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RESEARCH ARTICLE

Variation of the phenolic composition and α-glucosidase inhibition potential of seeds, soaked seeds, and sprouts of four wild forms and four varieties of common bean (*Phaseolus vulgaris*)

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Abstract

The determination of the changes in the composition of bioactive phenolic compounds of germinating seeds which accumulate high levels of these compounds could contribute to the understanding of the germination mechanism and the development of markers for the selection of plant genotypes. In the current study, the changes in the phenolic composition and α-glucosidase inhibition activity, taking place during the germination of four wild forms and four varieties of common bean (*Phaseolus vulgaris* L.) from Durango Mexico, were determined. A total of 66 phenolic compounds (19 phenolic acids, 18 isoflavones, 18 flavonol glycosides, 3 flavonol aglycones, 3 flavones, 2 dihydroflavonoids, 2 chalcones and one non-identified type) were found by HPLC-DAD, which were differentially accumulated by the seeds, 24 h-soaked seeds, and 4 day-sprouts of each genotype. The accumulation of the flavonol aglycones, myricetin, quercetin and kaempferol was distinctive of the wild seeds. Soaking not only caused leaching and degradation but also triggered the synthesis of new phenolic compounds whereas germination diversified the composition of isoflavones and flavonol glycosides. The seeds of all genotypes analyzed were important inhibitors of α-glucosidase, improving their potential after soaking and germination. The results suggested that the structure rather than the concentration of the flavonoids and phenolic acids determined the inhibitory potential of α-glucosidase of samples. The principal component analysis and cluster analysis revealed HPLC-DAD phenolic profiles as genotype-specific chemomarkers at any of the states (seeds, soaked seeds, and sprouts). The results have wide implications on agronomy and food quality.

Keywords

Common bean; phenolic profiles; α-glucosidase inhibition; varieties, wild forms

Introduction

In addition to the physiological and morphogenetic processes that take place during seed germination (1), changes in the synthesis and accumulation of specialized metabolites also can happen (2). These metabolites play important physiological roles in germinating seeds (3) and have agronomic (4, 5) and pharmaceutical importance (6, 7). For *Paseolus vulgaris*, one of the most economically important edible species of Fabaceae, in whose seeds, phenolics are the predominant bioactive compounds (8), the deter-

mination of the changes in its composition could support the understanding of its germination mechanism, as well as the development of markers for the selection of genotypes accumulating high levels of phenolics and the evaluation of the potential of common bean sprouts as a functional food.

Phenolics are among the major bioactive plant specialized metabolites which improve human health (6, 7, 9-11). One of the most relevant bioactivities of phenolic compounds is their capacity to inhibit the activity of enzymes involved in the metabolism of sugars (12), Thus, plant foods which are rich in these compounds are so important because they have the hypoglycemic potential. The great diversity of bioactivities of phenolic compounds encourages the constant exploration of plants as sources of these compounds.

The seed phenolic composition of *P. vulgaris* varies among the different cultivated genotypes (8, 13, 14), causing changes in their functional properties (13). This variation could be involved in changes in the germination potential and the functional properties of their sprouts. Some studies have been focused on the topic, like those for a black variety of common bean (15, 16). Despite the important contributions already done, there is still a lacuna in the undersatding relating to the phenolic changes taking place during germination of commercially important varieties of common bean. This lack of knowledge is greater for wild genotypes which may represent sources of valuable alleles for developing or improving the existing varieties.

Mexico is the center of origin of *P. vulgaris* and one of its main diversity and domestication centers where a lot of wild populations occur (17). In the north-central region occupied by the state of Durango, the fourth largest state (123 181 Km^2) of the country, due to the complex physiography and high climatic diversity (18), many wild populations of *P. vulgaris* with high genetic variability can be found (19). Despite their wide importance, only a few of these wild populations have been studied for their phenolic composition (20). So, the objective of the present study was to determine and compare the changes in the phenolic composition and α-glucosidase inhibition potential during the germination of the seeds of four wild forms and four varieties of common bean, from Durango, Mexico.

Materials and Methods

Plant material

Four wild forms of common bean were collected from October to November 2018 directly from their natural populations in Durango (Table 1). A sample of 100 seeds from each locality was deposited in the Germplasm Collection of the Interdisciplinary Research Center for Integral Regional Development, Durango unit of the National Polytechnic Institute (Mexico) (CIIDIR-IPN-Durango). Four cultivated varieties (*Frijozac, Negro Bola, Vaquita,* and *Cacahuate*), representing the 2018 harvest, were donated by the National Institute of Forestry, Agricultural and Livestock Research in the state of Durango (INIFAP Durango), Mexico. Seeds of each sample (10 g) were independently ground and sieved to 0.5 mm diameter particle size and stored in plastic bags at room temperature until analysis.

Imbibition

Seeds of each sample (10 g) were imbibed in distilled water (1:10 w/v), in dark for 24 h in an aired and dark incubator. Then, soaked seeds were dried at 40°C in a botanical air oven overnight, ground and sieved to 0.5 mm diameter particle size, and stored in plastic bags until analysis.

Germination

Seeds of each sample (10 g) were disinfected with 5% sodium hypochlorite, then three times rinsed with autoclaved distilled water and transferred to Petri dishes lined with wet filter paper. Seeds were germinated in an aired and dark incubator for 96 h at 27°C. Distilled water was spread on seeds every 24 h. Sprouts were dried in a botanical air oven at 40°C overnight and prepared as described for seeds and soaked seeds.

Quantification of total phenolics (TP)

Phenolic extracts were prepared from 5 g of dry tissue combined with 45 mL of 80% ethanol. Mixtures were sonicated for 1 h at room temperature and centrifuged (6000 rpm, 10 min). Supernatants represented the phenolic extracts. TP values were determined according to Skotti *et al.* (21), from a standard curve of gallic acid (slope: 8.2759, axis crossing point: 0.1013, r = 0.9970) and expressed as milligrams equivalents of gallic acid per gram of dry tissue (mg/g DT).

Phenolic profiles

Phenolic profiles were determined in a Perkin Elmer Flexar HPLC-DAD system, using a gradient method (22), with a Perkin Elmer Brownlee Analytical C18 column (4.6 × 250 mm, 5 µm). Chromatograms were registered at 260 and 340 nm. UV spectra of the resolved peaks were registered between 200 and 400 nm, using a Perkin Elmer Flexar diode array-detector (DAD). The injection volume was 50 µL and the flow rate was 0.8 mL/min. Structural information was obtained by interpreting the UV spectra according to the UV theory developed for flavones, flavonols, and phenolic acids (22), as well as by comparing the retention time (RT) and λ_{max} of the resolved compounds in the chromatograms with those of the following standards:

Table 1. Collections of common bean (*Phaseolus vulgaris*) seeds from four wild populations of Durango, Mexico.

Population	Register Number	Latitude N	Longitude W	Altitude (m)
El Mezquital	AIG-136	23° 27´ 48.1´´	104° 29´ 49.5´´	1400
Nombre de Dios	AIG-138	24° 05' 71''	104°14´ 23.0´´	1877
Nuevo Ideal	AIG-140	24° 45´11.9´´	105° 00´ 05.6´´	2037
Pueblo Nuevo	AIG-141	23° 51' 59.4''	105° 36' 29.7´´	1892

Vanillic acid (RT: 23.425; λ_{max}: 258, 290sh), gallic acid (RT: 5.667 min; λ_{max}: 270), rutin (quercetin-3-*O*-[rhamnosyl (1-6)-glucoside]; RT: 36.207 min; λ_{max}: 255, 265sh, 295sh, 356), hiperoside (quercetin-3-*O*-galactoside; RT: 34.951 min; λ_{max}: 256, 268sh, 304sh, 360), quercitrin (quercetin-3-*O*-rhamnoside; RT: 37.789 min; λ_{max}: 255, 263sh, 295sh, 350), myricetin (3,3',4',5,5',7-hexahydroxyflavone; RT: 42.500 min; λ_{max}: 254, 267sh, 300sh, 372), quercetin (RT: 48.19 min; λ_{max}: 256, 297sh, 371), baicalein (5,6,7-trihydroxyflavone; RT: 53.91 min; λ_{max}: 277, 321), morin (RT: 42.709 min; λ_{max}: 252, 262sh, 291sh, 318sh, 353), and naringenin (RT: 46.68 min; λ_{max}: 289, 325sh). Only the well-defined UV spectra were considered. The concentrations of compounds were estimated by area measurements, using a standard curve of rutin (slope: 6564.40, intercept: 127.33, r = 0.9965) for flavonols, and a standard curve of gallic acid (slope: 39870.00, intercept: 6372.9, r = 0.9985) for phenolic acids. Concentrations were expressed as micrograms per gram of dry tissue (µg/g DT). The total contents of flavones, isoflavones, dihydroflavonoids, flavonol glycosides, flavonol aglycones, and phenolic acids were estimated by adding the individual concentrations of compounds of the same type in one sample. Concentrations were reported as milligrams per gram of dry tissue (mg/g DT).

Inhibition of α-glucosidase and α-amylase activities

These assays were carried out according to Kim *et al.* (23) for the phenolic extract (10 µg/mL) of each sample, using *α*-glucosidase from *Saccharomyces cerevisiae*, and acarbose (1 mM) as a positive control. The results represented the percent inhibition relative to acarbose having 100% inhibition.

Data analysis

All the analyses were carried out for three independent pools of each sample. Data were subjected to an analysis of variance ($p < 0.05$) and means were separated by the Tukey test. To determine the contribution of each parameter to the discrimination of samples, data were submitted to a principal component analysis (PCA). To determine the similarity between samples, data were submitted to cluster analysis. Data analysis was carried out using Past 4.07b.

Results and Discussion

Total phenolics of seeds, soaked seeds, and sprouts

The significant ($p < 0.05$) variation in the content of total phenolics in the seeds analyzed is shown in Table 2. Differences in contents were less variable among the wild populations (between 7.32 and 10.00 mg/g DT) than among the varieties (between 4.69 and 13.09 mg/g DT). The variation was significant ($p < 0.05$) even between the two black beans analyzed (*Frijozac*: 11.27 mg/g DT and *Negro Bola*: 6.79 mg/g DT), which is in agreement with the proposal of Espinosa-Alonso *et al*. (24), about that the variation observed in the phenolic contents of common bean seeds is variable and more related to the genotype than to other factors, such as color. Comparatively, any of the seeds analyzed accumulated higher levels of total phenolics than other genotypes of common bean, such as *Arthopurpurea*, for which Alonso *et al.* (25) reported 2.07 g/Kg dry matter. The current results reveal the common bean genotypes analyzed as important sources of phenolic compounds. This is relevant due to the important bioactivities that these compounds have (7, 9).

Soaking for 24 h caused decreases in the concentration of total phenolics in all beans analyzed (Table 2). The percentage of decrease was highly variable among the wild beans, from 1.06 % for those from El Mezquital to 24.92 % for those from Nuevo Ideal. Among the varieties, the percentage of decrease was less variable (between 22.53% and 37.55%) and higher than the reduction found for 3 out of 4 wild common beans analyzed. Other varieties were found to respond differently, such as *Negro San Luis*, for which Guajardo-Flores *et al.* (16) reported that soaking caused no significant reduction in the total phenolics content. Characteristics of hilum, which is the water entrance point, cotyledon composition, and seed coat attributes influence the rate of water uptake (26). Thus, variations in these characteristics could partly explain the differences observed among the eight genotypes analyzed, those of the wild forms being able to prevent to a greater degree the loss of these compounds by leaching in the soaking water, like Guajardo-Flores *et al.* (15) reported that it occurred in the variety *Negro San Luis*. The soaked wild seeds from El Mezquital highlighted for their reduced loss of phenolics. Soaking bean seeds before cooking is a common

Table 2. Contents of total phenolics (mg/g DT) of dry seeds, 24 h-soaked seeds, and sprouts (4 days) of four wild and four varieties of common bean (*Phaseolus vulgaris*).

Sample	Dry Seeds	Soaked Seeds	Sprouts
El Mezquital (W)	10.00±0.57 c	9.89±0.40 e (-1.06)	2.54±0.02 a (-74.57)
Frijozac (V)	11.27±0.26 d	7.04±0.30 d (-37.55)	6.56±0.03 e (-41.82)
Nombre de Dios (W)	7.20±0.10 b	6.47±0.17 cd (-10.18)	3.34±0.11 b (-53.62)
Negro Bola (V)	6.79±0.30 b	5.26±0.22 b (-22.53)	11.66±0.35 f (+71.67)
Nuevo Ideal (W)	7.42±0.01 b	5.57±0.09 b (-24.92)	5.08±0.08 cd (-31.44)
Pueblo Nuevo (W)	7.32±0.37 b	5.96±0.26 b (-18.53)	5.66±0.04 d (-22.67)
Vaquita (V)	4.69±0.17 a	3.47±0.15 a (-25.99)	5.43±0.25 cd (+15.71)
Cacahuate (V)	13.09±0.22 e	10.11±0.29 e (-22.80)	5.03±0.09 c (-61.58)

W: Wild sample; **V**: Variety. The values represent the mean and standard deviation for three independent samples. Different letters in the same column mean significant differences ($p < 0.05$). Figures in parenthesis represent percentage decrease (-) or increase (+) compared to seeds.

practice to facilitate softening. However, this practice can reduce the functional properties of this food if the soaking water is not incorporated into the cooking process.

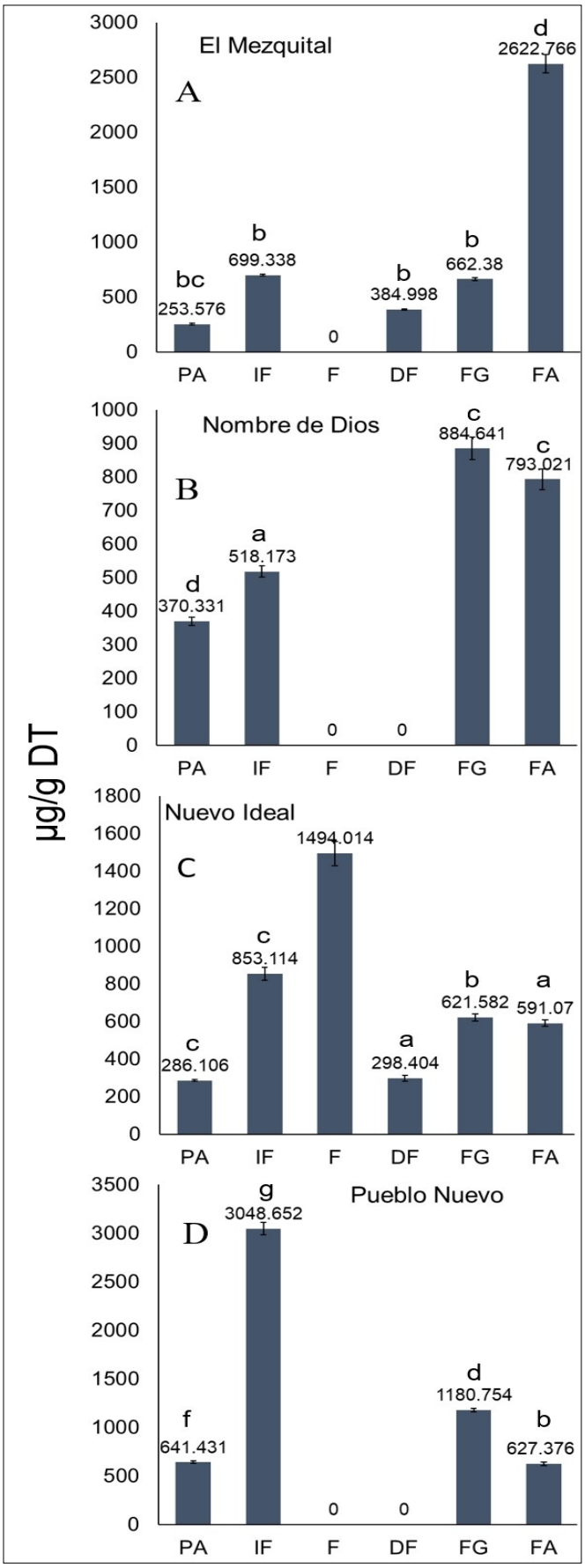
Variable decreases in the phenolic contents also occurred during the germination of all wild common beans analyzed (Table 2). Except for the sprouts of the varieties *Negro Bola* and *Vaquita*, which increased their levels of total phenolics by 71.67% and 15.71%, respectively, those of the other three varieties decreased. Other varieties have responded differently during germination, such as *Athropurpurea* (25), which reduced the level of these compounds after 24 to 72 h of germination, and *Negro San Luis*, which revealed no significant reduction in its total phenolic content (16). The different responses observed could be a consequence of the particular requirements for these compounds during germination by different genotypes of common bean, either wild or cultivated. The particular requirements may be the result of genetic differences emerged among the different genotypes of common bean, controlling responses to imbibition and germination. The variety *Negro Bola* has an important potential for the preparation of sprouts with a high content of total phenolics.

Phenolic compounds of seeds, soaked seeds, and sprouts

The results of the HPLC-DAD analysis revealed 66 different phenolic compounds accumulated in the seeds, soaked seeds, and sprouts of the eight genotypes analyzed of common bean. The structural information obtained from the 66 UV spectra (Supplementary Fig. S1) suggested that 19 (1, 3-8, 18, 20, 22, 23, 26, 27, 32, 33, 35, 37, 43, 55) were phenolic acids, 18 (2, 9-11, 13, 15, 16, 21, 29, 34, 40, 50, 51, 57, 59, 60, 63, 64) were isoflavones, three (12, 14, 47) were flavones, and two (19, 24) were dihydroflavonoids. Besides, 18 flavonol glycosides were found, distributed as one myricetin-3-*O*-glycoside (25), nine kaempferol-3-*O*-glycosides (28, 41, 42, 44, 46, 48, 53, 54, 56), three quercetin-3-*O*-glycosides (31, 39, 45), one kaempferol-3,7-*O*-diglycoside (52), and four flavonol glycosides to which a type could not be assigned (17, 30, 36, 49). Also, three flavonol aglycones were found: myricetin (58), quercetin (62), and kaempferol (66) to two chalcones (38, 61), and one non-identified type of phenolic compound (65). Phenolic acids and isoflavones, as well as several glycoside derivatives of myricetin, quercetin, and kaempferol, have also been found in other different varieties of common bean (13, 24, 27). The current results reveal a diverse and complex phenolic composition of seeds, soaked seeds, and sprouts of common bean, which is variable among genotypes, as will be seen in the following sections. This richness and complexity suggest important roles for phenolic compounds in these three developmental states of *Phaseolus vulgaris*. The knowledge of the phenolic composition of common bean seeds and the changes occurring during soaking and germination in different genotypes may support genetic improvement programs focused on developing new common bean varieties with enhanced levels of specific compounds.

Phenolic compositions of seeds

The seed phenolic composition was qualitatively and quantitatively variable among the 8 samples of common bean analyzed (Fig. 1). Phenolic acids and isoflavones were present in the 8 samples, and the highest level of these types of compounds was found in the variety *Negro Bola*; however, the wild beans from Pueblo Nuevo accumulated also high concentrations of both phenolic acids and isofla-



vones. For the seeds of other varieties, such as the dark bean *Tolosana*, no isoflavones were found (28). Flavones were poorly distributed among the genotypes, only the seeds from the wild bean from Nuevo Ideal accumulated important concentrations of these compounds.

From Fig. 1, two aspects must be highlighted, the first one, is the presence of flavonol aglycones in the four wild common beans and its absence in the four varieties analyzed. Our results are in agreement with what was reported for the variety *San Luis* (a black bean), for which Guajardo-Flores *et al.* (15) found no aglycone form. However, our results contrast with those of Aparicio-Fernandez *et al*. (29), who found myricetin in the black bean Jamapa and those of López *et al*. (28), who found quercetin and kaempferol in the cultivar *Tolosana* (a dark bean). The second aspect to highlight is that none of the four varieties accumulated dihydroflavonoids, which is in disagreement with the results of López *et al.* (28), who reported hesperetin and naringenin glycosides (two dihydroflavonoids) in the cultivar Tolosana. The current results compared to those of Aparicio-Fernandez *et al.* (29) and López *et al.* (28) suggest that *P. vulgaris* has high genetic variability, which causes differences in the phenolic composition between genotypes.

Our results revealed that, although the seed phenolic profile of the eight common beans shared compounds, as expected for genotypes of the same species, a particular profile was observed for each genotype, someone of them having unique compounds, such as the seeds from El Mezquital (compound 17), *Frijozac* (compound 23), Nombre de Dios (compound 55), Nuevo Ideal (compound 14), and Pueblo Nuevo (compounds 6, 16, 36 and 37), as it can be observed in the HPLC chromatograms shown in Fig. 2. The chromatograms also revealed that the seeds of the wild beans analyzed accumulated a higher richness of phenolics (between 12 and 15 compounds) than those of the varieties (between 7 and 9 compounds). Our results are in agreement with the proposal of Corso *et al.* (3) about that the diversity of specialized metabolites (like phenolics) has been reduced in most domesticated crops. The phenolic profiles have been reported to have a species-specific tendency for several plant species, such as species of *Physalis* (30), species of Verbenaceae (31), and species of Brassicaceae (32), among others. At level varietal, the potential of the foliar phenolic profiles to discriminate varieties was revealed by Reyes-Martínez *et al.* (33) for 25 varieties of common bean. Our results indicate that the seed phenolic profiles also have an important discriminatory potential at the level of variety and genotype, as revealed by the PCA and cluster analysis based on the matrix constructed with the profiles of each sample. The results of the PCA (Fig. 3A) separated the four wild beans from the four varieties, mainly by the contents of compounds 58 (myricetin), 62 (quercetin), 66 (kaempferol), 7 (a phenolic acid) and 24 (a dihydroflavonoid) in the wild genotypes. The three aglycone forms (myricetin, quercetin, and kaempferol) were only found in the four wild beans analyzed, suggesting that these aglycones can be a distinctive chemical attribute of the wild common beans; however, more wild genotypes should be

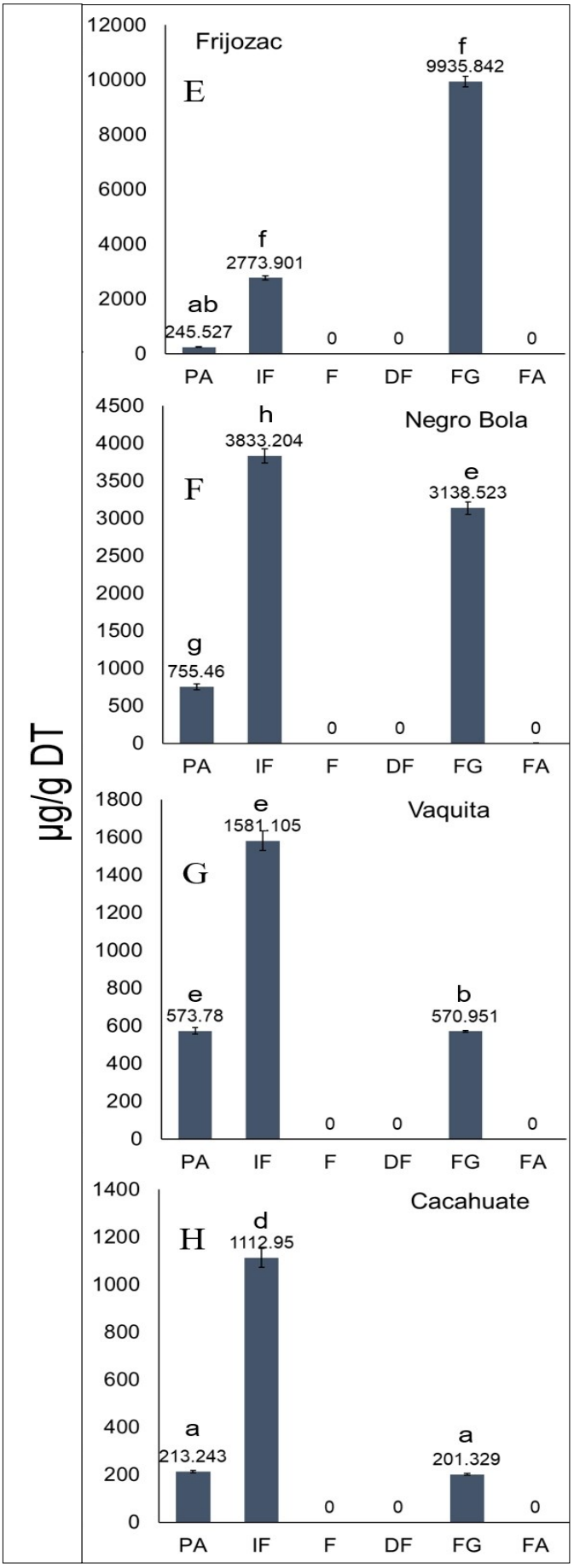


Fig. 1. Contents of phenolic acids (**PA**), isoflavones (**IF**), flavones (**F**), dihydroflavonoids (**DF**), flavonol glycosides (**FG**), and flavonol aglycones (**FA**) in the dry seeds of four wild (**A-D**) and four varieties (**E-H**) of common bean. Different letters above bars of the same type of compounds mean significant differences ($p < 0.05$) between genotypes.

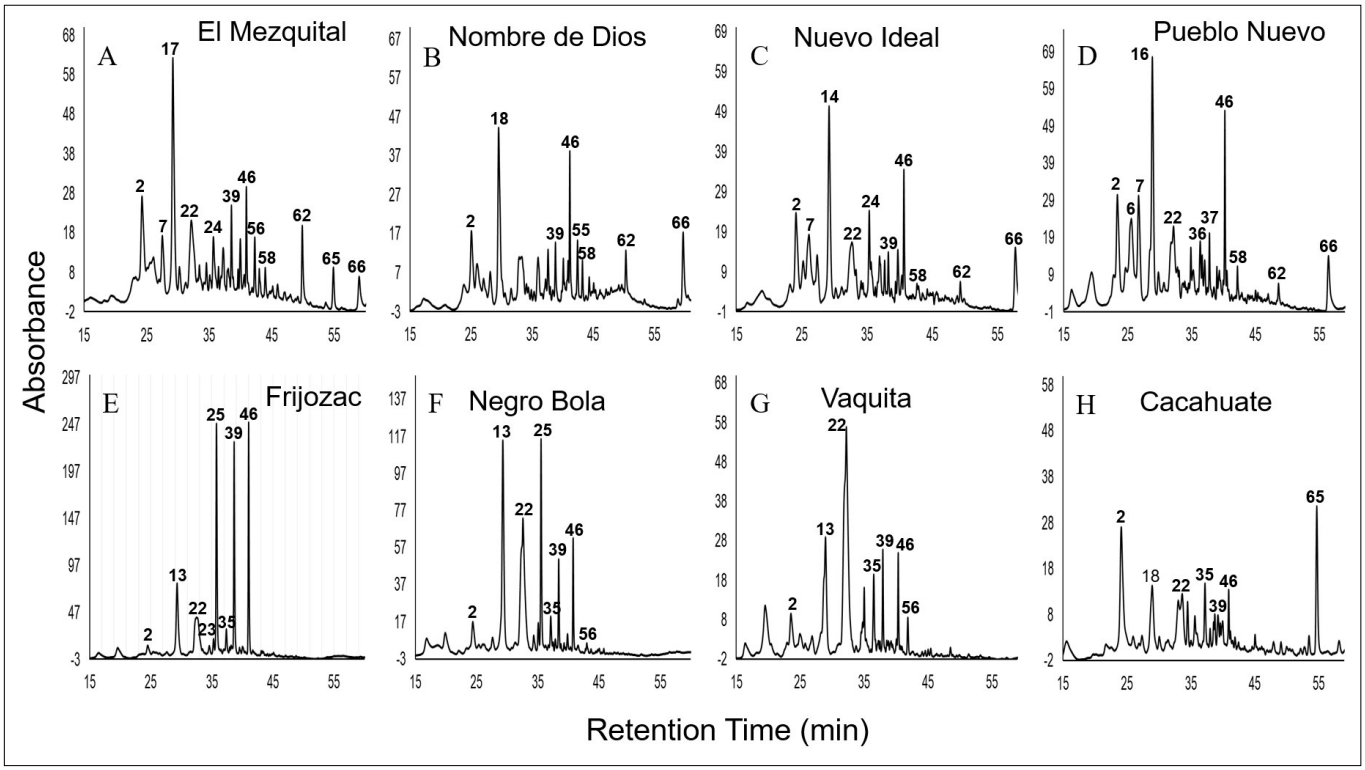
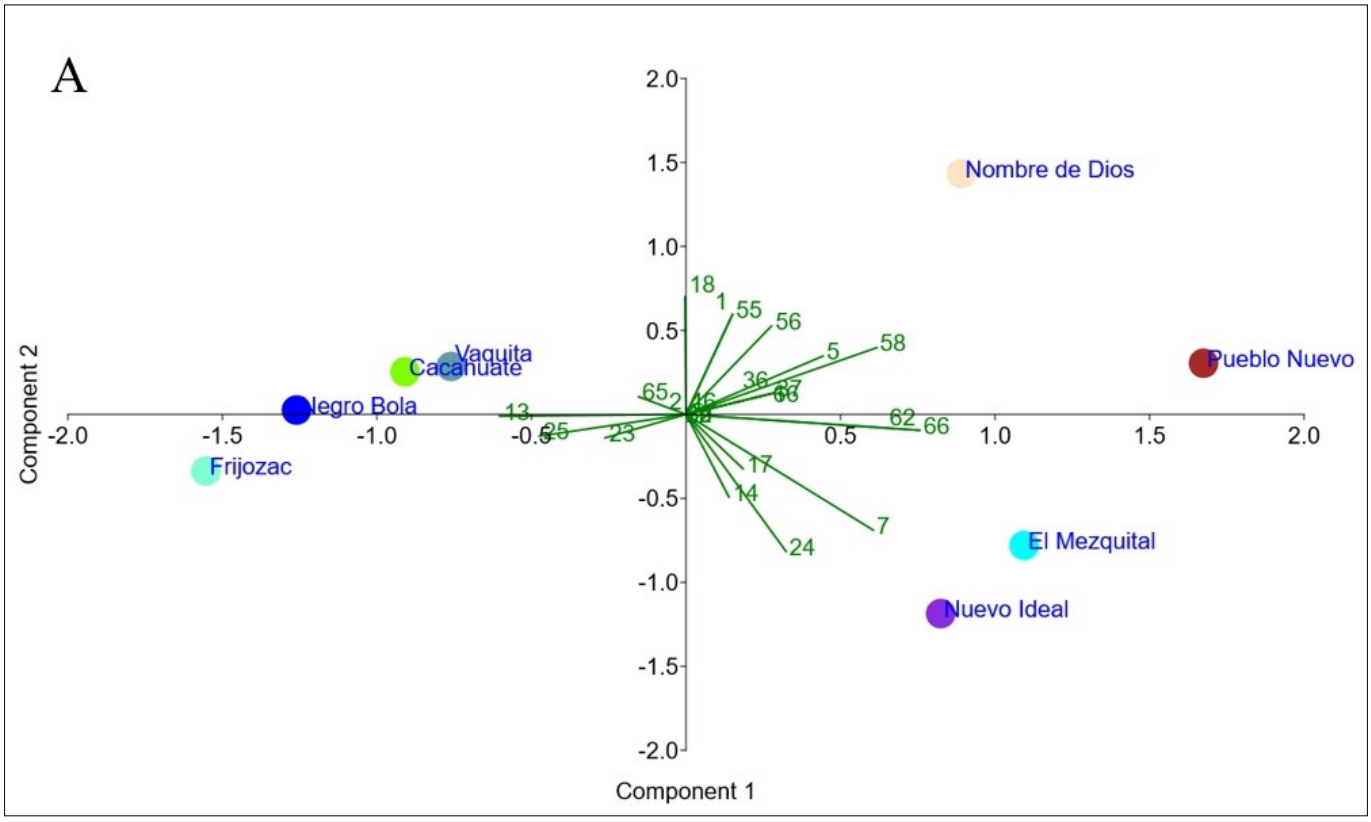


Fig. 2. HPLC chromatograms of the dry seed phenolic extracts of four wild forms (**A-D**) and four varieties (**E-H**) of common bean (*Phaseolus vulgaris*). The numbers of compounds correspond to those of Fig. S1.

analyzed. The results of the cluster analysis (Fig. 3B) revealed the formation of two major groups, one including the four wild beans and the other including the four varieties, showing the phenolic similarity between the elements in each of these two groups. However, the fact of every single sample formed a particular subgroup into each of the two main groups indicates that the seed phenolic profile of one genotype is not shared with another. The discriminatory potential of the seed phenolic profiles of common bean can even be higher, as they can differentiate plants grown under variable levels of soil moisture availability, as verified by Herrera *et al.* (34). The current results revealed the seed phenolic profiles of the common bean as important chemomarkers with agronomic and food quality implications, as it has been proposed for the phenolic profiles of other plant species (35).

Phenolic composition of soaked seeds

As for the phenolic composition of the dry seeds, that of



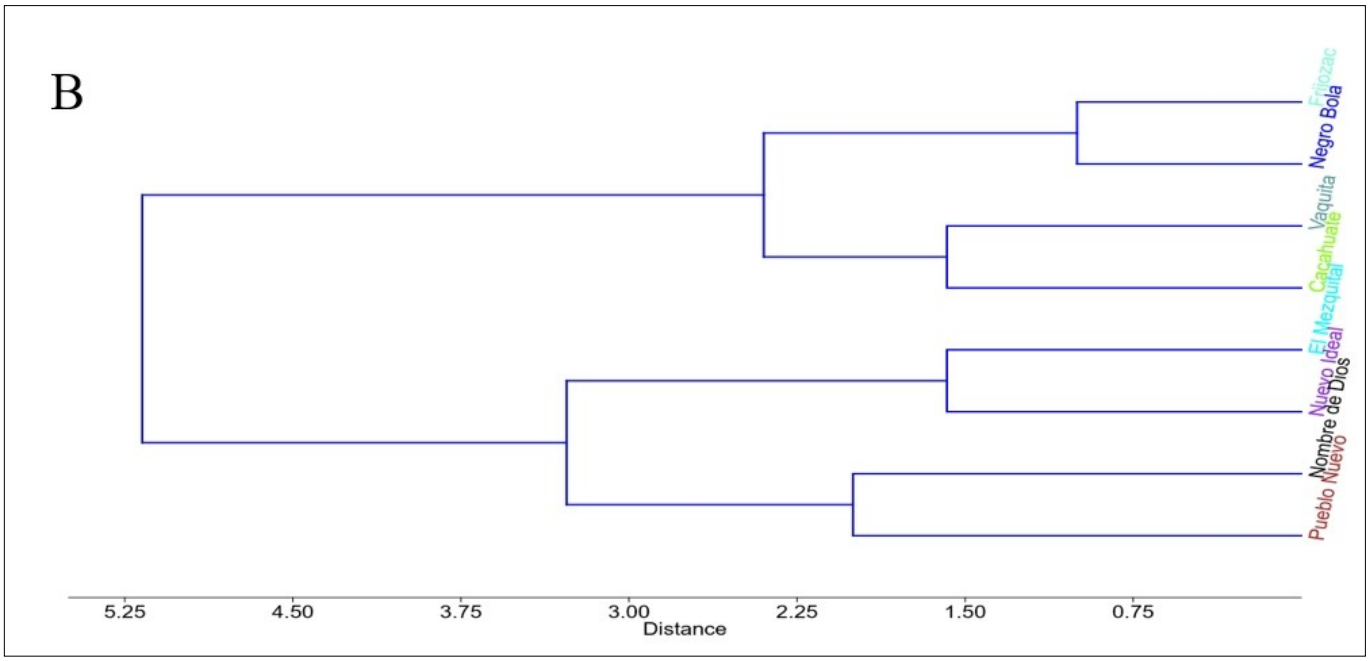
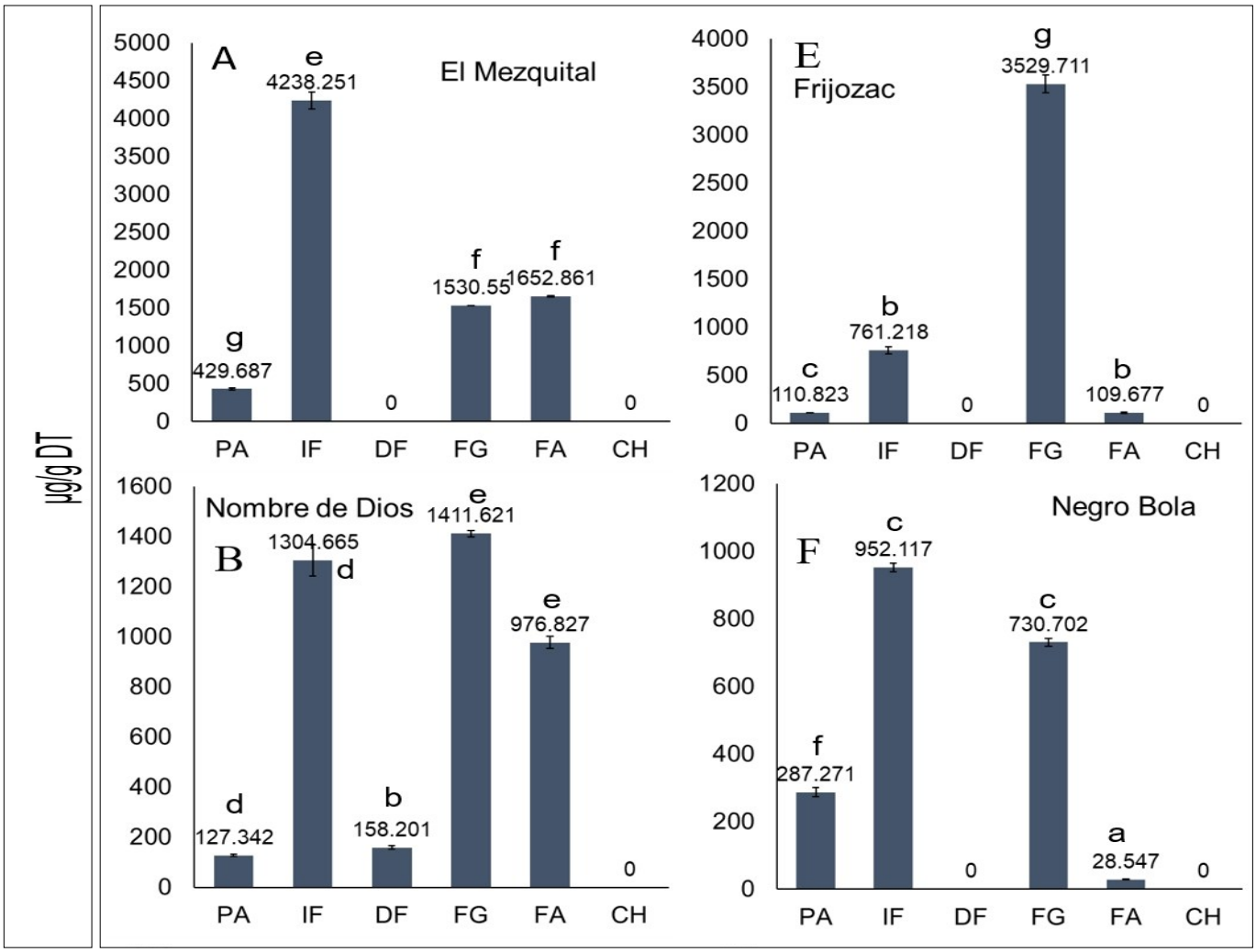


Fig. 3. Results of the PCA (**A**) and the cluster analysis (**B**) based on the phenolic composition of the dry seeds of four wild (El Mezquital, Nombre de Dios, Nuevo Ideal, and Pueblo Nuevo) and four varieties (*Frijozac*, *Negro Bola*, *Vaquita* and *Cacahuate*) of common bean (*Phaseolus vulgaris*).

the 24 h-soaked seeds was also qualitatively and quantitatively variable among the eight samples of common bean analyzed (Figs. 4, 5). Important differences in the concentration of different phenolics could be observed when compared with the dry seeds (Fig. 1), being the differences of variable magnitude ($p < 0.05$) between genotypes. Except for the samples from Nuevo Ideal and the variety *Vaquita*, phenolic acids, and isoflavones were important elements of the phenolic composition in most samples, just like in the dry seeds. Flavonol aglycones were accumulated at important levels in the soaked seeds of the wild forms, just like in their dry seeds. Contrary to what was found in the dry seeds, the soaked seeds of the two varieties of black bean (*Frijozac* and *Negro Bola*) accumulated



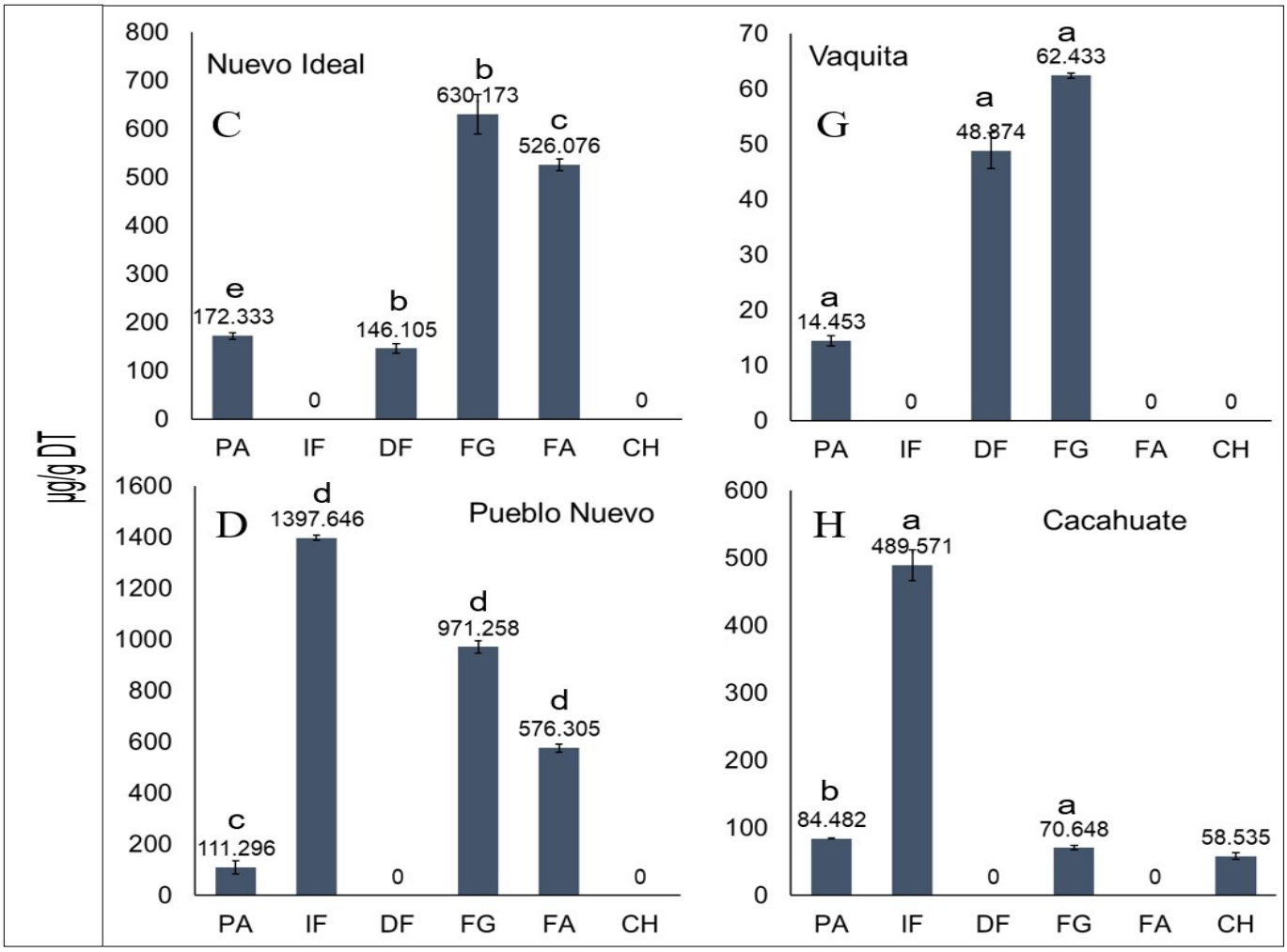


Fig. 4. Contents of phenolic acids (**PA**), isoflavones (**IF**), flavones (**F**), dihydroflavonoids (**DF**), flavonol glycosides (**FG**), and flavonol aglycones (**FA**) in the 24-h soaked seeds of four wild (**A-D**) and four varieties (**E-H**) of common bean. Different letters above bars of the same type of compounds mean significant differences ($p < 0.05$) between genotypes.

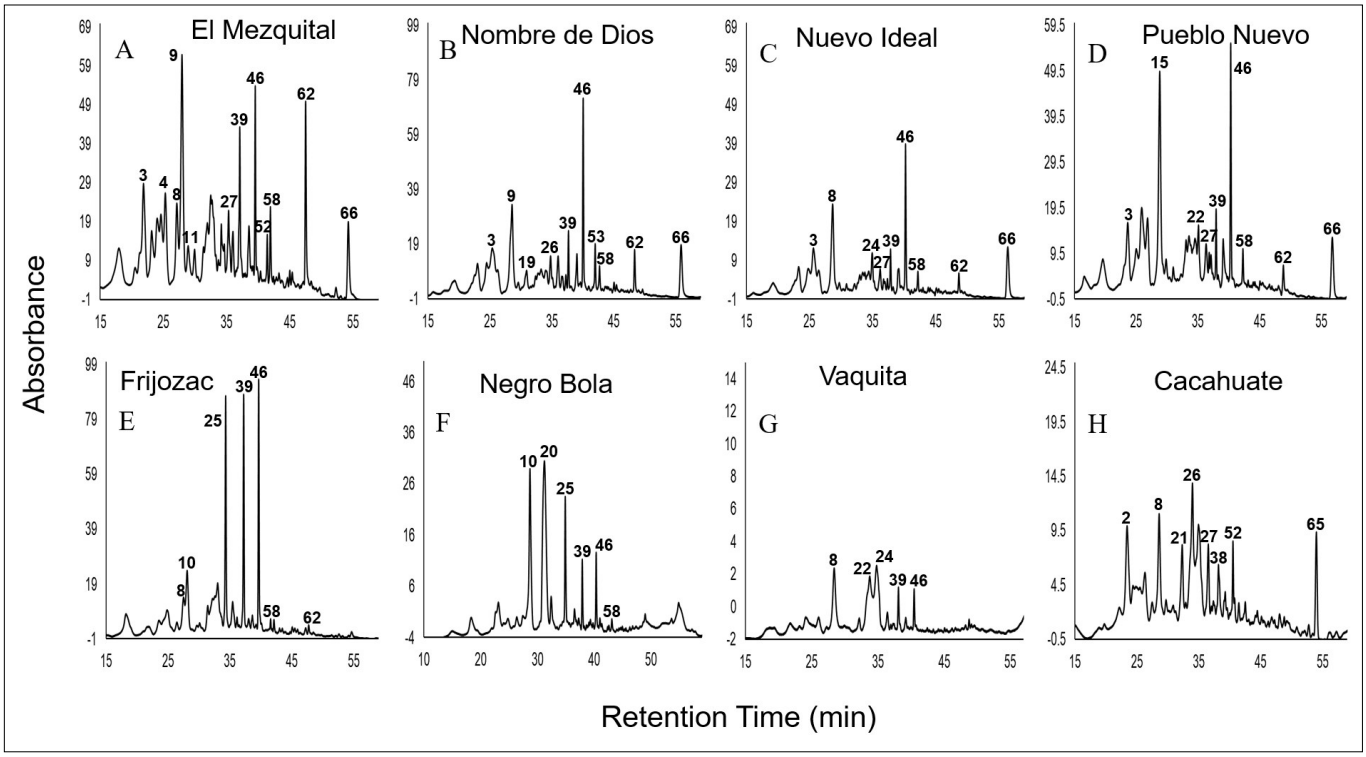


Fig. 5 HPLC chromatograms of the phenolics of the 24-h soaked seeds of four wild (**A-D**) and four varieties (**E-H**) of common bean (*Phaseolus vulgaris*). The numbers of compounds correspond to those of Fig. S1.

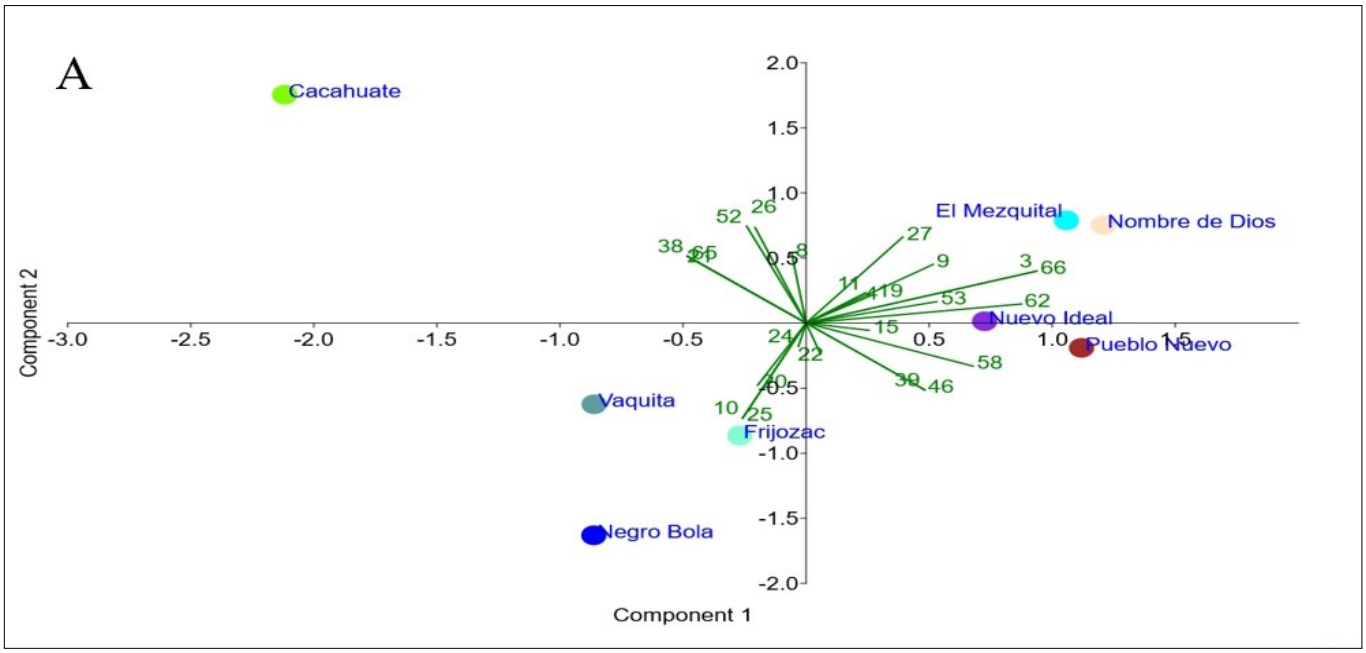
flavonol aglycones. Aglycones were also reported after soaking for the black bean *Negro San Luis* (15). Other changes observed after soaking concern flavones and dihydroflavonoids. Flavones were found in the dry seeds of the population of Nuevo Ideal (Fig. 1), but not in the soaked seeds from this same population. Dihydroflavo-

noids were found in the dry seeds from El Mezquital but not in the soaked seeds from the same population, were accumulated in the soaked seeds of Nuevo Ideal, just like in the dry seeds of this same population, and were found in the soaked seeds of Nombre de Dios and *Vaquita* but not in the dry seeds of these two populations.

The diminution of flavonol glycosides and the accumulation of flavonol aglycones in *Frijozac* and *Negro Bola* after soaking (Figs. 1, 4) suggest that imbibition for 24 h can enhance the activity of glycosidases, as Chiarello *et al.* (36) proposed that it occurs after soaking. However, the activity of glycosidases cannot explain either the increases of flavonol glycosides and the diminution of flavonol aglycones (compared with the respective dry seeds) in the soaked seeds from El Mezquital, the increase of both flavonol glycosides and flavonol aglycones in the soaked seeds from Nombre de Dios, the practically unchanged concentration of these two types of phenolics in the soaked seeds from Nuevo Ideal, or their reduction in the soaked seeds from Pueblo Nuevo (Figs. 1, 4). Our results suggest that the situation is more complex and, in addition to the improvement of glycosidase activities, both leaching and degradation of phenolics could also contribute to the changes in the phenolic compositions observed after soaking. Alonso *et al.* (25) and Guajardo-Flores *et al.* (15) proposed leaching as the cause of the changes found in the phenolic composition after soaking for the varieties *Athropurpurea* and *Negro San Luis*, respectively, while Mba *et al.* (37) proposed degradation of phenolics for five cultivars of common bean from Malawi. However, the increases here found in the concentration of some types of phenolics and the HPLC-DAD results, that will be discussed below, suggest that the activation of biosynthetic pathways of phenolic compounds also made an important contribution to the changes found in the phenolic composition of soaked bean seeds.

The comparison between the phenolic profiles of each sample of seeds soaked for 24 h (Fig. 5) with those of the respective dry seeds (Fig. 2), suggests that new compounds were synthesized, whereas some present in dry seeds were leached or degraded. For instance, compound 17 was found in the dry seeds from El Mezquital but not in their soaked seeds; compound 16 was found in the dry seeds from Pueblo Nuevo but not in their soaked seeds, and compounds 10 and 20 were found in the soaked seeds of the variety *Negro Bola* but was not previously found in their dry seeds. These changes in the phenolic composition suggest that the activation of enzymes of flavonoid synthesis could have been triggered during soaking. It has been reported that oxidative damages of DNA, RNA, and proteins gradually occur on non-germinating seeds, and during imbibition, metabolic pathways are rapidly activated to repair this damage and prepare seeds to germinate (38). The changes in the qualitative and quantitative phenolic composition found here for the soaked seeds of common bean could be associated with the preparation of seeds to germinate, as these compounds are recognized as important antioxidants (3). Phenylpropanoids and flavonoids are considered compounds involved in seed germination for several plant species (39). The increased concentrations here registered for some single phenolics during soaking cannot be explained by leaching or degradation but by synthesis and accumulation and suggest that they can play key roles in the germination of common bean seeds.

After soaking for 24 h, some genotypes accumulated unique compounds (4 and 11 in El Mezquital, 19 in Nombre de Dios, 15 in Pueblo Nuevo, 20 in *Negro Bola*, and 2, 21, 38, and 65 in *Cacahuate*) (Fig. 5). As found for the seeds, the richness of phenolic compounds was higher in the soaked seeds of the wild forms than in those of the varieties analyzed. The different compositions supported the discrimination between genotypes, being 3 (a phenolic acid), 58 (myricetin), 62 (quercetin), and 66 (kaempferol) the main responsible phenolic compounds for the discrimination between the varieties and the wild common beans analyzed (Fig. 6A). The phenolic similarity between the soaked seeds was higher among the wild forms than between any of them and the varieties analyzed, as was revealed by the cluster analysis of Fig. 6B. However, each



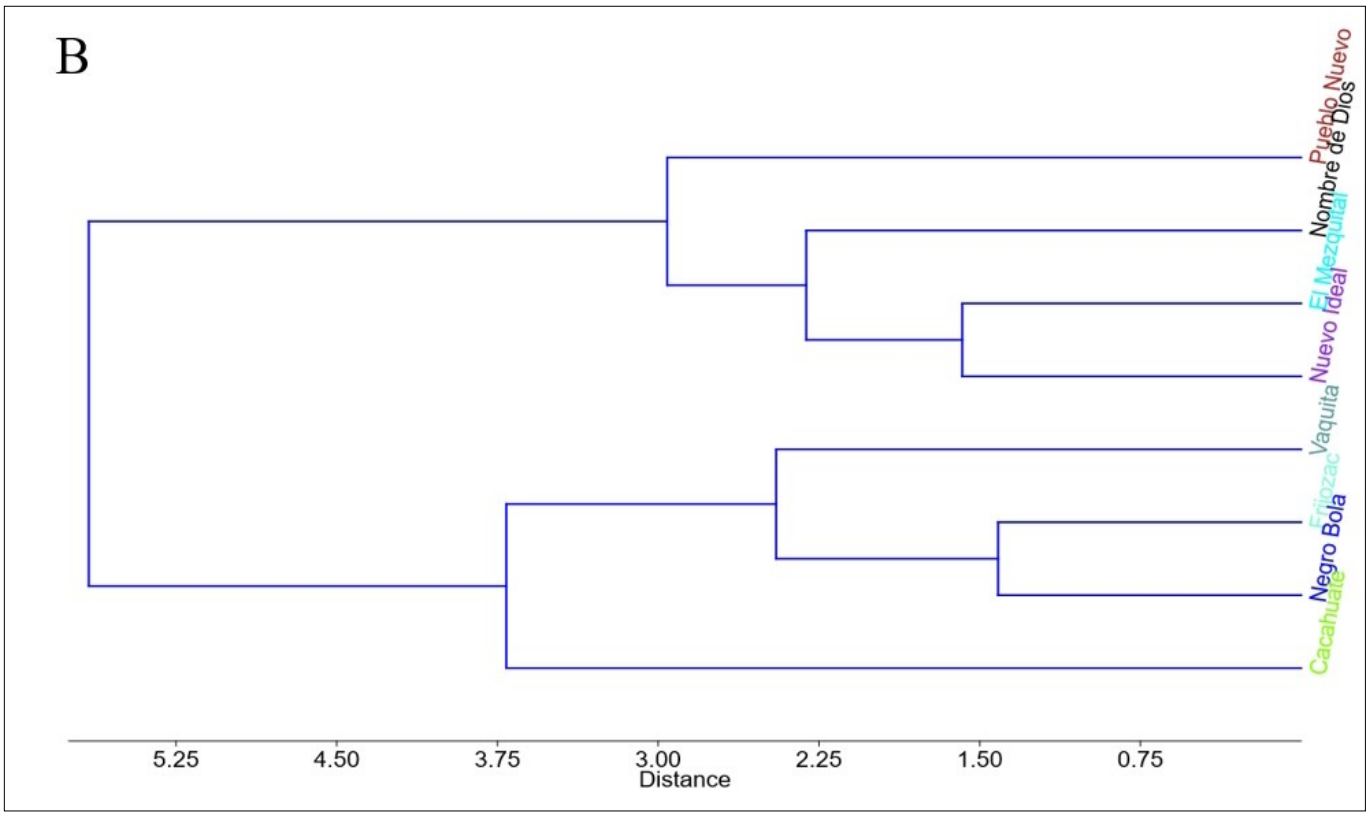
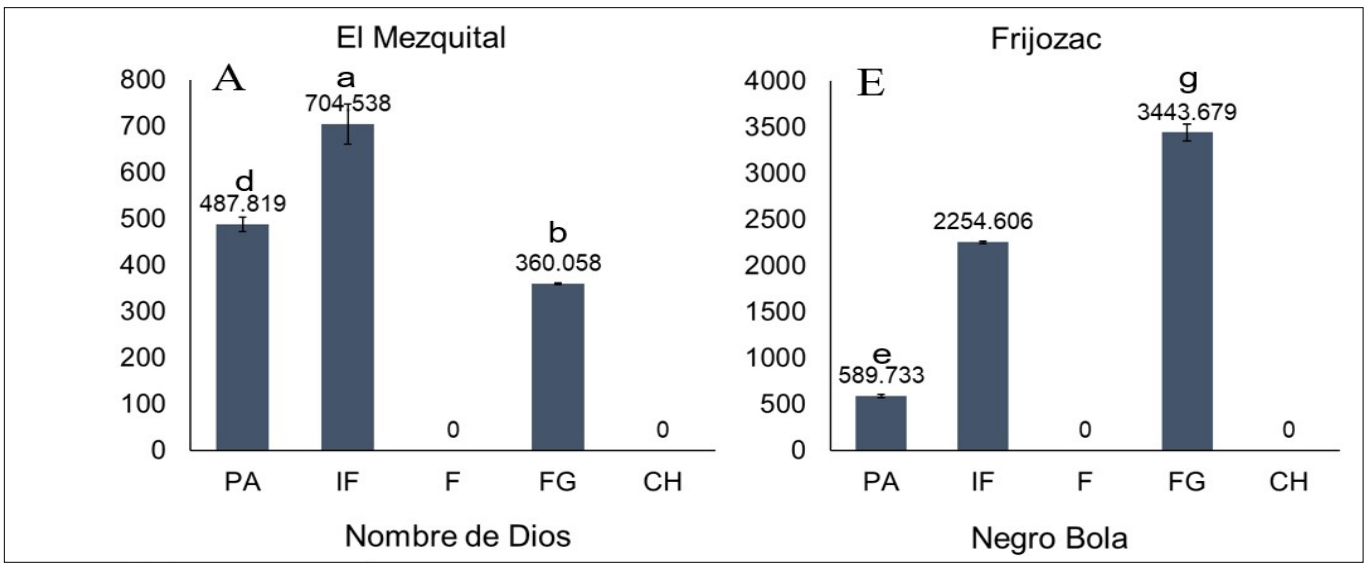


Fig. 6. Results of the PCA (**A**) and the cluster analysis (**B**) based on the phenolic composition of the soaked seeds of four wild (El Mezquital, Nombre de Dios, Nuevo Ideal, and Pueblo Nuevo) and four varieties (*Frijozac, Negro Bola*, *Vaquita* and *Cacahuate*) of common bean (*Phaseolus vulgaris*).

genotype displayed a unique phenolic composition, revealing an important potential of the phenolic profiles of 24 h-soaked seeds of common bean as chemomarkers with agronomic and food quality implications.

Phenolic composition of sprouts

Significant differences ($p < 0.05$) in the sprout phenolic composition among the eight genotypes of common bean analyzed were revealed (Fig. 7). Sprouts of the eight genotypes accumulated higher concentrations of phenolic acids than their respective soaked seeds; however, when compared with their respective dry seeds, the accumulation was variable among the eight genotypes. The accumulation of isoflavones was common to the eight genotypes analyzed; however, the magnitude of the accumulation was variable, highlighting the high level (2438.70 µg/g DT) of those of the natural population of Pueblo Nuevo. Some isoflavones were found in other varieties of common bean, like the 7-day sprouts of the cultivar *Tolosana* by López *et al*. (28). Flavones were found only in one (Pueblo Nuevo) out of the four wild sprouts, as well as in two varieties (*Negro Bola* and *Cacahuate*). The presence of flavones in sprouts contrasts with their absence in the soaked seeds (Fig. 4). The accumulation of flavonol glycosides was highly variable, highlighting the high level (3443.67 µg/g DT) of the sprouts of the variety *Frijozac*, although this value was considerably lower than in their respective seeds (9935.84 µg/g DT). The finding of flavonol glycosides in the sprouts of all genotypes of common bean analyzed is in disagreement with the results of Guajardo-Flores *et al.* (15), who reported the loss of glycosylated flavonols and the presence of their aglycones during sprouting. In fact,



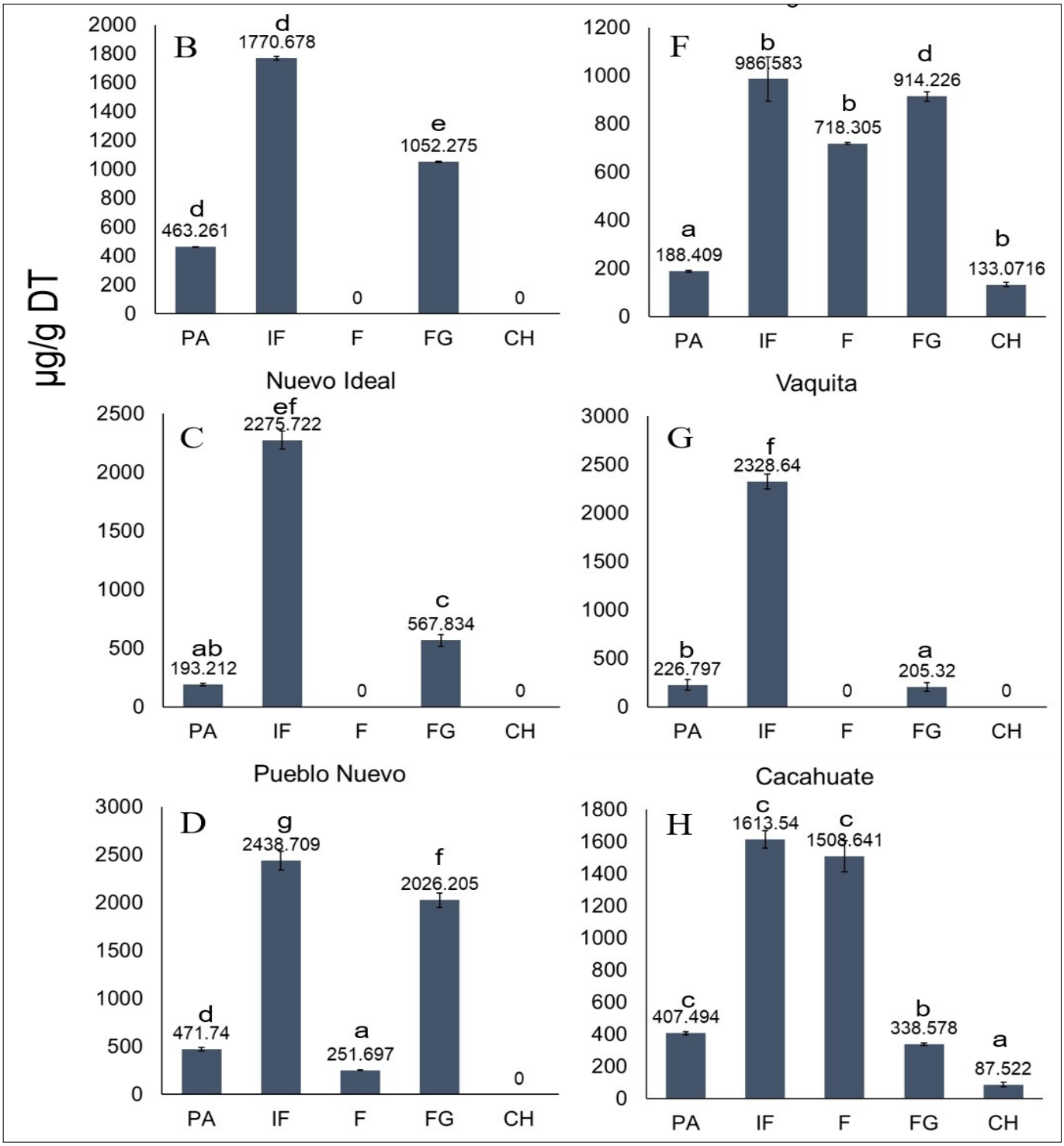


Fig. 7 Contents of phenolic acids (**PA**), isoflavones (**IF**), flavones (**F**), dihydroflavonoids (**DF**), flavonol glycosides (**FG**), and flavonol aglycones (**FA**) in the sprouts of four wild forms (**A-D**) and four varieties (**E-H**) of common bean. Different letters above bars of the same type of compounds mean significant differences ($p<0.05$) between genotypes.

no aglycone form was found in the sprouts of any of the wild forms and varieties of *P. vulgaris* here analyzed.

During sprouting, important changes in the phenolic profile of each genotype could be observed (Fig. 8), compared to the profiles of their dry seeds (Fig. 2) and soaked seeds (Fig. 5). These changes suggest that the metabolic pathways to produce phenolic compounds were improved. In sprouts, the proportion of glycosides of both kaempferol and quercetin was around 33% of total phenolics, which was around 1.73 and 1.37 times higher than in dry seeds and soaked seeds, respectively. These glycosides, besides diversifying the phenolic composition of sprouts, can play protective roles as antioxidants, as this activity has been well-recognized for them (3). In addition, these types of glycosides also can change the hydrophilicity and chemical stability of receptor molecules, improving the transportation of plant hormones (40) and contributing to the adequate hormone balance, which is needed for germination (41).

After germination, some genotypes accumulated unique compounds, such as El Mezquital (compounds 28, a phenolic acid; 33, a kaempferol-3-*O*-glycoside; and 49, a flavonol), *Frijozac* (compound 30, a flavonol), Pueblo Nuevo (compounds 41, a kaempferol glycoside, and 47,

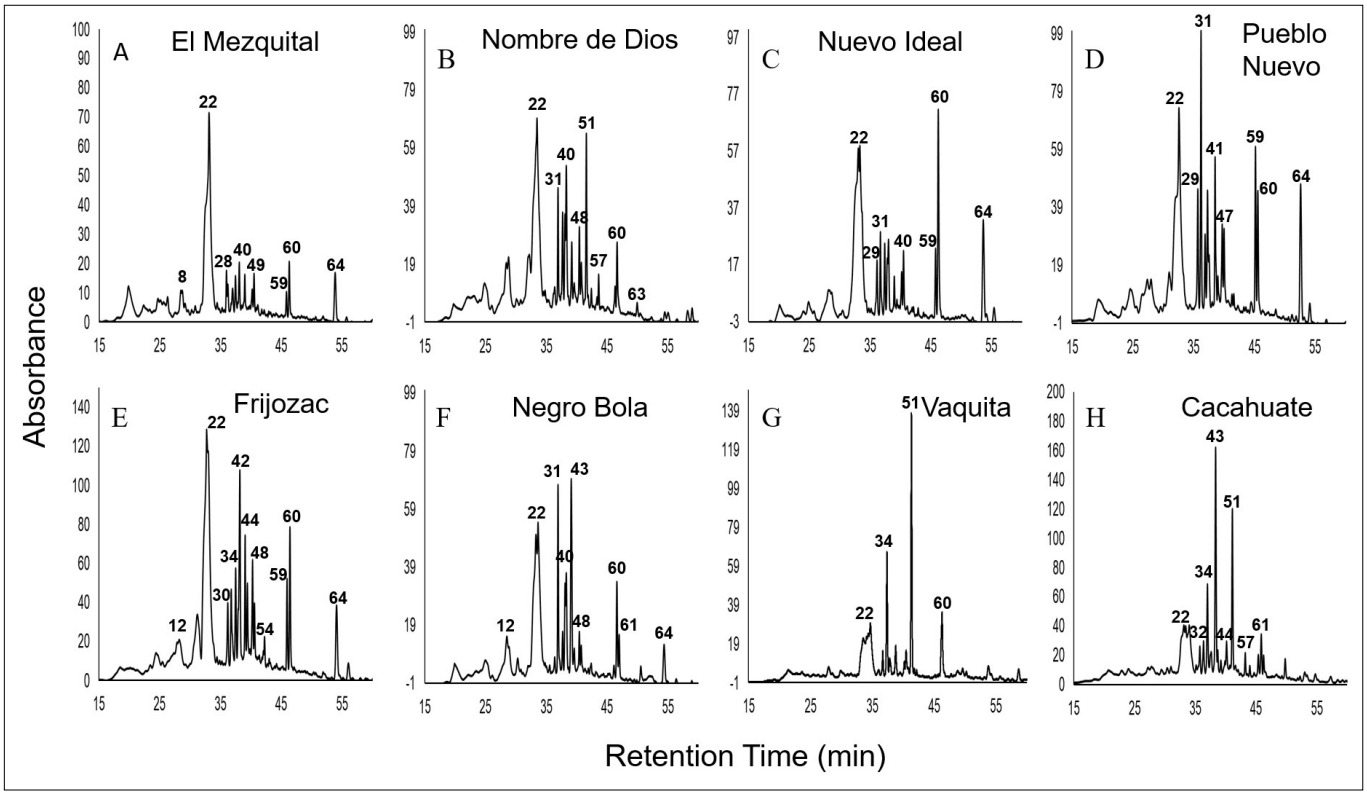
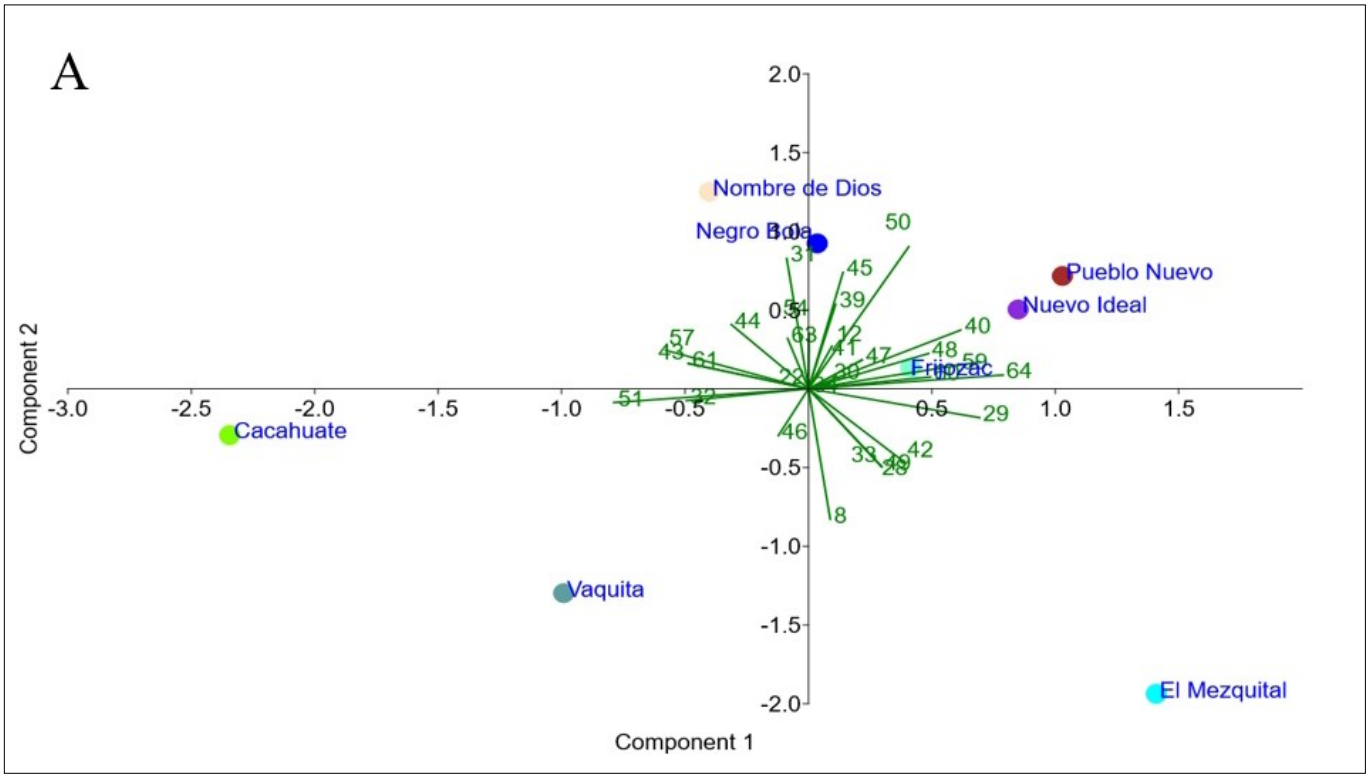


Fig. 8. HPLC chromatograms of the phenolics of the sprouts of four wild (**A-D**) and four varieties (**E-H**) of common bean (*Phaseolus vulgaris*). The numbers of compounds correspond to those of Fig. S1.

a luteolin glycoside), and Nombre de Dios (compound 63, an isoflavone). However, the HPLC phenolic profile of sprouts did not support the discrimination between wild forms and varieties of common bean, as revealed by the PCA and cluster analysis based on these profiles. The PCA results (Fig. 9A) suggested that compounds 8, 29, 40, 42, 50, and 64 were the main ones responsible for the discrimination between the sprouts of El Mezquital, *Frijozac*, Nuevo Ideal, and Pueblo Nuevo from those of Nombre de Dios, *Negro Bola*, *Vaquita* and *Cacahuate*. The results of the cluster analysis (Fig. 9B) suggested that domestication has not impacted the biosynthetic pathways of phenolic compounds of germinating seeds of common bean to such a degree that the phenolic composition can discriminate between wild or cultivated sprouts. However, the particular phenolic composition was able to discriminate between the sprouts of each genotype, as each of them formed a single clade. These results revealed that the sprout phenolic profiles of common bean are important chemomarkers to develop quality control tools with importance in agronomy and the food industry. The accurate registration of the variation in the phenolic composition



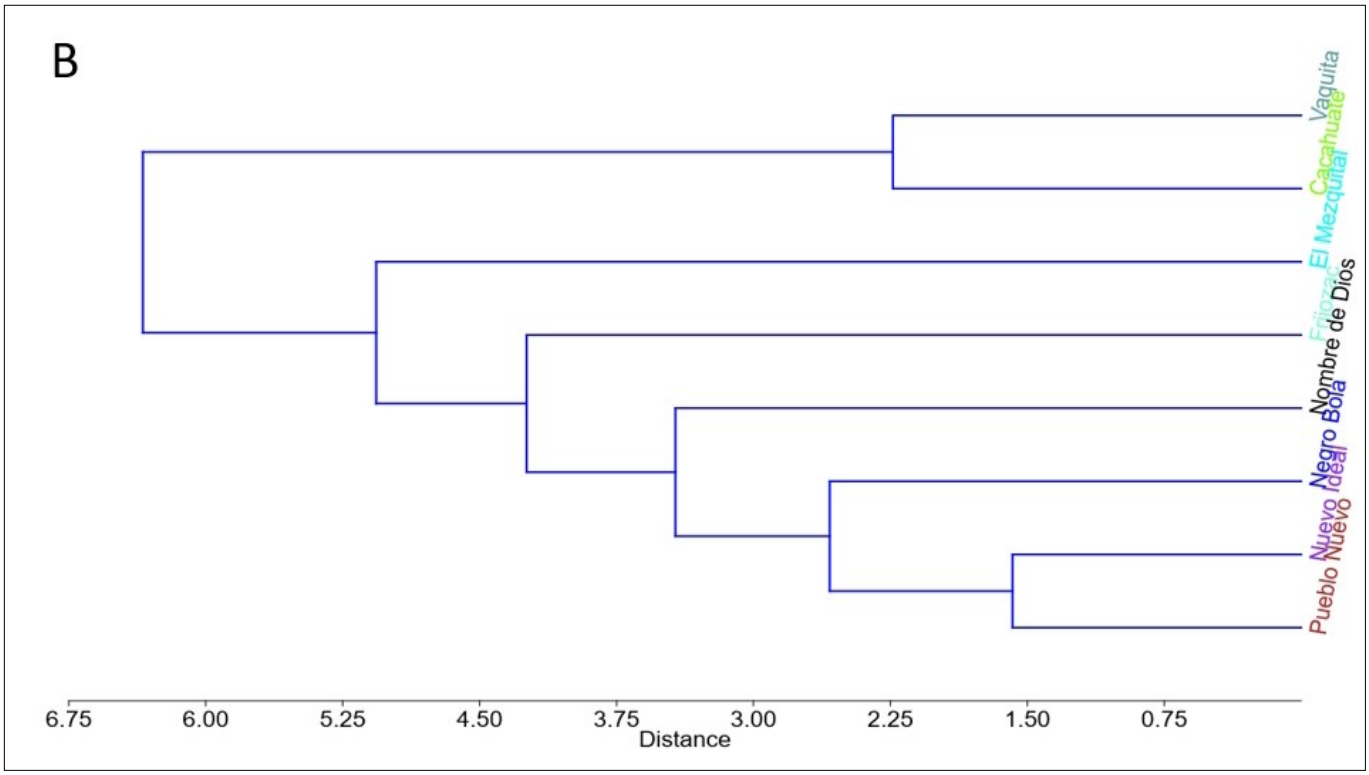


Fig. 9. Results of the PCA (**A**) and the cluster (**B**) analysis based on the phenolic composition of the sprouts of four wild forms (El Mezquital, Nombre de Dios, Nuevo Ideal, and Pueblo Nuevo) and four varieties (*Frijozac*, *Negro Bola*, *Vaquita* and *Cacahuate*) of common bean (*Phaseolus vulgaris*).

during imbibition and germination of individual genotypes of *P. vulgaris* is relevant, as chemical changes determine variations in the organoleptic and functional properties of plants (42).

Inhibition of α-glucosidase and α-amylase activities

The dry seeds, soaked seeds, and sprouts of the different genotypes of common bean analyzed exhibited different capacities of inhibition of α-glucosidase (Table 3). Important and variable activity as inhibitors of α-glucosidase were also reported for the seeds of 15 cultivars of common bean (between 20.7 and 82%) by Mojica *et al.* (14). Except for the variety *Vaquita*, soaking and germination significantly ($p < 0.05$) improved the inhibitory potential of seeds, the improvement being variable in the different genotypes (Table 3). Similar results were found by Donkor *et al*. (43) for germinated seeds of sorghum and rye, which exhibited higher potentials as α-glucosidase inhibitors than their respective seeds. It has been proposed that the α-glucosidase inhibitory potential of flavonoids results from its ability to bind amino acid residues in or near the active site, altering the active center of the enzyme (44) and that isoflavones, which have the B-ring attached to the 3 position, as well as 4'-OH flavonoids (such as kaempferol and quercetin derivatives) show high α-glucosidase inhibitory activity (45). We found that the soaked seeds and sprouts accumulated a higher proportion of both isoflavones (about 20% and 36%, respectively) and glycoside derivatives of both kaempferol and quercetin (32% and 33%, respectively) than seeds (12% and 23%, respectively), what could have contributed to the improvement of the inhibitory activity here found for the soaked seeds and sprouts of common bean.

The inhibition of α-glucosidase has been considered a strategy for the treatment of diabetes since it represents a mechanism to diminish the hyperglycemic state (46). Some authors have found an association between the concentration of phenolics and the inhibitory potential of α-glucosidase and other bioactivities of plant extracts (47). However, for the samples of common bean analyzed, the association between these two parameters was not apparent, since the sprouts, even having lower contents of phe-

Table 3. α-glucosidase inhibitions (%) of dry seeds, 24 h-soaked seeds, and sprouts of four wild forms and four varieties of common bean.

Sample	Dry seeds	Soaked seeds	Sprouts
El Mezquital (W)	73.56±0.40 bc A	93.79±0.65 ab B	98.13±1.73 b C
Frijozac (V)	86.23±1.19 de A	94.80±1.89 abc B	97.85±1.03 b B
Nombre de Dios (W)	66.37±1.89 ab A	98.75±0.61 d C	95.37±0.19 ab B
Negro Bola (V)	90.79±2.38 de A	98.98±0.77 d B	91.04±2.64 a A
Nuevo Ideal (W)	56.63±4.80 a A	96.81±1.44 bcd B	97.90±2.21 b B
Pueblo Nuevo (W)	81.98±3.40 cd A	99.55±2.07 cd B	98.24±0.53 b B
Vaquita (V)	95.38±3.79 e A	96.43±0.66 bcd A	97.48±3.00 b A
Cacahuate (V)	80.77±6.60 cd A	91.91±2.06 a B	95.42±2.42 ab B

nolic compounds than both dry seeds and soaked seeds (Table 2), showed greater α-glucosidase inhibitory activity (Table 3). Our results suggest that the structure of the flavonoids and phenolic acids found in the extracts of the sprouts analyzed could have done an important contribution to their α-glucosidase inhibition, in agreement with Tadera *et al.* (45) and Sun and Miao (48), who reported the relevance of the structure, mainly hydroxy and methoxy groups, in conferring the inhibitory activity to phenolics.

Conclusion

Phaseolus vulgaris has a complex and diverse phenolic composition, which varies among genotypes in any state, either dry seeds, soaked seeds, or sprouts. The presence of the flavonol aglycones myricetin, quercetin, and kaempferol was characteristic of the wild forms. Imbibition for 24 h, not only caused the leaching and degradation of phenolic compounds but also triggered the synthesis of some phenolic compounds. In soaked and sprouting seeds, glycosylation of kaempferol and quercetin, as well as the diversification of isoflavones, could have contributed to improving the α-glucosidase inhibition potential. The genotype-specific phenolic profiles, obtained by HPLC-DAD, of each state (seeds, soaked seeds, and sprouts) represent chemomarkers for genotypes of the species, which could support the development of chemical fingerprinting with implications in agronomy and food quality.

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Authors contributions

NAA conceived and designed the study, participated in the collections of plant material, performed the statistical analysis, and analysed and interpreted data. LWC, JAAR, ACR participated in the collections of plant material and carried out the total phenolics determinations. EADA, AIAC carried out the HPLC-DAD analysis. IRM, NNJ carried out the enzymatic inhibition determinations. All authors made a critical revision, providing intellectual content. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

Supplementary data

Fig. S1. UV spectra of the phenolic compounds found in the seeds, soaked seeds and sprouts of eight wild forms and eight varieties of common bean (*Phaseolus vulgaris*). S: Accumulated in seeds, I: Accumulated in soaked seeds, G: Accumulated in sprouts.

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