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Evaluation of foliar phenols of 25 Mexican varieties of common bean (*Phaseolus vulgaris* L.) as antioxidants and varietal markers

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The antioxidant properties and the foliar phenol composition of 25 Mexican varieties of *Phaseolus vulgaris* L. (common bean) were evaluated. *Phaseolus coccineus* was analysed with comparative aims. The high performance liquid chromatography with photodiode array detection analysis revealed 27 phenolics in the leaves of *P. vulgaris* (13 quercetin-3-*O*-glycosides, 8 kaempferol-3-*O*-glycosides, 2 myricetin glycosides and 4 phenolic acids) and 5 in *P. coccineus* (2 kaempferol-3-*O*-glycoside, 2 apigenin-7-*O*-glycoside and 1 luteolin-7-*O*-glycoside). All extracts showed high levels of phenols and flavonoids (0.964–5.601 mg g^{-1} dry tissue, and 0.287–1.418 mg g^{-1} dry tissue, respectively) and relevant antioxidant properties, suggesting that the leaves of the varieties of *P. vulgaris* are a significant source of natural antioxidants. The foliar phenol profiles were species-specific and, besides, the qualitative variation allowed discriminating among varieties of *P. vulgaris*. These profiles can represent an important varietal authenticity proof.

Keywords: *Phaseolus vulgaris*; antioxidant capacity; chemical markers; foliar flavonoids; phenolic acids

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is a legume cultivated all around the world but its diversification and domestication occurred in Mexico (Bitochi et al. 2013). The genus *Phaseolus* was originated at the American continent (Bitochi et al. 2013), it includes around 52 species; 40 being endemic to Mexico (Castillo et al. 2006). From many points of view, the most important species of *Phaseolus* is *P. vulgaris*. In Mexico, common bean grows under very contrast environmental conditions, suggesting that *P. vulgaris* has a highlighting biological capacity supported by an important genetic variability (Acosta-Gallegos et al. 2007).

Phenolic compounds are plant secondary metabolites, many of them, principally flavonoids, have biological properties with medical implications, antioxidant capacity being one of the most important properties because oxidants are involved in the development of cardiovascular disorders, neurodegenerative diseases and cancer (Ames et al. 1993). Flavonoids are important antioxidants that can be used in food industry, oppositely to the synthetic ones, which have been perceived as toxic and carcinogenic (Madhavi & Salunkhe 1996). The synthesis and

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accumulation of flavonoids in plant tissues have been reported with a species-specific tendency (Almaraz-Abarca et al. 2013), which make them important chemotaxonomic markers. The flavonoid composition of *P. vulgaris* has been mainly studied in the grains (seeds) because these are the edible structures (Romani et al. 2004). The foliar flavonoid composition of *P. vulgaris* has received little attention, probably because leaves are not consumed as food by humans. However, in Mexico and other countries of Latin America, the leaves of common bean are used as forage, and their accumulated flavonoids could be an important source of antioxidants for animal health. Chemical fingerprinting could assist in the authentication of varieties of common bean and in generating a better knowledge for using all the potential of this cultivated species. The aim of this study was to determine the foliar phenol composition of 25 cultivars of *P. vulgaris* developed in the National Research Institute of Forestry, Agriculture and Livestock (INIFAP Mexico), in order to evaluate the potential of these cultivars as source of antioxidant flavonoids, and to assess the significance of foliar phenol profiles as typification and authenticity tools to discriminate among cultivars of common bean.

2. Results and discussion

2.1. *Phenol and flavonoid contents*

The levels of total phenols varied significantly between varieties (Table 1). These levels were lower than those reported for the leaves of *Mesembryanthemum edule* (68.75 mg g^{-1} dry tissue), traditionally used in folk medicine in Africa (Falleh et al. 2011), and also lower than those reported for *Tamarix gallica* leaves (34.44 mg g^{-1} dry tissue), used for the treatment of many health disorders in Tunisia (Ksouri et al. 2009). Table 1 also shows the levels of flavonoids accumulated in the leaves of the analysed common bean varieties; the levels ranged significantly. The amounts of flavonoids accumulated in the samples of this study were lower than those reported by Falleh et al. (2011) for the leaves of *M. edule* (22.21 mg g^{-1} dry tissue), and closer to those reported for *T. gallica* leaves (3.91 mg g^{-1} dry tissue) by Ksouri et al. (2009).

2.2. *Phenol composition*

Patterns comprising 13 quercetin-3-*O*-glycosides, 8 kaempferol-3-*O*-glycosides, 2 myricetin glycosides and 4 phenolic acids were found in foliar tissues of *P. vulgaris*. The composition of the leaves of *Phaseolus coccineus* was very different, formed by two kaempferol-3-*O*-glycosides, two apigenin-7-*O*-glycosides and one luteolin-7-*O*-glycoside. The UV spectra and retention time of these 32 phenols are shown in Figures S1–S4. In *P. coccineus*, flavonols were found as minor components and flavones were the major components, and vice versa in *P. vulgaris*. Some varietal differences were found in *P. vulgaris* (Table S1). As example of that variation, the chromatograms of six varieties are shown in Figure S5. Compound **17** (quercetin-3-*O*-glycoside) was present in all the varieties of common bean and it was among the compounds accumulated at the highest levels (Table S1). Quercetin is a flavonoid with a broad spectrum of biological activity due to its *O*-orthodihydroxy structure in ring B, while kaempferol, which has a ring B bearing a single 4′-hydroxyl group, has a decreased antioxidant activity (Rice-Evans 1999). Glycoside derivatives of quercetin are among the major flavonoids in species recognised by their favourable effect on the human health, among them *Gingko biloba* (Mouren et al. 1994). This suggests that the main contribution to the antioxidant properties of the foliar extracts of common bean come from its quercetin derivatives; in each extract of *P. vulgaris*, the quercetin glycosides represented between 50% and 75%. Recently it was reported that kaempferol has inhibitory activity of human histone deacetylases; due to the acetylation of histones is related to cancer development, compounds having that activity are currently being studied as new preventive or therapeutic anticancer compounds (Berger et al. 2012). Our results indicated that

Table 1. Total phenols, total flavonoid content, free radical scavenging activity (EC_{50}), TAC and iron-reducing power of the foliar extracts of 25 varieties of *P. vulgaris* and *P. coccineus* (26 days).

Sample	Total phenols (mg GAE g^{-1} dry tissue)	Total flavonoids (mg QE g^{-1} dry tissue)	EC_{50} ($\mu g\ mL^{-1}$)	TAC (μg AA mL^{-1})	RP ($A_{700\,nm}$)
Bayo Victoria	5.601 ± 0.170 a	0.358 ± 0.025 ghi	2.450 ± 0.009 k	13.655 ± 0.264 cdf	0.901 ± 0.016 a
Pinto Bravo	5.268 ± 0.085 a	0.390 ± 0.040 fghi	3.256 ± 0.020 c	11.111 ± 1.554 fghi	0.758 ± 0.006 de
Azufrado Namiquipa	4.562 ± 0.331 b	0.556 ± 0.046 e	2.024 ± 0.011 q	8.184 ± 1.059 hi	0.508 ± 0.006 k
Negro Vizcaya	4.485 ± 0.152 b	0.321 ± 0.024 hi	3.719 ± 0.019 a	15.733 ± 0.446 cde	0.751 ± 0.011 e
Canario Regional	4.059 ± 0.173 bc	0.290 ± 0.023 i	3.349 ± 0.026 b	15.988 ± 1.732 bcde	0.620 ± 0.004 i
Negro San Luis	4.002 ± 0.068 bcd	0.351 ± 0.014 ghi	2.965 ± 0.018 e	19.932 ± 0.652 ab	0.765 ± 0.004 de
FM 2000	3.839 ± 0.269 cde	0.846 ± 0.094 c	1.796 ± 0.007 r	7.803 ± 2.277 i	0.754 ± 0.012 de
Azufrado Higuera	3.812 ± 0.076 cde	0.419 ± 0.003 fgh	2.350 ± 0.004 m	8.439 ± 0.847 hi	0.613 ± 0.003 i
Pinto Saltillo	3.576 ± 0.115 cde	0.287 ± 0.012 i	3.213 ± 0.002 c	12.553 ± 0.847 defg	0.583 ± 0.008 j
Flor de Mayo M-38	3.492 ± 0.273 def	0.360 ± 0.013 ghi	2.914 ± 0.024 f	16.242 ± 1.096 bcd	0.926 ± 0.002 a
P. coccineus	3.488 ± 0.091 def	1.034 ± 0.040 b	3.340 ± 0.012 b	9.329 ± 0.481 ghi	0.464 ± 0.004 lm
Pinto Centauro	3.450 ± 0.71 def	0.681 ± 0.020 d	2.444 ± 0.016 kl	14.843 ± 0.446 cdef	0.873 ± 0.009 b
Pinto Mestizo	3.381 ± 0.063 ef	0.296 ± 0.030 i	2.711 ± 0.015 hi	14.122 ± 1.101 cdef	0.692 ± 0.004 g
Flor de Junio Ana	3.001 ± 0.110 fg	0.440 ± 0.033 fg	2.399 ± 0.006 l	17.09 ± 1.209 bc	0.648 ± 0.019 h
Negro V8025	2.945 ± 0.154 fg	0.389 ± 0.024 fghi	2.692 ± 0.008 hi	11.238 ± 1.407 fghi	0.490 ± 0.002 kl
Flor de Junio Marcela	2.732 ± 0.155 gh	0.454 ± 0.040 efg	1.763 ± 0.010 r	22.094 ± 1.517 a	0.832 ± 0.006 c
Pinto Durango	2.616 ± 0.138 ghi	0.290 ± 0.006 i	3.065 ± 0.017 d	22.900 ± 1.376 a	0.776 ± 0.010 de
Negro Frijozac 101	2.613 ± 0.056 ghi	0.311 ± 0.020 i	2.717 ± 0.011 h	12.595 ± 1.834 defg	0.449 ± 0.003 m
Bayo Madero	2.294 ± 0.038 hij	1.418 ± 0.050 a	2.131 ± 0.008 p	22.688 ± 1.972 efgh	0.829 ± 0.001 c
Flor de Mayo Sol	2.133 ± 0.161 ijk	0.552 ± 0.055 e	1.474 ± 0.012 t	12.044 ± 0.264 efgh	0.839 ± 0.014 c
Negro Zacatecas	2.014 ± 0.214 jk	0.293 ± 0.005 i	2.665 ± 0.018 i	16.921 ± 1.328 bc	0.721 ± 0.010 f
Pinto Bayacora	1.749 ± 0.094 jkl	0.487 ± 0.014 ef	1.601 ± 0.022 s	11.068 ± 0.127 fghi	0.585 ± 0.007 j
Pinto Libertad	1.723 ± 0.482 kl	0.322 ± 0.026 hi	2.858 ± 0.017 g	12.637 ± 0.920 defg	0.703 ± 0.007 fg
Negro Altiplano	1.699 ± 0.109 kl	0.331 ± 0.017 hi	2.584 ± 0.006 j	11.620 ± 1.036 fghi	0.776 ± 0.006 de
Río Grande Querétaro	1.378 ± 0.135 lm	0.451 ± 0.013 efg	2.195 ± 0.013 o	8.269 ± 1.749 hi	0.662 ± 0.007 h
Vaquita	0.964 ± 0.048 m	0.309 ± 0.025 i	2.27 ± 0.015 n	12.510 ± 1.909 defg	0.721 ± 0.002 f
Quercetin			0.400 ± 0.006 v	Not evaluated	0.780 ± 0.004 d
Quercitrin			1.293 ± 0.018 u	22.448 ± 0.815 a	0.395 ± 0.004 d
Caffeic acid			0.403 ± 0.003 v	Not evaluated	Not evaluated
Ascorbic acid			Not evaluated	Not evaluated	0.653 ± 0.005 h

Notes: GAE, gallic acid equivalents; QE, quercetin equivalents; EC_{50}, efficient concentration at 50%; TAC, total antioxidant capacity; RP, iron-reducing power.
The values represent the mean and standard deviation of three independent analyses.
Different letters in the same column mean significant differences (Duncan's multiple range test; $p < 0.5$).

the phenol composition of leaves was different from the composition reported for seeds by Romani et al. (2004), who found several kaempferol and quercetin glycosides, isoflavones and anthocyanins in different cultivars of *P. vulgaris.*

2.3. *Antioxidant capabilities*

A linear reduction of DPPH* concentration associated with increase in the flavonol concentration in the extracts was observed ($0.9307 > r > 0.9987$). The EC_{50} values ranged significantly between varieties (Table 1). The antiradical activities were higher than those found for foliar extracts of *Smallanthus sonchifolius* (EC_{50} values between 50.7 and 11.3 $\mu g\,mL^{-1}$) reported by Arnao et al. (2011). The antiradical activities of the varieties Flor de Mayo Sol ($EC_{50} = 1.474\,\mu g\,mL^{-1}$) and Pinto Bayacora ($EC_{50} = 1.601\,\mu g\,mL^{-1}$) were similar to that of quercitrin ($EC_{50} = 1.293\,\mu g\,mL^{-1}$). The total antioxidant capacity (TAC) values for each sample are given in Table 1. Pinto Durango was the variety with the highest TAC ($22.9\,\mu g\,mL^{-1}$), this activity was similar to that of quercitrin, but lower than that reported by Falleh et al. (2011) for the leaves of *M. edule*. The phenols of the foliar extracts of *P. vulgaris* also showed an important capability to donate electron to free radicals, which contributes to stabilisation of reactive species and ending a free radical chain reaction, as it is shown by the values of absorbances at 700 nm (Table 1). All the extracts assayed showed higher iron-reducing power than quercitrin, 17 had higher reduction power than ascorbic acid and 6 varieties had reduction power values higher than quercetin ($A_{700\,nm} = 0.780$). Excepting the samples of Negro V8025 and Negro Frijozac 101 ($A_{700\,nm} = 0.490$ and 0.449, respectively), all the foliar extracts of *P. vulgaris* showed higher iron-reducing capacities than those found for rutin, an important natural antioxidant flavonoid, and its acylated derivatives (values of $A_{700\,nm}$ between approximately 0.020 and 0.500) reported by Lue et al. (2010).

2.4. *Variation in the foliar phenol profiles*

The foliar phenol profiles have been reported to have a species-specific tendency in several groups of plants like in *Agave* (Almaraz-Abarca et al. 2013), among others. This tendency was corroborated in this study for *P. vulgaris* and *P. coccineus* (Figures S5 and S6). Some chemical differences in the foliar phenol composition at level of varieties were found, as it is observed in the dendrogram resulted from a cluster analysis (Figure S6) based on a binary matrix of presence–absence constructed with the foliar phenol profile of each variety. Each variety had a typical foliar phenol profile; this makes those profiles important tools for the evaluation of variety authenticity for this crop plant at relatively early stages of development (26 days), mainly for varieties having very similar seeds (Figure S7). Pinto and Negro varieties shared more phenolic compounds, one to each other and were grouped in the same clade (B) by the cluster analysis, while the rest of the varieties formed a separated group (A) (Figure S6).

3. Conclusions

Varieties of common bean can synthesise and accumulate different phenolic compounds in leaves, majorly quercetin-3-*O*-glycosides, in a typical manner for each variety; this reveals the foliar phenol profiles of *P. vulgaris* as important chemical markers for the determination of authenticity of varieties. The high antioxidant capacities found for the foliar phenol extracts suggest that the leaves of that species are a valuable source of natural antioxidant phenols; this is relevant for animal health because the leaves of common bean are used to elaborate forage. The chemical variability of *P. vulgaris* offers the opportunity for developing new cultivars with

enhanced accumulation of compounds having health and nutritional effects for human and animals, through breeding programmes.

Supplementary material

Experimental details relating to this article are available online, alongside Table S1 and Figures S1–S7.

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