Characterization of the secondary metabolites in the seeds of nine native bean varieties (*Phaseolus vulgaris* and *P. coccineus*) from Querétaro, Mexico

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Abstract

Background: Beans (*Phaseolus* spp.) are one of the most important legumes due to their high nutritional value. The type and amount of beans' secondary metabolites varies according to their domestication status, species, and the site where they are grown. In the sate of Querétaro, the most commonly cultivated species are *P. vulgaris* L. and *P. coccineus* L., both of which can also be found in wild and disturbed areas.

Hypothesis: The aim of this project was to characterize the secondary metabolites in the seeds of the aforementioned species of *Phaseolus* with different domestication stages and from different geographical areas. The hypothesis is that wild beans collected in Mexican Plateau will have higher concentrations of secondary metabolites.

Study site and period of research: The seeds of six samples of domesticated *P. vulgaris* and three of wild and weedy *P. coccineus* populations were collected from the Mexican Plateau and the Sierra Madre Oriental in Querétaro, between 2013 and 2016.

Methods: It was an experiment with one factor and two levels (bean species) sampled randomized. The experimental unit was each sample, which was studied to obtain 17 chemical parameters, mainly secondary metabolites. From each sample 5 g were selected, minced and parameters were measured twice employing spectrophotometry and high performance liquid chromatography. Data was analyzed using a bootstrap method, discriminant analysis and by establishing simple correlations.

Results: The content of secondary metabolites from cultivated *P. vulgaris* seeds was higher, probably due to its favorable growth environmental and domestication status. Also, beans from the Mexican Plateau had a higher content of secondary metabolites than those from the Sierra Gorda. The analysis of these metabolites allowed for the identification of bean samples with the highest gallic, ellagic, ferulic and *p*-coumaric acids and vainillin content.

Conclusion: The studied samples had different metabolite content according to their species, domestication status and site of growth.

Key words: Beans, *Phaseolus*, phenols, proteins, Querétaro.

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Resumen

Antecedentes. El frijol (*Phaseolus*) es de las leguminosas más importantes a nivel mundial por su alta calidad y cantidad nutrimental. El contenido de metabolitos secundarios en las semillas de frijol varía de acuerdo a su estado de domesticación, especie y sitio de desarrollo o cultivo. En Querétaro, México las especies más cómmunte sembradas son *P. vulgaris* L. y *P. coccineus* L., las cuales también pueden encontrarse en poblaciones silvestres y malezoides.

Preguntas. El objetivo de éste estudio fue analizar los metabolitos secundarios en semillas del género *Phaseolus* con diferentes condiciones de domesticación y origen. Se considera que las semillas de *Phaseolus* silvestre tendrán el mayor contenido metabólico.

Métodos. Se empleó un factor (especie) con dos niveles, el muestreo fue aleatorizado y la unidad experimental fue cada muestra de semilla, sobre la cual se llevo a cabo la determinación de 17 parámetros químicos, como metabolitos secundarios. De cada muestra se tomaron 5 g que se molieron y las mediciones se tomaron por repetido. Se emplearon seis muestras de *P. vulgaris* y tres de *P. coccineus* recolectadas entre 2013 y 2016 en el Altiplano y la Sierra Madre Occidental en el estado de Querétaro. Para las cuantificaciones se empleó espectrofotometría (HPLC). El análisis de datos se llevó a cabo mediante el método de bootstrap, análisis discriminantes y estableciendo la correlación entre variables.

Resultados. Los metabolitos medidos tuvieron mayor concentración en semillas cultivadas (*P. vulgaris*) probablemente debido de las condiciones ambientales favorables de los campos de cultivo y por su nivel de domesticación. Además, las semillas del Altiplano tuvieron mayor concentración de metabolitos secundarios. El analisis bootstrap mostró que los ácidos gálico, elágico, ferúlico y *p*-coumárico y la vainillina son compuestos que permiten diferenciar entre las muestras.
Conclusión. Las muestras estudiadas presentan variación en el contenido metabólico según su especie, grado de domesticación y sitio de cultivo u origen.
Palabras clave: Fenoles, frijoles, *Phaseolus*, proteínas, Querétaro.

Author contributions:
Marcela Quiróz-Sodi: main idea of the experiment, seed collection, experimental execution, results interpretation, and writing of the article. Sandra Mendoza Díaz: reagents and equipment for the chemical analysis and interpretation, and manuscript review. Luis Hernández-Sandoval: main idea of the experiment, participation in seed collection, results interpretation, and manuscript review. Israel Carrillo-Ángeles: experimental design, data analysis and manuscript review.

The beans (*Phaseolus* spp.) belong to the botanical family Fabaceae. They are native to the Americas, and are the most cultivated legumes in Latin America and Africa. They are important as food, forage and nitrogen fixers (Cardador-Martínez *et al.* 2002, Peña *et al.* 2012). During the history of bean domestication, seeds have been selected based on qualities concerning farming and for cooking ease and taste. For example, cultivated varieties are selected according to seed size and local preference. Because selection focuses on these characteristics, helpful metabolites that protect plants against diseases and plagues are overlooked (Mejía *et al.* 2003, Lépiz *et al.* 2010, Bautista-Lozada *et al.* 2012, Peña *et al.* 2012). In medicine, agricultural, and nutritional research areas, *Phaseolus* varieties are generally improved to increase seed production, to achieve resistance to environmental conditions and pests and to fight degenerative illnesses (Cardador-Martínez *et al.* 2002, Mejía *et al.* 2003, Peña *et al.* 2012, Ramírez-Jiménez *et al.* 2014).

In the genus *Phaseolus*, metabolites are located in the reproductive organs, which contribute to securing reproductive processes and seed protection. These metabolites include phenolic pigments (flavonoids, anthocyanins, and tannins), lectins, trypsin and amylase inhibitors and phenolic acids (Soto-Figueroa 1994, Franco *et al.* 2002, Cardador-Martínez *et al.* 2002, Espinosa-Alonso *et al.* 2006, Reyes *et al.* 2008, Peña *et al.* 2012). In general, secondary metabolites, minerals, proteins, fibers and tannins are present in greater concentrations in wild beans than in cultivated beans (Benrey *et al.* 1998, Peña *et al.* 2012). A study developed by Nagl *et al.* (1997) suggests that species selection and genetic improvement in *Phaseolus*, have led to an increase in the quantity and quality of nutrients (vitamins and some proteins) and to the reduction of antinutrient and toxic components (lectins, phytohaemagglutinins, enzymatic inhibitors), which in nature are frequently used as a defense mechanism against herbivores.

Other characteristics like seed coat color are largely determined by their secondary metabolites content. Herrera-Flores *et al.* (2005) found that phenolic content varies among bean varieties and species and through different pods and seed maturity stages. They also registered the presence of polyphenols in *Phaseolus vulgaris, P. coccineus* and *P. polyanthus* Greenm. in parenchyma, whereas in *P. coccineus*, they found that, at day 35 of pod development, there is a wide space between parenchyma cells occupied by polyphenols. In addition, Soto-Figueroa (1994) found a close relationship between seed morphology and its phenolic content, which gives bean varieties different flavor. Furthermore, Beninger & Hosfield (2003) reported that antioxidant activity produces different colors in the coat of *P. vulgaris*.

Differences in nutrient and resource allocation generate variation in chemical and physical attributes between wild and cultivated plants (Gepts 2014). Broad research is concerned about how the different metabolites concentration between domesticated and wild plants varies affecting plant resistance, attractiveness and susceptibility to phytophages (Benrey *et al.* 1998). In Mexico, as in other countries, most of the bean studies are focused on the majority commonly cultivated species: (*P. vulgaris* L, *P. coccineus* L, *P. lunatus* L., *P. acutifolius* A. Gray and *P. dumosus* Macfad). Excluding wild and weedy varieties that could potentially contribute to the improvement of cultivated beans, and could also sustain the diversity of native beans (Lépiz *et al.* 2010).

Mexico has the greatest diversity of *Phaseolus* in the world, and the majority of the diversity is located in the western part of the country. This area is considered a center of domestication center of *Phaseolus vulgaris* (Chacón *et al.* 2005, Lépiz *et al.* 2010). The most commonly cultivated species is *P. vulgaris*. However, *P. coccineus* has excellent nutritive qualities and is found

mainly in the marginal highlands and high valleys of Mexico. Nonetheless, *P. coccineus* is not cultivated at the same intensity as *P. vulgaris* (Vargas-Vázquez *et al.* 2011). Traditional and native landraces are better adapted to different environmental conditions, and it is possible that there are more species and major distributions than reported in herbaria and germplasm banks (Cárdenas-Ramos 1997, Peña *et al.* 2012).

Although *Phaseolus* is a very important genus, information is lacking about wild and weed species, bean varieties and their usage in genetic improvement programs (Peña *et al.* 2012). A project exploring wild bean species was developed between 2003 and 2009 in several Mexican states (Jalisco, Michoacán, Colima, and Nayarit), where less species were currently found than previously reported (Lépiz *et al.* 2010). The authors strongly recommend field collections to conserve and study *Phaseolus* wild germplasm, emphasizing the importance of preserving genetic resources to generate better landraces and maintain the cultural richness of these crops in Mexico. Chacón *et al.* (2005) studied several cultivated *P. vulgaris* varieties and found differences in haplotypes between wild and domesticated seeds. They obtained bean samples from Chihuahua, Durango, Nayarit, Jalisco, Guanajuato, Michoacán, Puebla, Mexico City, Oaxaca and Chiapas, reporting six similar haplotypes from the samples from Jalisco, Michoacán, Puebla and Guanajuato. Additionally, Peña *et al.* (2012) point out the work of several authors evaluating partial chemical composition in Durango, Tlaxcala, Chihuahua and Oaxaca. They conclude that there is more genetic richness in wild bean populations and the genetic richness could be used to improve and complement cultivated bean consumption. Also, Vargas-Vázquez *et al.* (2011) studied the phenology and morphology of 98 *P. coccineus* samples from the Huasteco Karst region in the Sierra Norte of Puebla, and found that phenology was similar among the samples, whereas seed color was highly variable.

In the state of Querétaro, Mexico, there are 12 species of *Phaseolus*, where *P. vulgaris* (common bean) and *P. coccineus* (chimate bean) are the most commonly cultivated beans (CONABIO 2016, QMEX 2016). There are varying domestication stages of *P. coccineus* within the state, ranging from completely wild in the north, to domesticated at the south. Its distribution ranges from the humid forest areas in the northern part in the Sierra Madre Oriental (locally known as Sierra Gorda), to the dry environments in the southern areas of the Mexican Plateau.

The local populations prefer *negro* (black) beans, *P. vulgaris*, to *pinto* (mixed colors: brown, taupe pink, red, white, black) and *bayo* (pale pink and yellow) beans. It is possible to find other varieties such as *flor de mayo* (taupe pink), *mantequilla* (pale yellow), red and brown beans (Freytag & Debouk 2002, CONABIO 2016, QMEX 2016).

In Querétaro, little research regarding native beans has been conducted. One of the few studies is that of Díaz-Batalla *et al.* (2006), who quantified flavonoid and phenolic acids in seeds of fourteen varieties of *P. vulgaris*, with one sample native to Querétaro. They found that ferulic and *p*-coumaric acids and quercetin were the most abundant metabolites in of all the samples evaluated. The goal of this study was to describe the secondary metabolite profiles of some native seeds from *P. vulgaris* and *P. coccineus* with different domestication status in Querétaro. The proposed hypothesis was that secondary metabolites concentration will be higher in the seeds of *P. coccineus* from wild and disturbed areas.

Materials and methods

Distribution of species in Querétaro. Data about the genus *Phaseolus* was searched in records in the QMEX Herbarium, the Germplasm bank of the Universidad Autónoma de Querétaro, and the CONABIO databases (REMIB 2017). The information was compiled in a database, which briefly describes the bean species and varieties collected, their distribution, use, common names, and number of specimens collected.

Collection sampled for Phaseolus vulgaris *and* P. coccineus. The samples for the chemical analysis were collected from seven regions in Querétaro, two located at the Mexican Plateau and five from the Sierra Madre Oriental (Table 1). Nine samples were studied (Figure 1), six belonging to *P. vulgaris* (PV) and three belonging to *P. coccineus* (PC). The samples sizes of *P. vulgaris* ranged from 200 to 1,000 g of seeds, depending on the local availability. All of

Table 1. Data site of collection of the samples used in the experiment.

Species		*Phaseolus vulgaris* (PV)						*Phaseolus coccineus* (PC)		
Sample		PV1	PV2	PV3	PV4	PV5	PV6	PC1	PC2	PC3
Origin	Area	Mexican Plateau			Sierra Gorda			Mexican Plateau	Sierra Gorda	
	Site of collection	La Barreta	La Barreta	La Barreta	La Margarita	La Yesca	La Yesca	La Joya	Maravillas	La Yesca
	Geograpical coordinates	20° 49´ 46.93´´ N 100° 30´ 11.08 W	20° 49´ 46.93´´ N 100° 30´ 11.08 W	20° 49´ 46.93´´ N 100° 30´ 11.08 W	21° 21´ 24´´ N -99° 13´ 4´´ W	21° 13´ 14´´ N 99° 7´ 32´´ W	21° 13´ 14´´ N 99° 7´ 32´´ W	20° 48´33´´ N 100° 31´ 43´´ W	20° 56´ 10´´ N 99° 31´ 15´´ W	21° 13´ 14´´ N 99° 7´ 32´´ W
	Altittude (m asl)	2,127	2,127	2,127	1,627	1,596	1,596	2,450	2,134	1,596
	Variety	"Negro"	"Pinto"	Bayo	"Mantequilla"	"Negro"	"Pinto"	NA	NA	NA

the samples were collected from rainfed agriculture crops and provided by the landowners. The samples of *P. coccineus* were from three different wild and weedy populations. In these populations, five to fifteen plants with mature pods were selected. The pods were taken to the Laboratory of Botany at the Facultad de Ciencias Naturales (FCN), Universidad Autónoma de Querétaro (UAQ) in order to dry them at air temperature and obtain the seeds. The amount of *P. coccineus* seeds varied between 200 and 300 g. A 60 % backup of each sample was stored in the germplasm bank at the FCN-UAQ.

Secondary metabolites characterization of Phaseolus *seeds.* The characterization of the secondary metabolites consisted of the total content of phenolic compounds, tannins, anthocyanins, in addition to the amylase inhibitors and protein determinations for the methanolic and aqueous extracts. In accordance with the methodology described by Cardador-Martínez *et al.* (2002), a ratio of 1:10 was used to prepare the methanolic extracts. For the determination of the total amount of phenols, Folin Ciocalteu's technique was performed, using gallic acid as a standard in both, seed coats and cotyledons. The results were expressed as µg GAE/ml (Dewanto *et al.*

Figure 1. Seed samples of *Phaseolus* spp. and their collection areas. PV1, PV2, PV3, PV4, PV5 and PV6 (*P.vulgaris*). PC1, PC2 and PC3 (*P. coccineus*).

2002). Tannin content in seed coat was measured using the vanillin and (+)-catechin technique modified for microplate, and the results were reported as μg (+)-Cat/ml (Mejía *et al*. 2003). The seed coat anthocyanins were quantified employing the differential pH method with buffers of 0.025 M potassium chloride pH 1 and 0.4 M sodium acetate pH 4.5 (Giusti & Wrolstad 2001) and reported as μg of cyanidin-3-glucoside/ml. To obtain the HPLC profiles, the extracts were filtered through a 0.2 mm filter membrane and 50 μL were injected in triplicate into a reversed phase column (C18 Prep-Nova Pack HR, 60 Å, 6 um, 3.9-300 mm), using a Waters HPLC system (Waters Corporation, Milford, MA, USA) which consisted of a quaternary pump (model 600), a photodiode array detector (model 996), an in-line vacuum degasser (MetaChem Technologies Inc.), and a Rheodyne injector (4793). Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium[32] software program (Waters). Chromatographic conditions were λmax 280 nm; the gradient mobile system consisted of (A) water/1 % acetic acid and (B) acetonitrile at a flow rate of 0.8 ml/min. A linear gradient was used as follows: 85 % of solvent A for 2.5 minutes, 60 % of solvent A for 2.5 minutes, 70 % of solvent A for 5 minutes, 80 % of A for 2 minutes, 40 % of solvent A for 1 minute, 50 % of solvent A for 2 minutes and 85 % of solvent A for 3 minutes. Quantification was carried out by external standardization and full standard curves were constructed. A total of 13 commercial standards were measured: caffeic, chlorogenic, ellagic, ferulic, gallic, *p*-coumaric and sinapic acids, apigenin, catechin, kaempferol, quercetin, rutin, and vanillin. The results were reported as μg/ml (Díaz-Batalla *et al*. 2006, Ramírez-Jiménez *et al*. 2014).

Protein and amylase inhibitors were measured employing an aqueous extract of defatted bean flour in proportion 1:5, including seed coat and cotyledon (Campos *et al.* 1997). The protein quantification was performed by the Bradford's method modified for microplate with bovine albumin as standard (Bradford 1976). The amylase inhibitor content was determined by the spectrophotometric technique of disappearing substrate (Somogyi 1938) using a starch buffer substrate solution (0.05 % starch, 0.15 M NaCl, 0.1 M sodium acetate pH 5) and a developer solution (0.006 % I_2 + 0.06 % KI in HCl 0.02 M). The results were reported as Amylolytic Units (AU/ml).

Statistical analysis. The values of each chemical variable were compared between species (*P. vulgaris* and *P. coccineus*) and origin by using a bootstrap method implemented in the software Data Pilot 1.03 (Two Pilots Inc. 2003). This method generates multiple averages of subsamples to determine the probability that a group average is higher than the average of another group (Henson 2015). For one of the groups multiple averages were generated from the six samples of *P. vulgaris* and for the other group from the three samples of *P. coccineus*. On the other hand, two groups were generated according to their origin, one group from the four samples from the Mexican Plateau and the other one from samples from Sierra Gorda. With the set of variables differing between species and origin, a discriminant analysis was carried out in order to determine whether the nine samples were grouped by species or origin. For the selection of the variables used in the discriminant analysis, a Spearman Rho correlation coefficient for no normal variables was used. The explanatory power of each of the correlated variables was evaluated stepwise into the discriminant model (Kachigan 1986 Quinn & Keough 2003) and the significant model (Wilks' Lambda > 0.05 = X < 0.05) that maximized differences between species and origin was selected. The analysis was carried out in the software JMP 8.0 (SAS Institute Inc.).

Results

Distribution of species in Querétaro. Data about the genus *Phaseolus* was mostly obtained from the herbarium specimens with a total of 202 records and 13 species. *Phaseolus vulgaris* has 82 records and *P. coccineus* has 68, whereas the remaining species have 18 or less (*P. acutifolius, P. formosus* Kunth*, P. glabellus* Piper*, P. grayanus* Wooton & Standl.*, P. heterophyllus* Humb. & Bonpl. ex Wild*, P. leptostachyus* Benth.*, P. maculatus* Scheele*, P. microcarpus* Mart.*, P. pauciflorus* Sessé & Moc. Ex G.Don*, P. pedicellatus* Benth.*, P. pluriflorus* Maréchal, Mascherpa & Stainier). From the total records, 117 were placed in some variety or subspecies. At the infra-specific level, two subspecies (*P. coccineus* subsp. *coccineus*, *P. acutifolius* subsp. *latifolius* G.

Table 2. Common names used for *Phaseolus* spp. at the state of Querétaro.

SPECIES	COMMON NAME	SPECIES	COMMON NAME
P. vulgaris	Frijol americano	*P. vulgaris*	Frijol negro
P. vulgaris	Frijol faja de indio o San Franciscano	*P. vulgaris*	Frijol negro de mata
P. vulgaris	Frijol	*P. vulgaris*	Frijol negro de monte
P. vulgaris	Frijol bayacote	*P. vulgaris*	Frijol pintito
P. vulgaris	Frijol bayo	*P. vulgaris*	Frijol pinto
P. vulgaris	Frijol bolondito	*P. vulgaris*	Frijol pipiano
P. vulgaris	Frijol burro	*P. vulgaris*	Frijol rojo
P. vulgaris	Frijol criollo	*P. vulgaris*	Frijol sabino
P. vulgaris	Frijol ejotero	*P. vulgaris*	Frijol vaquita
P. vulgaris	Frijol Flor de mayo	*P. vulgaris*	Frijol verde
P. vulgaris	Frijol mantequilla	*P. vulgaris*	Frijoles morados varios
P. vulgaris	Frijol morado de mata	*P. coccineus*	Frijol chimate
P. vulgaris	Frijol moro	*P. coccineus*	Frijol chimate

F. Freeman), and four varieties (*P. leptostachyus* var. *leptostachyus* , *P. vulgaris* var. *vulgaris*, *P. vulgaris* var. *mexicanus* and *P. pedicellatus* var. *pedicellatus*) were found. The records were obtained in 18 municipalities, and the majority were from Landa de Matamoros (35), Querétaro (27), San Joaquín (19), Pinal de Amoles (17) and San Juan del Río (15). There were 56 collections from 1990 to 1999 and 60 from 2010 to the present. For the rest of the decades there were 31 collections or less. Samples were collected largely from pine and oak forests or from agriculture fields in the municipalities of San Juan del Río and Querétaro (Figure 2).

Twenty-six common names, mainly for *P. vulgaris* and *P. coccineus* were found from the species used for consumption (Table 2). Frequently their common names refer to the beans color (black, green, red, purple, pink, yellow), shape (*bolondito*: round), usage (*ejotero*: edible pods) or some other characteristics such as flavor or texture. The consumed part of the plant is the boiled pod (*P. coccineus*) and the cooked seeds, either whole or refried (*P. vulgaris* and *P. coccineus*).

Secondary metabolite characterization of Phaseolus *seeds.* The sampled seeds were quite different. Seeds from cultivated plants of *P. vulgaris* were the following colors: black, brown, light pink and yellow. The brightness of the color varied from intense to dull, and most of them had a smooth seed coat and a uniform color (Figure 1). The seeds of wild and weedy plants of *P. coccineus* were mainly black spotted with brown. They were shiny and had different shapes, from ovoid to quadrangular (Figure 1).

In general, the total phenolic content was higher in the seed coat than in the cotyledon; in *P. vulgaris* the mean was 20 % higher while in *P. coccineus* was 200 % higher. Samples PV2 and PV5 did not follow this pattern, and because of that the mean value declined. For the seed coat extract of *P. vulgaris* samples, PV5 showed the highest value (5.92 µg eq GAE/ml extract); and sample PV4 had the lowest value (1.47 µg eq GAE/ml extract). Both samples were from the Sierra Gorda. The tannin content ranged from 0.07 to 0.69 µg eq. (+) Cat/ml, with the highest value found in the Mexican Plateau´s *pinto* bean (*P. vulgaris*; 0.69 µg eq. + Cat/ml extract). For the sample PV4, *mantequilla* bean (*P. vulgaris*), tannins were not detected. The anthocyanins concentration of the bean seed coat ranged from 0.04 to 2.48 µg eq. Cyanidin-3-glucoside/ml; the lowest value was from PC2, *P. coccineus* and the highest for the sample PV5, *P. vulgaris*, respectively (Table 3).

In addition, seven phenolic acids (caffeic, chlorogenic, ellagic, gallic, ferulic, sinapic, and *p*-coumaric acids), an aldehyde (vainillin) and five flavonoids (rutin, (+)-catechin, quercetin, apigenin, and kaempferol) were determined by HPLC (Table 3). Sample PV1, *P. vulgaris* "negro" from Mexican Plateau, has the highest content of gallic, chlorogenic, ellagic, *p*-coumaric

Table 3. Mean values from chemical data from *P. vulgaris* and *P. coccineus* seeds evaluation. TPc (cotyledon total phenols), TPsc (seed coat coat total phenols), TAN (tannins), ANT (anthocyanins), GAL (galic) acid, CLO (chlorogenic acid), CAF (caffeic acid), ELA (ellagic acid), PCO (*p*-coumaric acid), FER (ferulic acid), CAT (cathechin), VAI (vainilin), API (apigenin), KAE (kaempferol), AMI (amylase inhibitors), PRO (protein).

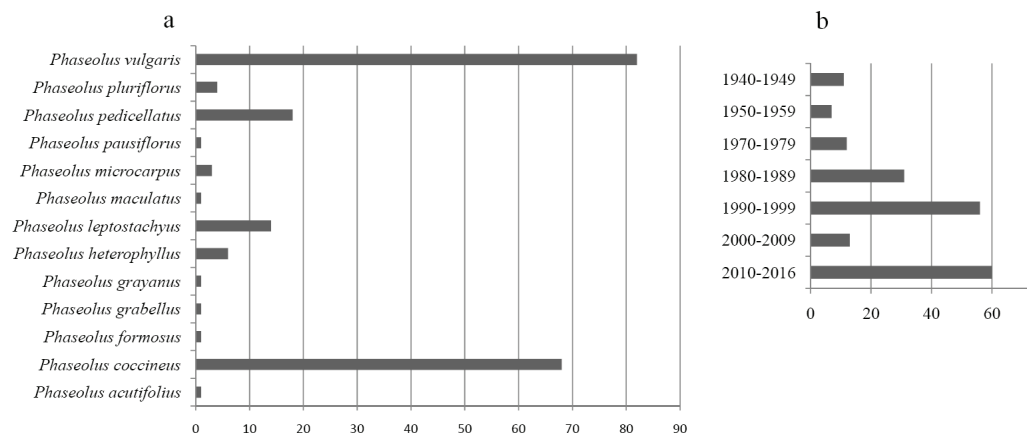| Species	*Phaseolus vulgaris* (PV)						*Phaseolus coccineus* (PC)					Significant value for media comparison by species (> 0.95)	Significant value for media comparison by origin (> 0.95)
Sample Origin	Mexican Plateau			Sierra Gorda			Mexican Plateau	Sierra Gorda		Media PV (DE)	Media PC (DE)		
Variety	PV1 "Negro"	PV2 "Pinto"	PV3 Bayo	PV4 "Mantequilla"	PV5 "Negro"	PV6 "Pinto"	PC1 NA	PC2 NA	PC3 NA				
TPc (µg eq GAE/ml extract)	0.73	17.02	1.23	0.96	7.19	1.24	1.06	2.79	1.16	4.72 (6.50)	1.67 (0.97)		
TPsc (µg eq GAE/ml extract)	2.90	3.90	5.50	1.47	5.92	2.50	2.53	3.40	4.26	3.69 (1.74)	3.40 (0.86)		
TAN (µg eq (+)- Cat/ml extract)	0.36	0.69	0.46	0.00	0.28	0.23	0.07	0.37	0.42	0.33 (0.23)	0.29 (0.18)		
ANT (µg Cyanidin-3-glucoside /ml extract)	0.78	0.68	0.04	0.29	2.48	0.18	0.44	0.12	1.22	0.74 (0.89)	0.59 (0.56)		
GAL (µg/ml)	0.31	0.21	0.17	0.12	0.11	0.15	0.10	0.12	0.13	0.17 (0.07)	0.12 (0.01)	*	*
CLO (µg/ml)	0.15	0.06	0.03	0.01	0.16	0.04	0.03	0.03	0.03	0.075 (0.064)	0.03 (0)	*	
CAF (µg/ml)	0.00	0.00	3.29	0.00	0.88	0.00	0.03	0.08	0.04	0.69 (1.31)	0.05 (0.026)		
ELA (µg/ml)	49.71	25.66	0.00	5.20	11.18	18.62	2.56	4.26	3.33	18.39 (17.87)	3.38 (0.85)	*	*
PCO (µg/ml)	0.86	0.49	0.00	0.12	0.08	0.32	0.76	0.23	0.15	0.31 (0.32)	0.38 (0.33)		
FER (µg/ml)	1.17	0.46	0.77	0.00	0.24	0.41	0.46	0.17	0.09	0.508 (0.411)	0.24 (0.19)		*
CAT (µg/ml)	0.08	0.02	0.10	0.01	0.13	0.01	0.02	0.01	0.02	0.058 (0.051)	0.02 (0.005)	*	
VAI (µg/ml)	0.00	0.01	0.00	1.59	0.26	0.10	0.03	0.01	0.01	0.32 (0.62)	0.02 (0.01)	*	
API (µg/ml)	1.02	0.76	3.14	0.63	0.17	0.29	0.80	0.34	0.19	1.001 (1.09)	0.44 (0.31)		*
KAE (µg/ml)	0.04	0.06	0.57	7.20	5.51	0.99	0.17	0.32	0.69	2.39 (3.13)	0.39 (0.26)	*	
AMI (UA/ml)	-276.00	-360.00	-432.00	-216.00	-168.00	-536.00	-656.00	-398.00	-208.00	-331.33 (138.48)	-420.67 (224.85)		
PRO (µg/ml)	0.40	0.39	0.45	0.40	0.61	0.62	0.39	0.31	0.56	0.47 (0.10)	0.42 (0.12)		

Figure 2. Number of collections of *Phaseolus* in Querétaro ordered by species (A) and year (B) registered at the QMEX Herbarium, the Germplasm bank of the Universidad Autónoma de Querétaro, and the CONABIO databases.

and ferulic acids and apigenin (53.22 µg/ml in total). Sample PV5, *P. vulgaris negro* from Sierra Gorda, possessed the highest concentration of chlorogenic acid, vainillin, and kaempferol (5.93 µg/ml in total). The highest content of caffeic acid and rutin was found in the seed coat extract of sample PV3, *P. vulgaris* "*bayo*" (3.29 µg/ml), and sample PV4, *P. vulgaris* "*mantequilla*" (0,035 µg/ml), respectively. Even rutin and quercitin were detected in some samples but in most of them this substances were undetectable. Sinapic acid was not detected in the assayed samples. Protein content was high in samples PV5 (0.61 µg/ml) and PV6 (0.62 µg/ml), both representing *P. vulgaris* from Sierra Gorda. Amylase inhibitors values were on average lower for *P. vulgaris* and 20 % higher in *P. coccineus*. The lowest content was recorded in the sample PV5 (-168 UA/ml) and the highest in PC1 (-656 UA/ml).

*Correlation analysis between samples and species (*P. vulgaris *and* P. coccineus*).* Differences were not statistically significant between species. However, the secondary metabolite concentration was higher in *P. vulgaris* than in *P. coccineus*, with the exception of *p*-coumaric acid. In contrast, when analyzing by origin most of the values are higher in Mexican Plateau seeds, with the exceptions of anthocianins, vainillin and amylase inhibitors.

Statistically, differences in of chemical characteristics were found in gallic, chlorogenic and ellagic acids, cathechin, vainillin and kaempferol, finding that all of them were higher in *P. vulgaris*. The chemical characteristics that had differences were gallic, *p*-coumaric and ferulic acids and apigenin. All of them were higher in the Mexican Plateau samples.

Pairs of chemical variables between which a correlation was detected when analyzed by species were ferulic acid- *p*-coumaric acid, tannins and apigenin, quercitin-gallic acid, chlorogenic acid, total phenolic compounds in cotyledon and amylase inhibitors (Figure 3). The significant model that maximized differences between species (Wilks $\lambda = 0.20$, 3 df, $P = 0.03$) included three variables: ferulic and gallic acids, anthocianins, kaempferol and total phenolic content in seed coat. The variables that revealed differences between the samples of different origins were catechin-chlorogenic and caffeic acids, whereas in the seed coat they were anthocianins and total phenolic content (Figure 4).

Discussion

Several factors affect the amount secondary metabolites in the seeds, including species, site of cultivation, storage and environmental conditions during growth (Luthria & Pastor-Corrales 2006). The selection of varieties is made according to the regional environmental conditions, consumer preferences and the objective of cultivating a particular variety. The concentrations of phenolic compounds in the seed coat and cotyledon was higher than those obtained by Luthria

Figure 3. Multivariate analysis by species. GAL (gallic acid), CLO (chlorogenic acid), CAT (catechin), ELA (ellagic acid), VAI (vainillin), KAE (kaempferol).* means significant pairwise correlation.

& Pastor-Corrales (2006). They reported content ranges between 0.019 and 0.048 μg GAE/ml in 15 varieties of *P. vulgaris*. In comparison to the results of Peña *et al*. (2012) and Mejía *et al*. (2003), which observed tannin concentrations higher than a hundred times, *i.e*., between 0.01 and 0.05 μg (+)- Cat/ml., whereas in this study the concentrations were lower. Also, the amounts of anthocyanins are much lower than the values reported by Puertas-Mejía *et al*. (2013) and Espinosa-Alonso *et al*. (2006), who reported quantities of 13.3 and 25.5, and 3.4 μg eq. cyanidin-3-glucoside/ml, respectively, in the common bean. These differences could be caused by the storage, drying and extraction methods, coupled with the intrinsic variability in the samples (Dai & Mumper 2010, Puertas-Mejía *et al*. 2013).

The phenolic acid content in *negro*, *pinto* and *bayo* seeds of *P. vulgaris* were similar to those reported by Díaz-Batalla (2006) in terms of vanillin and quercetin. In contrast, the amounts of *p*-coumaric and ferulic acids, and kaempferol were higher. Comparing the data from black bean *P. vulgaris* with thev values reported by Ramírez-Jiménez *et al.* (2014), the contents of gallic and chlorogenic acids as well as catechin and rutin were similar. Caffeic, *p*-coumaric and ferulic acid values were higher, and the rutin, ferulic acid and quercetin values lower. Luthria & Pastor-Corrales (2006) studied 15 varieties of common bean and they founded great variability in their secondary metabolites. Their reported concentrations of caffeic, *p*-coumaric and ferulic acid were lower than the values reported here; however, sinapic acid was detected in their study. Moreover, protein contents reported here are lower compared with the one obtained by Luthria & Pastor-Corrales (2006) (15.94 mg / ml).

The findings were high quantities of caffeic and gallic acids, kaempferol, rutin and cathechin in the cultivated bean *P. vulgaris*. We expected these findings because of the secondary metabolites antioxidant, antimutagenic, antiviral and antimicrobe functions, anti-inflammatory properties, as well as the ability to regulate triglicerids metabolism and prevent degenerative illnesses (Cardador-Martínez *et al.* 2002, Mejía *et al.* 2003). In the case of gallic and caffeic acids and cathechin and kaempferol this goal was achieved.

The metabolites found in higher concentrations were ellagic acid, kaempferol and apigennin. These have antioxidant and antimutagenic properties, help in insulin liberation and reduce
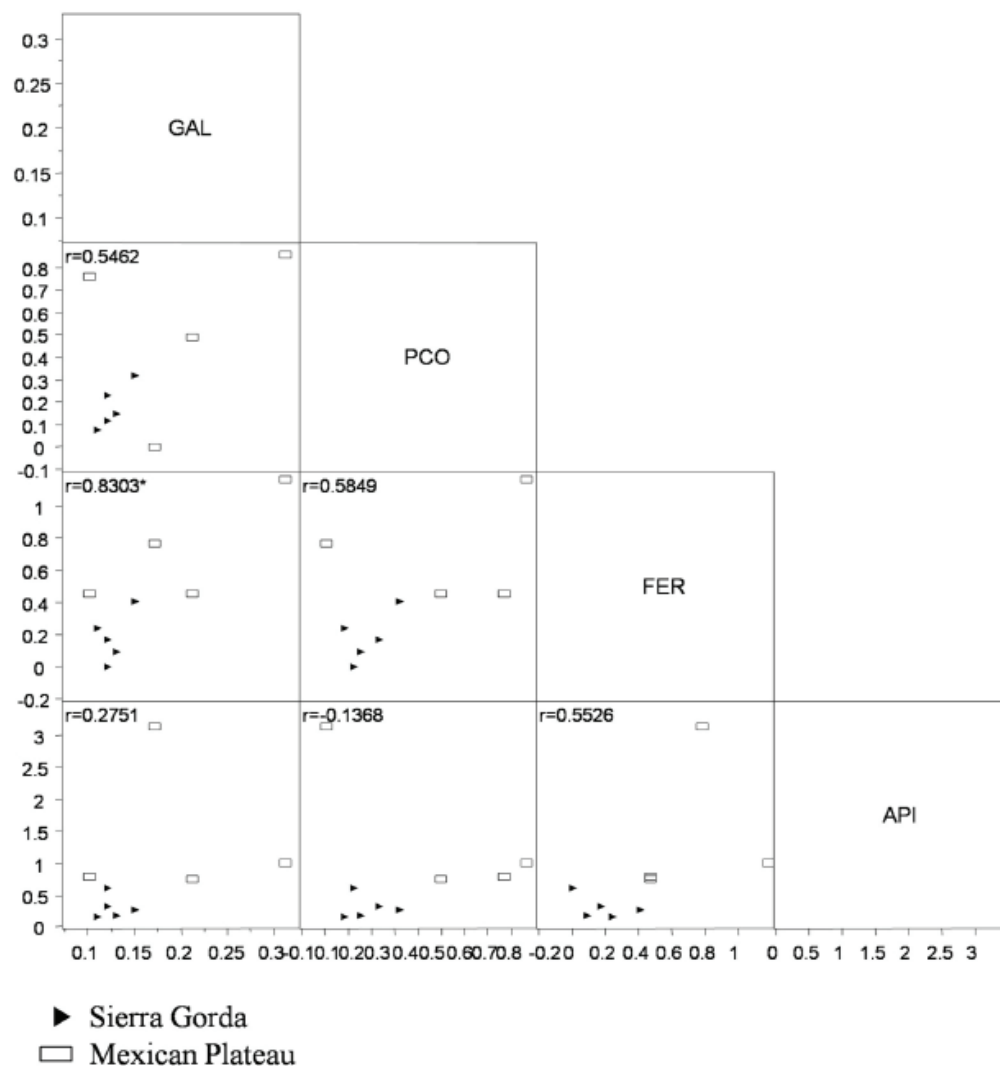
Figure 4. Multivariate analysis for chemical measurements by origin. GAL (gallic acid), PCO (*p*-coumaric acid), FER (ferulic acid), API (apigenin). * means significative pairwise correlation.

cardiovascular illnesses (Ramírez-Jiménez *et al.* 2014). When analyzing by species *P. coccineus* has the highest concentration of *p*-coumaric acid. When analyzing by origin, anthocianins, vainillin and amylase inhibitors were highest for samples from Sierra Gorda. Those metabolites mainly provide the fragrance to attract or deter pests and to reduce starch digestibility in insects (Mbogo *et al*. 2009). The concentration of secondary metabolites is highly diverse and could be affected not only by species type and domestication process, but also by storage, drying techniques and environmental conditions in crop fields, thus increasing the variability within each species and sample (Luthria & Pastor-Corrales 2006). According to our data, the gallic, ellagic, ferulic and *p*-coumaric acids and vainillin are the metabolites that differ most. For future research we widely suggest sampling in a gradient of origin that lends to identify the effects of this factor in the metabolites analyzed.

Acknowledgments

ship, the staff members and students who assisted in the project´s development; and Dr. Victor Steinmann for the English revision of the manuscript.

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