obtained in black beans were somewhat similar to those reported by Takeoka et al. (*7*) and higher than for other Mexican black cultivars (0.37−0.71 mg/g) (*9*). Also, for some black collections we found similar total anthocyanin contents as wild berry species (*22*) (data not shown).

It was observed that black collections with higher levels of total anthocyanins had lower levels of condensed tannins; thus, a low inverse correlation (−0.58, $p = 0.01$) for total anthocyanin and condensed tannins was obtained (data not shown). In summary, there was not a differential profile of total phenol, condensed tannin, and anthocyanin contents by region, because wild and weedy collections have a wide genetic variation. Total phenols, condensed tannins, and anthocyanins, assessed by colorimetric procedures, are shown in a supplementary figure included in the Supporting Information.

Some studies have tried to find an association between the seed coat color and the polyphenol content in cultivated beans; however, information about polyphenols in wild and weedy collections and their association with seed coat color is scarce. Inter- and intravariations of total phenols and condensed tannins content in relation to color group are shown in **Figure 1**. Intergroup variation for total phenols was discrete but increased for condensed tannins.

High total phenols and condensed tannins changed in the five groups as follows: black, 0.98−1.55 mg of GAE/g and 10.05−24.77 mg of CAE/g; mottled gray, 1.09−1.9 mg of GAE/g and 12.82−27.19 mg of CAE/g; caffeto, 1.01−1.78 mg of GAE/g and 12.99−28.72 mg of CAE/g; pale yellow, 1.26−2.11 mg of GAE/g and 19.11−35.70 mg of CAE/g; and mixtures, 0.91−1.59 mg of GAE/g and 9.49−23.02 mg of CAE/g, respectively (**Figure 1**).

There are controversies about the relationship between seed coat color and tannin content. It has been reported that the highest condensed tannins amount was found in more colored beans and the lowest in yellow and white seed coats beans (*6*, *11*); however, quite surprisingly, one study states that the highest antioxidant activity is in white seeds due to their condensed tannin content (*13*), and in another no relationship was found between seed coat color and content of condensed tannins (*24*).

Our results showed that total phenol and condensed tannin contents tended to increase according to clearness of seeds (**Figure 1**). We found a moderate direct correlation between both components in all samples (0.703, $p = 0.01$), which was higher for caffeto and pale yellow color groups (0.84 and 0.93, $p = 0.01$, respectively, data not shown). This result suggested that the main polyphenols found in these groups of color can be condensed tannins. In conclusion, we found high variability of total phenols, condensed tannins, and anthocyanins between analyzed collections and by means of a component analysis. It was found that collections or genotypes contributed more with total phenols (67.4%) and condensed tannin variation (67.9%) than color (12.5 and 24.7%, respectively). However, for total anthocyanins, color was the main component that contributed to the variation (57.7%), more than collections or genotypes (42.1%). This matter deserves more detailed studies.

Phenolic Acids Analyzed by HPLC. The range of total phenolic acids in wild and weedy beans was from 49.56 to 131.18 mg/kg of flour found in the mottled gray group (**Table 2**). These results are within the range as previously reported for 10 different legumes (18−163 mg/kg of flour) (*26*) and similar to those found for other wild and cultivated beans (*27*). However, we found a higher quantity of free phenolic acids in wild and weedy whole flour seed than in Carioca bean cotyledons (4.89 mg/kg of flour) (*28*), which suggests that phenolic acids are present mostly in seed coats.

The main phenolic acids found in analyzed beans were ferulic, vanillic, *p*-hydroxybenzoic, and sinapic acids and, in lower

Table 2. Phenolic Acids and Flavonoids in Wild and Weedy Common Beans Contents[a]

		phenolic acids									flavonoids				
sample	color[b]	PHBA	VA	CA	VAL	SYR	CMA	FA	SIN	total	DA	Q	KA	CM	total
Chiapas															
G-19026-C	II	13.95	12.49	0	6.92	0	5.66	21.22	9.17	**69.41**	1.19	4.17	4.02	0	**9.39**
Chihuahua															
G-22837	III	10.84	9.85	0	0	0	6.27	18.29	10.34	**55.58**	1.49	3.47	3.23	0	**8.18**
Durango															
G-10999	I	12.27	7.34	1.75	1.75	0	6.15	14.91	8.51	**52.68**	3.38	7.57	11.16	0	**22.11**
DgoSalt2	I	8.32	8.25	2.16	0.25	0	8.65	21.98	12.26	**61.87**	1.87	4.25	3.04	0	**9.16**
G-11024	III	16.80	13.77	2.67	0.53	0	6.67	24.99	12.75	**78.19**	1.73	8.08	34.26	0	**44.08**
DgoCCamp	III	8.86	7.20	0	2.29	0	4.14	20.29	13.17	**55.95**	0.94	11.12	15.10	0	**27.16**
DgoChInd	III	14.19	16.30	3.17	3.93	0	8.85	26.77	19.52	**92.72**	0.73	4.73	5.15	0	**10.60**
DgoPaura	III	16.06	12.28	3.89	2.18	0	7.85	27.86	20.24	**90.36**	1.59	6.85	13.15	0	**21.59**
DgoSBay2	III	15.04	8.63	2.06	4.41	0	7.47	20.52	14.01	**72.13**	1.51	7.14	17.47	0	**26.12**
G-10022	II	27.62	32.89	0	10.03	0	5.13	28.04	15.65	**119.36**	1.55	9.58	7.67	0	**18.80**
G-11025-B	IV	15.30	14.16	0.95	2.66	0	2.20	13.43	11.75	**60.45**	1.79	21.29	56.71	0	**79.79**
G-11028	M	9.47	7.96	0.61	2.96	0	6.41	14.10	12.25	**53.76**	1.26	13.58	10.81	0	**25.65**
G-11029	M	11.31	5.83	0	0.71	0	7.30	17.21	11.85	**54.21**	1.93	7.17	10.28	0	**19.38**
G-11034	M	16.11	23.87	0	2.22	0	3.32	19.00	11.81	**76.33**	1.42	58.58	7.84	0	**67.83**
DgoAgBla	M	15.28	25.83	0	3.53	0	4.57	31.21	17.99	**98.40**	1.02	7.36	4.83	0	**13.21**
Guerrero															
G-12878	I	11.36	18.63	2.52	3.73	9.28	5.95	26.40	12.97	**90.83**	1.82	9.25	13.23	0	**24.30**
G-12879-A	I	12.22	16.51	1.76	4.33	8.58	6.64	26.39	9.62	**86.06**	1.89	4.86	4.17	1.18	**12.10**
G-1002-A	II	10.28	18.30	3.09	7.63	0	2.98	18.70	8.50	**69.48**	2.15	5.80	10.46	1.23	**19.64**
G-12881-A	M	13.37	13.41	2.90	4.58	5.92	4.76	24.60	9.55	**79.08**	1.77	6.21	14.72	0.64	**23.33**
Jalisco															
G12865	I	6.45	22.73	11.83	3.23	0	4.04	29.31	16.25	**93.84**	2.19	6.48	6.87	0	**15.55**
G-12915-A	I	11.85	17.16	0	3.40	0	6.00	28.58	10.94	**77.94**	2.27	12.41	7.94	1.48	**25.64**
G-12930	III	9.98	18.39	1.65	3.52	5.44	6.39	17.30	8.53	**71.20**	2.93	2.99	4.57	0	**10.49**
G-12944	III	12.69	21.08	3.03	6.00	0	3.24	28.38	17.17	**91.59**	2.86	3.06	8.79	0	**14.71**
G-12957	II	9.31	24.78	2.71	0.94	0	7.62	27.04	15.67	**88.07**	2.18	2.55	17.21	0	**21.95**
G-12966	II	9.94	22.13	0	3.51	0	5.81	32.68	10.19	**84.27**	1.75	3.29	5.64	1.88	**12.54**
G-9995	II	10.92	22.67	1.56	0.26	0	3.95	15.93	6.41	**61.70**	1.46	4.22	8.61	0	**14.28**
G-12955	IV	10.46	28.51	4.57	3.03	0	2.14	28.46	17.35	**94.52**	2.33	3.97	4.33	0	**10.63**
G-12934	M	17.07	17.82	5.35	2.07	6.81	5.92	25.62	13.94	**94.62**	1.35	4.39	6.28	0	**12.02**
G-12935	M	14.54	16.90	2.50	12.82	3.67	6.20	30.89	18.85	**106.38**	1.55	5.18	9.36	4.23	**20.31**
G-12945	M	12.93	13.63	5.14	3.73	6.16	4.39	22.31	21.69	**90.00**	1.95	5.31	8.76	3.63	**19.65**
G-12952	M	8.36	17.98	3.13	4.21	0	2.70	24.76	16.16	**77.30**	2.04	4.42	7.97	1.99	**16.42**
G-13026	M	14.57	13.19	0	12.51	9.33	10.23	34.51	22.40	**116.74**	1.48	9.13	6.18	7.14	**23.93**
G-13029	M	13.43	20.40	1.05	0	3.88	6.44	35.79	21.81	**102.79**	1.94	7.67	9.17	0	**18.78**
Michoacán															
G-12888	I	15.62	15.26	5.63	3.99	0	3.87	24.97	13.95	**83.28**	3.09	20.33	8.57	1.12	**33.12**
G-12889	I	22.91	15.77	17.12	4.07	0	6.61	34.01	10.76	**111.25**	0.80	7.81	12.09	0	**20.70**
G-12895	III	16.38	6.49	4.34	1.59	0	3.44	10.33	7.59	**50.16**	2.00	7.98	9.57	0	**19.55**
G-12960	III	11.87	6.77	16.27	0	0	4.09	13.97	11.20	**64.16**	1.83	4.53	3.63	0	**10.00**
G-10019	II	11.83	28.29	6.40	0	0	3.30	15.60	14.00	**79.43**	2.54	9.58	9.71	0	**21.83**
G-12896	II	12.94	10.05	16.68	0	0	3.65	19.27	6.99	**69.58**	2.28	6.26	10.03	0	**18.56**
G-12896-B	II	19.44	12.31	13.38	0	0	5.02	19.44	11.88	**81.47**	1.98	5.93	98.57	0	**106.48**
JSG y LOS 151	II	8.79	13.58	28.25	0	0	5.03	17.47	9.49	**82.61**	2.79	6.33	5.35	0	**14.46**
Pátzcuaro	II	12.67	12.80	4.00	1.99	0	2.24	10.24	5.62	**49.56**	1.20	5.39	6.74	0	**13.33**
G-11050	M	16.56	14.55	12.48	0	0	4.17	13.77	6.54	**68.07**	1.72	20.53	22.95	0	**45.20**
JSG y LOS 80	M	12.64	15.42	24.99	0	0	7.50	25.78	15.13	**101.46**	3.62	5.02	72.35	0	**80.99**
Morelos															
G-12874-B	I	14.32	20.20	0.00	9.33	12.79	5.81	20.73	11.42	**94.61**	1.66	5.65	5.85	0	**13.16**
G-10010	II	13.66	34.15	1.88	3.90	11.92	5.99	42.37	17.31	**131.18**	0.41	2.75	2.19	2.37	**7.72**
G-10016	II	14.38	14.82	0.00	22.28	6.14	4.88	30.33	13.67	**106.51**	2.15	5.56	7.88	0	**15.58**
G-10012	M	13.36	18.15	3.09	2.39	12.84	6.33	23.98	15.15	**95.28**	2.36	3.50	3.55	0	**9.40**
IB-UNAM	M	12.47	13.61	1.94	9.63	0	2.51	21.75	11.02	**72.94**	2.06	4.93	6.36	0	**13.34**
Nayarit															
JSG y LOS 38	M	21.53	42.76	0.00	2.10	0	3.96	21.68	8.29	**100.32**	1.59	3.20	5.26	1.13	**11.18**
Oaxaca															
OaxSanMi	III	8.36	22.08	14.34	0	0	3.01	21.57	10.09	**79.44**	1.80	2.08	6.08	0	**9.96**
OaxNTila	III	11.94	20.31	16.69	0	6.69	1.74	17.72	7.87	**82.97**	2.00	4.33	9.22	1.03	**16.59**
G-12871	II	20.54	20.39	18.99	0	0	4.18	26.76	11.55	**102.41**	1.48	4.30	6.68	0	**12.46**
G-12876	II	13.01	13.39	18.07	0	0	5.01	22.97	13.27	**85.71**	1.07	3.10	4.43	1.83	**10.43**
OaxMonAlb	II	5.54	19.72	8.74	0	0	2.47	16.84	19.58	**72.88**	1.68	2.93	5.59	0	**10.20**
OaxNPort	II	16.82	22.49	13.41	0	0	4.13	23.08	14.10	**94.03**	1.64	6.25	10.38	0.62	**18.89**
OaxSanAnt	II	4.82	15.98	15.61	0	0	3.87	21.55	10.97	**72.80**	1.45	3.20	5.63	0	**10.28**
OaxTeita	II	22.71	28.10	18.99	0	0	2.45	28.06	9.52	**109.83**	1.84	5.48	10.13	0	**17.45**
MaOax	II	5.95	23.04	13.30	0	0	3.80	18.77	4.04	**68.89**	1.58	3.42	6.09	0	**11.10**
G-12875	M	12.40	12.69	14.56	0	0	4.94	19.40	12.38	**76.37**	1.83	4.78	8.44	0	**15.05**
Sinaloa															
G-12870-A	M	16.25	7.16	2.31	3.03	0	10.89	22.45	12.01	**74.09**	2.11	11.04	11.42	3.38	**27.95**

Table 2. (Continued)

		phenolic acids									flavonoids				
sample	color[b]	PHBA	VA	CA	VAL	SYR	CMA	FA	SIN	total	DA	Q	KA	CM	total
Zacatecas															
G-12987	I	17.37	12.33	0.00	3.23	6.96	8.10	27.41	15.91	**91.31**	1.45	5.80	6.92	0	**14.17**
Jamapa	I	16.83	16.70	20.56	0	13.30	3.39	20.85	11.52	**103.14**	2.49	24.20	17.49	0	**44.17**
Pinto	MC	19.91	12.41	23.04	3.86	0	4.36	25.66	15.70	**104.93**	2.00	2.29	23.52	0	**27.82**

[a] Values are means of two determinations expressed as mg/kg of bean flour (fresh basis). Phenolic acids: HBA, *p*-hydroxybenzoic acid; VA, vanillic acid; CA, caffeic acid; VAL, vanillin aldehyde; SYR, syringic acid; CMA, *p*-coumaric acid; FA, ferullic acid; SIN, sinapic acid. Flavonoids: DA, daidzein; Q, quercetin; KA, kaempferol; CM, coumestrol. [b] Seed color: (I) black; (II) mottled gray; (III) caffeto; (IV) pale yellow; (M) mixture; (MC) mottled cream.

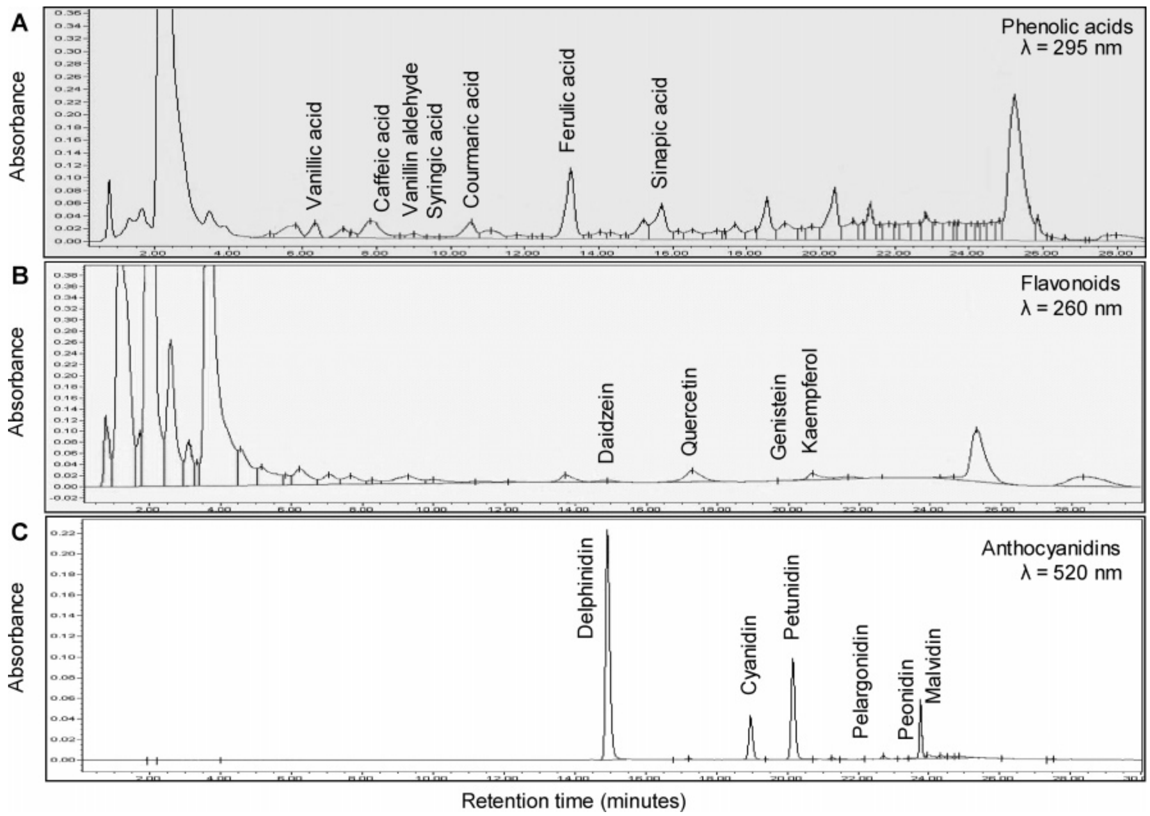

Figure 2. Typical chromatograms by DAD-HPLC of wild and weedy beans: (**A**) phenolic acids [vanillic, caffeic, syringic, coumaric, ferulic, and sinapic acids and vanillin aldehyde (measured at $\lambda = 295$ nm)]; (**B**) flavonoids [daidzein, quercetin, genistein (not found), and kaempferol (measured at $\lambda = 260$ nm]; **(C**) anthocyanins from black bean [delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin (measured at $\lambda = 520$ nm)].

quantities, vanillin aldehyde, caffeic, syringic, and *p*-coumaric acids (**Figure 2A**). We did not find a profile of phenolic acid content by region because of the large variation. Collections from Morelos had the highest range of phenolic acids (72.94 ± 131.18 mg/kg of flour, **Table 2**). To establish if phenolic acids had some influence on seed color, we evaluated the content of phenolic acids by color group and found an important intragroup color variation in phenolic acids and in the total phenolic acid content as well (**Table 2**). We did not observe a trend in phenolic acid content by color group; however, we found that mottled gray collections had higher contents of *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, vanillin aldehyde, and ferulic acid than other collections of different colors.

We found that Morelos G-10010, a mottled gray collection, had the best profile of total phenolic acids. Other important collections of different color were Michoacán G-12889 (111.25 ± 11.49 mg/kg) for black seeds, Dgo ChIndio (92.72 ± 8.51 mg/kg) for caffeto seeds, Jalisco G-12955 (94.52 ± 1.81 mg/kg) for pale yellow seeds, and Jalisco G-13026 (116.74 ± 1.25 mg/kg) for mixtures of seed colors. Meanwhile, the cultivated beans Pinto and Jamapa had the same phenolic acid amounts (104.93 ± 3.82 and 103.14 ± 3.72 mg/kg of flour, respectively) (**Table 2**). Some wild and weedy collections had a better phenolic profile than cultivated beans. Phenolic acids may not affect the natural color of seed coats, because the content of phenolic acids was not statistically different between color groups.

Flavonoids by HPLC. The total flavonoid content in wild and weedy beans was from 7.72 to 106.48 mg/kg of flour, found in the mottled gray group (**Table 2**), lower than reported for cultivated French bean (*29*) and Zolfino bean (*10*). Michoacán G-12896-B showed the highest content of flavonoids (106.49 ± 2.96 mg/kg) and Morelos G-10010 the lowest (7.72 ± 0.27 mg/kg), which at the same time had the highest phenolic acid content (**Table 2**). **Figure 2B** shows a typical graph of the flavonoids present in common beans. The main flavonoid found in wild and weedy beans was kaempferol, and Michoacán G-12896-B (mottled gray) contained the highest amount (98.57 ± 3.61 mg/kg of flour). Durango G-11034 (mixture of colors)

Table 3. Anthocyanidin Content in Wild, Weedy, and Cultivated Common Beans by HPLC[a]

color	name collection	delphinidin (mg/g)	petunidin (mg/g)	cyanidin (mg/g)	malvidin (mg/g)	pelargonidin (mg/g)	peonidin (mg/g)	total anthocyanins (mg/g)
I	Dgo Salt 2	0.44	0.08	0.01	0.05	0.02	0.01	0.60 klm
I	Dgo G-10999	0.33	0.06	0.07	0.03	0.02	0.02	0.53 mn
I	Gro G12879A	1.44	0.60	0.08	0.22	0.01	0.01	2.36 b
I	Jal G-12865	0.83	0.35	0.06	0.14	0.01	0.01	1.40 d
I	Jal G-12915A	0.67	0.42	0.03	0.19	0.01	0.01	1.33 d
I	Mich G-12888	1.21	0.27	0.15	0.08	0.03	0.01	1.73 c
I	Mich G-12889	0.67	0.07	0.01	0.03	0.02	0.02	0.82 i
I	Mor G12877B	0.63	0.17	0.05	0.07	0.01	0.01	0.94 gh
I	Zacatecas G-12987	0.29	0.06	0.04	0.03	0.01	0.01	0.44 ño
II	Gro G-1002A	0.09	0.01	0.04	0.02	0.01	0.01	0.19 g
II	Chiapas G-19026-C	0.29	0.05	0.07	0.03	0.01	0.01	0.46 ño
II	Jal G-9995	0.45	0.07	0.08	0.03	0.02	0.01	0.66 jk
II	Dgo G-10022	0.45	0.05	0.08	0.02	0.02	0.01	0.63 jkl
II	Nayarit JSG y LOS 38	0.38	0.02	0.11	0.03	0.03	0.01	0.57 lm
II	Oax Portillo	0.18	0.04	0.03	0.03	0.01	0.01	0.29 p
II	Oax Teita	0.72	0.09	0.12	0.04	0.02	0.01	1.0 fg
M	Dgo AgBla	0.89	0.14	0.09	0.08	0.03	0.01	1.23 e
M	Dgo G-11034	0.49	0.07	0.07	0.04	0.02	0.01	0.69 j
M	Gro G-12881	0.27	0.06	0.09	0.03	0.03	0.01	0.48 nñ
M	Jal G-12945	0.62	0.12	0.07	0.05	0.01	0.01	0.88 hi
M	Jal G-12934	0.71	0.14	0.08	0.06	0.02	0.01	1.03 f
M	Mor G-10012	0.26	0.03	0.06	0.02	0.02	0.01	0.40 o
M	Oax G-12875	0.42	0.03	0.09	0.02	0.02	0.01	0.58 klm
M	Sinaloa G-12870-A	0.34	0.04	0.01	0.03	0.02	0.01	0.45 ño
C	Jamapa	2.06	0.76	0.12	0.44	0.03	0.01	3.41 a

[a] Values represent the mean of two repetitions. Means in a column with different letters are significantly different ($p < 0.05$). Collections with different color: (I) black; (II) mottled gray; (M) mixtures; (C) cultivated (black bean).

contained the highest amount of quercetin (58.58 ± 2.54 mg/kg of flour). However, our results suggest that wild and weedy beans could have a higher antioxidant capacity due to their quercetin content compared with Zolfino and other types of beans that mainly contain glycosides of kaempferol (*10*, *29*).

Daidzein and coumestrol were found only in low levels in a few collections (**Figure 2B** and **Table 2**), whereas genistein was not detected at all. Common bean is not an important source of isoflavonoids compared with soybean; however, these compounds have been found in high levels in germinated samples (*27*).

Large variations in total flavonoid content were presented by collections from the same region; Durango (9.16−79.80 mg/kg of flour) and Michoacán (10.0−106.49 mg/kg of flour) samples reached the highest levels (**Table 2**). A higher intra-group color variation in flavonoid content was observed, similar to phenolic acids. The total flavonoid average in the pale yellow group was higher than that in the black, mottled gray, and caffeto groups, because Durango G-11025-B had a high level of kaempferol. The pale yellow group is represented by two collections; however, we found high differences within these collections and with other samples. It may be interesting to use more samples of this color group. **Table 2** shows that the collections with the best flavonoid profiles were Michoacán G-12888 (33.12 ± 3.90 mg/kg) for black seeds, Michoacán G-12896-B (106.49 ± 2.96 mg/kg) for mottled gray seeds, Durango G-11024 (44.08 ± 2.44 mg/kg) for caffeto seeds, Durango 11025-B (79.80 ± 3.83 mg/kg) for pale yellow seeds, and Michoacán JSG y LOS 80 (80.99 ± 8.80 mg/kg) and Durango G-11034 (67.83 ± 2.19 mg/kg) for mixture of colors, whereas Pinto and Jamapa showed lower flavonoid contents than wild and weedy beans (27.82 ± 6.17 and 44.17 ± 4.55 mg/kg of flour, respectively) (**Table 2**). Benninger and Hosfield (*6*) found slight differences in kaempferol 3-glucoside content from three different color genotypes; however, they suggested that anthocyanidin polymers may be playing a role in seed color rather than flavonoid monomers. Thus, the lack of differences found between color groups may be due to hydrolyzed aglycones quantified by us.

Anthocyanin Profile by HPLC. The anthocyanin profile was analyzed only in black, mottled gray, and heterogeneous color mixtures of wild and weedy collections and also in Jamapa (cultivated bean) (**Table 3**). Delphinidin, petunidin, and malvidin have been reported in black common bean (*7*−*10*). After hydrolysis, the six main anthocyanidins were detected (**Figure 2C**). It was assumed that the six anthocyanidins represent 100%; thus, the percentage ranges for all analyzed collections by individual anthocyanin were as follows: delphinidin, 48.5−81.0%; petunidin, 3.7−31.8%; cyanidin, 0.9−22.5%; malvidin, 3.9−14.2%; pelargonidin, 0.4−6.5%; and peonidin, 0.5−3.7%.

The anthocyanin contents found were higher in black seeds (group I) than in mottled gray (group II) and heterogeneous mixtures (M). Total anthocyanins ranged from 0.19 to 3.41 mg/g of flour, and the highest content was for Jamapa (3.41 ± 0.2 mg/g of flour), followed by Guerrero G-12879-A (2.36 ± 0.3 mg/g of flour), Michoacán G-12888 (1.73 ± 0.2 mg/g of flour), Jalisco G-12865 (1.40 ± 0.01 mg/g of flour), Jalisco G-12915-A (1.33 ± 0.01 mg/g of flour), DgoAgBla (1.23 ± 0.02 mg/g of flour), and Jalisco G-12934 (1.03 ± 0.02 mg/g of flour). Our results were higher than those reported by Romani et al. (*10*). Our estimated values were in the range from 2.12 to 37.88 mg/g of seed coat, in agreement with the findings of Takeoka et al. (*7*) and higher than found for a black Korean cultivar (*8*). Jamapa bean presented the following profile: delphinidin, 60.3%; petunidin, 22.3%; cyanidin, 3.4%; malvidin, 12.9%; pelargonidin, 0.7%; and peonidin, 0.3%. Interestingly, our results show that some wild and weedy beans have a better profile of anthocyanins than other black cultivated beans from Mexico (*9*).

If a comparison is made of total anthocyanins (spectrophotometric data) with HPLC anthocyanin contents, we can see that some samples assessed by HPLC presented values almost twice those of the former method, and the correlation factor was 0.8521 ($p = 0.01$) between both analyses. Thus, the spectrophotometric method may be used as a fast and rough procedure to estimate the anthocyanin content.

The benefit of anthocyanin consumption to health is well-known, and the U.S. intake is ≈180−215 mg per day, provided mostly by blue and red fruits, vegetables, and also red wine (*30*). However, these same sources are not available in all developing countries; an alternative source of anthocyanins is the intake of 100 g of black beans, which may incorporate the same or higher quantities (340 mg/day) of anthocyanins in the basic diet (*30*).

In conclusion, we found interesting profiles of polyphenols in wild and weedy collections. Durango G-11025 B (pale yellow) was one of the most important collections due to its high total phenol and tannin contents and also high levels of flavonoids; this material may be used in breeding programs. Jalisco G-12915-A, Guerrero G-12879-A, Michoacán G-12888, and Jalisco G-12865 (black collections) proved to be good sources of anthocyanins and could be considered for increasing anthocyanin levels in other cultivars with low anthocyanin contents. Michoacán G-12895 (total phenols and condensed tannins) and Dgo ChInd (phenolic acids) could be interesting for brown-yellow seed improvement, such as the Bayo bean type, widely consumed in central Mexico. Pale yellow seeds showed the highest content of tannins; this profile can be used to improve the Azufrado or Peruano yellow varieties consumed in northwestern Mexico. Mottled gray bean collections can be an important source of polyphenols, that is, Michoacán G-12896-B (flavonoids and tannins) and Morelos G-10010 (phenolic acids), also Oax NPort and Durango G-10022 (total phenols as tannins). In general, we believe that we are providing basic valuable information about the phenolic compound profile of wild and weedy bean collections, for preservation and rational utilization in genetic and food uses. Research on the genetic association between wild and weedy beans from different regions of Mexico and the genetic biodiversity in comparison with cultivated beans is pending.

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Supporting Information Available: Hunter *Lab* colors and phenols, condensed tannins, and total anthocyanins in bean collections. This material is available free of charge via the Internet at http://pubs.acs.org.

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