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Phenolic Compounds, Antioxidant Activity and Mineral Contents in Grains of *Phaseolus lunatus* L. and *P. coccineus* L. Landraces

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ABSTRACT

Background: *Phaseolus vulgaris* L. is widely studied for its bioactive compounds and nutritional properties while minimal attention has been given to *P. lunatus* L. or *P. coccineus* L. Here, a comparison of the phenolic compounds, minerals and antioxidant activity between landraces of *P. lunatus* and *P. coccineus* was made based on samples from populations cultivated by indigenous communities in Oaxaca, Mexico and with reference to improved varieties of *P. vulgaris*.

Methods: The phenolic compound contents and antioxidant activity in four samples of *P. lunatus*, four of *P. coccineus* and four improved varieties of *P. vulgaris* (control) were evaluated by spectrophotometry using reference standards. Through inductively coupled plasma-optical emission spectrometry, the mineral macro- and microelements contents were determined.

Result: Except for Fe, Zn and Na, significant differences and high levels of variability were observed in the phenolic compound and mineral contents and antioxidant activity of *P. lunatus*, *P. coccineus* and *P. vulgaris* across the populations evaluated. The highest concentration of phenolic compounds was recorded in the seed coat, followed by whole grains and cotyledons.

Key words: Bioactive compounds, Communitarian food systems, Indigenous communities, Optical emission spectrometry (OES), Plant genetic resources.

INTRODUCTION

Beans are among the most cultivated legumes and contribute greatly to food and socioeconomic resources worldwide. The largest area planted with beans is found in Asia, followed by Africa and America, with 18.9, 8.5 and 7.2 million ha, respectively; beans are planted not only in developing countries but also in countries where they are often consumed and imported (10.4 million tons) (FAO, 2021). From a global economic perspective, bean production is primarily focused on the cultivation of *P. vulgaris* L., *P. lunatus* L. and *P. coccineus* L. The wild forms of these species are distributed from Mexico to Central America (Mesoamerica), a region considered the origin, domestication and diversification center and they can be distinguished from different landraces preserved by small farmers (Soleri *et al*., 2013). Phenolic compounds perform various biological functions during plant growth and development, where biosynthesis is influenced by biotic and abiotic stress conditions, genetic factors and genetic-environmental interactions (Yang *et al*., 2018). A bean grain consists of the embryo, cotyledons and seed coat, each with different chemical compositions. For example, in the seed coat of *P. vulgaris* and *P. coccineus*, the polyphenol, flavonoid and anthocyanin contents were higher than those in cotyledons (Capistrán-Carabarin *et al*., 2019). Therefore, there is interest in determining the composition of the seed coat to study biological activity and applications in human health (Yang *et al*., 2018).

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P. vulgaris has received widespread attention for its bioactive and nutritional compounds and antioxidant activity (Alcázar-Valle *et al*., 2021). Previous studies have focused mainly on improved varieties of *P. vulgaris*, followed by *P. coccineus* (Quiroz-Sodi *et al*., 2018) and finally *P. lunatus* (Seidu *et al*., 2015; Palupi *et al*., 2022). Therefore, evaluating the variation in bioactive compounds and nutritional-nutraceutical content of *Phaseolus* seeds will help guide genetic improvement programs by providing nutritional assessments. The objective of this study was to compare the phenolic compounds, mineral contents and antioxidant activity of landraces of *P. lunatus* and *P. coccineus* based on samples of populations cultivated in indigenous communities of Oaxaca, Mexico and with reference to improved varieties of *P. vulgaris*.

MATERIALS AND METHODS

Germplasm evaluated

The evaluated collection consisted of four populations of *P. lunatus* from communities of Oaxaca and Yucatan and four populations of *P. coccineus* collected in Oaxaca and Puebla, Mexico, all during 2018 and 2019. A commercial variety, Michigan, two experimental varieties and a landrace of *P. vulgaris* were used as controls. All experimental activities and laboratory analyses were performed at the CIIDIR-Oaxaca research center from Instituto Politecnico Nacional, Mexico.

Evaluation of phenolic compounds and antioxidant activity

The sample preparations were performed according to García-Díaz *et al*. (2018) and Capistrán-Carabarin *et al*. (2019). The polyphenols in the seed coat, cotyledons and whole grains were determined according to the methods described by Singleton and Rossi (1965), where the absorbance of the reaction was measured at 750 nm with a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) and the results are reported in mg gallic acid equivalents per gram of dry weight (mg GAE g^{-1} dw). The flavonoid contents were evaluated based on the methods described by Zhishen *et al*. (1999), where the absorbance was measured at 510 nm in a spectrophotometer and the results are reported as mg equivalents of catechin per gram of dry weight (mg EC g^{-1} dw). The monomeric anthocyanin content was determined in the seed coat and whole grains based on the method described by Giusti and Wrolstad (2001) and the values obtained were expressed as mg equivalents of cyanidin-3-glucoside per gram of dry weight (mg C3G g^{-1} dw).

Antioxidant activity by DPPH and FRAP

The free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was evaluated following the procedure described by Brand-Williams *et al*. (1995), where the absorbance of the reaction was measured at 517 nm using a spectrophotometer and the results are expressed as equivalent micromoles of Trolox per gram of dry weight (μmol TE g^{-1} dw). In the FRAP method, the iron reduction capacity was measured as described by Benzie and Strain (1999), where the absorbance reaction was evaluated at 593 nm by a spectrophotometer and the results are expressed as equivalent micromolar of Trolox per gram of dry weight (μmol TE g^{-1} dw).

Determination of minerals

The mineral contents were evaluated following the methodology described by Martínez-Martínez *et al*. (2019). The quantification of micro- and macronutrients (Fe, Zn, Mn, P, Ca, Mg, K, Na and S) was performed by inductively coupled plasma-optical emission spectrometry (ICP-OES Thermo Scientific iCAP 6500 DUO, United Kingdom) based on multielement standards (High Purity® Standards, USA) and the results are expressed in mg of the element per 100 g of dry weight (mg 100 g^{-1} dw).

Statistical analysis

A database was compiled to evaluate the phenolic compounds, minerals and antioxidant activity in the seed coat, cotyledons and whole grains of each population from each spicie using three replicates per sample analyzed. Analysis of variance was performed using a completely random linear model assuming nesting of populations within species. In addition, multiple comparisons of means were performed by Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Variation in the contents of phenolic compounds and antioxidant activity

The analysis of variance revealed significant differences in polyphenols, flavonoids, anthocyanins and antioxidant activity in seed coat, cotyledons and whole grains among species (Fs > 217.0; $p \leq 0.01$) and within species or between populations (Fs > 31.0; $p \leq 0.01$). Among species, differential patterns were observed; for example, the phenolic compound contents and antioxidant activity were consistently lower in *P. vulgaris* than in *P. lunatus* or *P. coccineus*, except for anthocyanins and flavonoids in cotyledons. Most polyphenols and flavonoids and antioxidant activity were found in the seed coat, with the highest levels in *P. lunatus*, followed by *P. coccineus* and *P. vulgaris*; similarly, the concentrations of polyphenols and flavonoids in whole grains were highest in *P. coccineus*, followed by *P. lunatus* and *P. vulgaris*. In all cases, the highest concentrations of compounds and antioxidant activity were in the seed coat compared with whole grains and cotyledons (Table 1).

In whole grains of *P. coccineus*, Alvarado *et al*. (2019) observed a lower content of polyphenols (<1.68 mg GAE g^{-1}) but recorded higher values of anthocyanins in whole grains with a black and purple color (>1.07 mg C3G g^{-1}). *P. vulgaris* and *P. coccineus* had higher contents of phenols and anthocyanins in the seed coat and cotyledons, while the values observed for *P. lunatus* were like those determined by Alcázar-Valle *et al*. (2021) (Table 1).

The differences in phenolic compounds, antioxidant activity and mineral concentrations in grains among and within *P. lunatus*, *P. coccineus* and *P. vulgaris* differed slightly from the patterns found by Alcázar-Valle *et al*. (2021). For example, they did not find differences between *P. coccineus* and *P. lunatus* and in this study, the differences were evident in the seed coat and whole grains. The differences between values and response patterns are due, in part, to differences in the genotypes/landraces evaluated and genotype-environment interactions, as indicated by García-Díaz *et al*. (2018) for *P. vulgaris*.

In this study, the improved varieties of *P. vulgaris* used as controls had lower concentrations of polyphenols in the seed coat, cotyledons and whole grains than the populations of *P. lunatus* and *P. coccineus*; however, for the anthocyanin contents in the seed coat and whole grains, the behavior was reversed. Alcázar-Valle *et al*. (2021) observed the same pattern in their comparison of the same three species. In the samples of *P. coccineus* evaluated by Alvarado-López *et al*. (2019), the variation in polyphenols and flavonoids ranged from 1.29 to 2.07 mg GAE g^{-1} and from 1.08 to 1.61 mg QE g^{-1}, respectively and in this study, the variation ranged from 17.0 to 34.1 GAE g^{-1} and 1.13 to 2.32 mg EC g^{-1}, indicating that the populations evaluated have the potential to be used in plant breeding (Table 2).

Antioxidant activity

The highest concentrations of polyphenols, flavonoids and anthocyanins were recorded in the seed coat, followed by whole grains and finally cotyledons in the three species evaluated (Table 2). This pattern was also observed for antioxidant activity evaluated by the DPPH and FRAP methods (Table 3). In this case, the populations with high antioxidant activity in the seed coat were PL-018-4 (*P. lunatus*) and PC-022 (*P. coccineus*). Populations PL-014 and PL-015-1 exhibited excellent antioxidant activity in their

Table 1: Differences and similarities among *P. lunatus, P. coccineus* and *P. vulgaris* (control) grains in phenolic compounds and antioxidant activity.

Compounds and antioxidant activity evaluated	*P. lunatus*	*P. coccineus*	*P. vulgaris*
Total polyphenols (mg GAE g^{-1} dw)			
Seed coat	193.9a[1]	170.4b	89.0c
Cotyledons	3.66a	3.52a	2.06b
Whole grain	12.01b	22.22a	1.09c
Flavonoids (mg EC g^{-1} dw)			
Seed coat	12.82a	8.50b	3.17c
Cotyledons	0.09c	0.16b	0.20a
Whole grain	0.72b	1.66a	0.50c
Monomeric anthocyanins (mg C3G g^{-1} dw)			
Seed coat	4.80b	3.12c	7.19a
Whole grain	0.31b	0.19c	0.94a
Antioxidant activity (DPPH; µmol TE g^{-1} dw)			
Seed coat	1460.1a	1414.6b	783.6c
Cotyledons	26.2a	24.4b	15.1c
Whole grain	87.7b	201.1a	87.8b
Antioxidant activity (FRAP; µmol TE g^{-1} dw)			
Seed coat	569.0a	505.9b	216.0c
Cotyledons	5.15a	5.31a	2.39b
Whole grain	67.6b	113.1a	65.9b

[1]The means with the same letter in each row are not significantly different (Tukey's test, $p \leq 0.05$).

Table 2: Contents of polyphenols, flavonoids and monomeric anthocyanins in grains of *P. lunatus*, *P. coccineus* and *P. vulgaris*.

Samples	Total polyphenols (mg GAE g^{-1})			Flavonoids (mg CE g^{-1})			Anthoc. (mg C3G g^{-1})	
evaluated	Seed coat	Cotyledon	Whole grain	Seed coat	Cotyledon	Whole grain	Seed coat	Whole grain
P. lunatus								
PL-005	123.8f[1]	3.16cd	10.19f	5.9f	0.10cd	0.53de	2.89de	0.18de
PL-014	182.4cd	4.82a	12.46e	11.6c	0.09d	0.63d	4.59c	0.09de
PL-015-1	272.8a	4.03b	15.14d	20.8a	0.10cd	1.19c	1.75e	0.25de
PL-018-4	196.8bc	2.64e	10.24f	13.0b	0.07d	0.55de	9.96a	0.72c
P. coccineus								
PC-005	177.6d	3.04de	16.64cd	8.3e	0.16bc	1.31c	3.87cd	0.26de
PC-008	142.1e	2.86de	21.13b	8.6de	0.12cd	1.89b	3.90cd	0.27d
PC-022	208.9b	4.63a	34.06a	9.6d	0.21b	2.32a	1.89e	0.05e
PC-036	153.3e	3.55c	17.04c	7.4e	0.14b-d	1.13c	2.82de	0.20de
***P. vulgaris* (control)**								
PV-001	85.4gh	2.80de	0.73g	3.3g	0.11cd	0.66d	6.89b	1.33a
PV-002[2]	98.4g	2.13f	0.92g	3.7g	0.12cd	0.51de	7.70b	0.77bc
PV-003[2]	100.6g	2.08f	0.96g	2.8g	0.12cd	0.46de	9.43a	0.96b
PV-004[2]	71.4h	1.24g	1.73g	2.9g	0.44a	0.36e	4.73c	0.71c

[1]Means with the same letter in each column are not significantly different (Tukey's test, $p \leq 0.05$); [2]Improved variety.

Table 3: Antioxidant activity in grains of *Phaseolus lunatus, P. coccineus* and *P. vulgaris* (control) evaluated by the DPPH and FRAP methods.

Pop. ID/species	Seed coat		Cotyledons		Whole grain	
	DPPH[1]	FRAP[1]	DPPH	FRAP	DPPH	FRAP
P. lunatus						
PL-005	915.8g[2]	254.5fg	20.9c	3.03e	79.9e	58.4d
PL-014	1300.0e	574.8cd	37.5a	7.56a	104.6de	69.4cd
PL-015-1	1615.2c	627.6c	27.6b	5.63b	85.7e	76.2c
PL-018-4	2009.6a	819.2a	18.9c	4.38cd	80.5e	66.3cd
P. coccineus						
PC-005	1478.3d	503.9e	21.0c	4.00d	147.5c	126.8a
PC-008	1079.7f	265.9f	19.1c	4.79c	246.0b	127.6a
PC-022	1731.2b	736.6b	35.7a	8.25a	281.2a	73.5c
PC-036	1369.0e	517.4de	21.6c	4.18cd	129.8cd	124.3a
***P. vulgaris* (control)**						
PV-001	843.0g	197.3gh	18.4c	3.03e	86.3e	58.9d
PV-002[3]	892.4g	263.9f	14.3d	2.38ef	74.4e	56.3d
PV-003[3]	871.6g	260.3f	14.1d	2.02f	104.3de	92.0b
PV-004[3]	527.3h	142.5h	13.6d	2.14f	86.2e	56.5d

[1](μmol TE g^{-1} dry weight); [2]Means with the same letter in each column are not significantly different (Tukey's test, $p\leq0.05$); [3]Improved variety.

Table 4: Macro-and microelement mineral contents in the grains of three *Phaseolus* species.

Mineral	*P. lunatus*	*P. coccineus*	*P. vulgaris*
	Macronutrients (mg 100 g^{-1} dry weight)		
Ca	84.3c[1]	142.1b	157.1a
K	1681.3b	1716.4a	1589.0c
Mg	169.8b	183.0a	168.6b
P	397.2c	412.9b	488.2a
S	65.8b	78.9a	78.9a
Na	2.20b	6.55a	7.17a
	Micronutrients (mg 100 g^{-1} dry weight)		
Fe	5.46a[1]	5.39a	5.23a
Mn	1.42a	1.11c	1.25b
Zn	3.16a	3.55a	3.13a
Cu	0.545c	0.614b	0.942a

[1]Means with the same letter in each row are not significantly different (Tukey's test $p\leq0.05$).

cotyledons but low values in their whole grains. The improved varieties of *P. vulgaris* had lower antioxidant activity in their seed coat, cotyledons and whole grains, while the whole grains of *P. coccineus* had excellent antioxidant activity. This finding indicates that a larger seed size may result in a greater amount of seed coat and major antioxidant activity. Capistrán-Carabarín *et al*. (2019) recorded equivalent results and this specie offers food complementarity for its consumers but also is affected by changes during processing (Modgil *et al*., 2016).

The variation in the concentration of phenolic compounds and antioxidant activity are regularly associated with the presence or absence of intense colors in the seed coat, such as black, red, or purple. In addition, the profile of phenolic compounds in every fraction of the grain is influenced by genotype, environment and environment-genotype interactions (Alvarado-López *et al.,* 2019; García-Díaz *et al*., 2018) and in this study, it was evident that the species also affects the grain composition and antioxidant potential.

Mineral contents

Significant differences were observed in the mineral contents among species (Fs>2.5; $p\leq0.05$, 0.01) and among populations within species (Fs>1.0; $p\leq0.01$) for all the elements evaluated except Fe and Zn between species and Na between populations. The differences between species present three integral and complementary patterns in mineral micro- and macroelements: for the concentrations of Ca, P, S, Na and Cu, *P. lunatus* <*P. coccineus*≤*P. vulgaris*; for the concentrations of K and Mg, *P. vulgaris*≤*P. lunatus* < *P. coccineus*; and for the concentration of Mn, *P. coccineus* < *P. vulgaris* <*P. lunatus*. In this study, *P. lunatus* presented similar contents of Fe and Zn as *P. coccineus* and *P. vulgaris*, but the concentrations of the other six mineral elements were significantly lower in *P. lunatus* (Table 4). However, the values of K and P observed for *P. lunatus* were higher than those observed by Palupi *et al*. (2022), Jayalaxmi *et al*. (2016) and Seidu *et al*. (2015) and the values of P were similar to those reported by Giami (2006) for the same species, although methodological differences may have influenced these results. The species analyzed showed that the variation due to genotype and growth environment or crop location has a significant effect on the grain composition, as was reported for *P. vulgaris* (Bulyaba *et al*., 2020; Ribeiro and Maziero, 2022).

In the comparison of populations, the contents of Ca, K, Mg, Zn and Mg in the landraces of *P. lunatus* and *P. coccineus* were significantly higher than those in the improved varieties of *P. vulgaris* (control). A similar pattern was observed by Celmeli *et al*. (2018) comparing improved modern varieties and landraces and the first two species are potential sources

Table 5: Mineral element concentrations in grains of landraces and improved varieties of *P. lunatus* (PL), *P. coccineus* (PC) and *P. vulgaris* (PV).

Pop. ID[1]/ species	Macro and micronutrients (mg 100 g^{-1} dry weight)									
	Ca	K	Mg	P	S	Na	Zn	Fe	Mn	Cu
PL-005	100.9f[2]	1925.1ab	201.1a	509.8c	**94.7a**	1.35a	**4.49a**	**7.38a**	1.53b	0.708de
PL-014	79.1g	1903.3ab	186.9bc	483.4e	65.9a-d	1.55a	3.60a-c	7.07ab	1.51b	0.605e-g
PL-015-1	<u>73.5g</u>	1521.7de	<u>146.7e</u>	323.5j	61.3cd	3.03a	2.60cd	4.28cd	1.37c	0.466h
PL-018-4	83.6g	1451.6e	150.9e	<u>303.2k</u>	<u>47.5d</u>	2.71a	<u>2.25d</u>	<u>3.69d</u>	1.32c	<u>0.438h</u>
PC-005	146.5c	1729.8c	187.9b	377.8h	71.4a-d	8.46a	2.95b-d	4.74cd	0.91f	0.557f-h
PC-008	117.1e	1718.9c	183.0bc	454.2g	76.7a-c	5.53a	3.25b-d	5.87a-c	**1.72a**	0.514gh
PC-022	132.2d	**1960.1a**	**202.9a**	473.6f	77.8a-c	2.93a	3.88ab	5.87a-c	<u>0.77g</u>	0.705de
PC-036	164.9b	1521.8de	164.3d	362.8i	86.9a-c	8.60a	3.98ab	5.16cd	1.06e	0.664ef
PV-001	**221.6a**	<u>1431.8e</u>	162.3d	326.5j	63.7b-d	7.57a	3.97ab	4.84cd	1.73a	**1.104a**
PV-002[3]	137.5cd	1841.0b	180.4c	**583.7a**	90.2ab	4.99a	3.10b-d	5.57bc	1.09e	0.978ab
PV-003[3]	143.9cd	1470.5e	164.4d	491.1d	88.2a-c	7.83a	2.96b-d	5.62a-c	1.19d	0.885bc
PV-004[3]	133.3d	1606.9d	167.2d	535.9b	75.0a-d	8.01a	2.65cd	4.98cd	1.07e	0.836cd

[1]The prefixes PL, PC and PV indicate *Phaseolus lunatus*, *P. coccineus* and *P. vulgaris*, respectively; [2]Means with the same letter in each column are not significantly different (Tukey's test, $p \leq 0.05$); [3]Improved variety. Note: The **bold** numbers indicate the highest value and the <u>underlined</u> numbers indicate the lowest value for each mineral.

of genes for a plant breeding program or for direct consumption, according to the methods suggested by Ribeiro and Mezzomo (2020). Zn and Fe are essential mineral elements for human health and were exceptionally high in the PL-005 and PL-014 populations of *P. lunatus*, the PC-022 population of *P. coccineus* and the PV-003 population of the control species *P. vulgaris*, with 3.88 to 4.49 and 5.62 to 7.38 mg 100 g^{-1} Zn and Fe, respectively (Table 5). These values are lower than the Fe content reported by Seidu *et al.* (2015) and Palupi *et al.* (2022) in *P. lunatus* but within the ranges of Fe and Zn in *P. vulgaris* reported by Herrera-Hernández *et al.* (2018).

The evaluated populations of *P. lunatus* and *P. coccineus* had lower contents of Ca and P than the evaluated populations of *P. vulgaris.* The contents of K and Mg were greater in a population of *P. lunatus* (PL-005) and one of *P. coccineus* (PC-022) than in *P. vulgaris and* this pattern was also observed for Zn and Fe. PL-005 and PC-022 have excellent concentrations of K, Mg, S, Na, Zn and Fe and the traditional variety PV-001 (*P. vulgaris*) has excellent concentrations of Ca, Na, Zn, Fe, Mn and Cu (Table 5).

CONCLUSION

The present study focused on *P. lunatus* and *P. coccineus* as promising sources of nutritional and nutraceutical compounds with high antioxidant activity levels and mineral contents. Significant differences were recorded between *P. lunatus* and *P. coccineus* relative to *P. vulgaris* (control) in the contents of total polyphenols, flavonoids, macroelements and microelements (except for Fe and Zn) and antioxidant activity and there were also significant differences and high variability between populations evaluated for all the compounds (except Na). The populations of each species are complementary in terms of the nutrition they provide when whole grains are consumed. The highest concentrations of phenolic compounds and highest levels of antioxidant activity were observed in the seed coat, followed by whole grains and cotyledons, indicating that the seed coat is an important source of bioactive compounds for nutraceutical purposes.

Conflict of interesst: None.

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